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Covid-19 and diabetes, volume II

Edited by
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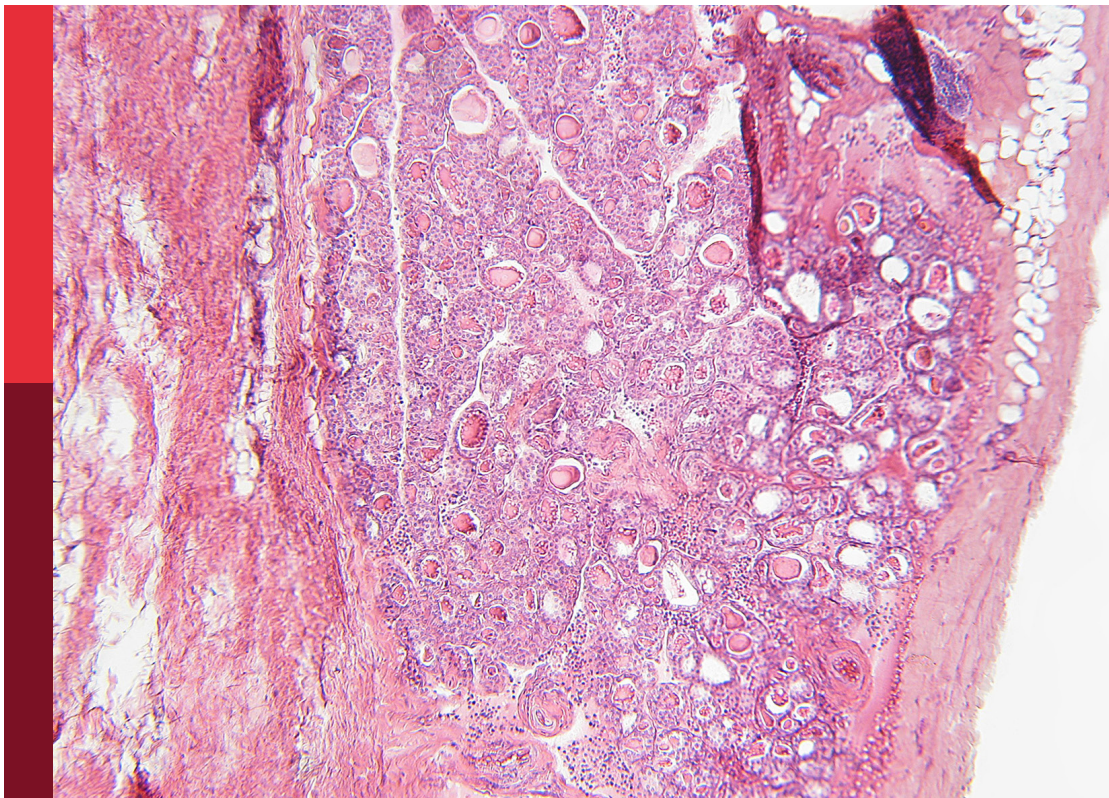

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Covid-19 and diabetes, volume II

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Editorial: COVID-19 and diabetes, volume II

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Editorial on the Research Topic
COVID-19 and diabetes, volume II

People with type 1 or type 2 diabetes mellitus have been highly affected by COVID-19 as they are at a higher risk of developing COVID-19-related complications. The relationship between diabetes mellitus and COVID-19 is complex and multidirectional, nonetheless our efforts to better understand its pathophysiology is evolving at a rapid pace. Individuals with diabetes mellitus are known to be more susceptible to contracting severe COVID-19 and SARS-CoV-2 infection. However, there is also evidence that COVID-19 may influence the pathophysiology of diabetes, affecting blood glucose control not only in those already predisposed or living with diabetes but also in those without the disease.

Diabetes mellitus is one of the main comorbidities contributing to a worse COVID-19 prognosis and outcome. Stidsen et al. showed that the risk of hospitalization due to COVID-19 was higher in patients with diabetes compared to non-diabetics during pandemic waves in Denmark. Understanding the pathophysiological relationship between severe COVID-19 outcome and diabetes remains to be a progressive area of research. A review by Landstra et al. suggested that the poor prognosis of COVID-19 in diabetic patients can be linked to the comorbidities and risk factors normally present in diabetic patients. Al-Sayyar et al. adapted a comparative approach between different respiratory tract infection in people with diabetes including Tuberculosis and Influenza to understand the susceptibility of severe COVID-19 in people with diabetes. They reviewed and proposed that several mechanisms which might be responsible for increased susceptibility of severe COVID-19 outcomes in people with diabetes are shared with previously described respiratory infections including Influenza A virus and Mycobacterium tuberculosis.

Xie et al. reviewed current understanding of the complex relationship between diabetes and COVID-19. They provided an update on the two-way interactions between diabetes and COVID-19 and treatment strategies for COVID-19 severe cases from the perspective of ACE2. This bidirectional relationship was investigated by Pelle et al. where they showed that the expression of ACE2 receptors on pancreatic beta cells allow the virus to target the pancreas directly which might lead to hyperglycemia in patients with and without diabetes. This topic was also studied in a review by Shao et al. where, in additional to the direct targeting of the pancreas by the virus, the presence of an abnormal immune response, including an inflammatory storm and imbalances in T cells, could be the cause. Computational and machine learning approaches were utilized to gain insight into the complex relationship between diabetes and severe COVID-19 outcomes. Bassani et al.

performed *in silico* evaluation of the possible impact Omicron variant of SARS-CoV-2 in people with diabetes. The adapted computational approach suggested a different clinical relevance of the Omicron variant in people with diabetes. They hypothesized that the affinity between the viral Spike protein and the human receptor ACE2 is higher for Omicron variant when compared to other circulating variants, at the time of the study, in both normal and hyperglycemic environments. A multicenter study in Italy and Spain by Mayneris-Perxachs et al. utilized logistic regression analyses and machine learning algorithms to analyze confounding factors influencing the prognostic value of hyperglycemia related to severe COVID-19 outcomes and found that blood hemoglobin substantially modulates the well-established influence of hyperglycemia on severe COVID-19 outcomes.

Much of the efforts were directed toward better understanding of the impact of antidiabetic agents on COVID-19 patients with diabetes. Metformin is the first-line medication for the management of diabetes. Miao et al. performed a multiracial, multiethnic, urban observational study and found no significance difference in-hospital mortality or length of stay due to COVID-19 in patients group on Metformin. On the other hand, Wong et al. showed that Metformin use was associated with lower mortality rates and lower incidence of hyper-inflammatory syndrome among COVID-19 patients with diabetes. This result agrees with a Meta-Analysis by Kan et al. showed possible association between Metformin use and reduced mortality in COVID-19 patients with diabetes. In a commentary on the previous study, Zhao et al. showed significant association of SGLT2i, DPP4i and GLP1RA use with reduction in COVID-19 mortality risk in people with diabetes. A Bayesian Network Meta-Analysis by Chen et al. assessed the impact of different antidiabetic agents on the infection outcome among COVID-19 patients with diabetes. They found that antidiabetic drugs including Metformin, DPP4i, SGLT2i, and GLP1RA were associated with reduced COVID-19 related mortality. However, the use of insulin was associated with unfavorable outcome. This was also implicated in a systematic review and meta-analysis by Yang et al. where a meta-analysis on 18 studies revealed that Insulin treatment may be associated with increased adverse outcomes in Patients with COVID-19 and diabetes. In a randomized clinical trial of hospitalized COVID-19 patients with diabetes from Palestine, Abuhasira et al. showed that the use of linagliptin did not show any clinical improvement when compared to patients receiving standard care. Additional research on a larger scale is necessary to verify and examine the specific mechanism underlining the effects of antidiabetic drugs in greater detail in order to improve the effectiveness of care given to COVID-19 patients with diabetes. It is important for doctors to consider the risks and benefits of different medications for diabetes, and to tailor treatment plans to the individual needs of each patient, taking into account the risk of hypoglycemia, acidosis, and gastrointestinal symptoms. The goal is to achieve optimal glycemic control in patients with SARS-CoV-2 infection and improve the outcome.

One of the explored strategies to reduce the risk of severe COVID-19 outcomes in people with diabetes was early identification and strong glycemic control. Early identification of people at risk of COVID-19 severe outcomes is very important to develop effective therapeutic approaches. Moin et al. performed detailed proteomic analysis on patients with differing severity of COVID-19 and identified potential coagulopathy proteins that may predict COVID-19 severe outcomes in people with diabetes. Bajpeyi et al. highlighted that unmanaged diabetes and blood glucose in patients encountering COVID-19 increased the risk of disease severity and prolonged hospitalization. This agrees with a study by Wang et al. where they showed that COVID-19 patients with unmanaged glucose levels, due to undiagnosed diabetes or new onset diabetes due to COVID-19 infection, increased the risk of severe outcomes when compared to people with diabetes and managed glucose levels. A study by Llanera et al. showed that CRP and age are useful predictors on severe outcomes in COVID-19 patients with diabetes. Wang et al. explored the use of other markers not linked directly to diabetes and showed that patients with severe COVID-19 outcomes had lower levels of HDL cholesterol and higher NHR at the time of admission to the hospital. These factors were also found to be associated with severe outcomes in COVID-19 patients with diabetes. Moin et al. hypothesized that levels of proteins related to platelets may be affected in people with type 2 diabetes, potentially making them more prone to thromboembolic responses to infections like COVID-19. In their case control study, they showed that people with T2D were at a higher risk for thromboembolic events due to platelet hyperactivation. If they were also infected with COVID-19, this risk may be further increased, and they may be more likely to have a severe course of the disease. In another study by same group, they showed that strict control of fasting blood glucose might lead to hypoglycemia which in turn increases the levels of sNRP1 and its ligands (VEGF and SEMA3A) and put patient with T2D or obesity at higher risk of COVID-19 infection.

One potential factor that could contribute to the differences in COVID-19 severity among individuals is host genetic background. In a Spanish pilot study, Iñiguez et al. examined whether variations in the angiotensin converting enzyme (ACE) gene, which plays a crucial role in regulating the renin-aldosterone-angiotensin system (RAAS), could be associated with COVID-19 severity. Their genetic analysis of 128 COVID-19 cases reveled hat having the *ACE2* variants rs4341 and rs4343 may increase the risk of a worse outcome from COVID-19. These *ACE2* genetic variations, may be useful indicators of the severity of COVID-19 in patients with underlying conditions such as hypertension, dyslipidemia, or diabetes. Understanding this genetic information could help in the more precise treatment of hospitalized patients infected with SARS-CoV-2.

COVID-19 has been linked to the development of autoimmune conditions. Multiple previous reports have highlighted increased prevalence of new onset of type 1 diabetes during the COVID-19 pandemic but the exact pathophysiological pathways is not entirely understood. Mishra et al. presented a case where COVID-19 is identified as a potential trigger for diabetic ketoacidosis and newly detected pancreatic autoantibodies. Cases of ketoacidosis were also identified following COVID-19 vaccination in patients with T1D. Yakou et al. presented case series of severe ketoacidosis after COVID-19 vaccination in a type 1 diabetes patients on insulin and SGLT-2 inhibitor treatments. They proposed that T1D patients who are receiving intensive insulin therapy and treatment with sodium-glucose transporter medications are at a high risk for developing ketoacidosis, hence, careful consideration should be given to administering the vaccine to this group of patients.

The COVID-19 pandemic and the subsequent lockdowns have had a significant impact on the lifestyles of people all over the world, particularly on those with chronic conditions such as type 1 diabetes (T1D) and type 2 diabetes (T2D). In a study from Saudi Arabia, Al-Daghri et al. showed that lockdowns implemented as a response to the COVID-19 pandemic had a negative impact on the lifestyle and daily routines of individuals with diabetes in Saudi Arabia. The negative impact included psychological problems such as symptoms of depression and physical impact including decreased activity. Thy suggested that novel strategies are needed to support the health and well-being of people with T1D and T2D during future pandemics and lockdowns, and promote healthy lifestyle adaptations in this high-risk group. In another study from Saudi Arabia, Alaqeel et al. conducted a multicenter retrospective cohort study and found that lockdown in Saudi Arabia has had a major effect on children with T1DM and has led to a higher occurrence of DKA, including in children with newly diagnosed T1DM. This may be due to a delay in seeking medical attention. An opinion by Muñoz et al. highlighted that the COVID-19 pandemic has caused chronic uncertainty and psychosocial distress, which can negatively affect the ability of young people with type 2 diabetes (T2D) to manage their condition and access prescribed treatments. They recommended the development of multidisciplinary treatment plans to encounter the psychological burden of the pandemic. On the other hand, Minuto et al. reported that the lockdown in Italy caused a positive and unexpected change in blood sugar control for young patients with type 1 diabetes (T1D). The changes in lifestyle, including a healthier and less stressful environment and the continuation of physical activity, resulted in improved blood sugar control that was proportionate to the patient's age. These conflicting results could be caused by social or geographic differences among the study participants. These differences could include things like the level of access to healthcare and medical treatment, cultural and societal factors, and environmental conditions.

The COVID-19 pandemic has highlighted the impact of comorbidities like diabetes on the severity of the disease. Diabetes has been shown to lead to worse COVID-19 outcomes and increased mortality. Additionally, COVID-19 infection can lead to new-onset diabetes or worsen glycemic control in people with pre-existing diabetes due to pancreatic damage caused by the virus, the body's stress response to infection, and the use of diabetogenic drugs like corticosteroids in severe cases. Measures taken to curb the spread of the pandemic, like lockdowns, can also negatively impact individuals with diabetes by limiting access to medical care, exercise opportunities and psychological burden.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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ORIGINAL RESEARCH
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Platelet Protein-Related Abnormalities in Response to Acute Hypoglycemia in Type 2 Diabetes

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Introduction: Patients with severe COVID-19 infections have coagulation abnormalities indicative of a hypercoagulable state, with thromboembolic complications and increased mortality. Platelets are recognized as mediators of inflammation, releasing proinflammatory and prothrombotic factors, and are hyperactivated in COVID-19 infected patients. Activated platelets have also been reported in type 2 diabetes (T2D) patients, putting these patients at higher risk for thromboembolic complications of COVID-19 infection.

Methods: A case-control study of T2D (n=33) and control subjects (n=30) who underwent a hyperinsulinemic clamp to induce normoglycemia in T2D subjects: T2D: baseline glucose 7.5 ± 0.3mmol/l (135.1 ± 5.4mg/dl), reduced to 4.5 ± 0.07mmol/l (81 ± 1.2mg/dl) with 1-hour clamp; Controls: maintained at 5.1 ± 0.1mmol/l (91.9 ± 1.8mg/dl). Slow Off-rate Modified Aptamer (SOMA)-scan plasma protein measurement was used to determine a panel of platelet proteins.

Results: Prothrombotic platelet proteins were elevated in T2D versus controls: platelet factor 4 (PF4, $p<0.05$); platelet glycoprotein VI (PGVI $p<0.05$); P-selectin ($p<0.01$) and plasminogen activator inhibitor I (PAI-1, $p<0.01$). In addition, the antithrombotic platelet-related proteins, plasmin ($p<0.05$) and heparin cofactor II (HCFII, $p<0.05$), were increased in T2D. Normalization of glucose in the T2D cohort had no effect on platelet protein levels.

Conclusion: T2D patients have platelet hyperactivation, placing them at higher risk for thromboembolic events. When infected with COVID-19, this risk may be compounded, and their propensity for a more severe COVID-19 disease course increased.

Clinical Trial Registration: https://clinicaltrials.gov/ct2/show/NCT03102801, identifier NCT03102801.

Keywords: type 2 diabetes, obesity, platelet, COVID-19, SARS-CoV-2

INTRODUCTION

Patients with severe COVID-19 infections have coagulation abnormalities indicative of a hypercoagulable state, with thromboembolic complications and increased mortality (1). What underlies this hypercoagulable state has not been fully elucidated, although it is likely due to a combination of endothelial injury (direct invasion by SARS-CoV-2), the inflammatory response mediated largely by cytokines, patient immobility and altered circulating clotting proteins (decreased antithrombin combined with increased prothrombotic proteins like D-dimer, factor VIII, fibrinogen and fibrin degeneration products) (1).

Platelets are essential for hemostasis, but they are also involved in detrimental sequelae such as myocardial infarction, stroke and deep vein thrombosis (2–5). Type 2 diabetes (T2D) patients have activated platelets that contribute to the increased cardiovascular disease in T2D (6, 7). Platelets are known mediators of inflammation, and release proinflammatory and prothrombotic molecules such as CD40 ligand and thromboxane A2 (8). Oxidative stress markers are generated in the euglycemic state (9), but it is recognized that glucose variability and in particular hyperglycemia may increase inflammatory markers and oxidative stress through effects on the polyol and hexosamine pathways and increased protein kinase C activation *via* diacyglycerol (10–12).

Thrombocytopenia has emerged as a complication of COVID-19 infection; this, together with increased D-dimer levels, is likely due to hyperactivation of platelets and the coagulation cascade (1, 13).

We hypothesized that circulating levels of platelet-related proteins would be altered in T2D, priming these patients for an upregulated thromboembolic response to infection, and that euglycemia would lead to normalization of these platelet-related proteins. We therefore determined levels of a panel of platelet-related proteins in plasma in T2D compared to control subjects, and subsequently compared these proteins in T2D at baseline to those when euglycemia was re-established.

METHODS

A case-control study of T2D (n=33) and control subjects (n=30), approved by Yorkshire and Humber Research Ethics Committee, was performed. The hyperinsulinemic clamp was performed as previously described (14). Subjects were matched for age (p=ns) though BMI was higher in T2D (p<0.0001). For T2D patient inclusion, only metformin as anti-diabetic therapy was allowed.

The duration of diabetes in the T2D cohort was 4.2 ± 0.4 years. All participants were Caucasian who fasted 10-hours before venipuncture. In the T2D cohort, the baseline glucose of 7.5 ± 0.3mmol/l (135.1 ± 5.4mg/dl) was reduced to 4.5 ± 0.07mmol/l (81 ± 1.2mg/dl) with the 1-hour clamp whilst the control subjects were maintained at 5.1 ± 0.1mmol/l (91.9 ± 1.8mg/dl).

Slow Off-rate Modified Aptamer (SOMA)-scan plasma protein measurement (15) was used to determine platelet-related proteins: platelet factor 4 (PF4), platelet glycoprotein VI (PGVI), P-selectin, plasminogen activator inhibitor I (PAI-1), plasmin and heparin cofactor II protein concentrations, expressed as relative fluorescent units (RFU).

Statistics were performed with GraphPad Prism 8.0.

RESULTS

As anticipated, HbA1c was elevated in T2D (51 ± 2 vs 37 ± 0.5 mmol/mol [6.8 ± 2 vs 5.5 ± 2%], p<0.0001) Platelet count and C-reactive protein did not differ between T2D and control subjects (p=ns) (**Table 1**).

The following prothrombotic platelet-related proteins were increased in T2D versus controls: PF4 (76548 ± 15982 vs 37645 ± 5704 RFU, p<0.05); PGVI (4251 ± 331 vs 3567 ± 192 RFU, p<0.05); P-selectin (14030 ± 773 vs 11255 ± 467 RFU, p<0.01); PAI-1 (2161 ± 225 vs 1341 ± 208 RFU, p<0.01).

By contrast, the antithrombotic platelet-related proteins increased in T2D versus controls were plasmin (530 ± 30 vs 466 ± 11 RFU, p<0.05) and heparin cofactor II (4331 ± 241 vs 3720 ± 242 RFU, p<0.05) (**Figures 1A–F**).

Contrary to our hypothesis, normalization of glucose in the T2D cohort had no effect on levels of any of the plasma platelet-related proteins.

Notably, age correlated with levels of PF4 (r=0.58, p=0.0004), P-selectin (r=0.51, p=0.002) and Heparin cofactor II (r=0.56, p=0.0007) in the T2D cohort only (**Figures 1G–I**).

Stratification of the T2D and control subjects into gender subgroups revealed some interesting gender-related differences in platelet-related protein levels (**Figure 2**). The T2D group was composed of 20 males and 13 females; in the control group, there were 14 males and 16 females. PF4 was increased in T2D females versus control females (p=0.012) and versus control males

TABLE 1 | Demographic and clinical characteristics of the study participants.

Baseline	Type 2 Diabetes (n = 23)	Controls (n = 23)	p-value
Age (years)	64 ± 8	60 ± 10	<0.0001
Sex (M/F)	12/11	11/12	0.77
Weight (kg)	90.9 ± 11.1	79.5 ± 8.8	<0.0001
Height (cm)	167 ± 14	169 ± 5	0.64
BMI (kg/m^2)	32 ± 4	28 ± 3	<0.0001
Systolic BP (mmHg)	132 ± 8	122 ± 8	0.001
Diastolic BP (mmHg)	81 ± 7	75 ± 6	0.003
Duration of diabetes (years)	4.5 ± 2.2	N/A	
HbA1c (mmol/mol)	51.2 ± 11.4	37.2 ± 2.2	<0.0001
HbA1c (%)	6.8 ± 1.0	5.6 ± 0.2	<0.0001
Total cholesterol (mmol/l)	4.2 ± 1.01.0	4.8 ± 0.77	0.014
Triglyceride (mmol/l)	1.7 ± 0.7	1.34 ± 0.6	0.055
HDL-cholesterol (mmol/l)	1.1 ± 0.3	1.5 ± 0.4	0.001
LDL-cholesterol (mmol/l)	2.23 ± 0.8	2.7 ± 0.87	0.051
CRP (mg/l)	3.10 ± 2.87	5.30 ± 1110.03	0.66
Platelet count (x10^9/l)	270 ± 77	240 ± 48	0.07

BMI, Body mass index; BP, Blood pressure; HDL-cholesterol, High density lipoprotein cholesterol; LDL-cholesterol, Low density lipoprotein cholesterol; CRP, C-reactive protein. HbA1c, Hemoglobin A1c.

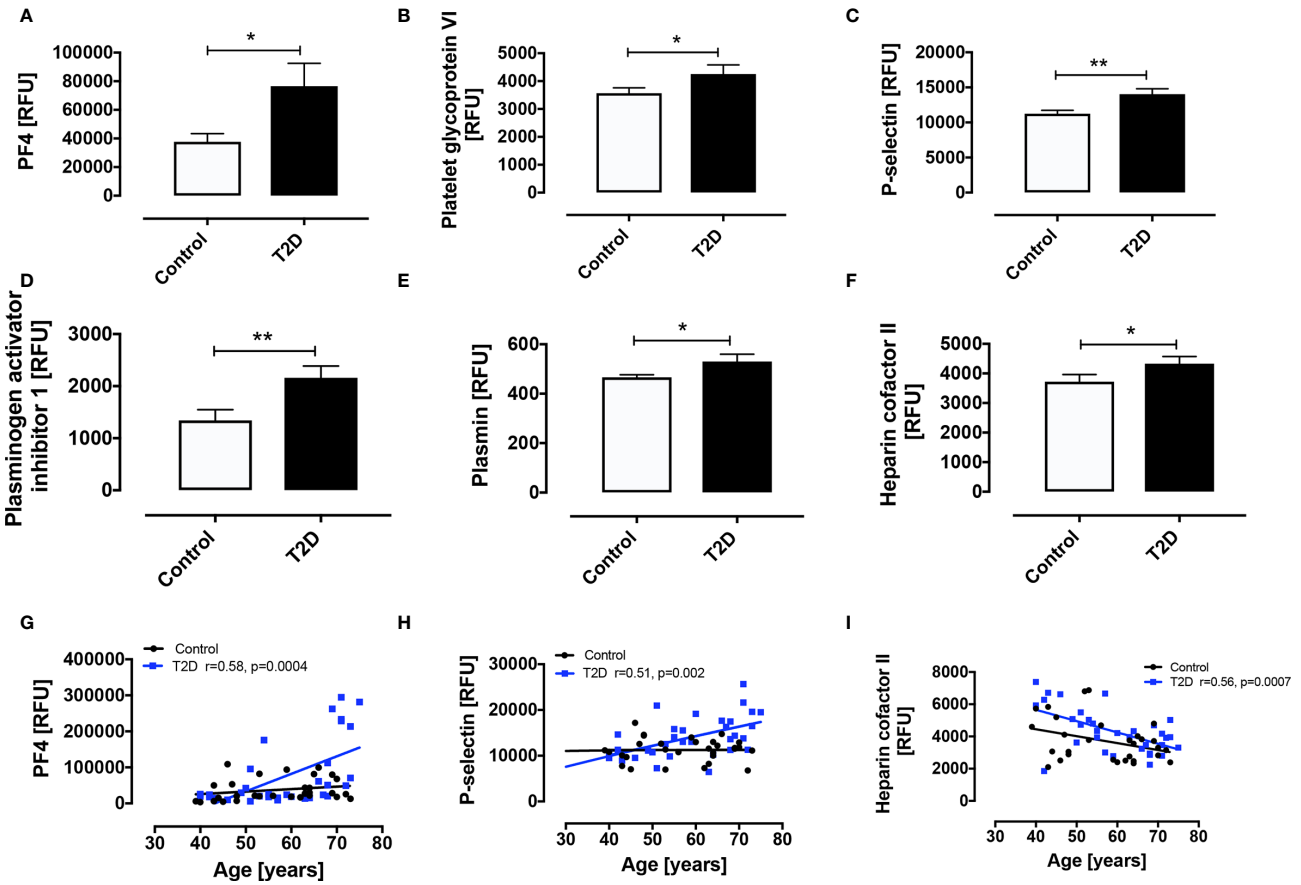

FIGURE 1 | Circulatory levels of platelet related proteins in obese type 2 diabetes (OT2D) and control subjects. Plasma protein levels of platelet factor 4 (PF4) **(A)**, platelet glycoprotein VI (PGVI) **(B)**, P-selectin **(C)**, plasminogen activator inhibitor I (PAI-1) **(D)**, plasmin **(E)**, plasmin and heparin cofactor II **(F)**. Age correlated with levels of PF4 (r = 0.58) **(G)**, P-selectin (r = 0.51) **(H)** and Heparin cofactor II (r = 0.56) **(I)** in the T2D cohort only. RFU: relative fluorescent units; *p < 0.05; **p < 0.01.

(p=0.035); there was a trend versus T2D males, but this did not reach significance (p=0.084). PGVI showed a trend towards increase in T2D females versus control females (p=0.071) though this did not reach significance. P-selectin was significantly elevated in T2D male versus control male (p=0.028) and versus control female (p=0.012) subjects; again, T2D females showed a trend towards increase versus control females, though this did not reach significance (p=0.085). PAI-1 was significantly elevated in both T2D females and T2D males versus control females (p=0.0001 and p=0.003, respectively); PAI-1 was also significantly elevated in control males versus control females (p=0.018). Plasmin was elevated in T2D females versus control females (p=0.018), control males (p=0.030) and T2D males (p=0.014). Heparin cofactor II was increased in T2D males versus control females (p=0.014).

DISCUSSION

This study shows that the levels of multiple platelet-related proteins are increased in T2D.

PF4 and P-selectin are released from activated platelets (16), PGVI is a platelet procoagulant mediator (17) and elevated PAI-1 is a thrombosis risk factor (18). By contrast, plasmin degrades fibrin clots (19) and heparin cofactor II inhibits thrombin (20).

Taken together, these results indicate that T2D patients have an elevated platelet activation baseline and, therefore, any additional insult such as infection would accelerate and enhance their hypercoagulable state.

COVID-19-infected individuals with acute respiratory distress syndrome (ARDS) are also in a hypercoagulable state, evidenced by increased fibrinogen and fibrin degradation products (D-dimers); this hypercoagulability may underlie organ failure and death (21).

The results of the hyperinsulinemic clamp showed that this enhanced platelet activation seen was not improved by returning to euglycemia in the T2D subjects. It is possible that the one-hour insulin clamp was insufficient to alter some of the proteins associated with platelet function to euglycemia and a longer time course may have resulted in changes, perhaps elicited through inflammatory or oxidative stress mechanisms due to the change in glucose levels (22).

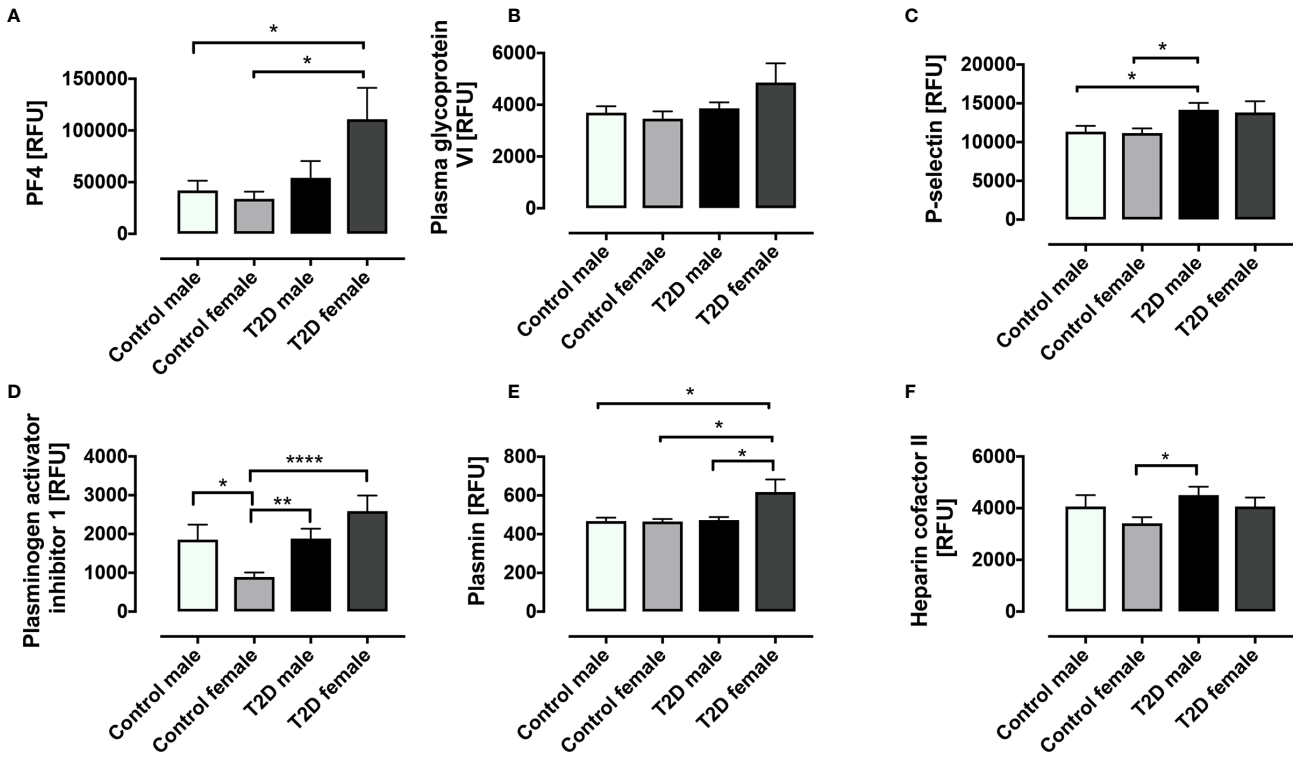

FIGURE 2 | Circulatory levels of platelet related proteins in obese type 2 diabetes (OT2D) and control subjects stratified according to gender. Plasma protein levels of platelet factor 4 (PF4) **(A)**, platelet glycopsrotein VI (PGVI) **(B)**, P-selectin **(C)**, plasminogen activator inhibitor I (PAI-1) **(D)**, plasmin **(E)**, plasmin and heparin cofactor II **(F)**. RFU: relative fluorescent units; *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$.

Interesting gender differences were also revealed by the gender-stratified subgroup analysis. In the case of PF-4, PAI-1 and plasmin, the elevation in these proteins in the T2D females drove the difference between T2D and controls in the whole group analysis; likewise, there was a trend for increase in T2D females for PGVI, though this did not reach significance. Conversely, for P-selectin and heparin cofactor II, it was the T2D males who predominantly drove the differences seen between T2D and controls in the whole group analysis. This is in keeping with other studies where proteomic analysis has shown a multitude of gender-related differences in platelet proteins (23–25), even suggesting mechanistic links to diseases with known gender disparities, such as the increased prevalence of cardiovascular disease in males (24).

Study strengths are that the T2D subjects had been diabetic for only a relatively short duration. Study limitations include small study numbers and, with more subjects enrolled, greater differences in platelet-related proteins between T2D and control subjects may have been apparent. Although T2D subjects were older and with higher BMI, this would not likely have altered protein levels. The lack of any changes from baseline in platelet-related proteins in response to normalization of glucose levels in the T2D subjects could perhaps be a consequence of the length of this clamp study being insufficient to reveal shifts in protein levels; therefore, future studies should be designed to maintain euglycemia for a longer period to better address this question. Furthermore, to conclusively determine the changes in platelet proteins in both T2D and controls in response to COVID-19 disease, it is necessary to analyze plasma from subjects documented to have SARS-CoV-2 infections and, ideally, to assess currently infected patients who have previously participated in clinical trials.

In conclusion, T2D patients have platelet hyperactivation, placing them at higher risk for thromboembolic events. When infected with COVID-19, this risk may be compounded, and their propensity for a more severe COVID-19 disease course increased.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Yorkshire and Humber Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AM and AB analyzed the data and wrote the manuscript. AA-Q performed the clinical studies. TS supervised clinical studies and edited the manuscript. SA contributed to study design, data interpretation and the writing of the manuscript. AB is the guarantor of this work. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The Impact of COVID-19 Pandemic Lockdown on the Incidence of New-Onset Type 1 Diabetes and Ketoacidosis Among Saudi Children

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Background: Overburdened healthcare systems during the coronavirus disease (COVID-19) pandemic led to suboptimal chronic disease management, including that of pediatric type 1 diabetes mellitus (T1DM). The pandemic also caused delayed detection of new-onset diabetes in children; this increased the risk and severity of diabetic ketoacidosis (DKA). We therefore investigated the frequency of new-onset pediatric T1DM and DKA in Saudi Arabia during the COVID-19 pandemic and compared it to the same period in 2019.

Methods: We conducted a multicenter retrospective cohort study, including patients aged 1–14 years admitted with new-onset T1DM or DKA during the COVID-19 pandemic (March–June 2020) and the same period in 2019. We assessed factors including age, sex, anthropometric measures, nationality, duration of diabetes, diabetes management, HbA1c levels, glycemic control, cause of admission, blood gas levels, etiology of DKA, DKA complications, length of hospital stay, and COVID-19 test status.

Result: During the lockdown, 106 children, compared with 154 in 2019, were admitted to 6 pediatric diabetes centers. Among the admissions, DKA was higher in 2020 than in 2019 (83% vs. 73%; P=0.05; risk ratio=1.15; 95% confidence interval, 1.04–1.26), after adjusting for age and sex. DKA frequency among new-onset T1DM and HbA1c levels at diagnosis were higher in 2020 than in 2019 (26% vs. 13.4% [P=<0.001] and 12.1 ± 0.2 vs. 10.8 ± 0.25 [P<0.001], respectively). Females and older patients had a higher risk of DKA.

Conclusion: The lockdown implemented in Saudi Arabia has significantly impacted children with T1DM and led to an increased DKA frequency, including children with new-onset T1DM, potentially owing to delayed presentation.

Keywords: type 1 diabetes mellitus, DKA, Saudi Arabia (KSA), COVID-19 pandemic, lockdown

INTRODUCTION

The prevalence of type 1 diabetes mellitus (T1DM) in Saudi Arabia is considered to be among the highest globally (1). One of the major and life-threatening complications of T1DM is diabetic ketoacidosis (DKA) as it may potentially lead to significantly higher rates of morbidity and mortality, along with impacting families and increasing the burden on healthcare systems (2, 3). Unfortunately, the incidence of DKA in Saudi Arabia remains as high as 40–70% among patients with new-onset T1DM, which is considerably high when compared with that in other countries (4, 5). This may have worsened during the coronavirus disease (COVID-19) pandemic when access to healthcare was insufficient.

Globally, the implemented lockdowns during the COVID-19 pandemic impacted access to healthcare for patients with chronic disorders including children with T1DM (6–8). Reports from the UK, Germany, the USA, Italy, China, and Spain documented an increase in the frequency and severity of DKA among children with T1DM (9–15). The government of Saudi Arabia had implemented early measures to reduce virus transmission prior to COVID-19 being declared a pandemic. However, in March 2020, after the World Health Organization declared the COVID-19 outbreak a pandemic, and owing to an increase in infected cases, Saudi Arabia imposed further preventive measures including a nationwide lockdown, banning international and national travel, closing of schools, and suspension of elective procedures and non-essential visits to hospitals (16, 17). The lockdown was lifted by end of June 2020, and ever since, other restrictions have been gradually minimized, as of January 2021 (18, 19).

The lockdown, local travel restrictions, virtual clinics instead of routine visits, suspension of non-essential hospital visits, and increased fear of acquiring COVID-19 infections from hospitals are the potential factors for delayed presentation of diagnosed and undiagnosed T1DM. In this study, we aimed to compare the frequency and severity of DKA in children with T1DM from six large pediatric diabetes centers in Saudi Arabia between March 1 and June 30 in 2019 and 2020 to examine the effect of the pandemic.

MATERIALS AND METHODS

Population and Sampling

We conducted a retrospective multicenter cohort study that included six pediatric diabetes centers in major cities of Saudi Arabia. We included patients aged 1–14 years, who were admitted with new-onset T1DM or DKA between March 1, 2020, and June 30, 2020, and compared their data to that of patients admitted in the same period of 2019. We excluded patients with pre-existing T1DM who were admitted for other reasons such as hypoglycemia, hyperglycemia without ketoacidosis, or surgical reasons. T1DM patients were identified either through electronic medical records using ICD-9 and ICD-10 codes for diabetes and insulin use during emergency room visits or inpatient admissions while some centers used their database registry to identify eligible patients. The data were collected using an electronic Google form.

The study was approved by the Institutional Review Board or the Independent Ethics Committee of each site prior to the initiation of the study. All aspects of the study were conducted in accordance with the International Council on Harmonization Good Clinical Practice principle.

Measures

We collected data on age, sex, anthropometric measures, nationality, duration of diabetes, diabetes management, latest HbA1c reports, average of the last 3 HbA1c reports, cause of admission, blood gas levels, etiology of DKA, DKA complications, length of stay in intensive care, and length of hospital stay. The diagnosis of diabetes was based on the American Diabetes Association criteria (20). T1DM was diagnosed if patients had at least one anti-pancreatic antibody positive, without features suggestive of type 2 diabetes and other forms of diabetes. The diagnosis of DKA was defined as a pH level <7.3 and/or bicarbonate level <15 mmol/L, along with ketonemia or ketonuria in the presence of hyperglycemia (blood glucose >11 mmol/L [≈200 mg/dL]) and severe DKA as pH <7.1 and/or serum bicarbonate <5 mmol/L (3).

Outcomes

The primary outcome was the frequencies of new onset T1DM and DKA during the period of March to June of 2019 and 2020. The secondary outcomes included the percentage of children admitted with severe DKA, parentage of DKA among new onset T1DM. Severe DKA was defined as a pH level less than 7.1 and/or bicarbonate level less than 5 mmol/L.

Statistical Analysis

We presented dichotomous data as frequencies (n) and proportions (%) and continuous data as mean ± standard error of the mean (SEM) for normally distributed data. We compared the frequencies of T1DM, DKA, and severe DKA observed during the aforementioned periods using the χ^2 test, and the data were adjusted using a generalized linear model for age and sex. We reported the effect estimate with risk ratio (RR) with 95% confidence intervals. Data analysis was performed using Stata/SE 16.0 for Mac.

RESULTS

A total of 260 children with T1DM were admitted to six pediatric diabetes centers in March–June 2019 and 2020 periods, of whom 154 children were admitted during 2019, and 106 children were admitted, in 2020. Overall, 200 (76.9%) children were admitted due to DKA, 112 of 154 patients (72.7%) in 2019 and 88 of 106 patients (83%) in 2020. Whereas overall admissions with new-onset T1DM was 98 (37.7%) in 2019 and 2020 (37% vs 38.7% respectively). The proportion of DKA among new-onset T1DM was significantly higher in 2020 (26%) than in 2019 (13.4%) [*P*=<0.001] (**Table 1**).

The mean age of the children was 9.8 ± 0.2 years, 120 (46.2%) were male, and 240 (95.4%) were Saudi Arabian (**Table 2**). Although the mean body-mass index (BMI) and BMI standard deviation score (SDS) were within the normal range, the study population showed higher BMI and BMI SDS in 2020, with a mean BMI z-score difference of −0.47 ± 0.28. Among 162 (62.3%) children with known T1DM, the mean diabetes duration was 4.8 ± 0.8 years. The mean HbA1c level was higher in 2020 than in 2019, with a mean difference of −0.7 ± 0.3% (*P*=0.13). HbA1c levels at diagnosis were significantly higher in 2020 than in 2019 (12.1 ± 0.2 vs. 10.8 ± 0.2.5) [*P*<0.001]). In children with established T1DM, most used multiple daily injections (91.9%), followed by insulin pump (6.5%), and two daily injections (4.9%). One child was reported to have developed cerebral edema in 2020, while 15 had developed acute kidney injury in 2019, and one in 2020 and no mortality secondary to DKA in both years.

The frequency of admission for new-onset T1DM was similar in both years. In contrast, admission due to DKA incidence was higher among newly diagnosed T1DM in 2020 (unadjusted RR=2.1; 95% CI, 1.3, 3.6) than in 2019, and (RR= 1.15; 95CI 1.04, 1.26) after adjusting for age and sex (**Table 3**). Females and older children had a higher risk of developing DKA and severe DKA. The frequency of severe DKA was similar in both years, after adjusting for age and sex. In 2020, two children who were diagnosed with severe DKA tested positive for COVID-19.

Risk factors leading to DKA among established cases include insulin omission (73%), infection (16%), insulin pump failure (1.2%), fasting (0.6%), improper insulin injection technique (0.6%), and other causes (0.6%).

DISCUSSION

To the best of our knowledge, this is the first multicenter study conducted in Saudi Arabia and the Middle East that compared the frequency of DKA during the COVID-19 pandemic to the same period in the previous year. Our study found a remarkable increase of 11% in DKA frequency among children with T1DM during the lockdown period. However, after adjusting for age and sex, there was no difference observed in DKA severity. Interestingly, despite a noticeable decrease in the frequency of new-onset T1DM in children, a significant increase was found in those children presenting with DKA, compared with those in 2019. A German study found increased incidence and severity of DKA during the pandemic on March 13, 2020, through May 13 compared to the same period of 2019 and 2018; however, the incidence of new-onset T1DM diagnosis remained the same (11). On the other hand, another study conducted in Italy between February 20 and April 14 in 2019 and 2020 found no difference in DKA incidence and new-onset T1DM diagnosis; however, an

TABLE 1 | DKA and severe DKA from March to June 2020, during the COVID-19 pandemic, compared with the same period in 2019.

		Overall N = 260	2019 N = 154	2020 N = 106	*P*-value
Overall	**Known T1DM**	162(62.3)	97(62.9)	65(61.3)	0.8
	New-onset T1DM	98 (37.7)	57 (37.0)	41 (38.7)	0.70
DKA*	**Overall**	200 (76.9)	112 (72.7)	88 (83.02)	0.05
	New-onset	35 (36.5)	15 (13.4)	23 (26)	<0.001
	Known T1DM	165(82.5)	97(86.6)	65(73.8)	0.89
Severe DKA*	**Overall**	47 (18.1)	24 (15.6)	23 (21.7)	0.20
	New-onset	11(23.4)	4(16.6)	7(30.4)	0.13
	Known T1DM	36 (76.6)	20(83.3)	16(69.5)	0.60

TABLE 2 | Baseline characteristics.

	Overall N = 260	2019 N = 154	2020 N = 106	*P*-value
Age, years	9.8 (0.2)	9.7 (0.24)	10.0 (0.3)	0.2
Sex, male*	120 (46.2)	69 (44.8)	51 (48.1)	0.6
Diabetes duration	4.8 (0.8)	5.1 (1.3)	4.4 (0.3)	0.6
BMI, z-score	−0.66 (0.1)	−0.85 (0.17)	−0.39 (0.22)	0.05
HbA1c, %	11.6 (0.1)	11.3 (0.2)	12.1 (0.2)	<0.001
HbA1c% among new-onset	11.5 (2.2)	10.87 (2.5)	12.14 (2.1)	<0.001
HbA1c% among known T1DM	11.70 (1.7)	11.57 (1.8)	12.0 (1.6)	0.13
Average of last 3 HbA1c, %	10.9 (0.2)	10.9 (0.2)	10.9 (0.2)	0.5
PICU length of stay, days	0.55 (0.04)	0.5 (0.1)	0.6 (0.1)	0.08
Hospital length of stay, days	2.88 (0.1)	2.9 (0.1)	2.9 (0.2)	0.5

Values are mean ± SEM unless indicated.
**N (%).*

TABLE 3 | Adjusted risk ratio of DKA and severe DKA from March to June 2020, during the COVID-19 pandemic, compared with the same period in 2019.

	DKA				Severe DKA			
	Risk ratio	95% CI		*P*-value	Risk ratio	95% CI		*P*-value
Visit period, 2020[a]	1.15	1.04	1.26	0.01	1.37	0.83	2.25	0.22
Age, years[b]	1.05	1.03	1.07	0.00	1.15	1.04	1.27	0.01
Sex, male[c]	0.76	0.68	0.85	0.00	0.56	0.32	0.97	0.04
Constant	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.22

[a]*Adjusted for current age and sex.*
[b]*Adjusted for visit period and sex.*
[c]*Adjusted for current age and visit period.*

increase in DKA severity was observed (10). A survey from 88 diabetes centers in the UK between March 1, 2020, and June 30, 2020, showed an increase in the incidence and severity of DKA at diagnosis, and about 20% of the centers have attributed this to delayed presentation, while the incidence of new-onset T1DM diagnosis remained the same (12).

Another interesting finding in our data was higher HbA1c during the pandemic among new-onset T1DM with or without DKA, which could be linked to delayed presentation. This finding has not been reported in previous studies, but UK multicenter study reported HbA1c in the pandemic with no comparison (14).

The possible causes of delayed presentation and increased DKA are likely to be similar to those reported in other countries, such as the focus of frontline healthcare workers on COVID-19-related clinical manifestations. In addition, parents avoided seeking medical care when children had milder symptoms with the concern of contracting COVID-19 at the hospital and owing to difficulties in accessing healthcare services due to the lockdown (6, 9).

Despite the relatively short period of lockdown, unfortunately, the BMI of children was found to be significantly higher in 2020 than in 2019. This finding was not reported in other T1DM cohorts investigated during the same period. However, studies on healthy children reported lower physical activity and increased screen time, which are considered major factors leading to childhood obesity (21).

Based on our data and those of other groups, we emphasize the recommendation of Elbarbary et al. that dissemination of awareness regarding T1DM symptoms in the public should be continued during a pandemic (9). Furthermore, telemedicine technology has been an effective tool during this pandemic to deliver care for known and new-onset diabetic patients. Thus, in such scenarios in the future, technology and telemedicine should be used to fill the gaps when the healthcare system is overburdened (6–9, 22).

There is a paucity of data regarding the severity of COVID-19 among children with T1DM (13). While adults with diabetes who contracted COVID-19 showed a severe disease course and poor outcome, studies in children with diabetes have demonstrated a milder course and better outcome (10, 22–24). Beliard et al. showed that DKA presentation among children with T1DM who were diagnosed with COVID-19 was not significantly different from those who were not (15). In the present study, COVID-19 cases among admitted patients were rare (2/106 patients). However, both patients had severe DKA with no complications related to COVID-19. Our findings are consistent with studies that showed a low prevalence of COVID-19 among children with diabetes (25).

Our study, however, has some limitations, the major one being that not all pediatric diabetes centers in Saudi Arabia were included; hence, we were unable to present region-adjusted incidence.

We have learned from our data as well as the previous studies that the pandemic has significantly impacted children with T1DM. The pandemic has caused a delay in the diagnosis of diabetes, which markedly increased DKA frequency and severity. This data emphasizes that healthcare access to diagnosed and undiagnosed children with diabetes should continue in the standard level of care during pandemics, in addition to the need for continuing awareness about T1DM to the public and among frontline healthcare practitioners.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

AAla, MA, RA, FA, and MD designed the research study. MH, AAln, AAls, AAls, AB, KA, YA, SA, and AT collected the data. AAla and RA analyzed the data. AAla, MA, RA, and FA wrote the first draft. All authors participated in the final draft. AAla and RA take final responsibility for this article. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPINION
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Impact of COVID-19 on Youth With Type 2 Diabetes: Lessons Learned From a Pediatric Endocrinologist and a Psychologist

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INTRODUCTION

The coronavirus disease (COVID-19) continues to devastate the diabetes community. People with type 2 diabetes (T2D) are at significantly higher risk for severe illness if infected by SARS-CoV2 (1, 2). It is unknown if youth with T2D are equally at risk for severe COVID-19. The Type 2 Diabetes Clinic at Children's Hospital Los Angeles serves more than 450 youth with type 2 diabetes. From the perspectives of a pediatric endocrinologist and psychologist, we have witnessed the physical and social-emotional impact of this pandemic on youth with T2D and their families.

PHYSICAL RISKS FOR YOUTH WITH T2D

The literature abounds with studies demonstrating diabetes, obesity, and hypertension as independent risk factors for severe COVID-19 infection (1, 3, 4). Unless explicitly stated, references to "diabetes" refer presumably to T2D, given its relative prevalence compared to other types of diabetes. To date, investigations have largely focused on the impact of SARS-CoV-2 on adults, with a paucity of information in youth. We recently showed that the incidence of youth who presented with diabetic ketoacidosis (DKA) when newly diagnosed with T2D nearly doubled since the start of the pandemic (5). Future multi-site studies will be needed to examine if our observation is generalizable to other centers that serve a large population of pediatric T2D. What remains unreported, is the impact of the pandemic on the physical health of youth with existing T2D.

Several characteristics and co-morbidities of youth with T2D increase their potential risk for severe COVID-19 infection. Pediatric T2D in the United States disproportionately affects ethnic and racial minority groups including Latinx and Black communities (6). A similar trend has been detected among people infected with COVID-19 (6, 7). Many youth with T2D develop uncontrolled hyperglycemia 12 to 18 months after they are diagnosed with diabetes (8). In some reports, hyperglycemia in individuals with T2D is associated with increased COVID-19 disease severity and mortality (9, 10). Most youth with T2D have additional co-morbidities, most commonly obesity and hypertension. If infected with SARS-CoV-2, these risk factors may increase their potential to develop severe COVID-19 illness.

Beyond the threat of SARS-CoV2 infection, the pandemic has adversely impacted the physical health of youth with T2D in our clinical practice. School closures have eliminated the benefits of school-based health care providers, who served as a critical safety net for many of our patients. For some, the lack of adult supervision has led to worsened medication adherence, poorly managed or unmanaged diabetes, and the development of diabetic ketoacidosis (DKA). The Stay-at-Home order, loss of access to physical education in school, and increased screen time further limits physical activity for these youth. Even though physical education has resumed in a virtual format for some, students who reside in apartments or multi-family homes frequently do not participate due to fear and/or embarrassment of disturbing their neighbors or housemates. In addition, many youths whose access to food was dependent on meals and snacks provided at school have increased their consumption of low-quality nutrition during this time. The lack of physical activity and increased caloric consumption has led to excessive weight gain and worsened glycemic control (11). Together, these factors may exacerbate existing risks for COVID-19 illness for these youth.

The global pandemic has also affected medical access for many pediatric patients with T2D. Although our hospital transitioned to telemedicine soon after the Stay-at-Home order was enacted, many of our patients' families have had difficulty accessing telehealth due to limited internet connectivity and/or technology literacy. In addition, most of our patients have state-subsidized insurance plans that require intermittent document updates based on in-person assessments. The closure of many government offices for in-person services and an overwhelmed telephone system has limited some families' ability to keep their insurance requirements up-to-date. In turn, the loss of health insurance benefits has contributed to delays in routine follow-up diabetes care visits and gaps in access to prescribed medications. The fear of exposure to COVID-19 associated with the use of public transportation or with the perceived risk in coming to the hospital has also contributed to missed or rescheduled appointments. As well, diabetes care visits have been postponed due to either the patient or family members testing positive for SARS-CoV2. The delay in accessing routine diabetes care has prevented timely medical intervention in glycemic management placing these youth at greater risk for poorly managed blood glucose and DKA.

PSYCHOSOCIAL RISKS FOR YOUTH WITH T2D

Youth with T2D are at higher risk for depression and anxiety compared to those without diabetes (12, 13). They often experience shame and embarrassment about their medical diagnosis due to stigma. Coupled with frequent reports of teasing and bullying, especially for those with overweight, youth with T2D are at risk for mood disorders and related symptoms such as social isolation and decreased energy and motivation. The COVID-19 pandemic has created additional stressors that compound existing psychosocial challenges for individuals with T2D. Together, these adversities can negatively impact diabetes management behaviors (14, 15).

With the economic downturn, many parents and guardians have reported job loss and/or a reduction in wages. The financial toll has resulted in a cascade of problems including homelessness or fear of eviction and food insecurity. For some, the financial strain has resulted in the fear of job loss by requesting time off and difficulty accessing transportation. These challenges have limited patient access to prescribed treatments including medical appointments and mental health services. Diminished access to this much-needed health care has exacerbated worries and feelings of helplessness and have contributed to the lack of appropriate care for an already vulnerable patient population. Youth experiencing heightened mood symptoms report struggles to follow prescribed diabetes management behaviors leading to a decrease in physical activity, increase in food consumption including unhealthy foods, and decreased motivation to take prescribed medications. Moreover, feelings of uncertainty associated with continuous public health warnings about the COVID-19 pandemic have fueled existing emotional vulnerabilities.

Our pediatric T2D community reports mounting stress amidst the ongoing uncertainties of the pandemic. Many are grieving the death of loved ones who succumbed to complications associated with COVID-19 illness. The youth with T2D and their families we serve report fears about exposure or re-exposure to SARS-CoV-2. Those who previously recovered from COVID-19 wonder if they will have the fortune to recover from mild or moderate symptoms if exposed to the virus again. Fears about risk for exposure lead to social isolation. In turn, some youth with T2D have reported that chronic social isolation during the pandemic has exacerbated feelings of sadness and loneliness. Youth also report worrying about their parent or guardian's risk for exposure to the virus, given that many adults in this community are essential workers and do not have the luxury to work from home. Despite the fear that they will be exposed to SARS-CoV-2 at work and subsequently exposure their child to this virus, many parents and guardians believe that they have no choice but to work to avoid job loss.

The closure of in-person schooling has also adversely impacted the mental health of our youth. Many youths benefit from school-based services including mental health support. While telehealth options are available, access is significantly limited for families who struggle to access virtual platforms. Some youth are grateful for a break from the potential for negative social interactions within the

school environment; however, they also report less opportunities for peer and academic support as well as everyday distractions. Furthermore, youth who prefer in-person and tactile educational opportunities report feelings of frustration, helplessness, and concern about their decline in academic performance. While some schools are resuming in-person educational opportunities, some families feel hesitant about returning to school due to concern around safety protocols and fear of SARS-CoV-2 exposure.

CONCLUSIONS AND RECOMMENDATIONS

The COVID-19 pandemic has far-reaching impacts in the care of youth with T2D. The demographic and co-existing co-morbidities may increase their risk for severe COVID-19 infection. While there continues to be much uncertainty about the course and sequelae of this virus, we must continue to assess and respond to the compounding physical and social-emotional impact of this pandemic on pediatric T2D populations. Furthermore, the disruption of the lockdown on the physical health and mental health of these youth must be considered in treatment planning as well as the development of public health policies.

To better understand the needs of youth with T2D, we reiterate the recommendations proposed by DiMeglio et al. (**Table 1**) (16). Specifically, improved data collection for diverse pediatric populations with T2D is needed to better understand the trends and outcomes for this population. It is not sufficient or acceptable to extrapolate from data gathered from adults. Careful interpretation of pediatric T2D data is critical to ensure that appropriate guidelines and precautions are in place within childcare and school settings, and for other activities involving physical interaction.

Psychosocial distress coupled with the chronic uncertainties of the COVID-19 pandemic can have a negative and significant impact on diabetes management behaviors and access to prescribed treatments for youth with T2D. Screening patients' mental health and basic necessities by health care providers and educators, in addition to multidisciplinary treatment planning, is necessary to address these needs.

TABLE 1 | Pediatric type 2 diabetes during COVID-19*.

NEEDS	RECOMMENDATIONS
• Prospectively-collected, longitudinal, retrievable public data on new onset incidence trends, new onset patient characteristics, and acute and long-term disease outcomes	• Collect data for youth with type 2 diabetes following established guidelines for epidemiologic data collections • Examine effects of obesity and hypertension on COVID-19 severity among youth with T2D • Improve data collection for racial and ethnic minority populations
• Thoughtful interpretations of emerging data reports, including thorough peer review	• Contextualize data to inform childcare decisions, including return to school and extracurricular • Provide a clear and unified voice for pediatric-specific type 2 diabetes concerns at the level of national and international organizations and public health messaging
• Access to multidisciplinary care and support including mental health, social work, and case management access	• Screen and provide appropriate support resources that target depression, food insecurity, financial stability • Support the use of accessible virtual platforms and alternate ways to provide youth with T2D with medical and mental health care in addition to any service required to sustain insurance benefits

Adapted sections with permission (18).

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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REVIEW
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COVID-19 and Diabetes: Understanding the Interrelationship and Risks for a Severe Course

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The relationship between COVID-19 and diabetes mellitus is complicated and bidirectional. On the one hand, diabetes mellitus is considered one of the most important risk factors for a severe course of COVID-19. Several factors that are often present in diabetes mellitus are likely to contribute to this risk, such as older age, a proinflammatory and hypercoagulable state, hyperglycemia and underlying comorbidities (hypertension, cardiovascular disease, chronic kidney disease and obesity). On the other hand, a severe COVID-19 infection, and its treatment with steroids, can have a specific negative impact on diabetes itself, leading to worsening of hyperglycemia through increased insulin resistance and reduced β-cell secretory function. Worsening hyperglycemia can, in turn, adversely affect the course of COVID-19. Although more knowledge gradually surfaces as the pandemic progresses, challenges in understanding the interrelationship between COVID-19 and diabetes remain.

Keywords: diabetes, COVID-19, SARS-CoV-2, coronavirus, severity, mortality, comorbidities, treatment

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INTRODUCTION

Diabetes mellitus is a complex chronic disease characterized by glucose dysregulation caused by an absolute or relative insulin deficiency. It includes various different types, with type 1 diabetes (T1D) and type 2 diabetes (T2D) as the most prevalent subtypes. T1D is characterized by autoimmune destruction of insulin-producing pancreatic β-cells, while T2D results from a combination of β-cell secretory defect and insulin resistance (1). The global burden of diabetes is high, with an overall prevalence of 9.3% and 463 million people suffering from the disease worldwide (2). It is often accompanied by various comorbidities and long-term complications, including obesity, hypertension, vasculopathy, a proinflammatory and hypercoagulable state and cardiovascular disease (CVD) (3–5).

Abbreviations: ACE(-2), Angiotensin converting enzyme (2); ARB, Angiotensin receptor blocker; BMI, Body mass index; CKD, Chronic kidney disease; COVID-19, Coronavirus disease 2019; CRP, C-reactive protein; CVD, Cardiovascular disease; DPP-4, Dipeptidyl peptidase 4; eGFR, Estimated glomerular filtration rate; GLP-1, Glucagon-like peptide 1; HbA1c, Glycated hemoglobin A1c; HR, Hazard ratio; ICU, Intensive care unit; IL, Interleukin; MERS-CoV, Middle-East respiratory syndrome coronavirus; OR, Odds ratio; SARS-CoV(-2), Severe acute respiratory syndrome coronavirus (2); SGLT-2, Sodium-glucose-co-transporter-2; T1D, Type 1 diabetes mellitus; T2D, Type 2 diabetes mellitus; WHO, World Health Organization.

In December 2019, the first cases of atypical pneumonia with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) were identified. A rapid global spread of the virus led the World Health Organization (WHO) to declare coronavirus disease 2019 (COVID-19) a pandemic on March 11th, 2020. Clinical presentation of COVID-19 is diverse and can vary from asymptomatic infection and mild upper respiratory tract symptoms to respiratory failure needing intensive care and death.

This review aims to provide an overview and assessment of the bidirectional relationship between COVID-19 and diabetes. On the one hand, diabetes and its associated comorbidities increase the risk of a more severe course of COVID-19 and increased mortality (6–10). Patients with diabetes are known to have an increased risk of infections, which is partly attributed to hyperglycemia causing immune dysfunction, among other effects (11–15). On the other hand, severe SARS-CoV-2 infection and its associated hyperinflammation contribute to hyperglycemia through an indirect negative effect on insulin target tissues and a potential direct negative effect on pancreatic β-cells (16). The resulting hyperglycemia can, in turn, worsen the prognosis of COVID-19 (17–20).

In this review, we will first discuss the primary risk of COVID-19 infection in patients with diabetes, then the risk of a severe course of COVID-19 with diabetes, followed by the potential additional risk of the most relevant comorbidities and other concomitant factors, and finally the role of glycemic control and the effect of the SARS-CoV-2 virus itself. All studies that were considered relevant for this review were found using PubMed and by cross-referencing.

RISK OF INFECTION WITH COVID-19

Patients with diabetes are known to have an increased risk of infections, in particular skin infections, genito-urinary tract infections and (bacterial) respiratory tract infections (14, 21). The hyperglycemic environment present in diabetes favors immune dysfunction through several pathways. The most important underlying mechanisms are a decreased production of interleukins in response to an infection, reduced chemotaxis and phagocytic activity, and immobilization of polymorphonuclear leukocytes. Hyperglycemia, including the resulting glycosuria, also increases the virulence of certain pathogens (11, 22, 23). In addition to an increased risk of infection, patients with diabetes also have a higher rate of infection-related hospitalizations as well as infection-related mortality. These risks are present in both T1D and T2D, but are greater in T1D (15). Importantly, it is well-known that the risk of infections is further increased with poorer glycemic control (12–14).

For SARS-CoV-2, clinical reports from all around the world found diabetes mellitus to be one of the most common comorbidities present in patients with COVID-19. In the beginning of the COVID-19 pandemic, this finding, along with the known increased infection risk for other infections, led to the assumption that patients with diabetes are at increased primary risk of COVID-19 infection. However, most of these reports describe patients in a hospitalized or even intensive care unit (ICU) setting, i.e. patients with a more severe course of the disease.

In the first largest consecutive case series from the United States of America (USA), describing 5,700 patients with COVID-19 admitted to 12 hospitals in the New York area, diabetes mellitus was the third most common comorbidity with 33.8% of patients suffering from the disease, after hypertension (56.6%) and obesity (41.7%) (24). This series, however, included both patients admitted to the general ward as well as to the ICU and does not distinguish between the two. A meta-analysis of six Chinese studies including 1,527 patients hospitalized with COVID-19 clearly demonstrated the difference between prevalence of diabetes in severe versus non-severe cases, describing a prevalence of diabetes of 11.7% in ICU cases, but 4.0% in non-ICU cases (25). In another study from China, prevalence of diabetes in 1,590 patients with COVID-19 was 8.2%, rising to 34.6% in patients with a severe course of the disease (26). In line with this finding, a report of the Chinese Center for Disease Control and Prevention on 44,672 COVID-19 cases which also included non-hospitalized patients, showed a lower prevalence of diabetes (5.3%) (27). Similarly, the US Centers for Disease Control and Prevention reported an overall prevalence of diabetes in patients with COVID-19 of 10.9%, with a prevalence of 6.4% in non-hospitalized COVID-19 patients (28). In a meta-analysis on 33 studies with a total of 16,003 patients, which included 30 studies from China, two from the USA and one from France, the pooled prevalence of diabetes in patients with COVID-19 was 9.8% (29). Finally, in Italy, a single-center study on 146 patients hospitalized with COVID-19 demonstrated a prevalence of diabetes of 8.9% (30). With an estimated prevalence of diabetes mellitus in the general population of 10.5% in the USA (31), 11.2% in China (32), 7.6% in France (33), and 8.3% in Italy (34), respectively, the prevalence of diabetes in patients infected with COVID-19 was not higher than in the general population.

In summary, based on these studies, the primary risk of infection with COVID-19 does not appear to be increased in patients with diabetes mellitus. In previous studies with other pathogens, the increased risk of infection in patients with diabetes also appeared to be present predominantly in bacterial, fungal or yeast infections and not in viral infections, such as COVID-19 (13). However, we do not know whether patients with diabetes behave differently with regard to willingness for SARS-CoV-2 testing and/or social distancing compared to patients without diabetes, which could affect incidence rates. Therefore, it is difficult to determine and compare the primary risk of infection. Since most studies involve patients with T2D and/or authors had not specified the type of diabetes, any possible difference between T1D and T2D in primary risk of COVID-19 infection is not yet known.

RISK OF A SEVERE COURSE OF COVID-19

During previous pandemics, numerous studies have shown that patients with diabetes are a key vulnerable group for a severe course of infections. During the 2009 H1N1 influenza pandemic, hospitalization of individuals with diabetes was up to six times higher as compared to individuals without diabetes (35, 36), risk of admission to the intensive care unit was four times higher (37), and risk of death two times higher (38). In the first SARS-CoV outbreak in 2002, diabetes was determined an independent risk factor for complications and death, with an odds ratio (OR) of 3.0 for death in patients with diabetes (39, 40). During the Middle-East respiratory syndrome coronavirus (MERS-CoV) outbreak, the prevalence of diabetes was 51% in patients who had MERS, and the OR for severe or critical disease ranged from 7.2 to 15.7 in patients with diabetes as compared to the overall population, with a mortality rate of 35% (41).

In the present SARS-CoV-2 pandemic, several studies and meta-analyses have investigated the impact of diabetes on severity of COVID-19. A severe course of COVID-19 is a broad concept, ranging from the need versus no need for hospitalization, ICU versus non-ICU admission, requirement versus no requirement of mechanical ventilation, fatal versus non-fatal disease and any composite outcome combining them. Although chosen endpoints vary per study, there was consensus in currently available literature that in order to be regarded as having a severe course of COVID-19, patients at least have to be hospitalized as a result of the disease. In the initial reports from China, the prevalence of diabetes was consistently higher among patients with severe versus non-severe disease (8, 42–44). As the pandemic progressed, larger studies from countries around the world provided more robust information.

In China, a nationwide study reported a higher prevalence of diabetes among patients with severe COVID-19 as compared to patients with non-severe disease (16.2% *vs* 5.7%) (45). The China Center for Disease Control and Prevention reported a diabetes prevalence of 5.3% among all 44,672 COVID-19 cases, but 19.7% among non-survivors, with a case fatality rate of 2.3% *vs* 7.3%, respectively (27). In the USA, among 5,279 patients diagnosed with COVID-19 in a prospective cohort study in New York City, prevalence of diabetes was higher in patients admitted to the hospital than patients not admitted (34.7 *vs* 9.7%) (7). The US Centers for Disease Control and Prevention reported diabetes prevalence rising with increasing severity of COVID-19, from 6.4% in non-hospitalized patients to 24.2% in hospitalized patients and 32.4% in ICU patients (28). The most recent report of the Istituto Superiore di Sanità (National Health Institute) in Italy, describing 85,418 patients who died from COVID-19, reported a prevalence of T2D of 29.3% (46).

Apart from reporting a higher prevalence of diabetes, several meta-analyses showed that the risk of diabetes was 2 – 4 fold higher in severe COVID-19 cases as compared to non-severe COVID-19 patients (29, 43, 47). In a meta-analysis of six Chinese studies, the pooled rate ratio of diabetes among patients with severe versus non-severe COVID-19 was 2.26 (30). A retrospective cohort study on 178 patients with diabetes hospitalized with COVID-19 in the USA found that patients with diabetes had a 59% higher risk of ICU admission, an approximately 97% increased risk of mechanical ventilation and twofold increased risk of death after adjustment for age, sex, ethnicity, BMI and any comorbidities (48). In a final large meta-analysis of 76 studies involving 31,067 patients with COVID-19, patients with diabetes were at significantly higher risk of severe infection (OR 2.38) as well as mortality (OR 2.21) (49).

Since most research had either been done in patients with T2D or the researchers did not specify the type of diabetes in their studies, it had long been unclear whether this higher risk of a severe course of COVID-19 was also present in T1D. Fortunately, more evidence on the risk in T1D is emerging and it demonstrates an increased risk for COVID-19-related mortality, ICU admission and hospitalization. A population-based study from the United Kingdom (UK) demonstrated that out of 23,804 COVID-19-related in-hospital deaths, 31.4% of patients had T2D and 1.5% had T1D. Unadjusted 72-day mortality rates per 100,000 people were 27 for individuals without diabetes, 138 for T1D and 260 for T2D. Interestingly, adjusted for age, sex, socioeconomic deprivation, ethnicity and geographical region, the OR for COVID-19-related mortality was even higher in patients with T1D compared to patients with T2D (3.51 *vs* 2.03) (9). Even more recently, a similar nationwide population-based study on 34,383 patients with T1D and 275,960 with T2D from Scotland showed similar increased risks for COVID-19-related mortality and ICU admission, with again higher risks for T1D as compared to T2D (T1D OR 2.40; T2D OR 1.37) (50). A final recent prospective cohort study from the USA confirmed these findings (51), and showed in their latest analyses on an even higher number of patients that there was a higher risk of hospitalization for COVID-19 in T1D (OR 4.60) as compared to T2D (OR 3.42) (52). The findings of these studies are summarized in **Table 1**.

In summary, patients with both type 1 and type 2 diabetes have an increased risk of a more severe course of COVID-19. While the risk for patients with T2D had already been established, recent evidence that emerged on the risk in T1D shows that patients with T1D appear to have an even slightly higher adjusted risk for adverse COVID-19-related outcomes. Although diabetes is an independent risk factor for disease severity (9, 48, 50–52), of course, patients with diabetes often have other comorbidities or concurrent factors that could add to their increased risk of severe COVID-19.

ROLE OF CARDIOVASCULAR RISK FACTORS, (MICRO)VASCULAR COMPLICATIONS AND PHARMACOLOGIC TREATMENT

Several mechanisms have been suggested as an underlying additional explanation for the more severe course of COVID-

TABLE 1 | Overview of the risk of adverse COVID-19-related outcomes in patients with type 1 and type 2 diabetes mellitus.

	Study population	Number of patients (n)	Outcome	T1D *vs* no DM OR (95% CI)	T2D *vs* no DM OR (95% CI)
Barron et al. (9)	United Kingdom, nationwide population-based *March 1st – May 11th 2020*	T1D: 263,830 T2D: 2,864,670 No DM: 58,244,220	Mortality	3.51 (3.16 – 3.90)[#] 2.86 (2.58 – 3.18)[$]	2.03 (1.97 – 2.09)[#] 1.80 (1.75 – 1.86)[$]
McGurnaghan et al. (50)	Scotland, nationwide population-based *March 1st – July 31st 2020*	T1D: 34,383 T2D: 275,960 No DM: 5,143,951	Mortality and/or ICU admission	2.40 (1.82 – 3.16)[&]	1.37 (1.28 – 1.47)[&]
Gregory et al. (51, 52)[Φ]	Nashville, Tennessee, USA, single-center* *March 17th – December 24th 2020*	T1D: 136 T2D: 1,100 No DM: 19,422	Hospitalization	4.60 (3.04 – 6.98)[€]	3.42 (2.94 – 3.99)[€]

COVID-19, coronavirus disease 2019; T1D, type 1 diabetes; T2D, type 2 diabetes; DM, diabetes mellitus; OR, odds ratio; CI, confidence interval; ICU, intensive care unit; USA, United States of America.

[Φ] Gregory et al. have published the initial findings of their prospective cohort study, followed by a report of further analyses on an even higher number of patients. Here, we report the findings of their latest analyses.

[*] Vanderbilt University Medical Center (VUMC), Nashville, Tennessee, USA.

[#] Adjusted for age, sex, deprivation, ethnicity and geographical region.

[$] Adjusted for age, sex, deprivation, ethnicity, geographical region and previous hospital admissions with coronary heart disease, cerebrovascular disease, or heart failure.

[&] Adjusted for age and sex.

[€] Adjusted for age, sex, ethnicity, hypertension, smoking, and body mass index (BMI).

19 in patients with diabetes. This chapter aims to provide an overview of prognostic factors associated with severity of COVID-19 in patients with diabetes. It focuses on the most relevant factors in the context of diabetes and COVID-19 such as demographic features, cardiovascular risk factors, (micro) vascular complications and pharmacologic treatments. Other comorbidities associated with diabetes mellitus are beyond the scope of this review (53). In assessing the risk of an individual for a more severe course of COVID-19, it is important to consider these prognostic features, summarized in the top half of **Figure 1**.

Age, Sex, Ethnicity, Socioeconomic Status

In the general population, a higher age, male sex, non-white ethnicity and lower socioeconomic status are demographic features that have all been associated with a more severe course of COVID-19 (7, 8, 42, 47, 54–58). As expected, these features also add to the risk of COVID-19 severity in patients with diabetes. Similar to the non-diabetic population, in a Chinese two-center retrospective study on 306 patients hospitalized with COVID-19, older age and male sex were found more frequently in non-survivors as compared to survivors among patients with diabetes (age 72 *vs* 63 years; 71.0% *vs* 43.4% male, respectively). Age ≥ 70 years was found to be an independent risk factor for in-hospital death for patients with diabetes as well as for patients without diabetes (hazard ratio (HR) 2.39 and 5.87, respectively) (59). In a recent larger French nationwide prospective cohort study on 1,317 patients with diabetes hospitalized with COVID-19 (of which 88.5% T2D), age was an independent risk factor for 7-day mortality as well (OR 2.39) (60). In that study, patients with T1D (n = 56) were separately described, confirming that patients with T1D who met the primary outcome (i.e. requirement of mechanical ventilation or death by day 7) were significantly older compared to patients who did not (65.3 *vs* 53.2 years) and prevalence of this primary outcome incrementally increased with increasing age in patients with T1D (12.0% for age < 55 years, 23.8% for age 55 – 74 years and 50.0% for age ≥ 75 years) (61). The UK population-based study on a total of 264,390 patients with T1D and 2,847,020 patients with T2D confirmed increased COVID-19-related mortality in both T1D and T2D for all of the demographic features. As compared to patients 60 – 69 years of age, patients 70 – 79 years of age were at increased risk for death (T1D HR 1.89; T2D HR 1.94), and patients ≥ 80 years of age were at even greater risk (T1D HR 4.79; T2D HR 4.52). For male sex, risk of mortality increased similarly for patients with T1D as compared to T2D (HR 1.61 for both). In T1D, COVID-19-related mortality was significantly higher in individuals of black and Asian ethnicities compared to white ethnicity (HR 1.77 and 1.57, respectively). For patients with T2D, risk of COVID-19-related mortality was higher in individuals of black and Asian, as well as mixed ethnicity (HR 1.63, 1.08 and 1.30, respectively). The researchers also identified a clear association between COVID-19-related death and socioeconomic status among patients with either type of diabetes, with mortality significantly higher in individuals in the most deprived quintile as compared to those in the least deprived quintile (T1D HR 1.93; T2D HR 1.46) (10). The Scottish population-based study on 319,349 patients with diabetes found similar associations for age (OR 1.076), sex (female OR 0.705) and socioeconomic status, with risk of ICU admission or mortality for COVID-19 decreasing with each quintile of increasing socioeconomic status (least deprived quintile *vs* most deprived quintile OR 0.379) (50). Additionally, risk for hospitalization in patients with diabetes was also found to be increased in the USA prospective cohort study for age (OR 1.93 for each 25 year increment), sex (OR 1.22 for male) and ethnicity (OR 2.01 for black; OR 1.78 for other) (51).

Hypertension and Cardiovascular Disease

Among the most commonly described comorbidities present in patients with COVID-19 are hypertension and cardiovascular disease (CVD), with hypertension being the most common in

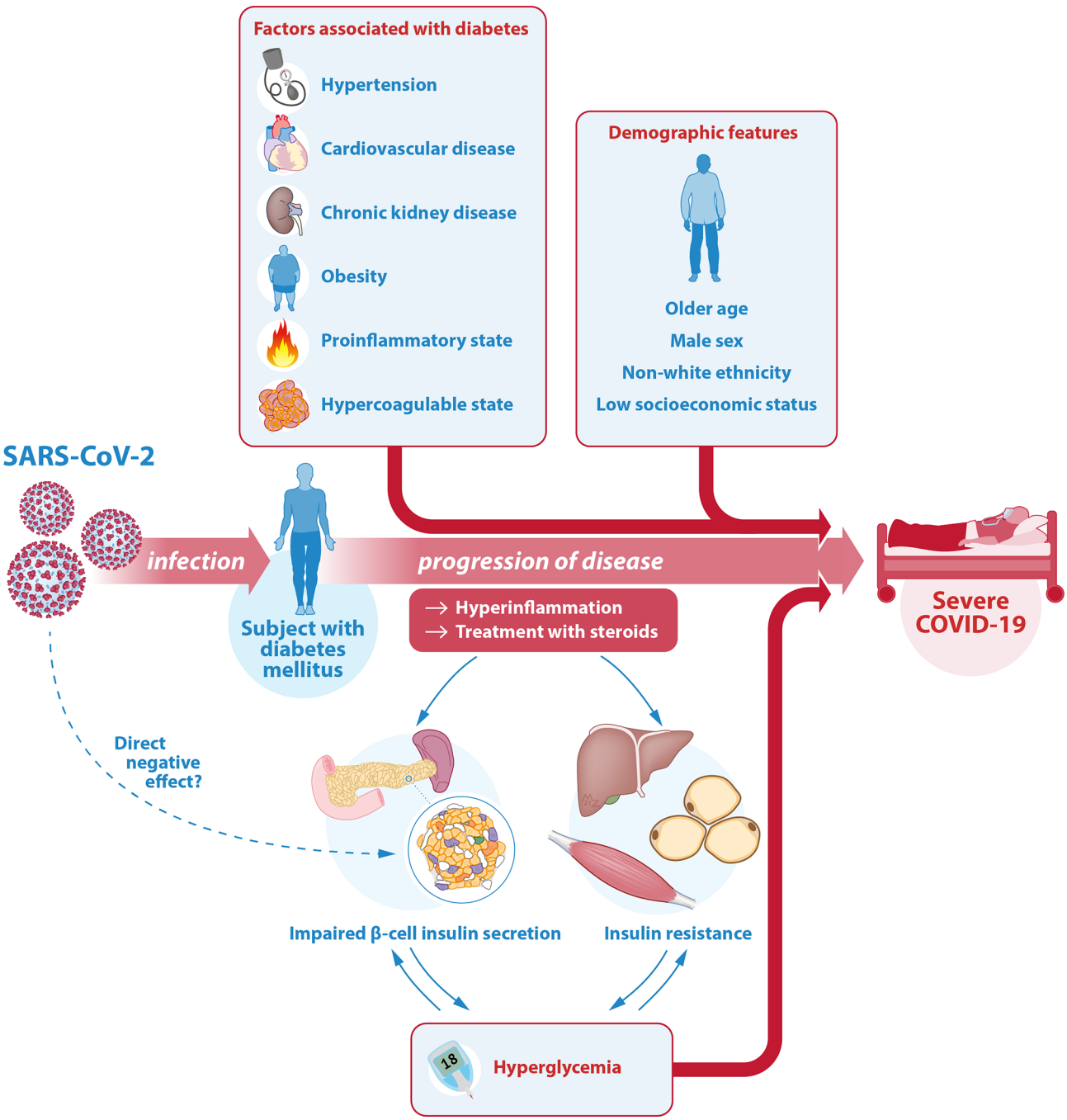

FIGURE 1 | Illustration of the interrelationship between SARS-CoV-2, COVID-19 and diabetes.
The relationship between COVID-19 and diabetes is complicated and bidirectional. Diabetes mellitus is one of the most important risk factors for severe COVID-19. In a patient with diabetes, the associated comorbidities and diabetes-related complications as well as certain demographic features can further contribute to this higher risk of a severe course of COVID-19. Another key factor is glycemic control. On the one hand, hyperglycemia is a strong risk factor for a more severe course of COVID-19. On the other hand, the hyperinflammation associated with severe COVID-19 as well as its treatment with steroids can cause or worsen hyperglycemia through an effect on insulin target tissues (predominantly liver, muscle and fat cells) reducing insulin sensitivity (insulin resistance), as well as on pancreatic β-cells causing insufficient insulin secretion. There may even be a direct effect of SARS-CoV-2 on the β-cell through the ACE-2 receptor, but this is controversial. Hyperglycemia itself can lead to glucose toxicity, thus further decreasing insulin sensitivity and insulin secretory function. Hereby, the risk of severe COVID-19 in patients with diabetes is increased even further. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; ACE-2, angiotensin converting enzyme 2.

numerous reports from around the world (6–8, 24–28, 42, 43, 45–47, 54, 56). Hypertension and CVD are also among the most commonly associated comorbidities with diabetes (3, 4). Considering this strong association, and the fact that both hypertension and CVD have been identified as strong independent risk factors for severity of COVID-19 in the general population (47, 56, 58), it can be safely assumed that they are important concomitant factors adding to the risk of severe COVID-19 in patients with diabetes. Several studies have confirmed this. In a Chinese study on hospitalized COVID-19 patients, 153 individuals with diabetes more frequently had a history of hypertension and CVD as compared to 153 individuals without diabetes who were age- and sex-matched (hypertension 56.9% *vs* 28.8%; CVD 20.9 *vs* 11.1%, respectively). Among non-survivors, 83.9% of patients with diabetes suffered from hypertension and 45.2% from CVD, compared to 37.5% and 18.8% of patients without diabetes. In this cohort, hypertension was independently associated with an increased risk for in-hospital COVID-19-related death in patients with diabetes (HR 3.10) (59). A multi-center retrospective cohort study from Italy confirmed hypertension to be associated with an additional increased risk of mechanical ventilation, ICU admission or death (OR 2.31) in patients with diabetes (62), and a prospective cohort study from the USA found increased adjusted risk for hospitalization (OR 1.39) (51), although this was not described in every cohort (10, 50). In the Chinese study, as opposed to hypertension, CVD was only found to be significantly related to in-hospital death in patients with diabetes in the univariable regression analyses (HR 3.79), but not in the adjusted multivariable analyses. In the French nationwide cohort study on patients with COVID-19, an unadjusted increased risk for death was identified for patients with diabetes and concomitant hypertension (OR 1.81), macrovascular complications (OR 3.58), microvascular complications (OR 5.25), and several other cardiovascular-related diseases (60). Because T2D and CVD are so strongly related, it has been suggested that the higher risk of severe COVID-19 for T2D and CVD may overlap and not be additive. In the only currently available case-control study from Italy on 79 patients with T2D hospitalized for COVID-19 and 158 age- and sex-matched T2D controls without COVID-19, no higher prevalence of CVD was found in cases with COVID-19 (63). However, in a multi-center retrospective cohort study from the same group, a single cardiometabolic risk factor did not significantly increase risk of critical COVID-19 or mortality, but cardiometabolic multimorbidity did significantly and independently increase this risk (OR 3.19) (62). In the UK population-based study, for T1D, previous stroke (HR 2.33) and previous heart failure (1.80) but not previous myocardial infarction were associated with COVID-19-related mortality. For T2D, all of these cardiovascular comorbidities were independently associated with increased risk of death (myocardial infarction HR 1.11; stroke HR 2.02; heart failure HR 2.09) (10).

Chronic Kidney Disease

Chronic kidney disease (CKD) is one of the well-known microvascular long-term complications of diabetes. Also, diabetes is the most common cause of chronic kidney disease (CKD). The prevalence of CKD among patients with T2D is around 40% (64, 65) and the lifetime risk of developing CKD in T1D around 20-25% (66). In patients hospitalized with COVID-19, CKD has been shown to be an independent risk factor for in-hospital death (58, 67). Recent studies have demonstrated that this association is also present in patients with diabetes. In the French nationwide cohort study on hospitalized COVID-19 patients, diabetic kidney disease (i.e. estimated glomerular filtration rate (eGFR) of ≤ 60 mL/min/1.73m^2 and/or proteinuria) was a predictor for early death in patients with diabetes (OR 3.19) (60). Additionally, the Italian case-control study on patients with T2D hospitalized with COVID-19 and T2D controls without COVID-19 confirmed a higher prevalence of CKD stage IIIb (eGFR < 45 ml/min/1.73m^2) in those hospitalized (OR 3.09) (63). In the UK population-based study, an eGFR < 60 mL/min/1.73m^2 was associated with increased COVID-19-related mortality in both T1D and T2D. Increasing stages of CKD were associated with incrementally increasing risks of COVID-19-related mortality, for eGFR 45 – 59 mL/min/1.73m^2 (T1D HR 2.07; T2D HR 1.39), eGFR 30 – 44 mL/min/1.73m^2 (T1D HR 2.46; T2D HR 1.76), eGFR 15 – 29 mL/min/1.73m^2 (T1D HR 3.71; T2D HR 2.31) and finally eGFR < 15 mL/min/1.73m^2 (T1D HR 8.35; T2D HR 4.91) (10). It is important to recognize CKD in patients with COVID-19 because impaired renal function warrants dosage reduction of some treatments used for COVID-19 (68).

Obesity

Obesity has been extensively linked to severity and mortality of COVID-19 (7, 58, 69–73). Several mechanisms have been suggested to explain the strong association between obesity and a severe course of COVID-19, i.e. ventilatory difficulty, the hypercoagulable and proinflammatory environment present in obesity, alterations in gut microbiota, nutritional deficits and immune dysregulation (74). Obesity is also linked to diabetes, in particular T2D, with an association so strong that the term diabesity has been used in the literature (75). Individuals with increasing body mass index (BMI) have an incrementally increasing risk of developing diabetes, already starting at an overweight BMI (25 – 29.9 kg/m^2; OR 1.59), through class 2 obesity (BMI 30 – 39.9 kg/m^2; OR 3.44) up to class 3 obesity (BMI ≥ 40 kg/m^2; OR 7.37) (76). With both diabetes and obesity providing proinflammatory, hypercoagulable and immune dysregulatory conditions, an additional effect of obesity on severity of COVID-19 in patients with diabetes can be expected. Indeed, obesity has been shown to increase the risk of a severe course of COVID-19 in patients with diabetes. In the French nationwide cohort study, BMI was independently associated with their primary outcome (tracheal intubation and/or death within 7 days; OR 1.24). Interestingly, this effect was not significant in COVID-19-related death alone (60). The USA prospective cohort study found each 8.9 kg/m^2 increment to be associated with an adjusted OR of 1.25 for hospitalization (51). An effect on COVID-19-related mortality was found in the UK population-based study, which identified a U-shaped association between BMI and mortality for both T1D and T2D. In that study, obesity was independently associated with

an even higher additional risk of death in patients with T1D compared to T2D. Compared with a BMI of 25.0 – 29.9 kg/m^2, a lower BMI of < 20.0 kg/m^2 as well as of 20.0 – 24.9 kg/m^2 were associated with increased COVID-19-related mortality (T1D HR 2.45 and 1.51; T2D HR 2.33 and 1.34, respectively). A higher BMI of 35.0 – 39.9 kg/m^2 as well as of ≥ 40.0 kg/m^2 also accounted for increased risk of COVID-19-related mortality for both types of diabetes (T1D HR 1.72 and 2.33; T2D HR 1.17 and 1.60, respectively). Interestingly, for T1D but not for T2D, a BMI of 30.0 – 34.9 kg/m^2 was additionally associated with mortality (HR 1.47) (10). This U-shaped association with a low BMI, in addition to an high BMI, also attributing to an increased risk of death may be an explanation for the lack of significance on mortality in the French cohort in which a BMI of < 25 kg/m^2 was set as the reference for statistical analyses.

Inflammation

In COVID-19, the most common post-mortem findings include profound inflammation of several tissues (77). Patients with severe COVID-19, in particular patients with acute respiratory distress syndrome, have a marked rise in inflammatory markers such as C-reactive protein (CRP), D-dimer, ferritin and interleukin 6 (IL-6) (8). An extreme form of hyperinflammation associated with severe COVID-19 is found in some patients who experience a cytokine storm, an uncontrolled state of hyperinflammation resulting in widespread tissue damage, multi-organ failure and death (78, 79). In several studies, high blood concentrations of inflammatory markers, cytokines and chemokines have been associated with COVID-19 severity and mortality (7, 8, 42, 80, 81). Diabetes is associated with a proinflammatory state, which may contribute to the risk of a more severe course of COVID-19 and a higher risk of experiencing a cytokine storm. The proinflammatory cytokines and toxic metabolites that are present in a cytokine storm are already chronically elevated in individuals with diabetes as part of the low-grade chronic inflammation (82–84). Although understanding the underlying pathogenesis of low-grade inflammation leading to a more rapid progression of COVID-19 and the associated cytokine storm is still subject of research, one of the most important pathophysiological mechanisms underlying the higher risk in patients with diabetes is thought to involve the proinflammatory NF-kappa-B pathway, which is chronically activated in patients with diabetes (82, 85). Additionally, the low-grade chronic inflammation present in individuals with diabetes is associated with exaggerated macrophage, monocyte and T-cell recruitment, and with decreased regulatory T-cell function, which promotes further inflammation in a continuous feedback cycle (86–89). Indeed, in several clinical studies in patients with diabetes and COVID-19, worse inflammatory profiles with higher inflammatory markers such as CRP, D-dimer, IL-6 and ferritin were identified compared to patients without diabetes (17, 19, 44, 90). Patients with diabetes were also at a higher risk of excessive uncontrolled inflammation responses (90). Additionally, elevated CRP and D-dimer levels, as well as higher IL-6, TNF-α and ferritin levels were found in non-survivors as compared to survivors with diabetes (44, 59). A high CRP was independently associated with increased risk of mortality (OR 1.87) for patients with diabetes hospitalized with COVID-19 (60). Among patients with diabetes, the group most vulnerable to hyperinflammation and cytokine storm in COVID-19 consists of the individuals with poor glycemic control, since hyperglycemia stimulates the synthesis and release of cytokines (91).

Coagulation

COVID-19 has been widely associated with thromboembolic events such as pulmonary embolism, deep-vein thrombosis (DVT), ischemic stroke and myocardial infarction, which represent a predominant cause of death in patients with severe COVID-19 (92–94). Similar to the proinflammatory state, diabetes is also associated with a hypercoagulable state. Patients with diabetes in general have an increased risk of thromboembolic events (95), which, in the case of COVID-19, can add to a high risk of death. Hypercoagulation in COVID-19 is thought to occur due to the profound inflammatory response and cytokine storm observed in some patients. Because patients with diabetes have a more pronounced inflammatory response (see paragraph *Inflammation*), they may be at greater risk to suffer from thromboembolic events in the case of COVID-19. Another important contributor in patients with diabetes may be hyperglycemia, which was previously shown to further exaggerate coagulation, as well as hyperinsulinemia, which inhibits fibrinolytic activity (96). In two studies, longer prothrombin times and higher D-dimer concentrations were found in COVID-19 non-survivors as compared to survivors in hospitalized Chinese patients with diabetes (44, 59). However, this was not the case in the French nationwide cohort study in which D-dimer levels were not a significant predictor of 7-day mortality in patients with diabetes hospitalized with COVID-19 (60). Apart from these studies describing biochemical outcome parameters such as D-dimer levels, no clinical studies have yet been performed on the prevalence of thromboembolic events in patients with diabetes as compared to patients without diabetes. It could be hypothesized that patients with COVID-19 and diabetes are at higher risk of thromboembolic events, especially when other risk factors such as older age, obesity, inflammation and immobilization due to hospital admission are present.

Medication

In addition to diabetes mellitus and the conditions that are associated with the disease, the treatments of these conditions might also increase the risk of a more complicated course of COVID-19, as well as account for drug interactions with treatments given for COVID-19. This gives rise to some specific considerations in patients with diabetes and/or associated comorbidities who are infected with SARS-CoV-2. The benefits, risks and recommendations for each of the treatments are summarized in **Table 2**.

Some studies reported that insulin use in patients with diabetes was associated with a greater COVID-19 related morbidity and mortality (50, 60, 97). Even though in two of these studies adjustment for confounders using multivariable regression models was performed (50, 60), worse outcomes in patients on insulin therapy could be related to confounding by indication, rather than pointing to a harmful effect of insulin (98). Patients on insulin therapy are more likely to have a more

TABLE 2 | Benefits, risks and recommendations for glucose-lowering and other commonly used treatments in patients with diabetes and COVID-19.

Medication	Benefits	Risks	Recommendations
a. Glucose-lowering drugs			
Insulin	Careful adjustment for improved glucose regulation possible, associated with better COVID-19 outcomes.	Hypoglycemia, especially in combination with (hydroxy)chloroquine. High dosages could be needed in critically ill patients, especially when treated with steroids.	Do not discontinue, initiate in patients with severe COVID-19.
Metformin	Anti-inflammatory properties, no hypoglycemia.	Lactic acidosis in critically ill patients.	Discontinue in patients with a severe course (impaired renal or hepatic function, sepsis, heart failure, respiratory distress).
Sulfonylureas	N/A	Hypoglycemia, especially in combination with (hydroxy)chloroquine.	Consider discontinuation based on COVID-19 severity, nutritional status and risk of hypoglycemia.
SGLT-2 inhibitors	No hypoglycemia.	Diabetic ketoacidosis and dehydration.	Discontinue in patients with a severe course (impaired renal or hepatic function, sepsis, heart failure, respiratory distress).
GLP-1 receptor agonists	Anti-inflammatory properties, no hypoglycemia.	Risk of dehydration with side-effects like vomiting and diarrhea.	Do not discontinue, encourage adequate fluid intake and regular meals.
DPP-4 inhibitors	Well-tolerated, no hypoglycemia, usable in wide range of renal function.	N/A	Do not discontinue.
Thiazolidinediones	Anti-inflammatory properties, reduction of insulin resistance.	Risk of fluid retention and aggravation of heart failure.	Consider discontinuation in patients with a severe course, particularly patients with heart failure.
b. Other commonly used drugs			
Statins	Possible beneficial effect on COVID-19-related outcomes, at least no detrimental effects.	N/A	Do not discontinue.
ACE-inhibitors/ARBs	Possible beneficial effect on COVID-19-related outcomes, at least no detrimental effects.	N/A	Do not discontinue.

N/A, not applicable; SGLT-2, sodium-glucose-co-transporter-2; GLP-1, glucagon-like peptide 1; DPP-4, dipeptidyl peptidase 4; ACE, angiontensin-converting enzyme; ARB, angiotensin receptor blocker.

severe and a longer duration of diabetes. In addition, glucose dysregulation, and thus increased use of insulin, occurs with more severe COVID-19 (see paragraph *Direct and Indirect Effects of SARS-CoV-2*). Indeed, better glycemic control is associated with better COVID-19-related outcomes (see paragraph *Glycemic Control*). In one study, treatment with insulin was associated with achievement of glycemic targets and improvement of COVID-19-related outcomes in patients hospitalized with the disease (99). The French nationwide cohort study also did not find any significant association between insulin use and increased mortality (60). Moreover, the Italian case-control study on patients with T2D hospitalized with COVID-19 and T2D controls without COVID-19 even demonstrated a lower prevalence of basal insulin use in those hospitalized (OR 0.18), after adjustment for cardiovascular, pulmonary and kidney disease (63). Consensus recommendations of experts from around the world include the recommendation that insulin therapy should not be stopped, as well as the recommendation to start insulin therapy in patients with severe COVID-19 (100). Of course, in patients with T1D, cessation of insulin therapy is never an option. Steroids, an important treatment pillar for patients with severe COVID-19, can result in marked hyperglycemia and the need for very high dosages of insulin.

Patients with COVID-19 that were treated with metformin have been reported to have better outcomes including reduced mortality and lower levels of inflammation in studies using univariable (97), as well as adjusted multivariable analyses (60). This was consistent with previous findings of anti-inflammatory properties of metformin (101), although improved COVID-19-related outcomes were not found in every study (50). However, metformin poses a known risk of lactic acidosis in critically ill patients, including patients with COVID-19 (102). It has therefore been recommended to discontinue metformin in patients with a severe course of COVID-19, i.e. in patients with sepsis, impaired renal or hepatic function, heart failure or respiratory distress (100, 103). However, because of the anti-inflammatory, cell-protective and important glucose regulatory effects of metformin without risk of hypoglycemia, it has been suggested to maintain metformin treatment in all patients hospitalized with COVID-19, provided they have not developed kidney or liver failure (104).

With contradictory evidence, it is still unclear whether sulfonylureas are associated with increased morbidity or mortality in patients with COVID-19. In the Scottish nationwide population-based study, the authors have found an increased risk of severe COVID-19 in patients using sulfonylureas (OR 1.310) after adjustment for age, sex, diabetes duration and type of diabetes (50). In an unadjusted univariable analysis from the French nationwide cohort study, this effect was not found (60). As sulfonylureas convey an increased risk of hypoglycemia, which increases during illness if oral intake is poor, discontinuation of sulfonylureas may be warranted in certain patients with COVID-19, based on the severity of COVID-19, nutritional status and risk of hypoglycemia.

Sodium-glucose-co-transporter-2 (SGLT-2) inhibitors do not carry a risk of hypoglycemia and have not been associated with

increased risk of severe COVID-19 in multivariable adjusted analyses (50). However, they are associated with a risk of diabetic ketoacidosis during illness (105, 106). To reduce the risk of metabolic decompensation, expert recommendations state to discontinue SGLT-2 inhibitors in patients with severe COVID-19 (100).

Glucagon-like peptide-1 (GLP-1) receptor agonists exert anti-inflammatory properties, which is known to have beneficial effect on low-grade inflammation associated with atherosclerosis (107, 108). Furthermore, GLP-1 receptor agonists have renoprotective effects (109, 110). Considering the well-known beneficial effect of both GLP-1 receptor agonists as well as SGLT-2 inhibitors on prevention of cardiovascular and kidney disease, both risk factors for a severe course of COVID-19, these drugs may be of preventive importance (111). Whether GLP-1 receptor agonists exert similar beneficial anti-inflammatory effects specifically in relation to inflammation in COVID-19 is not yet clear. In several animal studies, GLP-1 receptor agonists have been associated with attenuation of pulmonary inflammation and preservation of lung function in experimental lung injury as well as respiratory syncytial virus infection (112–115). In the French prospective study on patients with COVID-19, use of GLP-1 receptor agonists was, however, not associated with a change in mortality (60). In the Scottish nationwide population-based study, adjusted risk of severe COVID-19 was even found to be increased (50). GLP-1 receptor agonists do not pose a risk for hypoglycemia, but the gastro-intestinal side-effects of GLP-1 receptor agonists may cause or worsen dehydration. Adequate fluid intake and regular meals should be encouraged. Cessation of GLP-1 receptor agonists in patients with COVID-19 is not deemed necessary (100).

Dipeptidyl peptidase-4 (DPP-4) inhibitors have been subject to debate since the start of the COVID-19 pandemic. In previous coronaviruses, DPP-4 was found to be a co-receptor for viral entry into the cell (116). Therefore, it had been speculated that DPP-4 inhibitors might have a beneficial effect, interfering with this binding and therefore reducing viral entry (117, 118). One retrospective cohort study found reduced mortality and improved clinical outcomes in patients with T2D and COVID-19 using sitagliptin (119). However, multiple clinical studies did not show any morbidity or mortality benefit of DPP-4 inhibitors in univariable (60, 97, 120), adjusted multivariable (50), or propensity-score matched analyses (121). In line with these findings, a recent study on SARS-CoV-2 showed that it does not use DPP-4 as a co-receptor for viral entry (122). Because DPP-4 inhibitors are generally well-tolerated, have no risk of hypoglycemia and can be used for a wide range of kidney function, it is recommended that they are continued in patients with COVID-19 (100).

Thiazolidinediones exert anti-inflammatory and antioxidant effects and significantly reduce insulin resistance (123). Therefore, it has been speculated that thiazolidinediones could ameliorate prognosis of COVID-19 in patients with diabetes (124). However, this has not yet been confirmed by clinical studies (50). Important side-effects of thiazolidinediones include weight gain and fluid retention, associated with aggravation of heart failure (125). For this reason, until more clinical trials more accurately identify the risks and benefits associated with thiazolidinedione treatment in (severe) COVID-19, use of thiazolidinediones in patients with diabetes and COVID-19 remains controversial. Discontinuation may be warranted in patients with a severe course of COVID-19, especially in patients with heart failure.

In addition to glucose-lowering therapies, patients with diabetes often also use other comedications like statins. Statins are lipid-lowering drugs that have notable anti-inflammatory and cardiovascular protective properties. Because CVD is a comorbidity highly associated with worse outcomes of COVID-19, it can be hypothesized that use of statins might reduce this risk (126). However, statins increase the expression of angiotensin converting enzyme 2 (ACE-2), the main receptor that is used by SARS-CoV-2 to enter a cell (127). This raised concerns on statins potentially increasing the risk for COVID-19 severity. To this end, several studies have looked into the effect of statins on COVID-19 morbidity and mortality. A large Chinese retrospective study on 13,981 patients with COVID-19 showed that in-hospital statin use was associated with reduced 28-day mortality as compared to a propensity score matched non-statin group (5.2% *vs* 9.4%, respectively) (128). In a retrospective cohort of 717 patients with COVID-19 in Singapore, after propensity score matching, statin use was independently associated with lower ICU admission, but not with a decrease in mortality (129). In the French prospective study on 1,317 hospitalized patients with diabetes and COVID-19, statins were also not associated with a change in mortality in the unadjusted analyses (60). In adjusted multivariable analyses of the UK population-based study, statins were not significantly associated with change in COVID-19-related mortality among patients with T1D. In T2D, use of statins was associated with a decrease in mortality (HR 0.72) (10). Although based on the available evidence it is still unclear whether statins have a beneficial effect on COVID-19-related outcomes, there at least does not seem to be a detrimental effect. Therefore, statins do not need to be discontinued in patients with COVID-19.

Because of their effect on the upregulation of the ACE-2 receptor (130), similar concerns as with statins were initially raised for ACE-inhibitors and angiotensin receptor blockers (ARBs) (131). Several studies have since then shown that there is no detrimental effect of ACE-inhibitors and ARBs on the severity of outcomes in patients with COVID-19 (60, 132–136). Some of the studies even showed improved clinical outcomes (50, 133, 136), although these findings need to be replicated in larger randomized controlled settings in order to draw a more definitive conclusion on any potential beneficial effects. In addition, with regard to a potential higher primary risk of infection, in a retrospective study on 4,480 patients as well as in a separate study on 12,594 patients who were tested for COVID-19, both using propensity score matching, the use of ACE-inhibitors and ARBs was not associated with a higher incidence of COVID-19 (135, 137). These findings are supported by a position statement of the European Society of Cardiology, that strongly recommends continuation of treatment

with ACE-inhibitors and ARBs in patients with COVID-19 (138).

BIDIRECTIONAL RELATIONSHIP: THE ROLE OF GLYCEMIC CONTROL AND SARS-COV-2

The interrelationship between COVID-19 and diabetes is complex. Apart from diabetes mellitus being one of the most important risk factors for severe COVID-19, with several comorbidities and diabetes-related complications further adding to this risk, another key factor is glycemic control. On the one hand, poor glycemic control is an important independent risk factor for a severe course of COVID-19. On the other hand, a severe SARS-CoV-2 infection can contribute to hyperglycemia through several mechanisms, thereby further worsening the prognosis of patients with COVID-19. This chapter is aimed to provide an overview of this bidirectional relationship, which is illustrated in the bottom half of **Figure 1**.

Glycemic Control

Glycemic control as a predictor for worse outcomes of infections is not a new concept. During the 2009 H1N1 influenza pandemic, fasting plasma glucose levels were identified as an independent predictor for severity of H1N1 pneumonia (139). In the 2002 SARS-CoV outbreak, not only a history of diabetes (OR 3.0), but also fasting plasma glucose levels of ≥ 7.0 mmol/l (126 mg/dL) were found to be an independent predictor of mortality (OR 3.3) (39). Previous studies on the role of glycemic control in other infections have shown a U-shaped relationship between glycemia and infection-related hospitalization in 22,846 patients with T2D, with both low and high HbA1c levels increasing the risk of hospitalization, also after adjustment for confounding factors (14). In a population of 1,713 critically ill patients admitted to an intensive cardiac care unit, plasma glucose levels at admission were linearly associated with an increased risk for all-cause mortality (140).

In COVID-19, glycemic control also plays an important role in severity of outcomes. There are several different aspects to glycemic control that should be considered, i.e. glycemic control before hospital admission, at the time of hospitalization and during the in-hospital stay. An overview of important characteristics and outcomes of studies investigating the risk of adverse COVID-19-related outcomes according to glycemic control is presented in **Table 3**.

Glycemic Control Before Hospital Admission

Patients with a pre-admission HbA1c of ≥ 86 mmol/mol (10.0%) compared to patients with an HbA1c of 48 – 53 mmol/mol (6.5 – 7.0%) had increased COVID-19-related mortality in both T1D and T2D (HR 2.23 and 1.61, respectively) (10). Interestingly, for T2D, a pre-admission HbA1c of 59 – 74 mmol/mol (7.6 – 8.9%) and of 75 – 85 mmol/mol (9.0 – 9.9%) were additionally associated with higher COVID-19-related mortality as compared to an HbA1c of 48 – 53 mmol/mol (HR 1.22 and 1.36, respectively) (10). A separate study from the UK on 17,278,392 individuals with 10,926 COVID-19-related deaths confirmed this finding, identifying HbA1c < 58 mmol/mol (7.5%) to yield a lower risk of COVID-19-related death as compared to HbA1c ≥ 58 mmol/mol (HR 1.58 *vs* 2.61, respectively, adjusted for age and sex) (58). Contrarily, in a French a nationwide cohort study on 1,317 patients with diabetes hospitalized for COVID-19, HbA1c was not associated with the composite outcome of mechanical ventilation and death (60). Additional analyses from the recent USA prospective cohort study showed in 102 patients with T1D that risk of hospitalization for COVID-19 incrementally increased with increasing levels of HbA1c (OR 2.96 for the first and second quartile of HbA1c, OR 5.12 for the third quartile and OR 9.76 for the fourth quartile) (51, 52).

During the pandemic, countries around the world have responded with different lockdown strategies in an attempt to control the outbreak. With glycemic control before hospital admission as an important risk factor for a severe course of COVID-19, several studies have been conducted on the effect of the pandemic and subsequent lockdown on glycemic control. It had been hypothesized that the limited possibility for physical exercise, increased stress and disruption of daily routines would negatively affect glycemic control. To this end, a large group of 280 patients with T1D and 155 patients with T2D was studied in our center during the lockdown period. Interestingly, we found an improvement in glycemic control in the lockdown period as compared to before the lockdown. In both T1D and T2D, patients in the highest pre-lockdown tertile of HbA1c showed the biggest improvement in HbA1c (T1D -4.3 mmol/mol (-0.39%); T2D -6.8 mmol/mol (-0.62%)). Improved continuous glucose monitoring data in patients with T1D reflected this observation (142). Smaller studies during the lockdown in Italy as well as Spain additionally supported this finding, with improved glucose time in range (3.9 – 10.0 mmol/l; 70 – 180 mg/dL), reduced time above range (> 10.0 mmol/l (180 mg/dL)) and reduced time below range (< 3.9 mmol/l (70 mg/dL)) in patients with diabetes who stayed at home during the lockdown (143–145).

Glycemic Control at the Time of Hospitalization

Hyperglycemia at the time of admission to the hospital was also identified as an important risk factor for a more severe course of COVID-19. Even in patients without previous diagnosis of diabetes, a high fasting blood glucose level ≥ 7.0 mmol/l (126 mg/dL) at the time of admission with COVID-19 was independently associated with 28-day mortality (141). A retrospective multi-center study of 2,041 COVID-19 patients hospitalized in China found hyperglycemia ≥ 6.1 mmol/l (110 mg/dL) at the time of hospital admission to be an independent risk factor for progression to critical disease and death in patients with non-critical illness at that time (HR 1.30). In patients diagnosed with critical disease, hyperglycemia ≥ 6.1 mmol/l (110 mg/dL) was an independent risk factor for in-hospital mortality (HR 1.84) (18). A study from Italy on 271 patients

TABLE 3 | Overview of the risk of adverse COVID-19-related outcomes according to glycemic control.

	Study population	Number of patients (n)	Parameter of glycemic control	Outcome	Risk HR/OR (95% CI)
a. Glycemic control before hospitalization					
Holman et al. (10)	United Kingdom, nationwide population-based cohort *March 1st – May 11th 2020*	T1D: 265,090 T2D: 2,889,210	HbA1c 59 – 74 mmol/mol (7.6 – 8.9%) HbA1c 75 – 85 mmol/mol (9.0 – 9.9%) HbA1c ≥ 86 mmol/mol (10.0%)	Mortality	T1D: HR 1.16 (0.81 – 1.67)* T2D: HR 1.22 (1.15 – 1.30)* T1D: HR 1.37 (0.90 – 2.07)* T2D: HR 1.36 (1.24 – 1.50)* T1D: HR 2.23 (1.50 – 3.30)* T2D: HR 1.61 (1.47 – 1.77)*
Williamson et al. (58)	United Kingdom, nationwide population-based cohort *January 1st – May 6th 2020*	17,278,392	HbA1c ≥ 58 mmol/mol (7.5%)	Mortality	HR 2.61 (2.46 – 2.77)[#] HR 1.95 (1.83 – 2.08)[$]
Cariou et al. (60)	France, multi-center cohort *March 10th – March 31st 2020*	846	HbA1c 53 – 63 mmol/mol (7.0 – 7.9%) HbA1c 64 – 74 mmol/mol (8.0 – 8.9%) HbA1c ≥ 75 mmol/mol (9.0%)	Mortality	OR 1.55 (0.82 – 2.93)[&] OR 1.09 (0.52 – 2.28)[&] OR 0.84 (0.40 – 1.75)[&]
Gregory et al. (51, 52)[Φ]	USA, single-center cohort *March 17th – December 24th 2020*	T1D: 102	1st HbA1c quartile 2nd HbA1c quartile 3rd HbA1c quartile 4th HbA1c quartile	Hospitalization	OR 2.96 (1.11 – 7.86)[€] OR 2.96 (1.11 – 7.86)[€] OR 5.12 (2.12 – 12.35)[€] OR 9.76 (4.42 – 21.54)[€]
b. Glycemic control at the time of hospitalization					
Wang et al. (141)	China, multi-center retrospective *January 24th – February 10th 2020*	605	Fasting blood glucose level ≥ 7.0 mmol/l (126 mg/dL)	Mortality In-hospital complications	HR 2.30 (1.49 – 3.55)[~] OR 3.99 (2.71 – 5.88)[&]
Wu et al. (18)	China, multi-center retrospective *December 26th 2019 – March 15th 2020*	2,041	Hyperglycemia ≥ 6.1 mmol/l (110 mg/dL)	Critical disease and mortality overall Mortality in critical patients	HR 1.30 (1.03 – 1.63)[£] HR 1.84 (1.14 – 2.98)[£]
Copelli et al. (17)	Italy, single-center retrospective *March 20th – April 30th 2020*	271	Hyperglycemia ≥ 7.78 mmol/l (140 mg/dL)	Critical disease and mortality	HR 1.80 (1.03 – 3.15)[¥]
c. Glycemic control during in-hospital stay					
Bode et al. (20)	USA, multi-center retrospective *March 1st – April 6th 2020*	1,122	Diabetes and/or uncontrolled hyperglycemia (≥ 2 measurements > 10.0 mmol/l (180 mg/dL) within 24h)	Mortality	OR 6.12 (3.63 – 10.31)[&φ]
Zhu et al. (19)	China, multi-center retrospective *December 30th 2019 – March 20th 2020*	Total: 7,337 T2D: 952	Normoglycemia (glycemic variability during hospital stay 3.9 – 10.0 mmol/l (70 – 180 mg/dL) versus hyperglycemia (> 10.0 mmol/l (180 mg/dL)	Mortality	HR 0.14 (0.03 – 0.60)[ω]

COVID-19, coronavirus disease 2019; T1D, type 1 diabetes; T2D, type 2 diabetes; HR, hazard ratio; OR, odds ratio; CI, confidence interval; USA, United States of America.

[Φ] Gregory et al. have published the initial findings of their prospective cohort study, followed by a report of further analyses on an even higher number of patients. Here, we report the findings of their latest analyses.

** Adjusted for age, sex, socioeconomic deprivation, ethnicity, region of residence, duration of diabetes, body mass index (BMI), systolic blood pressure, prescription for antihypertensive drugs, serum total cholesterol, prescription for statins, smoking status, history of myocardial infarction, stroke, heart failure and eGFR.*

[#] Adjusted for age and sex.

[$] Adjusted for age, sex, obesity, smoking status, deprivation, cancer, reduced kidney function, asthma, chronic respiratory disease, chronic cardiac disease, hypertension, chronic liver disease, stroke, dementia, other neurological disease, organ transplant, asplenia, rheumatoid arthritis, lupus or psoriasis and any other immunosuppressive condition.

[&] Unadjusted.

[€] Adjusted for age, sex, ethnicity, hypertension, smoking, and body mass index (BMI).

[~] Adjusted for age, sex and CRB-65 score (measure of pneumonia severity).

[£] Adjusted for age, sex, hypertension, smoking, insulin treatment, glucocorticoids, chronic kidney disease, chronic obstructive pulmonary disease, cancer and admission white cell counts, lymphocyte counts, D-dimer, aspartate transaminase, alanine transaminase and creatinine.

[¥] Adjusted for age, sex, hypertension, cerebrovascular disease, chronic obstructive pulmonary disease, chronic kidney disease and cognitive impairment.

[φ] Calculated from data provided in the original paper.

[ω] Adjusted by propensity score matching, including age, sex, severity of COVID-19, hypertension, cardiovascular disease, cerebrovascular disease, chronic liver disease and chronic kidney injury.

hospitalized with COVID-19 shows a stepwise increase in unadjusted mortality rates from patients without diabetes or hyperglycemia through patients with known diabetes and finally patients with at-admission hyperglycemia (16.8%, 28.6% and 39.4%, respectively), although the difference between patients with diabetes and hyperglycemia bordered on statistical significance. After adjustments, only hyperglycemia at the time of admission remained as an independent predictor of mortality (HR 1.80) (17).

Glycemic Control During In-Hospital Stay

Additionally, glucose regulation during in-hospital stay is another important prognostic factor for worse outcomes in patients hospitalized with COVID-19. In 1,122 patients

hospitalized with COVID-19 in the USA, mortality rates were significantly higher in those with diabetes or uncontrolled hyperglycemia (i.e. ≥ 2 blood glucose measurements > 10.0 mmol/l (180 mg/dL) within a 24-hour period) during hospital stay as compared to those with normoglycemia (28.8% *vs* 6.2%, respectively) (20). In a Chinese retrospective multi-center study of 7,337 COVID-19 cases, of which 952 had T2D, patients with well-controlled blood glucose levels during hospitalization (glucose variability between 3.9 – 10.0 mmol/L (70 – 180 mg/dL)) were compared to patients with poorly controlled blood glucose levels (> 10.0 mmol/L (180 mg/dL)). Patients with good glycemic control during hospitalization had markedly lower mortality compared to poorly controlled patients, with an adjusted HR for mortality of 0.14 (19).

The strong influence of glycemic control and hyperglycemia on the severity of COVID-19 at all three different timepoints before as well as at the time of and during hospitalization, stresses the importance of glycemic control as a potential modifiable risk factor in patients with diabetes.

Direct and Indirect Effects of SARS-CoV-2

Several clinical studies have shown that patients with COVID-19 are prone to notable glucose dysregulation. In patients with COVID-19, an unusually high number of diabetic ketoacidosis and hyperglycemic hyperosmolar syndrome have been reported (60, 146–150). Interestingly, new-onset diabetes has also been reported in patients hospitalized with COVID-19 (151–154). In one study on eight patients with T2D that were critically ill with COVID-19 and admitted to the ICU, insulin requirements rapidly increased to extremely high doses with a mean peak insulin requirement of 201 units per day (2.2 units/kg/day) (155).

This glucose dysregulation found in patients infected with SARS-CoV-2 may be the result of several mechanisms. First, the hyperinflammation and cytokine storm present in certain patients with a severe course of COVID-19 can have a substantial effect on insulin resistance (156). In addition, high dose steroids are recommended in patients with severe or critical COVID-19 by the WHO, as it considerably reduces the risk of mortality (157). Treatment with steroids is also well-known to increase peripheral insulin resistance, thereby inducing hyperglycemia (158). Another phenomenon that could play a role is stress hyperglycemia. In acute illnesses, cortisol, epinephrine and glucagon are released as a stress response, stimulating gluconeogenesis in the liver and thereby causing transient hyperglycemia (159, 160). The hyperglycemia resulting from these processes is likely to lead to glucose toxicity of β-cells, thereby further decreasing insulin secretory function. High dose steroids as well as inflammatory cytokines can additionally impair insulin secretion (161–163). These mechanisms may individually or collectively cause or worsen glucose dysregulation in patients with COVID-19.

Apart from these indirect mechanisms, it has been suggested that SARS-CoV-2 might have a direct effect on the pancreatic β-cells through interaction with the ACE-2 receptor. In order to enter a cell, SARS-CoV-2 binds with its spike protein to the ACE-2 receptor that is present on human epithelial cells of several tissues, including the small intestine and pulmonary alveoli (both transmission routes for SARS-CoV-2). After binding to the ACE-2 receptor, the virus' spike protein is proteolytically cleaved by transmembrane serine protease 2 (TMPRSS2), which facilitates viral entry into the cells, where viral replication takes place leading to progression of the infection and cell-to-cell transmission (122). Previous research on the presence of the ACE-2 receptor in pancreatic β-cells and its role in coronavirus infection has been contradicting. Several studies have been performed after the 2002 SARS-CoV outbreak, since this virus, like SARS-CoV-2, also binds to the ACE-2 receptor. In a Chinese study, immunohistochemical staining of ACE-2 was performed on donated tissues of a 43-year old male brain-dead organ donor, including the pancreas. They showed clear staining of ACE-2 protein in the endocrine pancreatic islets of Langerhans, and nearly no staining in exocrine tissues (16). Another study used quantitative real-time polymerase chain reaction to quantitively map the transcriptional expression profile of ACE-2, and thereby found ACE-2 expression in the islets of Langerhans (164). However, neither of these studies have differentiated between the different cells present within the islets of Langerhans, i.e. the various endocrine cells as well as endothelial and ductal cells. In a recent study, immunohistochemistry showed ACE-2 expression particularly in the microvasculature, but also in ductal cells and in endocrine cells, where it was found to be preferentially expressed in β-cells (165). Conversely, a different study demonstrated ACE-2 expression in pancreatic microvascular and ductal cells, and only rarely in endocrine cells. They supported their finding by examining pancreata from three patients who died from COVID-19, where they found SARS-CoV-2 nucleocapsid protein in ductal epithelium, but not in endocrine cells (166). In an autopsy study on patients who died from SARS in the previous outbreak, SARS-CoV antigen and RNA were also not found in pancreatic endocrine cells (167). However, a recent study found that human pancreatic exocrine, but also endocrine cells express the viral entry receptor ACE-2, with highest staining coefficients in the pancreatic β-cells. The authors additionally showed that isolated human pancreatic islets, including β-cells, that were exposed to SARS-CoV-2 became infected and showed increasing intra- and extracellular viral RNA levels, pointing to SARS-CoV-2 viral replication. This was inhibited by co-culturing with remdesivir. In pancreatic β-cells that were infected, glucose-dependent insulin secretion was found to be impaired. These findings were supported by postmortem examination of different organs, including the pancreata, of four patients who died from COVID-19, staining for SARS-CoV-2 N-protein. This pancreatic histopathology revealed the presence of N-protein in both exocrine and endocrine cells in all four patients, indicating a persistent pancreatic SARS-CoV-2 infection during severe COVID-19 (168).

In summary, hyperglycemia is found in high numbers of patients with COVID-19, with mechanisms such as hyperinflammation, corticosteroid treatment, and glucose toxicity potentially contributing to this finding. Although current *in vitro* and *ex vivo* studies point to the possibility of SARS-CoV-2 infecting pancreatic β-cells, thereby impairing insulin secretion and having a direct effect on glucose

dysregulation, the clinical relevance of these findings has not been established.

DISCUSSION

In summary, patients that suffer from diabetes mellitus do not appear to have an increased primary risk of COVID-19 infection, although several factors such as differences in social distancing behavior or willingness for SARS-CoV-2 testing make a robust conclusion difficult. Patients with both type 1 and type 2 diabetes are at greater risk for a severe course of COVID-19 and mortality. This poorer prognosis is likely additionally linked to the comorbidities and other risk factors that are often concomitantly present with diabetes mellitus, but also to glycemic control. The interrelation between diabetes and COVID-19 is complicated and bidirectional, with COVID-19 causing hyperglycemia on the one hand, but hyperglycemia causing worse outcome of COVID-19 on the other hand. Diabetes itself, as well as the comorbidities often associated with diabetes additionally contribute to this risk of a severe outcome of COVID-19. This relationship is illustrated in **Figure 1**.

Although diabetes itself appears to be an independent risk factor for severe COVID-19, the most important factors that co-contribute to an increased risk of COVID-19 severity and mortality in patients with diabetes include older age, hypertension, CVD, CKD, obesity, a proinflammatory and hypercoagulable state, and glucose dysregulation. All of these factors should be acknowledged when assessing the risk of a more severe course of COVID-19 in patients with diabetes. Patients with T1D appear to have a relatively higher risk of severe COVID-19 compared to T2D. Interestingly, the risk profile for patients with T1D appears to be different compared to the risk profile of those with T2D as well, at least for glycemic control and BMI, with an HbA1c ≥ 86 mmol/mol (10.0%) in T1D but ≥ 59 mmol/mol (7.5%) in T2D associated with increased COVID-19-related mortality. In both T1D and T2D, a BMI of < 25 kg/m^2 increases the risk for death. However, in T1D a BMI of ≥ 30 kg/m^2 is already associated with increased mortality, while in T2D this risk increases at a higher BMI of ≥ 35 kg/m^2.

It is important to recognize that some of these risk factors are modifiable. For example, improved glucose regulation (i.e. better diabetes (self-)management) and a healthier BMI immediately contribute to a decreased risk of a severe course of COVID-19. It is imperative for health care professionals in the field as well as for patients with diabetes to be aware of the influence they herewith have on reducing their risk of COVID-19 severity as much as possible.

Although more knowledge is gradually surfacing as the pandemic progresses, challenges in understanding this interrelation between diabetes and COVID-19 remain. In particular, the clinical relevance of the potential direct effect of the virus on the function of pancreatic β-cells through the ACE-2 receptor, demonstrated in *in vitro* and *ex vivo* studies, requires further research. In addition, most research up until this moment has been performed in patients with T2D or in patients in whom the type of diabetes was not specified. The risk for T2D had therefore already been established. However, recently emerged evidence points to patients with T1D being at an even slightly higher risk of a more severe course of COVID-19 and increased mortality. Because of their completely different pathophysiology and the different comorbidities associated with either type of diabetes, future studies should distinguish between T1D and T2D to further understand specific risks.

AUTHOR CONTRIBUTIONS

EK initiated this study. CL designed the review structure, performed the literature search, selection and interpretation of data, and drafted the manuscript including tables and figures. CL and EK finalized and critically reviewed the manuscript and approved the final version. All authors contributed to the article and approved the submitted version.

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ORIGINAL RESEARCH
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Soluble Neuropilin-1 Response to Hypoglycemia in Type 2 Diabetes: Increased Risk or Protection in SARS-CoV-2 Infection?

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Introduction: Neuropilin-1(NRP1) is a cofactor that enhances SARS-CoV-2 coronavirus cell infectivity when co-expressed with angiotensin-converting enzyme 2(ACE2). The Renin-Angiotensin System (RAS) is activated in type 2 diabetes (T2D); therefore, the aim of this study was to determine if hypoglycaemia-induced stress in T2D would potentiate serum NRP1(sNRP1) levels, reflecting an increased risk for SARS-CoV-2 infection.

Methods: A case-control study of aged-matched T2D (n = 23) and control (n = 23) subjects who underwent a hyperinsulinemic clamp over 1-hour to hypoglycemia(<40mg/dl) with subsequent timecourse of 4-hours and 24-hours. Slow Off-rate Modified Aptamer (SOMA)-scan plasma protein measurement determined RAS-related proteins: renin (REN), angiotensinogen (AGT), ACE2, soluble NRP1(sNRP1), NRP1 ligands (Vascular endothelial growth factor, VEGF and Class 3 Semaphorins, SEM3A) and NRP1 proteolytic enzyme (A Disintegrin and Metalloproteinase 9, ADAM9).

Results: Baseline RAS overactivity was present with REN elevated and AGT decreased in T2D ($p<0.05$); ACE2 was unchanged. Baseline sNRP1, VEGF and ADAM9 did not differ between T2D and controls and remained unchanged in response to hypoglycaemia. However, 4-hours post-hypoglycemia, sNRP1, VEGF and ADAM9 were elevated in T2D ($p<0.05$). SEMA3A was not different at baseline; at hypoglycemia, SEMA3A decreased in controls only. Post-hypoglycemia, SEMA3A levels were higher in T2D versus controls. sNRP1 did not correlate with ACE2, REN or AGT. T2D subjects stratified according to ACE inhibitor (ACEi) therapies showed no difference in sNRP1 levels at either glucose normalization or hypoglycaemia.

Conclusion: Hypoglycemia potentiated both plasma sNRP1 level elevation and its ligands VEGF and SEMA3A, likely through an ADAM9-mediated mechanism that was not associated with RAS overactivity or ACEi therapy; however, whether this is protective or promotes increased risk for SARS-CoV-2 infection in T2D is unclear.

Clinical Trial Registration: https://clinicaltrials.gov, identifier NCT03102801.

Keywords: Neuropilin-1, type 2 diabetes, COVID-19, SARS-CoV-2, ACE inhibitors, ADAM9

INTRODUCTION

Neuropilin-1 (NRP1) is a cofactor that enhances SARS-CoV-2 coronavirus cell infectivity when co-expressed with ACE2 (1). SARS-CoV-2 uses the spike protein for cell entry, and its cleavage facilitates attachment to NRP1; therefore, tissues with increased membrane NRP1 have increased infectivity risk (1) and increased circulating NRP1 expression may raise infection risk. NRP1 is a 120–130 kDa surface-expressed glycoprotein, initially characterized as a neuronal receptor for specific secreted members of the semaphorin family (SEMA3) involved in axon repulsion (2). NRP-1 also serves as a receptor for a number of isoforms of vascular endothelial growth factor (VEGF). Soluble isoforms of NRP1 (sNRP1) also exist without transmembrane or cytoplasmic domains and function as natural ligand sequesters, inhibiting the interaction of VEGF-A or other growth factors with their specific receptors and with membrane-bound NRP1 (3). The generation of sNRP1 is mediated *via* proteolytic cleavage by a disintegrin or metalloproteinase-9 or 10 (ADAM9 or ADAM10) (4). NRP1 interacts with the RAS, a risk factor for COVID-19 disease (5), contributing to protection from angiotensin II-induced hypertension (6).

T2D is associated with high risk for acquiring SARS-Cov-2 infection, severe disease, acute respiratory distress syndrome and increased mortality (7). Diabetic patients have an overactive RAS with ACE2 being overexpressed in kidney (8) and the circulation (9), and ACE2 expression may be increased in lungs that is thought to increase susceptibility and severity to SARS-Cov-2 infection. Furthermore, patients with T2D exist in a state of chronic low-grade inflammation (10); both hypoglycaemia and hyperglycemia compound the risk of worse outcomes in hospitalized T2D patients with COVID-19 (11), likely due to the increase in inflammatory mediators that further promote risk for an acute cardiovascular event and/or multi-organ failure (12, 13).

Here, we hypothesized that underlying RAS activation may potentiate the levels of sNRP1 and its ligands (VEGF and SEMA3A) in hypoglycaemia-induced stress in type 2 diabetes (T2D), reflecting an increased risk for SARS-CoV-2 infection.

MATERIALS AND METHODS

Study Design

This prospective parallel study was performed in 46 subjects, adult T2D (n=23) and control (n=23). The duration of diabetes was <10 years and all T2D subjects were on a stable dose of medication (metformin, statin and/or angiotensin converting enzyme inhibitor/angiotensin receptor blocker) over the prior 3 months. For those with T2D, no medications for glycemic control except metformin were allowed. The study was performed at the Diabetes Centre at Hull Royal Infirmary. All participants provided written informed consent. The trial was approved by the North West-Greater Manchester East Research Ethics Committee (REC number:16/NW/0518), registered at www.clinicaltrials.gov (NCT03102801) and conducted according to the Declaration of Helsinki.

Hyperinsulinemic Clamp

The method for performing the insulin clamp has been published previously (14). A schematic diagram of the insulin clamp study, showing the intervention and blood sampling time points has been outlined in **Figure 1**. Briefly, after an overnight fast, bilateral ante-cubital fossa indwelling cannulae were inserted 30 to 60 minutes prior to the commencement of the clamp (8:30 AM). To induce hypoglycemia, soluble intravenous insulin (Humulin S; Eli Lilly, Liverpool. UK) was given in a pump starting at a dose of 2.5 mU/Kg body weight (BW)/min, with an increment of 2.5mU/Kg BW/min every 15 minutes until two readings of venous blood glucose measured by a glucose analyser (HemoCue glucose 201+, Sweden) of 2.2 mmol/L (<40 mg/dl) or a single reading of 2.0 mmol/L (36 mg/dL) was obtained (14). The blood sample schedule was timed subsequently with respect to the time point when hypoglycemia occurred (**Figure 1**). Following the identification of hypoglycemia, intravenous glucose was given in the form of 150 mL of 10% dextrose and repeat blood glucose checks were performed after 5 minutes if blood glucose was still <4.0 mmol/L. All patients achieved a blood glucose of 2.0 mmol/L (36 mg/dL) at the point of hypoglycemia.

Blood Sample Preparation and Biochemical Marker Analyses

Venous blood samples collected during the screening visit were analysed for serum insulin, total cholesterol, triglycerides, HDL cholesterol, C-reactive protein (CRP) and glycated haemoglobin (HbA1C).

Blood samples were separated immediately by centrifugation at 3500g for 15 minutes at 4°C, and the aliquots were stored at –80°C, within 30-minutes of blood collection, until batch analysis. Serum insulin was assayed using a competitive chemiluminescent immunoassay performed on the manufacturer's DPC Immulite 2000 analyser (Euro/DPC, Llanberies, UK), with a coefficient of variation of 6 and no stated cross-reactivity with proinsulin.

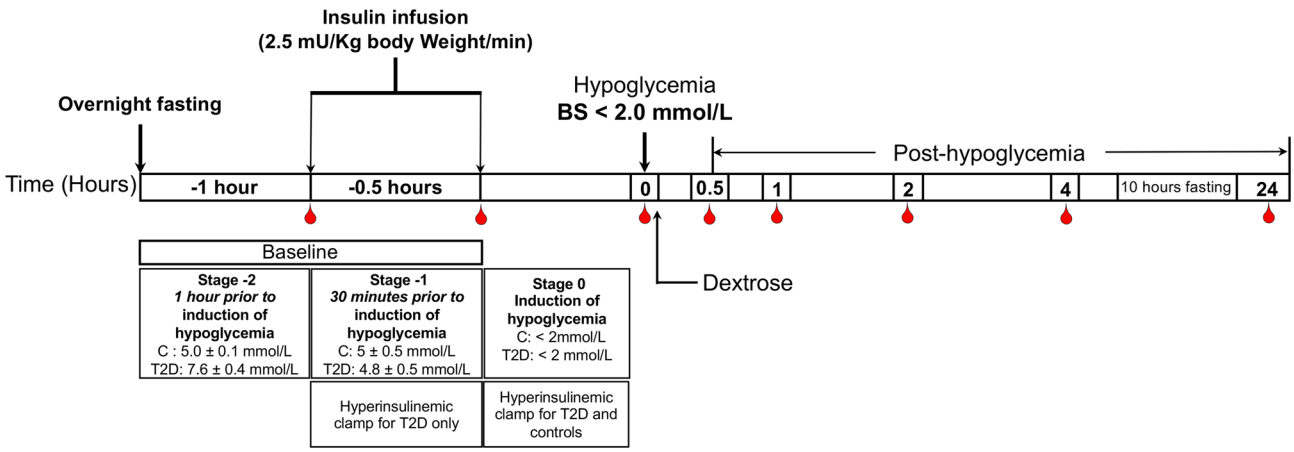

FIGURE 1 | Schematic diagram of the insulin clamp study, showing the intervention and blood sampling time points.

Fasting plasma glucose (FPG), total serum cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol levels were measured enzymatically using a Beckman AU 5800 analyser (Beckman-Coulter, High Wycombe, UK). LDL cholesterol was calculated using the Friedewald equation. Plasma whole blood samples were analysed for HbA1C on a Menarini Diagnostics HB9210 premier (A. Menarini Diagnostics Ltd, Winnersh-Wokingham, UK).

SOMA-Scan Assay

The SOMAscan assay used to quantify proteins was performed on an in-house Tecan Freedom EVO liquid handling system (Tecan Group, Maennedorf, Switzerland) utilizing buffers and SOMAmers from the SOMAscan HTS Assay 1.3K plasma kit (SomaLogic, Boulder, CO) according to manufacturer's instructions and as described previously (15–17). Initial Relative Fluorescent Units (RFUs) were obtained from microarray intensity images using the Agilent Feature Extraction Software (Agilent, Santa Clara, CA). Raw RFUs were normalized and calibrated using the software pipeline provided by SomaLogic.

Statistical analyses were performed on $\log_2$ RFU values using R version 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria) including base R package. Data handling and differential protein expression were analyzed using the autonomics and limma (18) packages. For differential protein analysis we applied limma models containing contrasts between timepoints, as well as contrasts between healthy and patients with diabetes at single timepoints. In both models, blocking by patient ID was performed to account for random effects. Batch effect correction was performed by adding batch as a covariate to the model. Limma obtained P values were corrected using the Benjamini-Hochberg method (19).

Statistical Analysis

There are no studies detailing the changes in NRP1 proteins in response to hypoglycaemia on which to base a power calculation. Sample size for pilot studies has been reviewed by Birkett and Day (20). They concluded that a minimum of 20 degrees-of-freedom was required to estimate effect size and variability. Hence, we needed to analyse the samples from a minimum of 20 patients per group. Data trends were visually evaluated for each parameter and non-parametric tests were applied on data that violated the assumptions of normality when tested using the Kolmogorov-Smirnov Test. Comparison between groups was performed at each timepoint using Student's t-test. A p-value of <0.05 was considered statistically significant. Statistical analysis was performed using Graphpad Prism (San Diego, CA, USA).

RESULTS

Study Participants

T2D (n=23) and control (n=23) subjects were matched for age (p=ns); T2D had higher BMI (p=0.0012); duration of disease was 4.5 ± 2.9 years (**Table 1**). Nine T2D subjects were treated with ACE inhibitor (ACEi) therapy. Systolic and diastolic blood pressure were higher in T2D ($p<0.001$). Renin was elevated and angiotensinogen decreased in T2D ($p<0.05$), indicating RAS overactivity; ACE2 was unchanged (**Table 2**) (5).

Changes of Plasma sNRP1 Levels in Response to Glucose Normalization and Hypoglycemia

Baseline levels of sNRP1 did not differ between T2D and controls (2298 ± 385 *vs* 2279 ± 488 RFU, T2D *vs* control, p=ns); normalization of glucose in T2D did not alter sNRP1 (2298.1 ± 80.3 *vs* 2279.1 ± 101.6 RFU of NRP1, T2D *vs* control, p=ns) (**Figure 2A**), and levels were unchanged in response to insulin induced normalization of glycemia (in T2D) (**Figure 2A**) and hypoglycaemia (both in control and in T2D) (**Figure 2B**). However, 4-hours post-hypoglycemia, NRP1 was elevated in

TABLE 1 | Demographic and clinical characteristics of the study participants.

Baseline	Type 2 Diabetes (n = 23)	Controls (n = 23)	p-value
Age (years)	64 ± 8	60 ± 10	<0.0001
Sex (M/F)	12/11	11/12	0.77
Weight (kg)	90.9 ± 11.1	79.5 ± 8.8	<0.0001
Height (cm)	167 ± 14	169 ± 5	0.64
BMI (kg/m^2)	32 ± 4	28 ± 3	<0.0001
Systolic BP (mmHg)	132 ± 8	122 ± 8	0.001
Diastolic BP (mmHg)	81 ± 7	75 ± 6	0.003
Duration of diabetes (years)	4.5 ± 2.2	N/A	
HbA1c (mmol/mol)	51.2 ± 11.4	37.2 ± 2.2	<0.0001
HbA1c (%)	6.8 ± 1.0	5.6 ± 0.2	<0.0001
Total cholesterol (mmol/l)	4.2 ± 1.01.0	4.8 ± 0.77	0.02
Triglyceride (mmol/l)	1.7 ± 0.7	1.34 ± 0.6	0.06
HDL-cholesterol (mmol/l)	1.1 ± 0.3	1.5 ± 0.4	0.001
LDL-cholesterol (mmol/l)	2.23 ± 0.8	2.7 ± 0.87	0.051
CRP (mg/l)	3.10 ± 2.87	5.30 ± 1110.03	0.66

BMI, Body mass index; BP, Blood pressure; HDL-cholesterol, High density lipoprotein cholesterol; LDL-cholesterol, Low density lipoprotein cholesterol; CRP, C-reactive protein; HbA1c, Hemoglobin A1c.

T2D (2476 ± 117 *vs* 2216 ± 94 RFU, T2D *vs* control, p=0.04) (**Figure 2B**). When T2D subjects were stratified according to ACE inhibitor (ACEi) therapies, there was no difference in sNRP1 levels in subgroups at either glucose normalization or hypoglycaemia (**Figure 2C**). sNRP1 did not correlate with any of the renin angiotensin system (RAS) proteins measured here: ACE2, REN or AGT (**Figure 3**).

Changes of Plasma Levels of NRP1 Proteolytic Enzyme ADAM9 in Response to Hypoglycemia in T2D

A Disintegrin and Metalloproteinase 9 (ADAM9) has been reported to be involved in proteolysis of NRP1 in human endothelial cells and that this process was stimulated by vascular endothelial growth factor (VEGF) (4). Therefore, plasma ADAM9 levels were determined and showed that ADAM9 did not differ at baseline between control and T2D subjects (1107 ± 60 *vs* 1255 ± 81 RFU of ADAM9, T2D *vs* control, p=ns) but were decreased following hypoglycaemia in T2D (1h and 2h post-hypoglycemia) (**Figure 4A**). Interestingly, in T2D, ADAM9 levels increased sharply 4-hours post-hypoglycemia compared to either the 2h post-hypoglycemia timepoint (1356 ± 81 *vs* 1019 ± 68 RFU of ADAM9 4h post-hypo *vs* 2h post-hypo, p<0.01) or baseline (1356 ± 81 *vs* 1107 ± 60 RFU of ADAM9 4h post-hypo *vs* baseline, p<0.05) (**Figure 4A**). Elevated ADAM9 levels were also observed 24h post-hypoglycemia both in control and T2D subjects (**Figure 4A**).

Changes of NRP1 Ligands (VEGFA and SEMA3A) in Response to Hypoglycaemia in T2D

Soluble isoforms of NRP1 (sNRP1) also exist without transmembrane or cytoplasmic domains, expressing only the extracellular domains which allow them to bind NRP1 ligands (21); therefore, the plasma levels of NRP1 ligands (VEGFA and SEMA3A) were determined in response to insulin-induced hypoglycemia. Neither VEGFA nor SEMA3A was different at baseline in T2D compared to controls (9037 ± 188 *vs* 9113 ± 212 RFU of VEGF, T2D *vs* control, p=ns; 970 ± 43 *vs* 935 ± 45 RFU of SEMA3A, T2D *vs* control, p=ns). Normalization of hyperglycemia did not alter plasma VEGF or SEMA3A levels in T2D (9145 ± 275 *vs* 9037 ± 188 RFU of VEGF, euglycemia *vs* baseline in T2D, p=ns; 965 ± 51 *vs* 970 ± 43, RFU of SEMA3A, euglycemia *vs* baseline in T2D, p=ns). While insulin-induced hypoglycemia did not alter plasma VEGF levels in either control or T2D subjects (**Figure 4B**), plasma SEMA3A levels significantly decreased at hypoglycemia in control cases compared to baseline (799 ± 27 *vs* 935 ± 45 RFU of SEMA3A in control, hypoglycemia *vs* baseline, p=0.01) (**Figure 4C**). Post-hypoglycemic SEMA3A levels (from 0.5h post-hypo to 24h post-hypo) did not differ from baseline or hypoglycemia in either controls or T2D; however, SEMA3A levels were generally elevated in T2D, showing significance at 0.5h, 2h and 4h post-hypoglycemia (p<0.05) compared to controls (**Figure 4C**). Interestingly, in a similar manner to ADAM9, VEGF levels also increased sharply 4-hours post-hypoglycemia compared to the 2h post-hypoglycemia, hypoglycemia or baseline timepoints (4h *vs* 2h: 9947 ± 225 *vs* 9026 ± 185 RFU of VEGF, p=0.002; 4h *vs* hypoglycemia: 9947 ± 225 *vs* 8894 ± 208 RFU of VEGF, p=0.001;

TABLE 2 | Circulating levels of renin angiotensin system (RAS)-related proteins at baseline and in response to hypoglycemia in control subjects and in subjects with T2D.

T2D subjects					
Renin (RFU)		**Angiotensinogen (RFU)**		**ACE2 (RFU)**	
Baseline (BL)	Hypoglycemia (H)	Baseline (BL)	Hypoglycemia (H)	Baseline (BL)	Hypoglycemia (H)
1730 ± 566	1601 ± 511	3786 ± 174	4027 ± 261	285 ± 29	286 ± 29
P=0.5 (BL *vs* H in T2D)		P=0.7 (BL *vs* H in T2D)		P=0.9 (BL *vs* H in T2D)	
Control subjects					
Renin (RFU)		**Angiotensinogen (RFU)**		**ACE2 (RFU)**	
Baseline (BL)	Hypoglycemia (H)	Baseline (BL)	Hypoglycemia (H)	Baseline (BL)	Hypoglycemia (H)
675 ± 71	666 ± 70	5005 ± 573	5248 ± 558	281 ± 18	277 ± 17
P=0.5 (BL *vs* H in control)		P=0.5 (BL *vs* H in control)		P=0.8 (BL *vs* H in control)	
P=0.02 (T2D *vs* control at BL)		**P=0.04 (T2D *vs* control at BL)**		P=0.7 (T2D *vs* control)	

Data is expressed as Mean ± SEM. RFU, relative fluorescent units; BL, baseline; H, hypoglycemia.

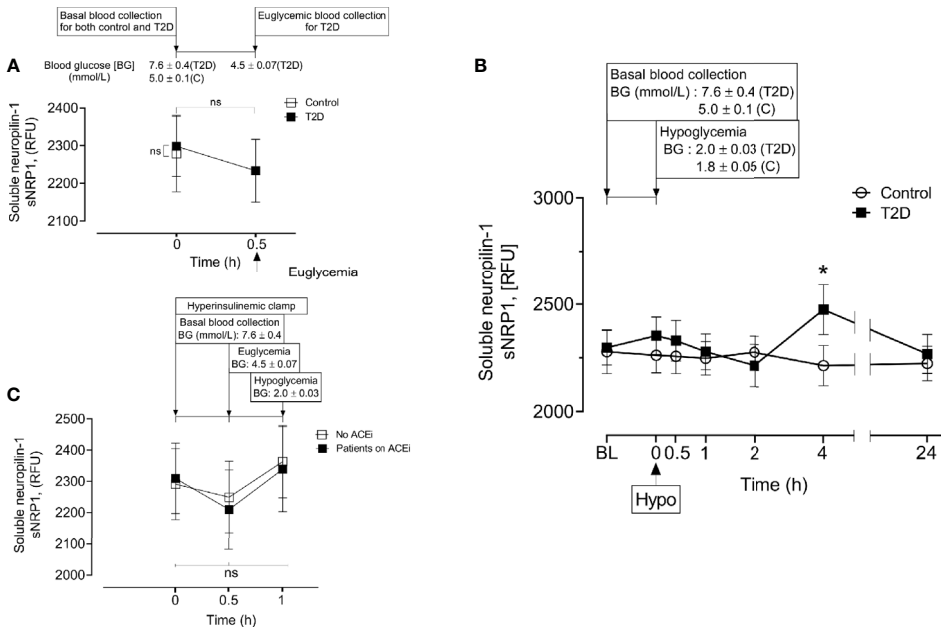

FIGURE 2 | Circulatory levels of Neuropilin-1 (soluble neuropilin-1, sNRP1) in plasma before, during and after iatrogenic induction of hypoglycemia. Blood sampling was performed at baseline (BL), euglycemia (30 min before hypoglycemia in type 2 diabetes (T2D) only), at hypoglycemia (0 min) and post-hypoglycemia (30 minutes, 1-hour, 2-hours, 4-hours and 24-hours). At baseline (BL), blood glucose (BG) was 7.6 ± 0.4 mmol/L (for T2D) and 5.0 ± 0.1 mmol/L (for control, **C**). At glucose normalization, BG was 4.5 ± 0.07 mmol/L (for T2D). At the point of hypoglycemia, BG was 2.0 ± 0.03 mmol/L (for T2D) and 1.8 ± 0.05 mmol/L (for control). **(A)** Effect of glucose normalization on plasma sNRP1 levels in T2D (black squares) and control (open squares) subjects. **(B)**, Changes in sNRP1 levels in response to hypoglycaemia and post-hypoglycemic timepoints in control (white circles) and in T2D (black squares) subjects. **(C)**, Effect of glucose normalization and hypoglycaemia on plasma levels of sNRP1 in T2D patients who were treated with ACE inhibitors (ACEi) (black squares) and those without ACEi therapy (white squares). *p < 0.05, T2D *vs* control; Hypo, hypoglycaemia; RFU, relative fluorescent units; ns, not significant.

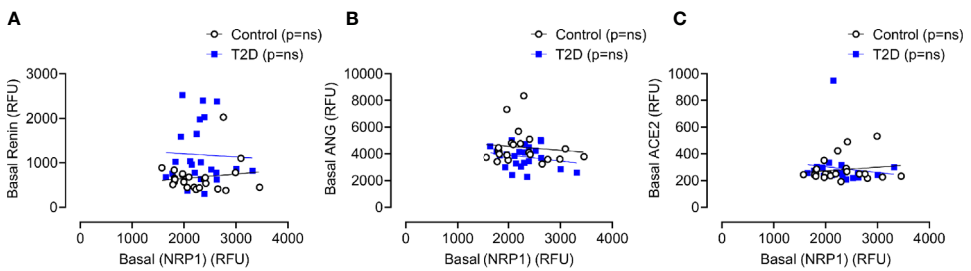

FIGURE 3 | Correlation of plasma levels of soluble NRP1 (sNRP1) with basal levels of renin angiotensinogen system (RAS) proteins. Correlation of soluble NRP1 (sNRP1) with Renin **(A)**, angiotensinogen (ANG) **(B)** and angiotensin-converting enzyme 2 (ACE2) **(C)**. No correlation of sNRP1 with these RAS proteins was found in either T2D or control subjects. Control subjects, open black circles; T2D subjects, solid blue squares; ns, not significant.

4h *vs* baseline: 9947 ± 225 *vs* 9037 ± 188 RFU of VEGF, p=0.003) (**Figure 4B**).

Plasma RAS and sNRP1 Related Proteins in Response to Hyperglycemia in T2D

To determine whether hyperglycemia affects the levels of RAS or sNRP1 associated proteins, we performed correlation analysis between the change in blood glucose from hyperglycemia (baseline) to normoglycemia with basal levels of plasma RAS-related proteins (Renin, ANG and ACE2) (**Supplementary Figure 1A**) and plasma sNRP1 related proteins (sNRP1, VEGF, SEMA3A and ADAM17) (**Supplementary Figure 1B**). Our data indicate that none of the proteins differed in response to hyperglycemia in T2D.

Gender Stratification

We next sought to determine whether sNRP1 related proteins differ between males and females in response to changes in

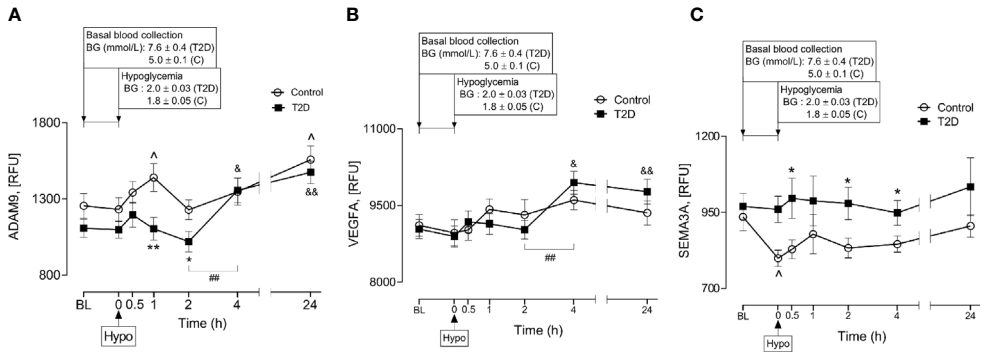

FIGURE 4 | Plasma levels of A Disintegrin and Metalloproteinase 9 (ADAM9), class 3 semaphorins (SEMA3A) and vascular endothelial growth factor (VEGF) before, during and after iatrogenic induction of hypoglycemia. Changes in plasma ADAM9 **(A)**, VEGF **(B)** and SEMA3A **(C)** levels in response to hypoglycaemia and post-hypoglycemic timepoints in control (white circles) and T2D (black squares) subjects. *p < 0.05, **p < 0.01, control *vs* T2D; ^p < 0.05, control hypoglycaemia *vs* control post-hypoglycemia timepoints; $^{\&}$p < 0.05, $^{\&\&}$p < 0.01, T2D hypoglycaemia *vs* T2D post-hypoglycemia timepoints; $^{\#\#}$p < 0.01, T2D 2-h post-hypoglycemia *vs* T2D 4-h post-hypoglycemia. Hypo, hypoglycemia; RFU, relative fluorescent units.

glycemia. Stratification of plasma levels of sNRP1, VEGF, SEMA3A and ADAM17 based on gender demonstrated no significant differences in their levels at baseline or hypoglycemia in control subjects (**Supplementary Figures 2A, B**); nor at baseline, normoglycemia, or hypoglycemia in T2D subjects (**Supplementary Figures 2C–E**).

DISCUSSION

Here we report the levels of soluble neuropilin-1 (sNRP1), NRP1 proteolytic enzyme ADAM9 and NRP1 ligands, VEGF and SEMA3A, in response to insulin-induced hypoglycemia in obese patients with T2D. Our data demonstrate that soluble sNRP1, VEGF or SEMA3A and ADAM9 levels did not differ from control subjects in the basal condition in obese T2D; however, an alteration of their levels was observed in response to insulin-induced hypoglycemia. An elevation of sNRP1 levels in association with its proteolytic enzyme as well as ligands post-hypoglycemia suggests the possible connection of glycemic control with NRP1 cleavage in obese T2D subjects.

NRP1, a transmembrane glycoprotein, was initially identified as a receptor for class 3 semaphorins (SEMA3A), which are negative mediators of neuronal guidance (2, 22). NRP1 also functions as a high-affinity co-receptor for a number of vascular endothelial growth factor (VEGF) isoforms, in particular $VEGF_{165}$, and enhances its activity in functions such as endothelial cell migration (23, 24). NRP1 is a single spanning transmembrane glycoprotein consisting of a large extracellular domain, a very short transmembrane domain and a short cytoplasmic domain. The NRP1 extracellular domain is divided into: (i) an A domain consisting of two a-domain repeats (a1a2) homologous to the complement proteins C1r and C1s, (ii) a B domain consisting of two b-domain repeats (b1b2) (25). In addition to the membrane form, a naturally occurring soluble NRP-1 protein (sNRP1) containing only the extracellular a1/a2 and b1/b2 domains is generated by alternative splicing of the NRP-1 gene (26) and this is capable of binding $VEGF_{165}$ and SEMA3A (27). Therefore, sNRP1 is thought to function as a natural inhibitor of the membrane NRP1 by sequestering its ligands (3). Our data also demonstrate a simultaneous increase of sNRP1 and VEGF (4h post-hypoglycemia), suggesting a possible sNRP1 and VEGF interaction after the induction of hypoglycemia in obese T2D. sNRP1-induced ligand sequestration (by binding with VEGF) may block the interaction of VEGF and membrane-bound NRP1 (21); therefore, this action may reduce angiogenesis in severe COVID-19 disease and decrease the risk of SARS-CoV-2 cellular infectivity. Additionally, akin to sNRP1 antagonism of VEGF165 as an antiangiogenic agent (3), sNRP1 may bind to SARS-CoV-2 virus, preventing its binding to tissue NRP1 thus hindering or preventing its tissue entry.

There is evidence to suggest that increased sNRP1, seen here following insulin-induced transient hypoglycaemia but also as a consequence of strict glycemic control, might offer protection against COVID-19 disease severity. Optimizing glycemic control during hospitalization has been associated with a reduction in the risk of severe disease and death in patients with COVID-19 (28). Moreover, in a subset comparison of age and risk-matched COVID-19 patients with pre-existing T2D and poor glucose control (blood glucose >10.0 mmol/L), there was a reduction in mortality from 11.1% to 1.1% with significantly lower d-dimer, C-reactive protein (CRP) and interleukin 6 (IL6) when glucose was maintained in a range of 3.9-10 mmol/L (29). Since our data also demonstrated an increased plasma sNRP1 level 4-h post insulin-induced hypoglycaemia, it is highly likely that glycemic control-mediated improvement of severity in COVID-19 patients is associated with elevated sNRP1. However, further studies with blood sampling from COVID-19 patients should be performed to measure sNRP1 levels and to study the correlation between sNRP1 and COVID-19 risk factors (for example, CRP, d-dimer and IL6) in response to glycemic control in order to elucidate the mechanism.

The mechanism increasing sNRP1 levels in obese T2D in response to hypoglycemia is unclear; however, post-hypoglycemic ADAM9 levels were also higher at similar

timepoints as the elevated sNRP1 levels in T2D cases. ADAM9 is a disintegrin and metalloproteinase involved in a wide array of cellular processes, especially those involving cell to cell interactions, adhesion, cell-matrix interactions, growth factor and cytokine signalling (30–32). Membrane-bound NRP1 is proteolytically cleaved by ADAM9 (or ADAM10) to produce its soluble form, sNRP1 (4). Here, ADAM9 was increased at 4-hours post-hypoglycemia in T2D and at 24-hours post-hypoglycemia in both T2D and control subjects. This suggests that ADAM9-mediated shedding of membrane bound NRP1 may potentiate elevated levels of sNRP1 over a prolonged period of time following a hypoglycaemic event.

Our data also demonstrate post-hypoglycemic elevation of plasma SEMA3A in T2D. SEMA3A levels have been reported to be significantly elevated in the vitreous fluid of patients with diabetic macular edema (33) and proliferative diabetic retinopathy (PDR) (34) *via* NRP1. Serum SEMA3A levels have also correlated with the phenotypes of diabetic retinopathy (35). Since secreted semaphorins (SEMA3 class) generally require NRPs as obligate co-receptors to interact with their surface receptors (36), it is likely that sNRP1 would also interact with SEMA3A in obese T2D in response to hypoglycemia. Thus, hypoglycemia-induced sNRP1 may pose a risk for diabetic microvascular complications which, in turn, may enhance the poor outcome of COVID19 severity in obese patients with T2D. However, apart from the connection of sNRP1 and risk of SARS-Cov-2, hypoglycemia-induced elevation in sNRP1 might also indicate a possible link between glucose counter-regulation and VEGF-NRP1 signalling in liver and kidney in T2D because sNRP1, but not NRP1, mRNA is expressed in liver hepatocytes and kidney distal and proximal tubules. On the other hand, NRP1 but not sNRP1, is expressed in liver veins and glomerular capillaries (3).

This study further showed that, whilst plasma renin was elevated, and angiotensinogen suppressed in T2D (5), that there were comparable levels of ACE2 and sNRP1 proteins between T2D and control subjects. In T2D, the sNRP1 levels did not change in response to normoglycemia or hypoglycemia regardless of whether or not the T2D subjects were on antihypertensive medication (ACEi). This suggests that there is RAS overactivation in T2D, and this was not affected by either acute normoglycemia or hypoglycaemia. However, sNRP1 levels were elevated post-hypoglycemia in T2D, suggesting that this hypoglycemic insult may result in a delayed but increased SARS-CoV-2 susceptibility. However, it is not precisely known how long that potential susceptibility window may last as blood sampling was not undertaken between the 4-hour and 24-hour post-hypoglycemia timepoints.

Whilst hyperglycemia may lead to more severe COVID-19 disease, the risk of hypoglycemia has also been shown to be increased during the COVID-19 pandemic, especially for those patients on the hypoglycemic agents sulphonylureas and insulin (37) and, paradoxically, that may mean that those T2D patients with the tightest, most optimal control (but with greater risk of hypoglycaemia) may be at higher risk of infection. Hydroxychloroquine may induce hypoglycemia in subjects with and without diabetes (38) and it has been shown that prophylactic hydroxychloroquine (HCQ) increases hypoglycemia in T2D (37); this may, therefore, conversely increase the subsequent risk of SARS-CoV-2 susceptibility. Moreover, an Indian study of patients with T2D revealed that the COVID-19 lockdown has been shown to increase the risk of hypoglycemia in patients with T2D, especially those receiving sulfonylureas (SU), insulin and HCQ, and especially in patients with associated co-morbidities (37). Notably, diabetic kidney disease (DKD), a co-morbidity already associated with increased risk of hypoglycaemia in non-COVID-19 infected patients due to factors such as reduced insulin clearance and degradation (39, 40), was the co-morbidity most frequently associated with hypoglycaemia during lockdown (37).

Study strengths include that these T2D subjects had a short disease duration and were relatively medication naïve. Study limitations include the small population under study and that measurement of circulating sNRP1, ADAM9, VEGF and SEMA3A may not reflect tissue levels; also, correlation of sNRP1 was with renin proteins rather than renin activity. In addition, the SOMAscan assay is designed as a discovery platform and measures relative protein concentrations using only external controls. Without internal controls and standard curves, it remains unclear which measurements are within the linear dynamic range (41); therefore, from a population point of view the same trends and associations will be present, but validation would be needed to individualise the results for treatment that was not done for the proteins reported here.

In conclusion, this data shows that strict blood glucose control associated with increased hypoglycemia potentiates sNRP1 levels *via* a mechanism involving ADAM9-mediated proteolytic cleavage of NRP1 that may continue to enhance sNRP1 levels following the hypoglycemic episode, independent of RAS activation or ACEi therapy. This post-hypoglycemia elevation of plasma sNRP1 may place patients with pre-existing obesity and T2D at increased risk for severe COVID19 disease; however, studies to clarify the role of sNRP1 in COVID19 patients are needed to determine if it promotes risk or affords protection for SARS-CoV-2 infection in T2D.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by North West-Greater Manchester East Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AM and AB analyzed the data and wrote the manuscript. AA-Q contributed to study design, performed experiments, collected, analyzed, and interpreted data and edited the manuscript.

TS supervised clinical studies and edited the manuscript. SA contributed to study design, data interpretation and the writing of the manuscript. AB is the guarantor of this work. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.665134/full#supplementary-material

Supplementary Figure 1 | Correlation of change of blood glucose from normoglycemia to hyperglycemia with basal levels of renin angiotensinogen system (RAS) proteins, Renin, angiotensinogen (ANG) and angiotensin-converting enzyme 2 (ACE2) **(A)** and sNRP1 related proteins, sNRP1, VEGF, SEMA3A and ADAM17 **(B)** in T2D. No correlation of hyperglycemia with those proteins was found T2D subjects. Part A: Renin, open black circles; angiotensinogen (ANG), open blue squares; ACE2, open red diamonds. Part B: NRP1, open black circles; SEMA3A, open blue squares; ADAM9, open red diamonds; VEGF, open green triangles.

Supplementary Figure 2 | Gender stratification of RAS proteins or sNRP1 related proteins at baseline or at hypoglycemia. No significant differences between male and female in the levels of sNRP1, VEGF, SEMA3A or ADAM17 at baseline and hypoglycemia in control **(A, B)**; and at baseline, normoglycemia and hypoglycemia in T2D **(C–E)**.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Type 2 Diabetes Coagulopathy Proteins May Conflict With Biomarkers Reflective of COVID-19 Severity

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Objective: Detailed proteomic analysis in a cohort of patients with differing severity of COVID-19 disease identified biomarkers within the complement and coagulation cascades as biomarkers for disease severity has been reported; however, it is unclear if these proteins differ sufficiently from other conditions to be considered as biomarkers.

Methods: A prospective, parallel study in T2D (n = 23) and controls (n = 23). A hyperinsulinemic clamp was performed and normoglycemia induced in T2D [4.5 ± 0.07 mmol/L (81 ± 1.2 mg/dl)] for 1-h, following which blood glucose was decreased to ≤2.0 mmol/L (36 mg/dl). Proteomic analysis for the complement and coagulation cascades were measured using Slow Off-rate Modified Aptamer (SOMA)-scan.

Results: Thirty-four proteins were measured. At baseline, 4 of 18 were found to differ in T2D *versus* controls for platelet degranulation [Neutrophil-activating peptide-2 ($p = 0.014$), Thrombospondin-1 ($p = 0.012$), Platelet factor-4 ($p = 0.007$), and Kininogen-1 ($p = 0.05$)], whilst 3 of 16 proteins differed for complement and coagulation cascades [Coagulation factor IX ($p < 0.05$), Kininogen-1 ($p = 0.05$), and Heparin cofactor-2 ($p = 0.007$)]; STRING analysis demonstrated the close relationship of these proteins to one another. Induced euglycemia in T2D showed no protein changes *versus* baseline. At hypoglycemia, however, four proteins changed in controls from baseline [Thrombospondin-1 ($p < 0.014$), platelet factor-4 ($p < 0.01$), Platelet basic protein ($p < 0.008$), and Vitamin K-dependent protein-C ($p < 0.00003$)], and one protein changed in T2D [Vitamin K-dependent protein-C, ($p < 0.0002$)].

Conclusion: Seven of 34 proteins suggested to be biomarkers of COVID-19 severity within the platelet degranulation and complement and coagulation cascades differed in T2D *versus* controls, with further changes occurring at hypoglycemia, suggesting that validation of these biomarkers is critical. It is unclear if these protein changes in T2D may predict worse COVID-19 disease for these patients.

Clinical Trial Registration: https://clinicaltrials.gov/, identifier NCT03102801.

Keywords: type 2 diabetes, hypoglycemia, COVID-19, biomarkers, proteomics

INTRODUCTION

Proteomic pathways of platelet degranulation and the complement and coagulation cascades have recently been suggested as biomarkers of disease severity, being derived from a cohort of patients with differing severity of COVID-19 disease, including non-survivors (1). These data were in accord with another recently reported plasma proteomic analysis that also identified biological pathways involved in platelet degranulation and the coagulation cascade (2) in COVID-19 disease. Biomarkers to predict COVID-19 disease progression and outcome that allow a measured and appropriate proactive intervention may be key in improving patient treatment (3); however, they do need to be distinct from levels found in an uninfected population. In this regard, protein expression showing altered platelet function resulting in a prothrombotic potential together with changed markers of coagulation (4) has been reported in patients with type 2 diabetes (T2D) (5). With the increasing prevalence of diabetes, particularly with those patients having a worse COVID-19 outcome, this is of particular relevance (6).

Therefore, we undertook platelet degranulation and complement and coagulation cascade proteomic analysis in subjects with and without T2D to compare with these coagulopathy pathways described recently using proteomics in COVID-19 disease (1).

METHODS

Type 2 diabetes (T2D) (n = 23) and control subjects (n = 23) were enrolled in a case-controlled study, approved by Yorkshire and Humber Research Ethics Committee. A hyperinsulinemic clamp was performed as reported (7); all subjects were Caucasian and underwent a 10-h fast prior to the clamp. T2D: T2D baseline glucose 7.6 ± 0.4 mmol/L (136.8 ± 7.2 mg/dl), reduced to 4.5 ± 0.07 mmol/L (81 ± 1.2 mg/dl) for 1-h, following which the blood glucose was decreased to ≤2.0 mmol/L (36 mg/dl). Controls: baseline 4.9 ± 0.1 mmol/L (88.2 ± 1.8 mg/dl) (**Supplementary Figure 1**). The duration of diabetes was <10 years and all T2D subjects were on a stable dose of medication (metformin, statin, and/or angiotensin converting enzyme inhibitor/angiotensin receptor blocker) over the prior 3 months. For those with T2D, no medications for glycemic control except metformin was allowed. All had normal renal and hepatic biochemical indices and no prior history of cancer nor any contraindication to insulin infusion to achieve hypoglycemia (ischemic heart disease, epilepsy, seizure history, drop attacks, history of adrenal insufficiency, and treated hypothyroidism).

Proteins that were described for platelet degranulation (18 of 27 proteins) and the complement and coagulation cascades (16 of 19 proteins) (1) were measured using the Slow Off-rate Modified Aptamer (SOMA)-scan plasma protein measurement (7), shown in **Table 1**.

Statistical Analysis

There are no studies detailing the changes in platelet degranulation or complement and coagulation proteins in response to hypoglycemia on which to base a power calculation. Sample size for pilot studies has been reviewed by Birkett and Day (8). They concluded that a minimum of 20 degrees-of-freedom was required to estimate effect size and variability. Hence, we needed to analyze the samples from a minimum of 20 patients per group. Data trends were visually evaluated for each parameter and non-parametric tests were applied on data that violated the assumptions of normality when tested using the Kolmogorov-Smirnov Test. Comparison between groups was performed at each timepoint using Student's t-test. A p-value of <0.05 was considered statistically significant. Within-group comparisons are as follows: changes from baseline, and from hypoglycemia, to each subsequent timepoint were compared using Student's t-test. The sample size was too small to adjust for baseline covariates. Statistical analysis was performed using Graphpad Prism (San Diego, CA, USA).

For the proteomic analysis we fitted an intercept-free general linear model as a function of a subgroup (i.e. condition: timepoint), while taking the patient ID as a random effect using the R package limma. Subsequently, we computed the p value for two contrasts: baseline to hypoglycemia for both T2D and controls, and false discovery rate (FDR) corrected at a value of <0.05 as the cutoff for significance.

TABLE 1 | Demographic and clinical characteristics of the study participants.

Baseline	Type 2 Diabetes (n = 23)	Controls (n = 23)	p-value
Age (years)	64 ± 8	60 ± 10	<0.0001
Sex (M/F)	12/11	11/12	0.77
Weight (kg)	90.9 ± 11.1	79.5 ± 8.8	<0.0001
Height (cm)	167 ± 14	169 ± 5	0.64
BMI (kg/m^2)	32 ± 4	28 ± 3	<0.0001
Systolic BP (mmHg)	132 ± 8	122 ± 8	0.001
Diastolic BP (mmHg)	81 ± 7	75 ± 6	0.003
Duration of diabetes (years)	4.5 ± 2.2	N/A	
HbA1c (mmol/mol)	51.2 ± 11.4	37.2 ± 2.2	<0.0001
HbA1c (%)	6.8 ± 1.0	5.6 ± 0.2	<0.0001

BMI, Body mass index; BP, Blood pressure; HbA1c, Hemoglobin A1c; N/A, not applicable.

RESULTS

As reported previously (5), T2D subjects had higher BMI (p = 0.001) and higher blood pressure (p < 0.003) with a duration of diabetes 4.5 ± 2.2 years (**Table 1**).

For the 46 protein biomarkers described by Shu et al. (1), 34 were available for measurement in the Somalogic platform: 4 of 18 were found to differ at baseline in T2D for platelet degranulation [Neutrophil-activating peptide 2 (NAP-2) (p = 0.014), Thrombospondin-1 (THBS1) (p = 0.012), Platelet factor 4 (PF4) (p = 0.007), and Kininogen-1 (KNG1) (p = 0.05)], whilst 3 of 16 proteins differed for the complement and coagulation cascades [Coagulation factor IX (F9) (p < 0.05), Kininogen-1 (KNG1) (p = 0.05), and Heparin cofactor 2 (SERPIND1) (p = 0.007)] (**Table 2**). All proteins that differed between T2D and controls were higher at baseline in T2D, apart from Kininogen-1 that was lower at baseline in T2D.

Those proteins that significantly differed between T2D and controls share a close relationship to one another, as shown by the protein-protein interaction tool STRING (Search Tool for the Retrieval of Interacting Genes) pathways (**Figure 1**).

Following the hyperinsulinemic clamp inducing euglycemia in T2D, no difference from baseline to euglycemia was seen for any of the proteins (data not shown). However, when the clamp was continued to hypoglycemia, four proteins changed in controls from baseline [Thrombospondin-1 (p < 0.014),

TABLE 2 | Proteins identified as being altered in COVID-19 disease categorized according to **(A)** platelet degranulation; **(B)** complement and coagulation cascades in non-COVID infected type 2 diabetes and control subjects at baseline.

A. PLATELET DEGRANULATION **Target Full Name**	**Target**	**UniProt**	**Entrez Gene Symbol**	**T-test Baseline Control *vs* T2D**
Transgelin-2	Transgelin-2	P37802	TAGLN2	0.608
Neutrophil-activating peptide 2	NAP-2	P02775	PPBP	0.014
Fibrinogen gamma chain	Fibrinogen g-chain dimer	P02679	FGG	0.364
Fibrinogen	Fibrinogen	P02671, P02675 P02679	FGA	0.333
Thrombospondin-1	Thrombospondin-1	P07996	THBS1	0.012
Platelet factor 4	PF-4	P02776	PF4	0.007
von Willebrand factor	vWF	P04275	VWF	0.986
Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	alpha-1-antichymotrypsin complex	P01011	SERPINA3	0.892
Fructose-bisphosphate aldolase A	aldolase A	P04075	ALDOA	0.377
Metalloproteinase inhibitor 1	TIMP-1	P01033	TIMP1	0.079
Plasminogen	Plasminogen	P00747	PLG	0.398
Kininogen-1	"Kininogen, HMW"	P01042	KNG1	0.05
Alpha-2-antiplasmin	a2-Antiplasmin	P08697	SERPINF2	0.277
Fibronectin	Fibronectin	P02751	FN1	0.995
CD109 antigen	CD109	Q6YHK3	CD109	0.074
Alpha-2-macroglobulin	a2-Macroglobulin	P01023	A2M	0.924
Kallistatin	Kallistatin	P29622	SERPINA4	0.079
Apolipoprotein A-I	Apo A-I	P02647	APOA1	0.466

B. COMPLEMENT AND COAGULATION CASCADES **Target Full Name**	**Target**	**UniProt**	**Entrez Gene Symbol**	**T-test Baseline Control *vs* T2D**
Fibrinogen gamma chain	Fibrinogen g-chain dimer	P02679	FGG	0.364
Fibrinogen	Fibrinogen	P02671, P02675 P02679	FGA	0.333
Complement factor I	Factor I	P05156	CFI	0.132
von Willebrand factor	vWF	P04275	VWF	0.986
Coagulation factor IX	Coagulation Factor IX	P00740	F9	0.049
Complement factor H	Factor H	P08603	CFH	0.675
Complement component C9	C9	P02748	C9	0.993
Vitronectin	Vitronectin	P04004	VTN	0.294
Plasminogen	Plasminogen	P00747	PLG	0.398
Kininogen-1	"Kininogen, HMW"	P01042	KNG1	0.05
Alpha-2-antiplasmin	a2-Antiplasmin	P08697	SERPINF2	0.277
Complement component C4B		P0C0L4 P0C0L5	C4B	0.337
Plasma serine protease inhibitor	PCI	P05154	SERPINA5	0.608
Heparin cofactor 2	Heparin cofactor II	P05546	SERPIND1	0.007
Vitamin K-dependent protein C	Protein C	P04070	PROC	0.50
Alpha-2-macroglobulin	a2-Macroglobulin	P01023	A2M	0.924

All proteins that differed between T2D and controls were higher at baseline in T2D apart from Kininogen-1 that was lower in T2D.

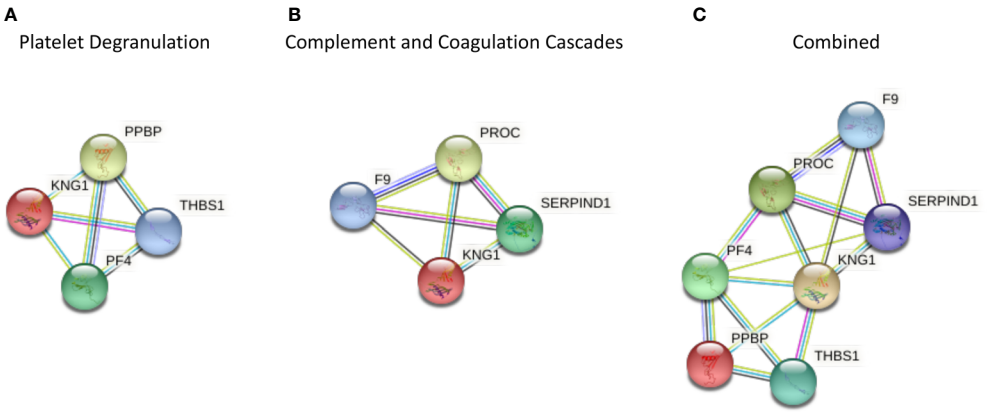

FIGURE 1 | The protein-protein interaction tool STRING 11.0 (Search Tool for the Retrieval of Interacting Genes) was used to visualize the significantly different proteins in type 2 diabetes (T2D) compared to controls, and for all of the proteomic proteins in COVID-19 disease severity described by others (1) (https://string-db.org/). Interactions between proteins are evidence-based and collated from databases, experiments, neighborhood, gene fusion, co-occurrence, text mining, co-expression, and homology. Here, we determined the relationships between the platelet degranulation **(A)** and complement and coagulation cascade proteins **(B)** presented in the study by Shu et al. (1) that were significantly different between non-COVID infected T2D and control subjects. **(A)** Platelet degranulation proteins that differed significantly between T2D and control subjects, indicating their relationship to one another. **(B)** Complement and coagulation cascade proteins that differed significantly between T2D and control subjects, indicating their relationship to one another. **(C)** Combined platelet degranulation and complement and coagulation cascade proteins that differed significantly in T2D, indicating their relationships to one another.

platelet factor 4 ($p < 0.01$), Platelet basic protein ($p < 0.008$), and Vitamin K-dependent protein C ($p < 0.00003$)], but only one in T2D [Vitamin K-dependent protein C ($p < 0.0002$)].

In view of the discrepancy in BMI between T2D and control subjects, correlations with the individual proteins *versus* BMI were undertaken. Only Coagulation factor IX was positively correlated with BMI, in T2D subjects only ($p = 0.04$, $r = 0.43$) (**Supplementary Figure 2**).

DISCUSSION

From the potential biomarkers identified by Shu for platelet degranulation and the complement and coagulation cascades that were purported to be predictors of severity in COVID-19 disease (1), 7 of the 34 proteins that were available in the SOMA-scan proteomic platform differed at baseline in patients with T2D compared to controls. This would potentially suggest that T2D patients with COVID-19 disease would be classed as having more severe disease, as some of their protein biomarker levels would be already altered, potentially leading to the introduction of early intensive therapy that may not be warranted or appropriate. Therefore, the significant difference seen in T2D compared to controls indicates the need for validation of such markers in the non-COVID-19 infected T2D population before they can be considered as biomarkers for COVID-19 and its severity. Conversely, that these biomarkers already differ in T2D may predict that more severe COVID-19 disease will result and be an indicator that more proactive intervention is required, as patients with T2D have a known propensity for increased COVID-19 disease severity (5).

In a wider context, exclusive of COVID-19, the results of this study also show that the complement and coagulation proteins investigated were not affected by glycemic changes until frank hypoglycemia was induced, showing that glycemic variability that is known to affect proteins through oxidative stress was not an issue here (9).

Neutrophil-activating peptide 2 is a heparin sulfate degrading enzyme (10) basally increased in T2D and its elevation is seen in acute coronary syndrome and it is thought to have a role in platelet-mediated vascular inflammation (11).

Thrombospondin-1 is a component of extracellular matrix that acts as a negative regulator of angiogenesis and is associated with cardiovascular disease where its elevation is seen in cardiac insults such as myocardial infarction (12). Here, it was basally increased in T2D *versus* controls that is in accord with its elevation and association with induced delay in re-endothelialization following arterial injury in diabetes (13), likely through increased inflammation (14) that is seen in T2D.

Platelet factor 4 was increased basally in T2D, as has been reported previously, and has been shown to be discriminatory for diabetes nephropathy and neuropathy (15).

Kininogen-1 plays a role in the kallikrein-kinin system (cooperating with the renin-angiotensin system); it has been suggested as a possible urinary biomarker for diabetic nephropathy (16) and may effect renal protection (17). Here, basal Kininogen-1 was higher in controls than T2D and this has been reported by others where it was suggested that its reduction may reflect bradykinin formation that has potent inflammatory effects (18). Taken together, this data suggests that basal elevation in T2D for Neutrophil-activating peptide 2, Thrombospondin-1, and Platelet factor 4 and a reduction in Kininogen-1 may contribute towards the vascular pathology of T2D.

Coagulation factor IX is a result of activation of the intrinsic coagulation pathway leading to fibrin formation and is not

reported to be different in T2D *versus* controls, the converse to what was observed here (19); however, its elevation in T2D may potentially increase the risk of a vascular event. Notably, this was the only factor that correlated positively with BMI, indicating that obesity may be a factor in its expression.

Heparin cofactor 2 is a vascular protective factor that primarily acts as an anticoagulant (20) and, in this context, its elevation would be protective.

Hypoglycemia resulted in alteration of the complement and coagulation cascades with an increase in thrombospondin, platelet factor 4, platelet basic protein (NAP-2 or PPBP), and vitamin K-dependent protein C. Vitamin K-dependent protein C functions as a natural anticoagulant to downregulate thrombin generation in the clotting cascade with cardioprotective properties (21), and therefore its elevation shown here may be seen to be protective.

These data show that protein changes in response to glucose variability above and below the physiological range and back to normoglycemia do occur but, to date, have scarcely been investigated despite their potential importance.

It is unclear from the Shu report what differing therapeutic agents were used for each of the COVID-19 severity categories (1), and therefore whether these treatment regimes may affect protein expression. This may be particularly relevant for hydroxychloroquine therapy, that has been associated with hypoglycemia in both diabetes and non-diabetes patients (22), and here we show that proteomic biomarker changes may result from hypoglycemia particularly in those without diabetes where four proteins changed in controls whilst only one protein differed from baseline in T2D.

Limitations of the study include that the SOMAlogic panel only included 34 of the 46 proteins in the platelet degranulation and complement and coagulation cascades that were reported by Shu et al. (1), and that the proteomic analysis used in each study differed so direct comparison to the Shu study (1) or others (2) was not possible. Of note, however, most proteins were common to the differing proteomic platforms used. In addition, as all study subjects were Caucasian, the results presented here may not be generalizable to other ethnic groups.

In conclusion, 8 of 34 protein biomarkers contained within the platelet degranulation and complement and coagulation cascades differed in T2D, with further changes at hypoglycemia that may be modified by COVID-19 therapy, suggesting that validation of these biomarkers is critical. It is unclear if these protein changes in T2D may be predictors of more severe COVID-19 disease in these patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Newcastle & North Tyneside Ethics committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AM and AB analyzed the data and wrote the manuscript. AA-Q contributed to study design, performed experiments, collected, analyzed, and interpreted data, and edited the manuscript. TS supervised clinical studies and edited the manuscript. SA contributed to study design, data interpretation, and the writing of the manuscript. All authors contributed to the article and approved the submitted version. AB is the guarantor of this work.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.658304/full#supplementary-material

Supplementary Figure 1 | Comparison of blood glucose levels in plasma before, during, and after iatrogenic induction of hypoglycemia. Blood sampling was performed at baseline (BL), at hypoglycemia (0 min), and post-hypoglycemia (30 min, 1-h, 2-h, 4-h, and 24-h) for controls (white circles) and for T2D (black squares). At baseline (BL), blood glucose was 7.5 ± 0.4 mmol/L for T2D subjects and 5.0 ± 0.1 mmol/L for control subjects. At point of hypoglycemia, blood glucose was 2.0 ± 0.03 mmol/L for the T2D cohort and 1.8 ± 0.05 mmol/L for the control cohort. Statistics: ****$p < 0.0001$, control *vs* T2D.

Supplementary Figure 2 | Correlation of Coagulation factor IX with BMI. Coagulation factor IX showed a positive correlation with BMI in subjects with type 2 diabetes (T2D) ($p = 0.04$, $r = 0.43$). No such correlation was seen in the control cohort.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The Effect of Lockdown and Physical Activity on Glycemic Control in Italian Children and Young Patients With Type 1 Diabetes

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Aims: The purpose of the study was to evaluate the impact of the lockdown established by the Italian government to limit the spread of Coronavirus disease (COVID-19) on glycemic control in a large sample of patients with type 1 diabetes (T1D) based on age, type of insulin therapy, number of telemedicine visits and physical activity.

Material and Methods: We retrospectively evaluated glycemic control in young T1D patients using the DexcomG6® system before the Italian lockdown (February 10–23, 2020—Time 0) and during lockdown (April 17–30, 2020—Time 1). Data on age, type of insulin therapy, number of telemedicine visits and physical activity of 202 patients with T1D and a median age of 18.2 years (range: 6–39) were collected.

Results: Data showed a significant improvement of TIR from 54.58% at T0 to 59.09% at T1 ($p \leq 0.0001$). Glycemic control improved significantly in patients ≥14 years old, showing the best outcome in the "university students and young adults" group (55.40% at T0 and 61.37% at T1, $p \leq 0.001$). All patients reduced physical activity during lockdown; in the 56 patients of "intense physical activity" group both at T0 and T1 TIR increased from ±56.91 to 64.11% ($p \leq 0.0001$).

Conclusions: Overall, the lockdown led to an unexpected improvement in glycemic control of young patients with T1D. A healthier and stressless lifestyle changes in association with the maintenance of physical activity resulted in a significant age-proportional improvement in glycemic control.

Keywords: type 1 diabetes, COVID-19, continuous glucose monitoring, children, glycemic control, physical activity

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INTRODUCTION

At the beginning of 2020, Coronavirus rapidly spread in many areas of the world, causing great public health concerns (1, 2). Coronavirus disease (COVID-19), if associated to diabetes mellitus, is characterized by higher mortality in adult patients (3–6). However, some reports suggest that in T1D patients younger than 25 years, the infection shows less severe and fairly similar clinical characteristics to healthy children and young people of the same age, who rarely require hospitalization (7–11). The contagiousness, clinical characteristics and mortality of infections due to the new COVID variants are not yet fully understood and may be different from those reported in the studies mentioned. To reduce the spread of Coronavirus, some governments have established restrictive policies. In particular, the Italian Government has ordered the first emergency measures starting from February 23, 2020 (suspension of school, sports activities and meetings) and a national quarantine starting from March 9, 2020 (movement restricted except for necessity). These restrictions may have negatively impacted glycemic control in T1D patients by reducing physical exercise and encouraging a sedentary lifestyle (12). Furthermore, during the lockdown period, all scheduled outpatient and hospital activities were suspended except for emergencies and telemedicine became the only and powerful tool to assess glycemic control (13).

Continuous glucose monitoring (CGM) is widely recognized as an extremely useful tool to monitor and improve glycemic control in T1D patients. It is well established that parameters indicating a good metabolic control are no longer limited to the concept of HbA1c alone, but they are extended also to "time in range" (TIR = 70–180 mg/dl), "time below range" (TBR <70 mg/dl), and "time above range" (TAR >180 mg/dl) (14–18).

Despite the concerns of diabetologists, some studies unexpectedly showed that glycemic control in T1D patients remained stable or improved during the lockdown period (19–34). However, these studies included both small and over-selected samples of patients, with different treatment schedules or adult patients. In contrast, one study in which non-CGM users were included showed worsening of glycemic control in children with type 1 diabetes (33).

The aim of our study was to evaluate the glycemic control in a large sample of young T1D patients during the lockdown period with particular attention to the role of age, type of insulin therapy, number of telemedicine visits and physical activity.

MATERIAL AND METHODS

Study Design and Study Population

This retrospective observational cohort study included young T1D patients followed at the G. Gaslini Hospital, Regional Diabetes Center (IRCCS Istituto Giannina Gaslini, University of Genova, Italy), aged 6–39 years old, who were using the Dexcom G6® CGM system with a percentage of use> 70%. Patients with T1D diagnosed after February 10, 2019 and patients with psychiatric disorders, pregnancy and in chronic therapy with drugs that interfere with glycemic control (such as corticosteroids or chemotherapy) were excluded.

Because of the retrospective nature of the study the ethic approval and informed consent already signed by parents and/or patients at the disease onset and renewed yearly, in which they agree on the use of clinical data for research purposes, were used. In addition, all parents and patients provided a specific informed consent for the collection of data.

The study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice.

Study Outcomes

The primary outcome was to compare the percentage of TIR (70–180 mg/dl), TAR (>180 mg/dl), and TBR (<70 mg/dl) in the period immediately preceding the lockdown (Italian DPCM of March 9, 2020) and during the lockdown period in young T1D patients using the Dexcom G6® CGM system for glucose monitoring. The secondary endpoints were to evaluate changes in glycemic control in relation to age, type of insulin therapy (multiple daily injection—MDI or continuous subcutaneous insulin infusion—CSII), number of telemedicine visits and physical activity.

Data Collection

We gathered data on glycemic control from the Dexcom Clarity software® reports of observation in the frame time of 2 weeks. We decided to compare data of the 14 days before the lockdown (February 10–23, 2020—Time 0) and data of 14 days during the lockdown period (April 17–30, 2020—Time 1).

We identified 202 patients (53% male) who fulfilled the study eligibility criteria. The following data were collected for each patient: demographic data (age and gender), age at clinical onset of T1D, type of insulin therapy (MDI or CSII), number of telemedicine visits in the period from Time 0 (T0) to Time 1 (T1). In addition, we collected information on physical activity before and after lockdown (T0 and T1). We defined "none physical activity" as the total lack of physical activity, "regular physical activity" as exercise time of less than 3 h per week and "intense physical activity" as exercise time of at least 3 h per week.

For the stratified data analysis, we divided patients into age groups according to the Italian career school path: "primary school children" (≥6 yrs; <10 yrs), "first grade secondary school children" (≥10 yrs; <14 yrs), "second grade secondary school students" (≥14 yrs; <18 yrs), and "university students and young adults" (≥18 yrs).

Statistical Methods

Data are described as mean and standard deviation (SD) or median and IQR for continuous variables, and as absolute and relative frequencies for categorical variables. The normal distribution of the variables was assessed using the Kolmogorov–Smirnov test. Non-parametric analysis (Mann–Whitney U-test, Kruskal–Wallis test, Wilcoxon test) for continuous variables and the Chi square or Fisher's exact test for categorical variables were used to measure differences between groups. Multivariate

analysis was performed to analyze the association between glycemic control (glycated hemoglobin and TIR) and exercise subgroup, age, number of telemedicine visits, gender and type of insulin therapy before and during the lockdown. P values ≤0.05 were considered statistically significant, and all P values were based on two tailed tests. Statistical analysis was performed using SPSS for Windows (SPSS Inc., Chicago, Illinois USA).

RESULTS

The median age of the 202 participants at observation was 17 years (range: 6–39 yrs) with a mean of 18.30 ± 6.43 yrs; median age at T1D onset was 8 years (range: 1–20 yrs) with a mean of 8.10 ± 4.18 yrs; median duration of disease was 9 years (range: 1–31 yrs) with a mean of 10.20 ± 6.72 yrs. Among the 202 patients included, 168 (83.2%) used CSII and 34 (16.8%) used MDI; 112 patients (55.4%) made a single telemedicine visit, 48 (23.8%) did not perform any visit and 42 (20.8%) made two or more telemedicine visits during the lockdown period. The population studied was divided into the following age groups: 7 (3.5%) "primary school children", 42 (20.8%) "first grade secondary school children", 58 (28.7%) "second grade secondary school students" and 95 (47.0%) "university students and young adults" (**Table 1**). No patient contracted COVID infection during the study period.

Exercise time was collected at T0 for 194/202 patients, of whom 38 (19.6%) did not engage in physical activity, 27 (13.9%) engaged in regular physical activity (<3 h per week) and 129 (66.5%) engaged in intense physical activity (≥3 h per week).

TABLE 1 | Demographic data, type of insulin therapy and weekly exercise time at T0.

	(n = 202)
Age at evaluation yrs, *mean ± SD*	18.30 ± 6.43
median (range)	17 (6–39)
Age at T1D onset yrs, *mean ± SD*	8.10 ± 4.18
median (range)	8 (1–20)
	N (%)
Gender	
Female N (%)	95 (47)
Male N (%)	107 (53)
Type of insulin therapy	
CSII N (%)	168 (83.2)
MDI N (%)	34 (16.8)
Telemedicine visit	
None N (%)	48 (23.8)
Single visit N (%)	112 (55.4)
2 or more visits N (%)	42 (20.8)
School grade	
Primary school N (%)	7 (3.5)
First grade secondary school N (%)	42 (20.8)
Second grade secondary school N (%)	58 (28.7)
University students/young adults N (%)	95 (47.0)
Physical activity, exercise time	
No physical activity N (%)	38 (19.6)
Physical activity <3 h per week N (%)	27 (13.9)
Intense physical activity ≥3 h wks N (%)	129 (66.5)

CSII, continuous subcutaneous insulin infusion; MDI, multiple daily injection.

The mean percentage of TIR increased from 54.58 ± 16.7 % at T0 to 59.09 ± 17.7% at T1 (p ≤0.0001), which corresponded to +4.5 percentage points; this mean difference amounted to 1.1 h per day spent in TIR (**Table 2**). The difference in the percentage of TAR (>180 mg/dl) was −4.0 percentage points (42.48 ± 17.78% at T0 *vs* 38.50 ± 18.45% at T1, p ≤0.0001), a difference that amounted to 1.0 h per day. The difference in the percentage of TAR >250 mg/dl was −3.5 percentage points (16.25 ± 12.31% at T0 *vs* 12.81 ± 11.59% at T1, p ≤0.0001), a difference that amounted to 48 min per day. The difference in the percentage of TBR (<70 mg/dl) was −0.4 percentage points (2.83 ± 2.91% at T0, 2.38 ± 3.06% at T1, p ≤0.002), a difference that amounted to 6 min per day. The difference in the percentage of TBR <54 mg/dl was −0.1 percentage points (0.59 ± 0.92% at T0, 0.48 ± 1.06% at T1, p ≤0.0001).

Data also showed a significant decrease of mean glycemic values (176.16 ± 29.87 mg/dl at T0 and 170.18 ± 30.14 mg/dl at T1, p ≤0.0001) and estimated HbA1c (7.76%—61 mmol/mol at T0 and 7.56%—59 mmol/mol at T1, p ≤0.0001) (**Table 2**).

Multivariate analysis was performed to determine the prognostic factors affecting glycemic control (glycated hemoglobin and TIR). Age, exercise subgroup, number of telemedicine visits, gender and type of insulin therapy before and during the lockdown were not significant.

Regarding the different age groups analyzed, glycemic control improved, although not statistically significant, in "primary school children" group (TIR 58.33 ± 21.14% at T0 and 61.49 ± 19.76% at T1) and remained almost stable in the "first grade secondary school children" group (TIR 55.74 ± 16.30% at T0 and 56.84 ± 16.91% at T1). On the other hand, glycemic control significantly improved in the "second grade secondary school students" group (51.96 ± 16.73% at T0 and 56.71 ± 19.42% at T1, p ≤0.005) and even more in the "university students and young adults" group (55.40 ± 16.53% at T0 and 61.37 ± 16.62% at T1, p ≤0.001) (**Table 3**). A statistically significant reduction in the weekly sports hours was observed in all groups: in "primary school children" group (4.36 ± 0.94 h at T0 and 0.14 ± 0.38 h at T1, p = 0.02), in "first grade secondary school children" group (6.01 ± 4.06 h at T0 and 1.82 ± 2.32 h at T1, p ≤0.0001), in "second grade secondary school students" group (5.14 ± 4.20 h at T0 and 2.72 ± 3.40 h at T1, p ≤0.0001and in "university students and young adults" group (3.74 ± 4.33 h at T0 and 2.7 ± 3.49 h at T1, p <0.03) (**Figure 1**).

All patients, regardless of age group, reduced the weekly hours of physical activity during the lockdown and changed their sports habits; 86.8% of patients who did not exercise at T0 continued to be sedentary, 29.6% of patients who were engaged in regular physical activity became sedentary and 40.7% started exercising intense physical activity. In addition, 27.9% of patients who did intense physical activity became sedentary, 28.7% reduced their weekly sports hours and 43.4% maintained their habits. At T1, stratifying by age groups, there was an important reduction in physical activity in the "first and second grade secondary school students" groups (10 to 18 years old), and a

slighter reduction in the "university students and young adults" group (>18 years old). Only one child out of seven in the "primary school children" group maintained physical activity (**Table 4**).

The pre-lockdown (T0) differences in glycemic control in relation to exercise were not statistically significant, although TIR was proportionally higher in relation to the weekly hours of physical activity: TIR 51.56 ± 16.74% in the "none activity" group, 55.51 ± 14.41% in the "regular physical activity" group and 55.63 ± 17.18% in the "intense physical activity" group; the same difference in glycemic control is observed during the lockdown: TIR 54.12 ± 17.01% in the "none activity" group, 59.69 ± 19.30% in the "regular physical activity" group and 63.98 ± 16.29% in the "intense physical activity" group. Furthermore, there is a statistically significant difference in glycemic control between the "no activity" group (n 77—TIR 54.12 ± 17.0 %), and the "intense physical activity" group (n 71—TIR 63.98 ± 16.3 %) at T1 (p value 0.0001) (**Table 5**).

We analyzed the data relating to the 129 patients belonging to the "intense physical activity" group at T0; we observed that the 56 subjects included in the "intense physical activity" group both at T0 and at T1 showed a statistically significant improvement in glycemic control in the lockdown period compared to the "none activity" group (54.76 ± 16.04% *vs* 64.11 ± 16.29%, p = 0.006) (**Table 6**). Moreover, the mean percentage of TIR increased from 56.91 ± 17.13% at T0 to 64.11 ± 16.29% at T1 (p ≤0.0001).

TABLE 2 | Primary outcome: Glycemic control at T0 and T1.

	T0 (N = 202)	T1 (N = 202)	p-value
Coefficient of variation (%)	36.53 ± 5.76	34.76 ± 5.76	≤0.0001
SD (mg/dl)	64.26 ± 14.26	59.15 ± 13.78	≤0.0001
HbA1c (%)	7.76 ± 1.04	7.56 ± 1.05	≤0.0001
TIR (%)	54.58 ± 16.67	59.09 ± 17.65	≤0.0001
TAR (%)	42.48 ± 17.78	38.50 ± 18.45	≤0.0001
TAR >250 mg/dl (%)	16.25 ± 12.31	12.81 ± 11.59	≤0.0001
TBR (%)	2.83 ± 2.91	2.38 ± 3.06	≤0.002
TBR <54 mg/dl (%)	0.59 ± 0.92	0.48 ± 1.06	≤0.01
Mean glucose value (mg/dl)	176.16 ± 29.87	170.18 ± 30.14	≤0.0001
Sport (hours/week)	4.64 ± 4.24	2.46 ± 3.22	≤0.0001

SD, standard deviation; TIR, time in range (70–180 mg/dl); TAR, time above range (>180 mg/dl); TAR >250, time above range (>250 mg/dl); TBR, time below range (<70 mg/dl); TBR <54, time below range (<54 mg/dl).

TABLE 3 | Secondary outcome: Glycemic control in relation to age groups at T0 and T1.

	Age ≥6 y <10 y (N = 7)			Age ≥10 y <14 y (N = 42)			Age ≥14 y <18 y (N = 58)			Age ≥18 y (N = 95)		
	T0 Mean ± SD Median (IQR)	T1 Mean ± SD Median (IQR)	p-value	T0 Mean ± SD Median (IQR)	T1 Mean ± SD Median (IQR)	p-value	T0 Mean ± SD Median (IQR)	T1 Mean ± SD Median (IQR)	p-value	T0 Mean ± SD Median (IQR)	T1 Mean ± SD Median (IQR)	p-value
Coefficient of variation (%)	36.99 ± 9.19	34.07 ± 5.25	0.24	37.55 ± 6.02	34.13 ± 5.68	**≤0.0001**	36.92 ± 5.24	36.13 ± 5.34	0.31	35.82 ± 5.66	34.27 ± 6.02	**0.001**
SD (mg/dL)	61.14 ± 15.91	55.29 ± 13.34	0.07	65 ± 14.01	58.43 ± 11.74	**≤0.0001**	67.33 ± 15.52	63.40 ± 15.08	**0.002**	62.28 ± 13.27	57.16 ± 13.43	**≤0.0001**
HbA1c (%)	7.54 ± 1.08	7.33 ± 1.12	0.15	7.66 ± 1.01	7.62 ± 0.96	0.53	7.95 ± 1.09	7.76 ± 1.31	**0.03**	7.71 ± 1.02	7.42 ± 0.89	**≤0.0001**
TIR (%)	58.33 ± 21.14	61.49 ± 19.76	0.50	55.74 ± 16.30	56.84 ± 16.91	0.28	51.96 ± 16.73	56.71 ± 19.42	**0.005**	55.40 ± 16.53	61.37 ± 16.62	**≤0.0001**
TAR (%)	39.21 ± 22.25	35.27 ± 20.99	0.13	40.31 ± 16.77	40.96 ± 17.78	0.97	45.38 ± 18.01	40.84 ± 20.75	**0.02**	41.92 ± 17.79	36.22 ± 16.98	**≤0.0001**
TAR >250 mg/dl (%)	12.07 ± 11.14	9.56 ± 11.15	0.50	16.36 ± 12.86 11.65 (16.40)	12.74 ± 9.99 10.70 (11.85)	**0.01**	18.63 ± 12.13	16.22 ± 15.09 120 (21.60)	**0.004**	15.06 ± 12.18 12.30 (16.70)	11.01 ± 9.30 8.50 (15.70)	**≤0.0001**
TBR (%)	2.49 ± 3.09	3.24 ± 3.25	0.12	3.34 ± 3.36 2.50 (3.70)	2.20 ± 2.51 1.40 (2.70)	**0.002**	2.60 ± 2.41 1.80 (3.00)	2.32 ± 2.14 1.60 (3.00)	0.39	2.77 ± 3.0 1.80 (3.60)	2.42 ± 3.70 1.30 (2.30)	**0.04**
TBR <54 mg/dl (%)	0.79 ± 1.60 0.20 (0.20)	0.87 ± 1.32 0.40 (0.60)	0.35	0.80 ± 1.17 0.40 (0.80)	0.49 ± 0.94 0.20 (0.70)	**0.03**	0.52 ± 0.79 0.20 (0.50)	0.40 ± 0.50 0.20 (0.60)	0.58	0.53 ± 0.79 0.20 (0.50)	0.49 ± 1.32 0.10 (0.40)	0.06
Mean glucose (mg/dl)	169.7 ± 30.87	163 ± 31.34	0.09	173.43 ± 29	172 ± 27.43	0.43	181.6 ± 31.41	175.9 ± 37.64	0.02	174.6 ± 29.23	166.4 ± 25.54	**≤0.0001**
Sport (hours/week)	4.36 ± 0.94	0.14 ± 0.38 0.00 (0.00)	**0.02**	6.01 ± 4.06 5.00 (3.75)	1.82 ± 2.32 1.00 (2.00)	**≤0.0001**	5.14 ± 4.20 4.00 (4.00)	2.72 ± 3.40 2.00 (4.00)	**≤0.0001**	3.74 ± 4.33 3.00 (5.40)	2.77 ± 3.49 2.00 (4.00)	**0.03**

SD, standard deviation; TIR, time in range (70–180 mg/dl); TAR, time above range (>180 mg/dl); TAR >250, time above range (>250 mg/dl); TBR, time below range (<70 mg/dl); TBR <54, time below range (<54 mg/dl). bold = statistically significant values.

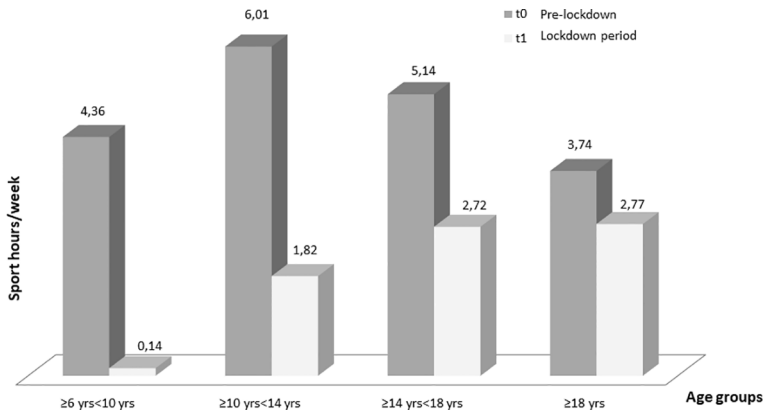

FIGURE 1 | Changes in weekly sports hours for each age at T0 (before the lockdown) and at T1 (lockdown period). Only one children of seven in "primary school children" group maintained physical activity (**Table 4**).

TABLE 4 | Study population divided by age and by sport group at T0 and T1 (N = 194).

	T0			T1		
	No sport (N = 38)	**Sport <3 h (N = 27)**	**Sport ≥3 h (N = 129)**	**No sport (N = 77)**	**Sport <3 h (N = 46)**	**Sport ≥3 h (N = 71)**
	N (%)	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**
Age ≥6 y <10y (N = 7)	0	0	7 (100)	6 (85.7)	1 (14.3)	0
Age ≥10 y <14 y (N = 40)	2 (5)	4 (10)	34 (85)	14 (35)	18 (45)	8 (20)
Age ≥14 y <18 y (N = 57)	8 (14)	8 14)	41 (72)	23 (40.4)	10 (17.5)	24 (42.1)
Age ≥18 y (N = 90)	28 (31.1)	15 (16.7)	47 (52.2)	34 (37.8)	17 (18.9)	39 (43.3)

DISCUSSION

In our study we analyzed the data of 202 young T1D patients in the period before and during the lockdown. All CGM parameters of glycemic control improved significantly during the lockdown period (TIR, TAR, TBR, median blood glucose and estimated HbA1c), despite the significant reduction in hours of physical activity. This improvement could be due to changes in daily rhythms caused by the closure of schools, universities, sport trainings and extra-school activities, and most likely both from greater parental controls and ease of diabetes management (waiting times between insulin administration and the start of meal, accurate carbohydrate count) (35, 36). Therefore, the negative effect due to the reduction in weekly sports activity was completely offset and overcome by the positive "lockdown effect", an effect in fact opposite to what was initially predicted by clinicians, but in line with the data available in the literature. Studies in the adult population showed improvement in glycemic control (19–28) unlike those on the pediatric and adolescent population which showed discordant data. Tornese et al. (29) showed glycemic improvement in a small and selected sample of patients (13 subjects, hybrid closed loop—HCL users). Brener et al. (30) showed a relatively stable glycemic control in pediatric patients with T1D and an association between a lower socioeconomic cluster and TIR in younger patients. Cristophoridis et al. (31) demonstrated that TIR did not differ significantly before and during the lockdown period; this study was conducted on a larger number of selected patients (34 subjects, predictive low glucose suspend—PLGS users). Di Dalmazi et al. (32) showed the improvement in CGM metrics in children and adults during lockdown, whereas TIR remained unchanged in teenagers. A study conducted in India by Verma et al. (33) showed a worsening in the glycemic trend of pediatric patients; only SMBG data monitoring were analyzed in this study and glycemic control deteriorated mainly due to unavailability of inuslin/glucostrips during the lockdown period. Another study conducted in India by Shah et al. (34) showed a stable glycemic control in children during lockdown, highlighting importance of stronger family support system leading to more steady daily routine. Therefore, we decided to collect data from a large population of children, adolescents and young adults, not overly selected for the type of therapy, and to stratify the data analysis by age groups, to clarify the effect of age on glycemic control during the lockdown.

The data show a reduction in physical activity across the study population. In Italy, the restrictions imposed by the government have greatly reduced the possibilities of participating in sports outside. Gyms, swimming pools, sports fields and playgrounds have been closed. In addition, the sports clubs have suspended activities and training. The only sports allowed were in-home activity or running alone in the neighborhood. There were no major changes in climatic conditions between T0 and T1 (+4°C in mean temperature,

TABLE 5 | Secondary outcome: Glycemic control in relation to physical activity at T0 and T1 (N = 194).

	T0						T1					
	No sport (N = 38)	**Sport <3 h (N = 27)**	**Sport ≥3 h (N = 129)**	**p-value No sport *vs* Sport <3 h**	**p-value No sport *vs* Sport ≥3 h**	**p-valueSport <3 h *vs* Sport ≥3 h**	**No sport (N = 77)**	**Sport <3 h (N = 46)**	**Sport ≥3 h (N = 71)**	**p-value No sport *vs* Sport <3 h**	**p-value No sport *vs* Sport ≥3 h**	**p-value Sport <3 h *vs* Sport ≥3 h**
	Mean ± SD median (IQR)	**Mean ± SD median (IQR)**	**Mean ± SD median (IQR)**				**Mean ± SD median (IQR)**	**Mean ± SD median (IQR)**	**Mean ± SD median (IQR)**			
Coefficient of variation (%)	36.47 ± 5.88	34.48 ± 5.99	36.92 ± 5.73	0.17	0.68	**0.04**	35.39 ± 5.47	33.06 ± 5.91	35.09 ± 6.04	0.02	0.58	0.09
SD (mg/dl)	66.71 ± 15.53	61.07 ± 15.04	63.96 ± 13.97	0.19	0.40	0.28	63.04 ± 14.18	55.72 ± 12.94	57.14 ± 13.47	**0.01**	**0.02**	0.78
HbA1c (%)	8.02 ± 1.13	7.77 ± 0.87	7.67 ± 1.05	0.56	0.15	0.49	7.85 ± 1.11	7.53 ± 1.12	7.28 ± 0.89	0.11	**0.002**	0.26
TIR (%)	51.56 ± 16.74	55.51 ± 14.41	55.63 ± 17.18	0.42	0.25	0.86	54.12 ± 17.01	59.69 ± 19.30	63.98 ± 16.29	0.07	**0.0001**	0.28
TAR (%)	49.90 ± 18.13	42.83 ± 15.38	41.07 ± 18.23	0.63	0.21	0.56	44.57 ± 18.04	38.32 ± 20.42	33.24 ± 16.52	0.09	**0.001**	0.19
TAR >250 mg/dl (%)	18.94 ± 14.45 15.25 (22.60)	13.97 ± 10.04 11.40 (16.10)	15.78 ± 12.19 12.30 (17.80)	0.25	0.30	0.64	16.07 ± 13.09 13.50 (18.50)	12.16 ± 12.16 8.55 (17.30)	9.94 ± 8.91 8.70 (10.60)	**0.05**	**0.002**	0.63
TBR (%)	2.71 ± 3.13 2.05 (3.70)	1.53 ± 2.14 0.70 (1.30)	3.11 ± 2.96 2.10 (3.50)	0.07	0.25	**0.001**	2.32 ± 2.64 1.30 (2.80)	2.0 ± 2.24 1.20 (3.10)	2.66 ± 3.95 1.60 (2.80)	0.53	0.70	0.29
TBR <54 mg/dl (%)	0.41 ± 0.59 0.20 (0.40)	0.41 ± 1.01 0.10 (0.30)	0.67 ± 0.98 0.30 (0.70)	0.06	0.29	**0.003**	0.51 ± 0.96 0.20 (0.50)	0.29 ± 0.41 0.10 (0.40)	0.58 ± 1.44 0.10 (0.50)	0.23	0.62	0.48
Mean glucose (mg/dl)	183.47 ± 33.66	176.19 ± 24.79	173.59 ± 30.18	0.50	0.15	0.51	178.45 ± 31.89	169.3 ± 32	162.3 ± 25.45	0.11	**0.002**	0.30

SD, standard deviation; TIR, time in range (70–180 mg/dl); TAR, time above range (>180 mg/dl); TAR >250, time above range (>250 mg/dl); TBR, time below range (<70 mg/dl); TBR <54, time below range (<54 mg/dl). bold = statistically significant values.*

TABLE 6 | Analysis of the data at T1 of the 129 patients belonging to the "intense physical activity" group at T0.

	T1(N = 129)					
	No sport (N = 36) Mean ± SD	**Sport <3 h (N = 37) Mean ± SD**	**Sport ≥3 h (N = 56) Mean ± SD**	**p-value no sport *vs* <3 h**	**p-value no sport *vs* ≥3 h**	**p-value sport <3 h *vs* ≥3 h**
Coefficient of variation (%)	36.13 ± 5.42	33.42 ± 4.96	35.52 ± 6.38	0.02	0.52	0.06
SD (mg/dl)	63.64 ± 14.04	56.84 ± 11.90	57.55 ± 13.83	0.05	0.07	0.96
HbA1c (%)	7.77 ± 1	7.59 ± 1.2	7.25 ± 0.88	0.36	**0.01**	0.17
TIR	54.76 ± 16.04	58.17 ± 20.46	64.11 ± 16.28	0.29	**0.006**	0.16
TAR	42.84 ± 17.29	39.66 ± 21.79	32.72 ± 16.35	0.35	**0.006**	0.12
TAR >250 (mg/dl)	15.31 ± 11.24	13.42 ± 12.78	9.80 ± 8.76	0.25	**0.01**	0.31
TBR	2.41 ± 2.79	2.18 ± 2.27	3.02 ± 4.31	0.66	0.43	0.24
TBR <54(mg/dl)	0.57 ± 1.11	0.32 ± 0.44	0.68 ± 1.59	0.42	0.86	0.50
Mean glucose (mg/dl)	176.30 ± 28.71	171 ± 34.33	161.4 ± 25.21	0.34	**0.01**	0.19

bold = statistically significant values.

and +3 rainy days between T0 and T1). The stratified analysis of data by age group raises some interesting considerations. In the group of "primary school children" a reduction in sports activity was observed during the lockdown. Despite the small number of patients in this group and the unclear reasons for such reduction, it is reasonable to believe that it was not possible for the parents to have their children carry out the physical activity that was regularly scheduled in sports center and the children did not

participate consistently in online workouts. Furthermore, younger patients are already normally under strict control of adults and the chances of not respecting the behaviors for the correct management of diabetes are rare even outside lockdown conditions; this could explain the less beneficial "lockdown effect" on glycemic control in this age group than in the others.

The data show that glycemic improvement increases proportionally to the age of the patients. Daily stress has a negative impact on glycemic control and is greater in puberty, adolescence and adulthood (36) and although quarantine was a stressful psychological condition, it seems that the young patients have reacted with high levels of resilience to this situation (37). In addition, teenagers and young adults are less controlled by parents, may not follow adequate behaviors for the correct management of therapies and have a much worse dietary lifestyle (dinners out of home, aperitifs, snacks) than children. It is also interesting to point out that during the lockdown the Italian families rediscovered the pleasure of cooking simpler and healthier products at home (during the lockdown in the Italian supermarkets the stocks of yeast and flour ran out); therefore, the consumption of ready-made, processed foods and fast-foods has decreased and the whole population seems to have followed a healthier Mediterranean diet (38).

The study shows some interesting aspects on the role of physical activity on glycemic control during the lockdown. The beneficial effects of sport in young T1D patients are widely known (12), although this is not reflected in the data of this study. The sub-analysis of 129 patients included in "intense physical activity" group at T0 (≥3 h per week) showed the effect of changing sport habits during the lockdown: patients who stopped exercising at T1 did not improve their glycemic control, while those who continued to do physical activity improved TIR in proportion to the weekly sports hours practiced. The sub-analysis of 56 patients included in "intense physical activity" group both at T0 and at T1 shows a significant improvement in glycemic control during the lockdown (+7.2% points of TIR). Therefore, the beneficial "lockdown effect" appears to be completely lost in patients who have adopted a sedentary lifestyle during the lockdown, while it is extremely enhanced in patients who have continued to exercise.

The role of technology in diabetes management is well known in our Regional Pediatric Diabetes Center and more than the 75% of our patients were already CGM users before the lockdown. In contrary to what was hypothesized in some other studies with a much smaller number of patients, the role of telemedicine does not seem relevant in obtaining better glycemic control during lockdown in T1D young patients (39–42). Our experience shows that the use of CGM and telemedicine has certainly played an important role for diabetes teams in maintaining contact with their patients and in continuing to support them in managing the disease, despite the fact that the number of visits does not correlate with better glycemic control. Our data allow us to conclude that glycemic improvement is not due to the increased support of the diabetes staff; in fact, the patients who required more telemedicine visits were those characterized by a worse metabolic control and who therefore needed more support.

Several studies have shown the role of socio-economic status in glycemic control during lockdown, highlighting the correlation of socio-economic deprivation and reduction of time in range in adults and the correlation between age, socio-economic status and glycemic control in children with T1D (30). In this study we did not collect data on the socio-economic status of patients and their families. Our clinical experience has shown greater difficulty in maintaining good glycemic control during lockdown in patients with lower socioeconomic status. In this complicated period, the role of technology in managing diabetes has become very important and this has prompted our center to develop technological tools more accessible to all families. To optimize the use of CGM and guarantee everyone access to the television service, we recently launched, in collaboration with our patient associations, a project to distribute smartphones to families who did not have the possibilities to acquire them.

It is well known that prolonged lockdown periods resulted in weight gain in children and adults. This issue was also evaluated in T1D patients during lockdown periods. Several studies showed weight gain during the lockdown. However, the weight gain did not change the glycemic control in the patients (43). We decided not to collect the data relating to the weight of the patients because the method of data collection (telemedicine, referenced data) did not seem sufficiently reliable for a statistical analysis.

The limitations of this study are the single-center nature and the very small sample size of "primary school children" group. However, this study is the first report that analyzed data from a large heterogeneous and not over-selected population of pediatric and young T1D patients, with a focus on the role of many factors to consider in the management of diabetes. We believe that our data and considerations may be useful to health care professionals caring for children and adolescents during the COVID-19 pandemics. The study clearly shows the aspects on which specialists should work more with patients in the unfortunate case we there will be a second lockdown: cooking at home, following a healthier and more balanced diet and not interrupting scheduled physical activity, and being ready for a physical activity tailored to the patient's age and personal needs. Moreover, why not think about how to work better on these aspects independently of the lockdown?

In conclusion, this study showed that the lockdown led to an overall improvement in glycemic control of young T1D patients. The healthier lifestyle and the potential reduction of stress contributed to the completely unexpected "lockdown effect", which resulted in a significant improvement in glycemic control and diabetes management.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

NM designed the study and wrote the manuscript. MB designed the study and wrote the manuscript. CMo researched data and

reviewed the manuscript. FV researched data. CMe researched data. FP researched data. MCab researched data. MCal did statistical analysis. Gd'A reviewed the manuscript and contributed to the discussion. MM reviewed the manuscript and contributed to the discussion. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Insulin Treatment May Increase Adverse Outcomes in Patients With COVID-19 and Diabetes: A Systematic Review and Meta-Analysis

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Background and Objective: Recently, insulin treatment has been found to be associated with increased mortality and other adverse outcomes in patients with coronavirus disease 2019 (COVID-19) and diabetes, but the results remain unclear and controversial, therefore, we conducted this meta-analysis.

Methods: Four databases, namely, PubMed, Web of Science, EMBASE and the Cochrane Library, were used to identify all studies concerning insulin treatment and the adverse effects of COVID-19, including mortality, incidence of severe/critical complications, in-hospital admission and hospitalization time. To assess publication bias, funnel plots, Begg's tests and Egger's tests were used. The odds ratios (ORs) with 95% confidence intervals (CIs) were used to access the effect of insulin therapy on mortality, severe/critical complications and in-hospital admission. The association between insulin treatment and hospitalization time was calculated by the standardized mean difference (SMD) with 95% CIs.

Results: Eighteen articles, involving a total of 12277 patients with COVID-19 and diabetes were included. Insulin treatment was significantly associated with an increased risk of mortality (OR=2.10; 95% CI, 1.51-2.93) and incidence of severe/critical COVID-19 complications (OR=2.56; 95% CI, 1.18-5.55). Moreover, insulin therapy may increase in-hospital admission in patients with COVID-19 and diabetes (OR=1.31; 95% CI, 1.06-1.61). However, there was no significant difference in the hospitalization time according to insulin treatment (SMD=0.21 95% CI, -0.02-0.45).

Conclusions: Insulin treatment may increase mortality and severe/critical complications in patients with COVID-19 and diabetes, but more large-scale studies are needed to confirm and explore the exact mechanism.

Keywords: insulin treatment, COVID-19, adverse outcomes, diabetes, mortality, complications

INTRODUCTION

Since the rapid outbreak of coronavirus disease 2019 (COVID-19) in 2019, the worldwide fight against the epidemic is still ongoing. As of January 30, 2021, the number of confirmed cases and deaths reached 114140104 and 2535520, respectively (1). The concomitant occurrence of two worldwide epidemic diseases, COVID-19 and diabetes, has introduced a series of problems. Diabetes is known as among the most serious comorbidities of COVID-19 to deteriorate disease development and outcomes. Recently, mechanisms underlying why diabetic COVID-19 patients are more prone to severe outcomes include higher glucose levels, impaired innate and adaptive immune response, impaired coagulation, obesity and hypertension (2).

Good glucose control has been demonstrated to exert a protective effect in the context of the development and outcomes of COVID-19 (3–5), and strict glucose management on admission for COVID-19 cases has been suggested regardless of diabetes status (6). The role of glycemic control strategies, such as several antidiabetic drugs including metformin and DPP-4 inhibitors in the efficacy and safety of COVID-19 and diabetes has been established (7, 8). However, whether insulin injection has some effect on the development of COVID-19 remains inconsistent. Notably, early research found that insulin treatment may be associated with adverse outcomes such as death (9) and intensive care unit (ICU) admission (10); however, evidence regarding the relationship between insulin treatment and COVID-19 remains inadequate and unconvincing. Therefore, we conducted a systematic review and meta-analysis to determine the association between insulin injection and the outcomes of COVID-19 to provide certain clinical information for these patients.

METHODS

This meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines, as previously described (11).

Article Search Strategy

We searched for eligible articles from January 25, 2021 and to February 20, 2021. The PubMed (2013-2020, 20 February), Cochrane Library (1960-2020, 20 February), EMBASE (1960-2020, 20 February) and Web of Science (1950-2020, 20 February) databases were searched in this study. Searches for all published articles related to both insulin treatment and COVID-19 were performed. The following search terms were used: "Insulin", "Insulin treatment", "COVID-19", "SARS-CoV-2", and "2019 novel coronavirus". The search strategy was as follows: [(insulin) OR (insulin treatment)] AND [(COVID-19) OR (SARS Cov-2) OR (coronavirus) OR (2019 novel coronavirus)]. Additional papers were identified by performing manual searches of the references of relevant articles and tracking citations to obtain more relevant studies. All articles published by February 20, 2021 with no language restrictions were included.

Selection Criteria

Two reviewers (YY and ZC) independently reviewed all eligible studies and selected those suitable for inclusion. Disagreements were settled by reaching a consensus or with the help of a third reviewer (JZ). All articles included in this meta-analysis met the following criteria: (1) population: the subjects were patients with both COVID-19 and diabetes; (2) intervention: insulin treatment; (3) comparator/control: the control group involved non-insulin treatment, including oral antidiabetic medications, no treatment or diet control; (4) importantly, the outcomes of our study refer to the mortality and other complications of COVID-19, and the form of the outcomes must be original data of COVID-19 deaths and complications or calculated adjusted/unadjusted odds ratios (ORs) or risk ratios (RRs); and (5) study design: clinical studies. Articles were excluded if they met the following criteria: (1) articles lacking information or data necessary for the purpose of this meta-analysis and (2) articles published as letters, reviews, editorials, or conference abstracts.

Data Extraction

All relevant articles were imported into EndNote X9 software and reviewed independently by two authors (YY and ZC). Discrepancies between the authors were resolved with the help of a third reviewer (JZ). The following information was extracted from the selected studies by the two independent investigators: author, publication year, country, study design, mean or median age, sample size, population and COVID-19 outcomes. All extracted data were then imported into Excel.

The severity of the disease was classified into 4 categories according to the Guidelines for the Diagnosis and Treatment of New Coronavirus Pneumonia (fifth edition) as follows (12): 1) *mild type*: patients with mild clinical symptoms and no pulmonary changes on CT imaging, 2) *common type*: patients with symptoms of fever and signs of respiratory infection, with pneumonia changes on CT imaging, 3) *severe type*: patients presenting with any of the following items: (a) respiratory distress, respiratory rate≥30/min, (b) oxygen saturation of finger ≤ 93% in resting condition, and (c) arterial partial pressure of oxygen (PaO2)/oxygen concentration (FiO2)≤300 mmHg (1 mmHg=0.133 kPa) or 4) *critical type*: patients meeting any of the following criteria: (a) respiratory failure requiring mechanical ventilation, (b) shock and (c) ICU admission requirement due to multiple organ failure. Since only 32 mild cases were available and these cases would normally not be hospitalized, these cases were removed from the analysis (**Figure 1**).

Quality Assessment of Studies

The quality of the included studies was assessed by the Newcastle-Ottawa Scale (NOS) (13). We assessed the quality of all relevant studies based on the type of study, sample size, participant selection, representativeness of the sample, adequacy of follow-up, comparability (exposed-unexposed or case-control), and method of ascertaining the cases and controls. The possible range of NOS scores is from 0 to 9; studies that scored 4-6 represent a modest risk of bias, and those that scored <3 indicate the highest risk of bias. A study with a score of 6 or higher was defined as a high-quality study.

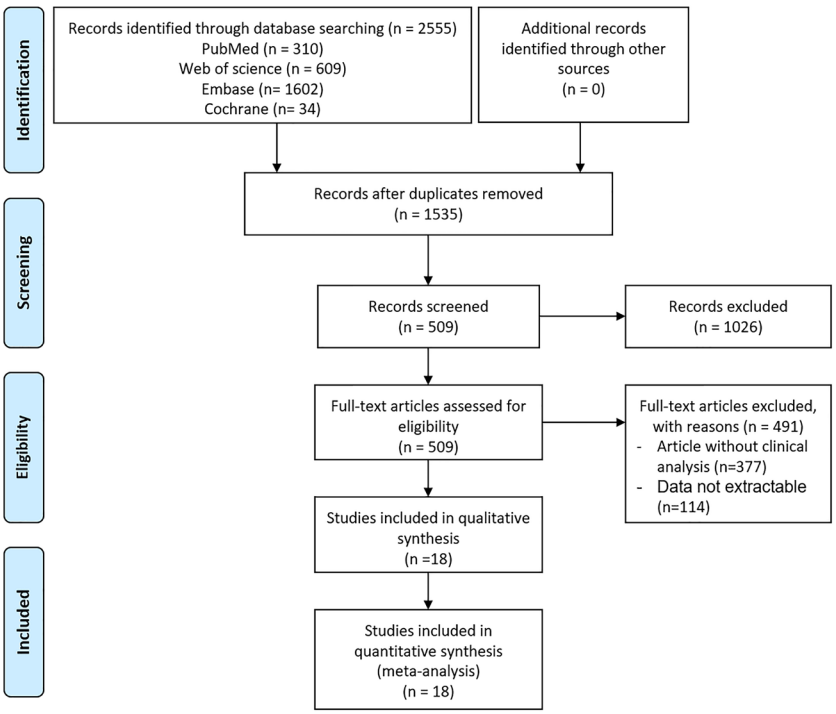

FIGURE 1 | Flow diagram of the study selection process.

Statistical Analysis

All analyses were performed using Stata (Version 13.0). The correlation between insulin treatment and mortality or incidence of complications of COVID-19 was expressed as the pooled ORs and 95% confidence interval (CI). ORs > 1 represented a direct association, and those < 1 represented an inverse association. Regarding the hospitalization time, the standardized mean difference (SMD) and 95% CI were used to analyze the results. A random-effects model was used for all results of our meta-analysis. I^2 statistics were used to assess the degree of heterogeneity as follows: 25%, 50%, and 75% represented low, moderate, and high degrees of heterogeneity, respectively. Subgroup analyses were performed using the following variables to analyze the heterogeneity: sample size (>80 or <80), period of insulin treatment (before and after admission), type of diabetes (T1DM, T2DM and Diabetes), and control groups (with or without antidiabetic drugs). Additionally, Begg's and Egger's tests and funnel plots were used to detect potential publication bias, with a p-value <0.05 suggesting the presence of bias.

RESULTS

Search Results and Study Characteristics

A flowchart of the study selection process is shown in **Figure 1**. After a preliminary search of the selected electronic databases, in total, 2555 studies were identified. After 1020 duplicates were eliminated, 1535 studies were selected by screening the titles and abstracts. After further excluding 1026 studies based on the titles and abstracts, 509 articles remained. Of these 509 articles, 491 were excluded for the following reasons after the full text was read: (1) not enough participant information was provided (n=377); and (2) the original data regarding insulin use were not provided (n=114). Finally, 18 articles (9, 10, 14–29) related to the use of insulin treatment and COVID-19 were included in this meta-analysis. The basic characteristics of the studies are presented in **Table 1**. Among the 18 studies included in this analysis, 2 studies were performed in France, 4 studies were performed in China, 5 studies were performed in the USA, 2 studies were performed in Italy, 1 in Russia, 1in Spain, 1 in Saudi Arabia, 1 in Iran and 1 in Iraq (**Table 1**). All included studies were published in 2020 or 2021. Additionally, as the different outcomes of COVID-19 were analyzed in our study, information regarding the association between insulin treatment and mortality is included in **Table 2**, and the outcomes of severe/critical complications or in-hospital admission are shown in **Table 3**.

Quality Assessment

NOS mainly consist of the following three aspects: sample selection, comparability of cases and controls, and exposure. All included studies had NOS scores higher than 6, indicating the high quality of our studies. The details of the risk of bias are described in **Table 1**.

TABLE 1 | Description of eligible studies reporting the association between insulin treatment and the outcomes of COVID-19.

No.	Author	Country	Age	Type of study	Sample size	NOS	Population
1	Bo Yu (14)	China	66 (57–73)	a retrospective study	689	8	patients with COVID-19 and T2DM
2	Cariou (15)	France	69.8 ± 13.0	a nationwide multicentre observational study	1317	7	patients with COVID-19 and diabetes
3	Yuchen Chen (16)	China	56.0 (39.0–67.0)	a retrospective study	904	8	patients with COVID-19 and diabetes (mostly T2DM)
4	Shayan Riahi (9)	USA	66.42 ± 12.67	a retrospective cohort	166	7	patients with COVID-19 and diabetes
5	Luis M. Pérez (10)	Spain	74.9 ± 8.4	a nationwide cohort study	2666	7	patients with COVID-19 and T2DM
6	Shivani Agarwal (18)	USA	68	cohort	1126	8	patients with COVID-19 and diabetes
7	Marina V (19)	Russia	NA	a retrospective study	309	7	patients with COVID-19 and T2DM
8	Marco Mirani (20)	Italy	71 (64–78)	cohort	90	8	patients with COVID-19 and T2DM
9	Shayesteh Khalili (21)	Iran	65.7 ± 12.51	cohort	127	7	patients with COVID-19 and diabetes
10	Justin J (22)	USA	65.7 ± 15.5	a retrospective study	46	8	patients with COVID-19 and diabetes
11	Huadong Yan (23)	China	49.18 (14.16)	Case-Control Study	717	8	patients with COVID-19 and T2DM
12	Ayman A (24)	Saudi Arabia	57.6 ± 13.9	cohort	806	8	patients with COVID-19 and T2DM
13	Achille Cernigliaro (25)	Italy	NA	a retrospective study	172	7	patients with COVID-19 and diabetes
14	Hussein Nafakhi (26)	Iraq	60 ± 10	a retrospective study	67	7	patients with COVID-19 and diabetes
15	Adèle Lasbleiz (27)	France	62.1 ± 14.0	cohort	2168	8	patients with COVID-19 and diabetes (mostly T2DM)
16	Michelle A (30)	USA	75.6 (10.8)	retrospective cohort study	775	8	patients with COVID-19 and T2DM
17	Grenye O'Malley (28)	USA	NA	cross-sectional study	113	7	patients with COVID-19 and T1DM
18	Ting Guo (29)	China	62.1 (42-76)	a retrospective study	19	7	patients with COVID-19 and diabetes

NA, data not available.

TABLE 2 | Description of studies reporting the association between insulin treatment and the mortality of COVID-19.

No.	Study	Sample size	Period of insulin treatment	Type of diabetes	Control groups	or	ci1	ci2
1	Bo Yu 2021 (14)	689	after admission	T2DM	with or without antidiabetic drugs	5.38	2.75	10.54
2	Cariou 2020 (15)	1317	before admission	diabetes	antidiabetic drugs	1.71	1.2	2.43
3	Yuchen Chen 2020 (16)	904	before admission	diabetes	with or without antidiabetic drugs	4.461	1.223	16.266
4	Shayan Riahi 2020 (9)	166	before admission	diabetes	antidiabetic drugs	2.486	1.204	5.134
5	Luis M. Pérez 2020 (10)	2666	after admission	T2DM	antidiabetic drugs	1.45	1.11	1.88
6	Shivani Agarwal 2020 (18)	1126	before admission	diabetes	without antidiabetic drugs	2.3	1.32	4.01
7	Marina V 2020 (19)	309	before admission	T2DM	antidiabetic drugs	2.67	1.42	5.02
8	Marco Mirani 2020 (20)	90	before admission	T2DM	antidiabetic drugs	3.05	1.57	5.95
9	Shayesteh Khalili 2020 (21)	127	before admission	diabetes	antidiabetic drugs	0.242	0.061	0.967
10	Justin J 2020 (22)	46	before admission	diabetes	with or without antidiabetic drugs	11.873	2.218	63.555
11	Achille Cernigliaro 2020 (25)	172	before admission	diabetes	antidiabetic drugs	1.87	0.89	3.89
12	Michelle A 2020 (30)	775	before admission	T2DM	with or without antidiabetic drugs	1.197	0.731	1.962

Primary Outcomes

Insulin Treatment and COVID-19 Mortality

The results of this meta-analysis of the use of insulin and COVID-19 mortality are shown in **Figure 2**. In general, the use of insulin is associated with increased mortality due to COVID-19 (OR=2.10 95% CI, 1.51-2.93); high heterogeneity was observed (I^2 statistic=71.5%, p<0.001) (**Figure 2**). The results of Egger's (p = 0.2) and Begg's tests (p = 0.193) and inspection of the funnel plots showed that there was no publication bias among the studies (**Figure 3A**). A sensitivity analysis was conducted by omitting one study at a time and showed that the results were stable (**Figure 4A**).

Insulin Treatment and COVID-19 Severe/Critical Complications

The overall analysis included 8 studies that reported quantitative data regarding severe/critical COVID-19 illness. All extracted data were divided according to the severe/critical illness status based on the standard illness severity level (12). The overall risk of severe/critical COVID-19 illness was higher in the insulin treatment group than the control group (OR = 2.56, 95% CI, 1.18–5.55, I^2 = 85.5%, p<0.001) (**Figure 5**). Begg's test (p = 0.902), Egger's test (p = 0.308) and the symmetric distribution funnel plots (**Figure 3B**) indicated that publication bias did not exist. The results of the sensitivity analysis conducted by excluding one study at a time did not change (**Figure 4B**).

Insulin Treatment and In-Hospital Admission for COVID-19

Overall, insulin therapy was not associated with in-hospital admission for COVID-19 (OR=1.23; 95% CI, 0.65-2.33) (**Figure 6A**). Strong heterogeneity existed among the 5 included articles (I^2 statistic=87.6%, p<0.001). Interestingly, the subgroup based on the type of diabetes significantly decreased

TABLE 3 | Description of studies reporting the association between insulin treatment and the severe/critical complications or in-hospital admission of COVID-19.

No.	Study	Sample size	Complications	Period of insulin treatment	Type of diabetes	Control groups	or	ci1	ci2
1	Bo Yu (14)	689	ICU admission	after admission	T2DM	with or without antidiabetic drugs	11.47	3.4	38.66
2	Bo Yu (14)	689	Invasive mechanical ventilation	after admission	T2DM	with or without antidiabetic drugs	6.73	3.39	13.37
3	Yuchen Chen (16)	904	Poor prognosis	before admission	Diabetes	with or without antidiabetic drugs	3.58	1.37	9.35
4	Luis M. Pérez (10)	2666	ICU admission;	after admission	T2DM	antidiabetic drugs	1.32	1.02	1.71
5	Huadong Yan (23)	717	severity	before admission	T2DM	antidiabetic drugs	2.71	1.6	5.5
6	Hussein Nafakhi (26)	67	extensive lung injury	before admission	Diabetes	antidiabetic drugs	0.4	0.3	5
7	Hussein Nafakhi (26)	67	Partial recovery with persistent symptoms	before admission	Diabetes	antidiabetic drugs	0.3	0.2	4
8	Ting Guo (29)	19	ICU admission	before admission	Diabetes	without antidiabetic drugs	115	4.115	3213.5
No.	**Study**	**Sample size**	**Complications**	**Period of insulin treatment**	**Type of diabetes**	**Control groups**	**or**	**ci1**	**ci2**
1	Luis M. Pérez (10)	2666	In-hospital admission	after admission	T2DM	antidiabetic drugs	1.23	0.95	1.6
2	Ayman A (24)	806	In-hospital admission	before admission	T2DM	antidiabetic drugs	1.46	1.03	2.07
3	Achille Cernigliaro (25)	172	In-hospital admission	before admission	Diabetes	antidiabetic drugs	2.13	1.01	4.49
4	Adèle Lasbleiz (27)	2168	In-hospital admission	before admission	Diabetes	antidiabetic drugs	3.8	1.9	8.1
5	Grenye O'Malley (28)	113	In-hospital admission	after admission	T1DM	without antidiabetic drugs	0.137	0.053	0.352

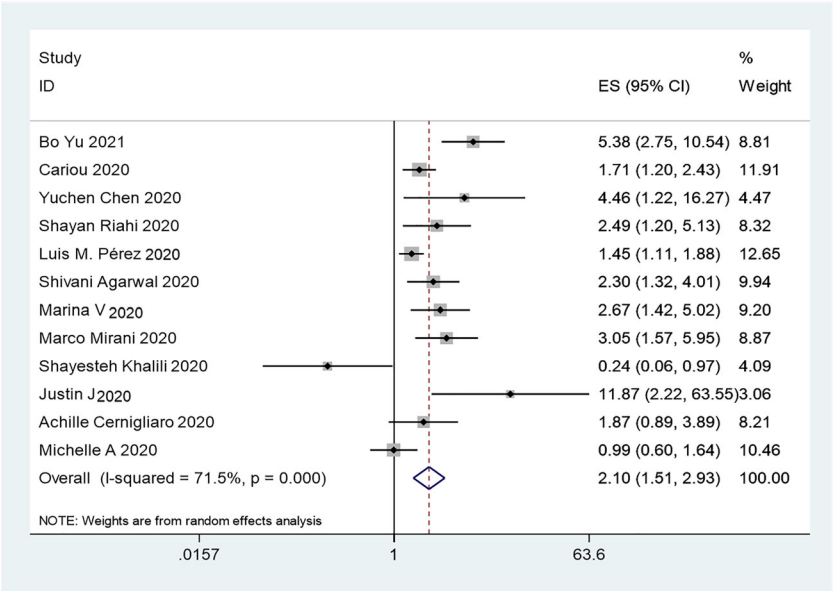

FIGURE 2 | Forest plots of OR for the association between the insulin treatment and the mortality of COVID-19.

the heterogeneity in each subgroup (**Figure 6B**). In the T2DM and Diabetes subgroups (patients with diabetes of which type was not clear in the extracted study), insulin treatment significantly increased in-hospital admission for COVID-19 (T2DM, OR=1.31; 95% CI, 1.06-1.61; Diabetes, OR=2.86; 95% CI, 1.62-5.05), which was the opposite of the results in the T1DM subgroup (OR=0.14; 95% CI, 0.05-0.35). However, due to the limited number of included T1DM studies, the effects of insulin treatment on patients with COVID-19 and T1DM require further solid evidence. According to the results of the funnel analysis (**Figure 3C**), Begg's test (p = 1.000), and Egger's test (p = 0.861), publication bias could be excluded. The sensitivity analysis also indicated the stability of our results (**Figure 4C**).

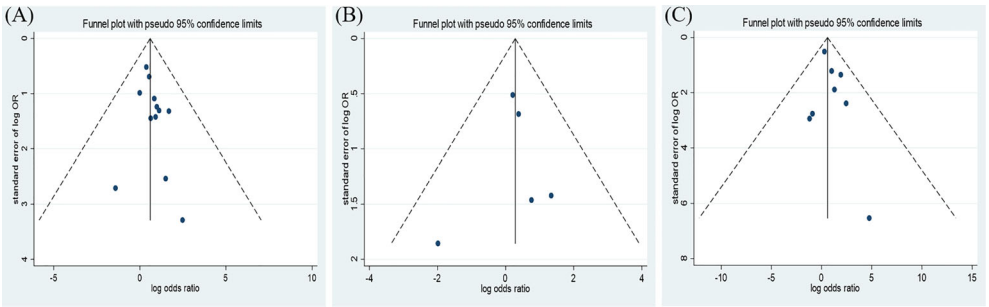

FIGURE 3 | Funnel plot of the association between the insulin treatment and the mortality **(A)**, complications **(B)**, and the in-hospital admission **(C)** of COVID-19.

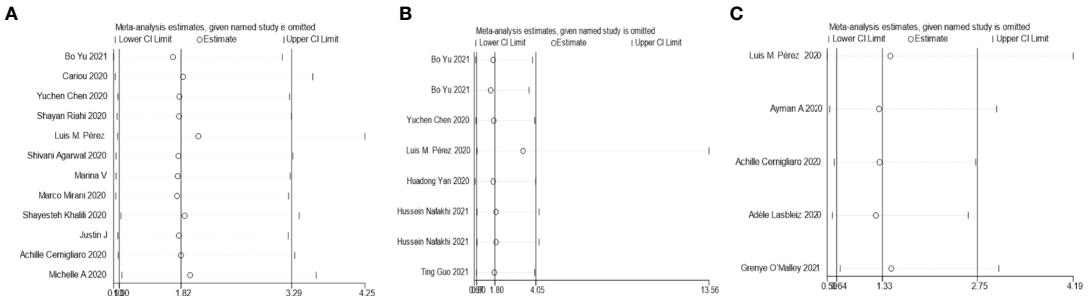

FIGURE 4 | Sensitivity analysis for the effect insulin treatment on the mortality **(A)**, complications **(B)**, and the in-hospital admission **(C)** of COVID-19.

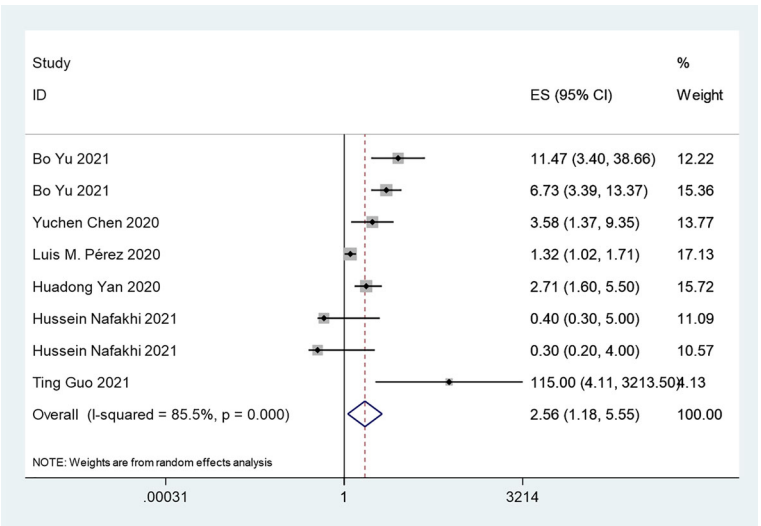

FIGURE 5 | Forest plots of OR for the association between the insulin treatment and the complications of COVID-19.

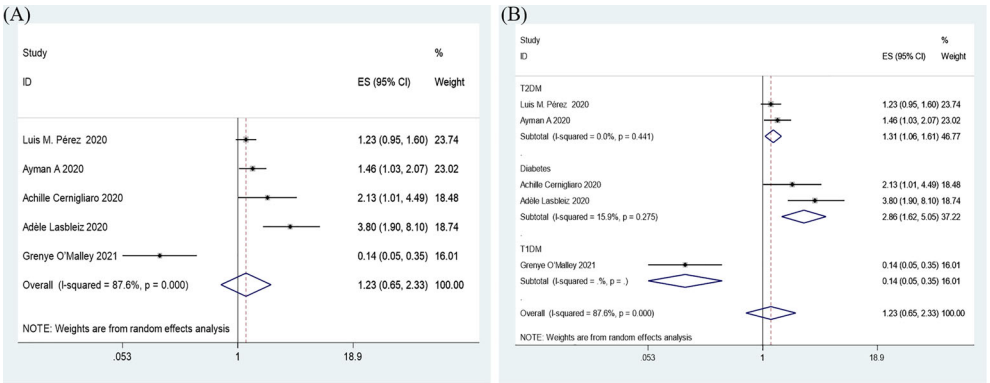

FIGURE 6 | Forest plots of OR for the overall result **(A)** and subgroup analysis based on type of diabetes **(B)** of the association between the insulin treatment and the in-hospital admission of COVID-19.

Insulin Treatment and COVID-19 Hospitalization Time

Only 2 studies mentioned the association between insulin treatment and COVID-19 hospitalization time (SMD=0.21 95% CI, -0.02-0.45). In general, insulin use did not affect the hospitalization time of COVID-19 patients (**Figure 7**). However, additional studies are needed to support this conclusion. Due to the limited number of included studies, further funnel plots or sensitivity analyses were not conducted.

Secondary Outcomes

The overall data showed that insulin treatment led to increased adverse COVID-19 outcomes, including higher mortality, with moderate heterogeneity (I^2 = 69.2%, p<0.001) (**Figure 2**). To eliminate heterogeneity, further prespecified subgroup analyses based on the sample size, period of insulin treatment, type of diabetes and type of control group, were conducted (**Tables 2**, **3**). However, moderate/high heterogeneity remained in all subgroups (**Figures S1A–D**).

High heterogeneity also existed in the analysis of the effect of insulin treatment on severe/critical complications (I^2 = 85.5%, p<0.001) (**Figure 5**). We conducted further subgroup analyses based on the sample size (**Figure S2A**), period of insulin treatment (**Figure S2B**), and type of diabetes (**Figure S2C**), but moderate/high heterogeneity remained in all subgroups. Additionally, in the subgroup analysis based on the type of control group, heterogeneity was also present in the antidiabetic drug group (I^2 = 74.3% p=0.002), while there was decreased heterogeneity in the with and without antidiabetic drug groups (I^2 = 12.9% p=0.317) (**Figure S2D**). Overall, this

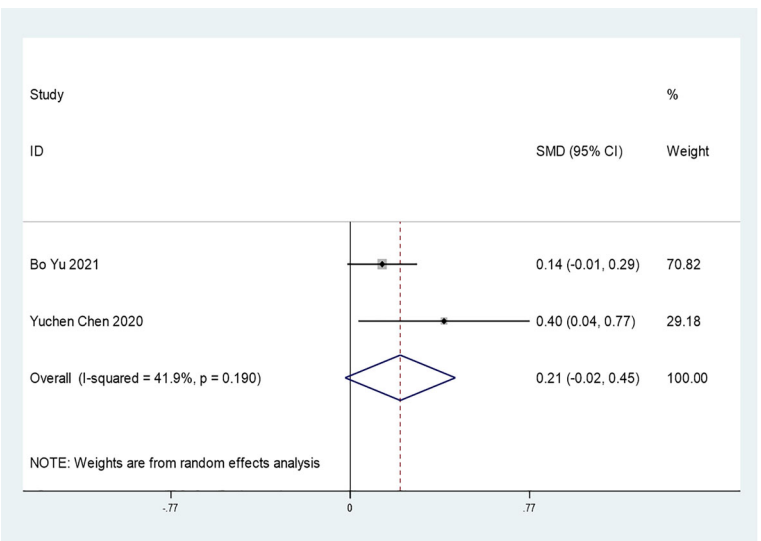

FIGURE 7 | Forest plots of SMD for the association between the insulin treatment and the hospitalization time of COVID-19.

meta-analysis still showed moderate/high heterogeneity after the subgroup analysis, indicating that the included articles failed to provide reliable information for further analysis.

DISCUSSION

Diabetes has been demonstrated to be among the most common comorbidities and is significantly associated with the mortality and severity of COVID-19 (16). Glucose-lowering medications have been proposed as a treatment for COVID-19. In contrast to preconception, insulin treatment has been demonstrated to be related to increased mortality and ICU admission for COVID-19 (14). In the current study, insulin treatment was associated with increased mortality and incidence of severe/critical complications in patients with COVID-19 and diabetes. Our study may provide evidence of the adverse effect of insulin treatment among patients with COVID-19 and diabetes, especially among those with type 2 diabetes (T2DM), as the subjects in most included studies suffered from T2DM. However, considering the limited number of studies concerning type 1 diabetes (T1DM) in our meta-analysis, the association between insulin treatment and adverse outcomes in patients with COVID-19 and T1DM are needed to be investigated in more large-scale clinical studies.

Association Between Insulin Treatment and COVID-19

Our meta-analysis mainly indicates that insulin treatment may be associated with increased mortality (**Figure 2**) and severe/critical complications in T2DM and COVID-19 patients (**Figure 5**). Insulin treatment may also increase in-hospital admission of patients with T2DM and COVID-19 (**Figure 6B**), while there may be no significant relationship between insulin treatment and hospitalization time (**Figure 7**). The patients in the included studies of this meta-analysis had used insulin before catching COVID 19, so the results in our meta-analysis should be interpreted with caution as insulin is usually given to patients in a late stage of diabetes, since it is difficult to rule out the negative effect of advanced diabetes on the poor outcome.

Mechanism Underlying the Relationship Between Insulin Treatment and Worse COVID-19 Outcomes

Although the mechanism underlying the association between insulin use and the poor outcome of COVID-19 remains unclear, there are several possible explanations for these results. First of all, diabetes itself contributed to a low grade of chronic inflammation, which characterized by impaired innate and adaptive inflammation system. This disorder of inflammation would lead to the dysregulation of the immune response with higher level of proinflammatory factors and lower anti-inflammatory cytokines. Insulin treatment was demonstrated to increase macrophage-produced inflammatory cytokine levels in the context of lipopolysaccharide-induced sepsis (31). Additionally, insulin was associated with increased lung inflammation in a sepsis model of diabetic rats (32), while lung cells are the main locations for COVID-19 inflammation. Therefore, we speculate that insulin treatment may participate in the development of COVID-19 by promoting proinflammatory system to aggravate inflammation disorder and pulmonary disease.

Moreover, insulin, an anabolic hormone to improve energy storage, is to increase appetite and weight gain, which contributed to obesity with inhibited lipolysis in adipose and muscle tissues (33, 34). Obesity people is characterized by chronic inflammation and impaired innate immunity with ample production of proinflammatory factors including tumor necrosis factor (TNF-α) and interleukin-6 (IL-6), which also comprised one of risk factors for COVID-19. Consistent with this, obesity and its impaired inflammatory imbalance contributed by insulin treatment may also be one of the mechanisms underlying the relationship between insulin use and COVID-19 outcomes.

Furthermore, hypoglycemia is among the most common adverse effects of insulin treatment, especially in the context of intensive care (35, 36). Insulin treatment increased the incidence of hypoglycemia in patients with COVID-19 and type 2 diabetes mellitus (T2DM) (14), which may impair the glucose homeostasis and glucose control to affect the COVID-19 outcomes. However, there must be other unknown mechanisms underlying the effect of insulin treatment on the outcomes of COVID-19, as the effect still remains in patients without hypoglycemia during hospitalization.

Although the potential mechanisms of the deleterious effect of insulin treatment on COVID-19 progress are discussed above, considering the irreplaceable role of insulin in controlling glucose and diabetes complications, it may be not advisable to give up insulin therapy before finding better or safer hypoglycemic agents for COVID-19 and T2DM patients. Moreover, Critical unwell patients with COVID-19 and diabetes have high insulin requirements and poorer time in optimal target range for blood glucose during peak inflammatory response (37), which indicating the insulin treatment is necessary during the late stage of COVID-19 disease. Additionally, the effects of insulin usage in COVID-19 progression are also affected by the blood glucose level and severity of diabetes (16, 17). Therefore, the results from the current meta-analysis should be interpreted with caution as the complicated situation during different stages of diabetes and insulin effectively in controlling glycemic levels, and more convinced well-designed randomized controlled trials are required to provide more precise conclusions for insulin usage in COVID-19 progression.

Theoretical and Practical Significance

Our study is to summarize the effects of insulin therapy and clinical in outcomes of patients with COVID-19 and diabetes. Additionally, we raised an interesting and significant question regarding the mechanism underlying the relationship between insulin and the development of COVID-19, as there are no reasonable explanations. Therefore, the timing or dose of insulin

treatment in patients with COVID-19 and diabetes may be an urgent public problem that must be addressed to control the development of COVID-19.

Limitations of the Study

There are several limitations in our study. First, the high heterogeneity in the results cannot be ignored, and most of the study included was observational study, many biases could not be controlled. Second, the type of diabetes was unclear in several included studies, potentially rendering our conclusion not generalizable to all types of diabetes. Notably, one report focusing on the protective effect of insulin treatment on COVID-19 outcomes involved patients with T1DM and, revealed the opposite conclusion from our results (including mostly T2DM patients), indicating that a different approach to insulin therapy may be needed according to the type of diabetes. Therefore, our study is more likely to be applicable to T2DM patients, while guidance for insulin treatment in COVID-19 patients with different types of diabetes, especially T1DM, needs more clinical research. Third, the causal relationship between insulin treatment and COVID-19 outcomes remains vague due to the limited amount of prospective data in our included studies. Moreover, this systematic review and meta-analysis was not registered in PROSPERO, representing another limitation of our study. Last but not least, the results from this study are only based on observational studies which have many confounders, therefore the evidence generated from this study is not strong enough. More randomized clinical trials are still needed to confirm the results from this study.

Conclusion

In summary, our study revealed that insulin treatment is associated with increased mortality and other severe/critical complications of COVID-19. Our findings could provide clinical guidance for insulin treatment in patients with COVID-19 and diabetes. Further randomized controlled trial was needed to confirm this finding.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Conceptualization, YY. Methodology, ZC, JZ and YY. Software, YY. Validation, JZ, YY and ZC. Formal analysis, YY and ZC. Investigation, YY. Resources, JZ. Data curation, ZC. Writing—original draft preparation, YY. Writing—review and editing, JZ. Visualization, JZ. Supervision, ZC and JZ. Project administration, JZ. Funding acquisition, JZ. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.696087/full#supplementary-material

Supplementary Figure 1 | Outcomes of the subgroup analysis of the sample size **(A)**, period of insulin treatment **(B)**, type of diabetes **(C)**, kind of control groups **(D)** of the insulin treatment with mortality in patients with T2DM by forest plots.

Supplementary Figure 2 | Outcomes of the subgroup analysis of the sample size **(A)**, period of insulin treatment **(B)**, type of diabetes **(C)**, kind of control groups **(D)** of the insulin treatment with sever/critical complications in patients with T2DM by forest plots.

Supplementary Figure 3 | Outcomes of the subgroup analysis of the period of insulin treatment **(A)**, kind of control groups **(B)** of the insulin treatment with the in-hospital admission by forest plots.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ACE Gene Variants Rise the Risk of Severe COVID-19 in Patients With Hypertension, Dyslipidemia or Diabetes: A Spanish Pilot Study

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Coronavirus disease 19 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection continues to scale and threaten human health and public safety. It is essential to identify those risk factors that lead to a poor prognosis of the disease. A predisposing host genetic background could be one of these factors that explain the interindividual variability to COVID-19 severity. Thus, we have studied whether the rs4341 and rs4343 polymorphisms of the angiotensin converting enzyme (ACE) gene, key regulator of the renin-aldosterone-angiotensin system (RAAS), could explain the different outcomes of 128 COVID-19 patients with diverse degree of severity (33 asymptomatic or mildly symptomatic, 66 hospitalized in the general ward, and 29 admitted to the ICU). We found that G allele of rs4341 and rs4343 was associated with severe COVID-19 in hypertensive patients, independently of gender ($p<0.05$). G-carrier genotypes of both polymorphisms were also associated with higher mortality ($p< 0.05$) and higher severity of COVID-19 in dyslipidemic ($p<0.05$) and type 2 diabetic patients ($p< 0.01$). The association of G alleles with disease severity was adjusted for age, sex, BMI and number of comorbidities, suggesting that both the metabolic comorbidities and the G allele act synergistically on COVID-19 outcome. Although we did not find a direct association between serum ACE levels and COVID-19 severity, we found higher levels of ACE in the serum of patients with the GG genotype of rs4341 and rs4343 ($p<0.05$), what could explain the higher susceptibility to develop severe forms of the disease in patients with the GG genotype, in addition to hypertension and dyslipidemia. In conclusion, our preliminary study suggests that the G-containing genotypes of rs4341 and rs4343 confer an additional risk of adverse COVID-19 prognosis. Thus, rs4341 and rs4343 polymorphisms of ACE could be predictive markers of severity of COVID-19 in those patients with hypertension, dyslipidemia or diabetes. The knowledge of these genetic data could contribute to precision management of SARS-CoV-2 infected patients when admitted to hospital.

Keywords: COVID-19, angiotensin converting enzyme, polymorphisms, hypertension, dyslipidemia, diabetes

INTRODUCTION

At the time of writing this paper, the COVID-19 has resulted in a pandemic with more than 187 million of confirmed cases around the world that has caused more than 4 million deaths (1) (July 2021). Most of the patients infected by SARS-CoV-2 are asymptomatic or present mild-moderate symptoms. However, in our midst, approximately 10% of patients require hospital admission (2) and 20% of hospitalized patients develop severe respiratory diseases which may quickly progress to respiratory failure, shock and multiorgan dysfunction (3). In hospitalized COVID-19 patients, more than 30% may require intensive care treatment and around 39% of ICU admitted patients die (4). Thus, it is essential to identify those individuals more likely to develop severe forms of the disease for an early precision management. Older age, male sex and pre-existing conditions such as hypertension, obesity, diabetes and chronic kidney disease are risk factors predisposing to serious disease. However, many individuals with these features do not develop severe symptoms, and the causes are not fully understood (3, 5).

The renin-angiotensin-aldosterone system (RAAS), one of the major blood pressure regulatory pathways, could be involved in the pathogenesis of COVID-19, since SARS-CoV-2 uses angiotensin-converting enzyme-2 (ACE2) as the binding receptor to enter the cell. The physiological homeostasis of this system is regulated by the balance of the angiotensin converting enzyme (ACE) and ACE2. ACE converts angiotensin I (Ang I) to angiotensin II (Ang II) which, through its interaction with the angiotensin II type 1 receptor (AT1R), induces a strong vasoconstriction and triggers proinflammatory, proapoptotic and profibrotic pathways in the lung and other organs (6). In RAAS, ACE2 contributes to the inactivation of Ang II, by hydrolyzing it to Ang-1-7, and, therefore, physiologically counters Ang II/AT1R effects, at the same time that stimulates vasodilation and promotes anti-inflammatory, antifibrotic and antithrombotic actions *via* the Ang-1-7/Mas receptor axis (6). In conditions where ACE2 bioavailability is decreased, Ang II-AT1R pathway is potentiated, aggravating COVID-19-induced inflammation and lung injury. Also, high levels and activity of ACE would further increase the activity of the AngII/AT1R pathway. Moreover, advanced age, cardiovascular diseases, hypertension and metabolic diseases such as type 2 diabetes and obesity also lead to further dysfunctions in the renin-angiotensin-aldosterone system (RAAS), likely rendering patients with these underlying comorbidities more susceptible to further repercussions of ACE/ACE2 imbalance (7–9) and references therein].

Genetic polymorphisms in ACE, such as rs4343, rs4341 and the ACE I/D polymorphism, have been shown to affect ACE levels and activity, and confer susceptibility to hypertension (10), type 2 diabetes (11), overweight (12), nephropathy (13, 14) and certain cardiovascular (15, 16) and autoimmune diseases (17). More specifically, the DD genotype of the ACE I/D polymorphism has been associated to higher levels of serum ACE and higher levels of circulating IL6 in patients with myocardial infarction. In contrast, the II genotype has been associated to lower circulating ACE levels, and robust coagulation, and/or enhanced fibrinolysis (7). Since some of these processes have been reported to be involved in the pathogenesis of COVID-19, the DD genotype could predispose to complications of COVID-19 due to higher baseline ACE levels and its consequences (7). Indeed, an association of the DD genotype of the ACE I/D polymorphism with severe COVID-19 has been reported in hypertensive males (18). However, analyzing the I/D polymorphism is laborious and time-consuming and some authors have described a preferential amplification of the D allele (19). Rs4341 and rs4343 polymorphisms are in complete linkage disequilibrium with the ACE I/D polymorphism (19, 20), better reflect circulating ACE levels than direct ACE Alu I/D genotyping (21) and can be easily evaluated by TaqMan assays, therefore they could be better prognostic markers. In the present study we investigated the association of rs4341 and rs4343 polymorphisms of the ACE gene with COVID-19 outcomes in patients with different degree of severity. Identifying genetic variants that influence the severity of COVID-19 could be useful to better understand the physiopathology of the disease or identify new therapeutic targets, through the identification of effector transcripts of the genetic variants that underlie the phenotype. Besides, it could facilitate the early identification of patients genetically susceptible for severe COVID-19 to better monitor them and give them a more appropriate clinical management that may improve their outcomes.

Abbreviations: COVID-19, coronavirus disease 19; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ACE, angiotensin converting enzyme; RAAS, renin-aldosterone-angiotensin system; ICU, intensive care unit; ACE2, angiotensinconverting enzyme-2; Ang I, angiotensin I; Ang II, angiotensin II; AT1R, angiotensin II type 1 receptor.

MATERIAL AND METHODS

Subjects

In this study, 128 SARS-CoV-2 positive patients (confirmed by PCR from nasopharyngeal swabs) from the first epidemic wave (17 April -29 May 2020), were recruited at the Hospital Universitario San Pedro, Logroño, Spain. These patients were grouped into the following severity of illness categories: i) asymptomatic or mildly symptomatic patients not requiring hospital admission (n=33), ii) severe COVID-19 patients requiring hospitalization in the normal ward (n=66) and iii) critically ill patients admitted to the intensive care unit (ICU) (n=29).

Asymptomatic/mildly symptomatic group was composed by patients with any of the following non-life-threatening symptoms (n=26) such as fever, myalgia, fatigue, rhinorrhea, vomits, sickness, diarrhea, dry cough, cephalalgia, anosmia, ageusia (they had no lung involvement in chest X-ray and had not required oxygen supplements) and also by asymptomatic individuals (n=7) diagnosed with RT-PCR-confirmed SARS-CoV-2 infection (for being a close contact to a COVID-19 positive case). Both types of patients were analyzed in a single group since none of them required hospital admission.

Severe COVID-19 patients hospitalized in the normal ward were patients with symptoms requiring oxygen therapy or patients presenting a high decompensation of their underlying diseases due to the SARS-CoV-2 infection. ICU group were patients that required invasive mechanical ventilation.

Relevant clinical data were obtained from the medical records of patients. Comorbidities were diagnosed by physicians of the Hospital Universitario San Pedro according to the guidelines of different medical Societies. More specifically, the body mass index (BMI) of the patients was calculated as kg/m^2. Patients were classified by BMI according to the criteria of the World Health Organization (WHO) and the Spanish Society for the Study of Obesity (SEEDO) (22), with obesity defined as a BMI of 30 kg/m^2 or higher for both sexes. Patients were also classified by blood pressure (BP) values and plasma lipoprotein and triglycerides levels according to the criteria of the European Society of Cardiology, with hypertension defined as office systolic BP values ≥140 mmHg and/or diastolic BP values ≥90 mmHg (23) and dyslipidemia defined as total cholesterol levels ≥250 mg/dl (>200 mg/dl for secondary dyslipidemia or diabetic patients) or low-density lipoprotein-cholesterol (LDL-C) ≥130 mg/dl and/or triglycerides levels ≥200 mg/dl (>150 mg/dl for secondary dyslipidemia or diabetic patients) (24). Similarly, patients were classified according to the guidelines of the WHO and the American Diabetes Association as having diabetes mellitus type 2 if they met one of the following criteria: fasting plasma glucose level of ≥126 mg/dL (≥7.0 mmol/L), 2-hours value of ≥200 mg/dL (≥11.1 mmol/L) in 75 g oral glucose tolerance test (OGTT) or random plasma glucose level of ≥200 mg/dL (≥11.1 mmol/L) (25). Classification of patients with heart conditions (heart failure, coronary artery disease and cardiomyopathies) was performed according to the guidelines of the European Society of Cardiology (26) and diagnosed on the bases of features of systolic or diastolic heart dysfunction in echocardiography, biochemical tests, magnetic resonance imaging at rest, and chest X-ray. Chronic kidney disease was defined according to the Spanish Society of Nephology as albuminuria (ACR ≥ 30 mg/g), urine sediment abnormalities, electrolyte and other abnormalities due to tubular disorders, abnormalities detected by histology, structural abnormalities detected by imaging, history of kidney transplantation, or glomerular filtration rate <60 ml/min/1.73 m^2. Dementia and/or other neurological conditions category included patients with Alzheimer Disease, senile dementia or Parkinson disease, diagnosed according to the guidelines of the Spanish Society of Neurology (SEN) (27). The criterion for the diagnosis of COPD was defined by a post-bronchodilator FEV1/FVC ratio lower than 0.7 according to the Spanish COPD Guidelines (GesEPOC) (28). The "Others" category included patients with cancer, hepatic chronic disease or peptide ulcer.

Mortality was evaluated as patients' death within 90 days from SARS-CoV-2 infection detection by PCR (COVID-19 disease onset).

This study was performed following the Helsinki Declaration and was approved by an independent ethical committee for clinical research (*Comité de Ética de Investigación con medicamentos de La Rioja*, CEImLAR, reference number PI-412). All patients or their representatives/relatives gave their consent to participate in the study.

Genomic DNA Isolation and Genotyping

Genomic DNA was extracted from buffy coat layer using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany). The rs4341 and rs4343 genotyping of the DNA samples was carried out in an Applied Biosciences (ABI) 7300 RT-PCR system using predesigned TaqMan SNP Genotyping Human Assays from ABI (Foster City, CA) according to the manufacturer's instructions. The ID assays used were C:29403047_10 and C:11942562_20 for rs4341 and rs4343 respectively.

Circulating ACE Levels Determination

Serum ACE levels were measured by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit from R&D (Human ACE Quantikine ELISA Kit, R&D, Minneapolis, USA).

Statistical Analysis

Categorical data are presented as counts (percentages) and were analyzed using the χ^2 test or Fisher's exact test. Quantitative values are expressed as mean ± standard deviation. Normal distribution of quantitative variables was checked using the Shapiro-Wilk test. Quantitative data were evaluated with the Kruskal-Wallis test followed by the Mann-Whitney U test. *P* values <0.05 were considered statistically significant. Statistical analysis was performed using GraphPad Prism 6 (GraphPad Prism®, La Jolla, California, USA).

Allele and genotype frequencies, Hardy-Weinberg equilibrium test, and association of the rs4341 and rs4343 polymorphisms with COVID-19 outcome were analyzed using SNPStats online software (https://snpstats.net/) (29). To reduce the potential confounding effects from age, gender, BMI and number of comorbidities, an adjustment for these factors was performed.

RESULTS

Distribution of Demographic and Clinical Characteristics

Table 1 describes the characteristics and demographic data of SARS-CoV-2 infected patients. Mean age of asymptomatic/mildly symptomatic patients was lower than those with more serious illness (p<0.001). A higher frequency of males was found in both groups of hospitalized patients when compared to asymptomatic/mildly symptomatic patients (p<0.01 Hospital ward *vs* asymptomatic/mildly symptomatic; p<0.001 ICU *vs* asymptomatic/mildly symptomatic). A higher frequency of hypertension (p < 0.001) and dyslipidemia (p < 0.001) was also found in both groups of hospitalized patients when compared to asymptomatic/mildly symptomatic patients, and a similar tendency was observed when examining the frequency of type 2 diabetes mellitus (p<0.01), heart conditions (p<0.05) and dementia or other neurological conditions (p<0.001), although the difference only reached statistical significance for hospitalized patients who did not required ICU admission. A higher proportion of patients with chronic kidney

TABLE 1 | Clinical and demographic characteristics of COVID-19 patients.

	Asymptomatic or mildly symptomatic N = 33	Hospital Ward N = 66	ICU N = 29	*P*-value
Age (years)	43.58 ± 8.7	70.8 ± 20.4***	65.1 ± 10.8***	**<0.0001**
Male	8 (24.2%)	34 (51.5%)**	22 (75.9%)***	**0.0003**
Hypertension	0 (0%)	30 (45.4%)***	12 (41.4%)***	**<0.0001**
Dyslipidemia	0 (0%)	13 (19.7%)**	7 (24.1%)**	**0.0140**
Type 2 diabetes mellitus	0 (0%)	12 (18.2%)**	3 (10.3%)	**0.0287**
Heart conditions (heart failure, coronary artery disease, cardiomyopathies)	1 (2.9%)	12 (18.2%)*	5 (17.2%)	0.0964
Obesity[a]	3 (9.1%)	14 (22.6%)	11 (39.3%)**	**0.0197**
Chronic kidney disease	0 (0%)	4 (6.06%)	4 (13.8%)*	0.0813
Dementia and/or other neurological conditions	0 (0%)	19 (28.8%)***	3 (10.3%)$^{\phi}$	**0.0009**
COPD (chronic obstructive pulmonary disease)	0 (0%)	7 (10.61%)	3 (10.3%)	0.1518
Others	1 (2.9%)	5 (7.58%)	3 (10.3%)	0.4974
Deceased	0 (0%)	11 (16.7%)*	9 (47.37%)***$^{\phi\phi}$	**<0.0001**

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs Asymptomatic/mildly symptomatic cases; $^{\phi}p < 0.05$, $^{\phi\phi}p < 0.01$ vs Hospital ward group.
[a]*Obesity: BMI ≥ 30 kg/m2.*
Significant differences appear in bold case.

disease was also detected in ICU admitted group when compared to asymptomatic/mildly symptomatic group (p<0.05). Besides, we found a higher prevalence of obesity in the group of patients admitted to the ICU when compared to asymptomatic/mildly symptomatic patients group (p<0.01). The percentage of obese patients in the hospital ward group was also higher than in the asymptomatic/mildly symptomatic group, although the difference was not statistically significant. We did not observe significant differences in the prevalence of chronic obstructive pulmonary disease (COPD) among the 3 groups studied. The mortality rate was higher in patients with severe symptoms compared to patients with mild or no symptoms who did not require hospitalization (p<0.05 Hospital ward *vs* asymptomatic/mildly symptomatic; p<0.001 ICU *vs* asymptomatic/mildly symptomatic). Besides, the number of patients who died within 90 days after COVID-19 onset was significantly superior in the ICU group than in the hospital ward group (p<0.01).

Genotype Distribution Among Groups With Different COVID-19 Outcome

The genotypes of both polymorphisms were distributed in concordance with Hardy-Weinberg equilibrium in all groups (for rs4341: asymptomatic/mildly symptomatic *P*=0.16, hospital ward group *P*=1, ICU group *P*=0.72; for rs4343: asymptomatic/mildly symptomatic *P*=0.29, hospital ward group *P*=0.81, ICU group *P*=0.72).

We did not observe significant differences in the genotype and allele frequencies of rs4341 and rs4343 under any of the inheritance models between the 3 groups of SARS-CoV-2 infected patients (**Figure 1**). When we performed the analysis stratifying by sex no significant differences were observed when comparing rs4341 and rs4343 genotypes of male and female patients (**Figure 2**). When evaluating the association of both polymorphisms with the age of the patients and its interaction with the COVID-19 outcome no significant differences were observed either (data not showed).

Genotype Distribution Among Groups With Different COVID-19 Outcome in Relation to Comorbidities

The association of the rs4341 and rs4343 polymorphisms with the risk of severe COVID-19 was also evaluated in relation to comorbidities. When we analyzed only hypertensive patients admitted to the ICU, a higher frequency of the G allele in both polymorphisms compared to hypertensive patients hospitalized in the ward was observed (p<0.05) (**Figures 3A, C**). A similar, but not statistically significant tendency was observed in the frequency of the GG genotype (**Figures 3B, D**). When examining the genotypes of dyslipidemic patients divided according to COVID-19 severity, a higher frequency of G allele (p<0.01) and G-containing genotypes (p<0.05) was found in dyslipidemic patients admitted to the ICU than in those admitted to the hospital ward (**Figure 3**). The same differences were observed when evaluating the allele and genotype distribution of rs4341 and rs4343 in diabetic patients (p<0.001 for allele frequencies, p<0.01 for genotype frequencies) (**Figure 3**). These genotype frequencies observed in hypertensive, dyslipidemic and diabetic patients did not differ by sex (data not shown). A higher frequency of GG was not associated to a higher severity in obese patients with SARS-CoV-2 infection (data not shown). No associations were also found among rs4341 and rs4343 genotypes and COVID-19 severity in relation to heart conditions, renal disease, COPD or dementia and/or other neurological conditions (data not shown).

Finally, a higher incidence of G-containing genotypes of both polymorphisms was detected among deceased COVID-19 patients who had been admitted to the ICU compared to survivals (p<0.05) (**Figure 4**).

The G allele of rs4341 and rs4343 polymorphisms is a risk factor for metabolic disorders per se, so to investigate whether the increased risk of severe COVID-19 is due to the potentiation of the metabolic dysfunctions by these alleles, it is independent of the presence of metabolic dysfunctions, or both the comorbidities and the G allele act synergistically, an additional

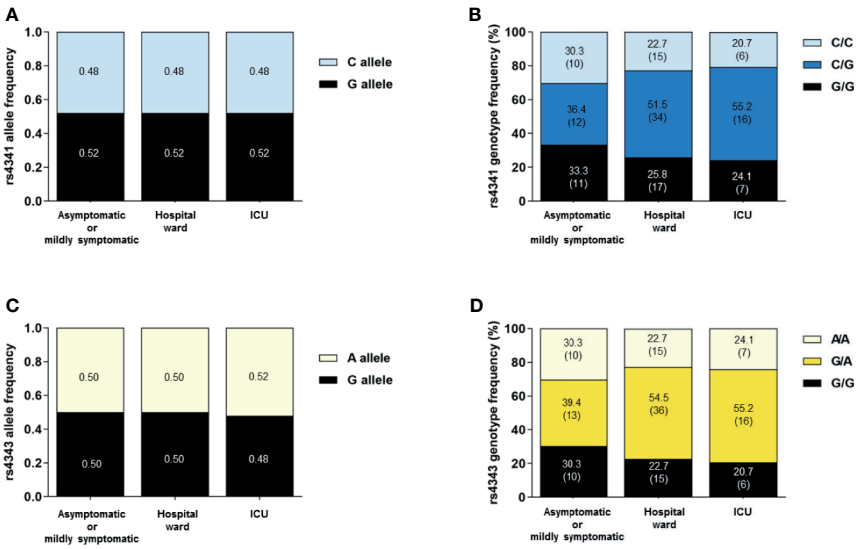

FIGURE 1 | Allele and genotype distribution of the rs4341 and rs4343 polymorphisms among SARS-CoV-2 infected patients. Allele frequencies of rs4341 **(A)** and rs4343 **(C)** polymorphisms. Data are presented as proportions. Genotype frequencies of rs4341 **(B)** and rs4343 **(D)** polymorphisms. Data are presented as percentages. The number of patients is indicated in brackets. All data were adjusted for age and sex.

analysis was performed adjusting for age, sex, BMI and number of comorbidities. After adjusting for these additional variables, a significant higher frequency of patients with the GG genotype of rs4341 and rs4343 was found in COVID-19 patients with hypertension (**Table 2**). A higher frequency of the GG genotype was also found in dyslipidemic and diabetic patients even after adjusting for BMI and number of metabolic comorbidities in addition to age and sex (**Table 2**). A higher presence of G-containing genotypes was also found among deceased COVID-19 patients who had been admitted to the ICU after adjusting for the above indicated possible confounding factors (**Table 3**).

Association of Serum ACE Levels With rs4341 and rs4343 Genotypes

Serum ACE levels were significantly higher in patients with the rs4341 GG genotype than in individuals with GC ($p<0.05$) or CC genotypes ($p<0.001$), with the CC genotype being the one associated with the lowest levels of ACE (**Figure 5A**). The same result was observed for the rs4343 polymorphism (**Figure 5B**). This higher elevation of ACE levels in individuals with the GG genotype was also confirmed in hypertensive patients ($p<0.05$), although this elevation was not directly associated with the severity of COVID-19 (**Figures 5C, D**). A similar result was found when examining dyslipidemic patients, although the differences did not reach statistical significance ($p=0.07$), probably due to the small sample size of some groups and to the absence of patients with CC/AA genotype in the ICU group (**Figures 5C, D**). No association was found between ACE serum levels and the genotype of the rs4341 and rs4343 polymorphisms in diabetic patients, nor with its severity (**Figures 5C, D**).

DISCUSSION

Patients with SARS-CoV-2 infection can experience a wide range of clinical manifestations, ranging from no symptoms to critical illness and even death. However, the causes are still unknown. In this context, and although the sample size of the infected people is small, we found that COVID-19 severity was associated to male gender, as previously described (30). Similarly, older age and a higher number of comorbidities was found in both hospitalized groups compared to asymptomatic patients, in concordance with the literature (3, 31). However, we did not observe a higher mean age or a higher prevalence of hypertension, dyslipidemia, diabetes mellitus, obesity and cardiovascular disease in patients admitted to the ICU compared to those admitted to the ward, suggesting that other mechanisms/pathways could be involved in the severity of COVID-19 illness. In fact, several studies have suggested that variabilities in the genotype distribution of ACE polymorphisms could explain the variable prevalence and clinical outcomes of COVID-19 among different regions of the world (32–34). Thus, we investigated the association of two ACE polymorphisms involved in diabetes and hypertension (rs4341 and rs4343) (10, 11) with COVID-19 outcome in patients from La Rioja. In our study, we did not find differences for rs4341 and rs4343 frequencies among the three groups of patients analyzed when it was assessed without considering the comorbidities. However, we observed that having G allele in rs4341 and rs4343 worsens the outcome of hypertensive, dyslipidemic, and diabetic COVID-19 patients. Moreover, we found that, unlike hospitalized patients in the ward, all diabetic patients admitted to the ICU had the GG genotype. The number of type 2 diabetic patients in this group was very small (only 3 patients), so this result should be viewed with caution. However, our results clearly revealed that rs4341 and

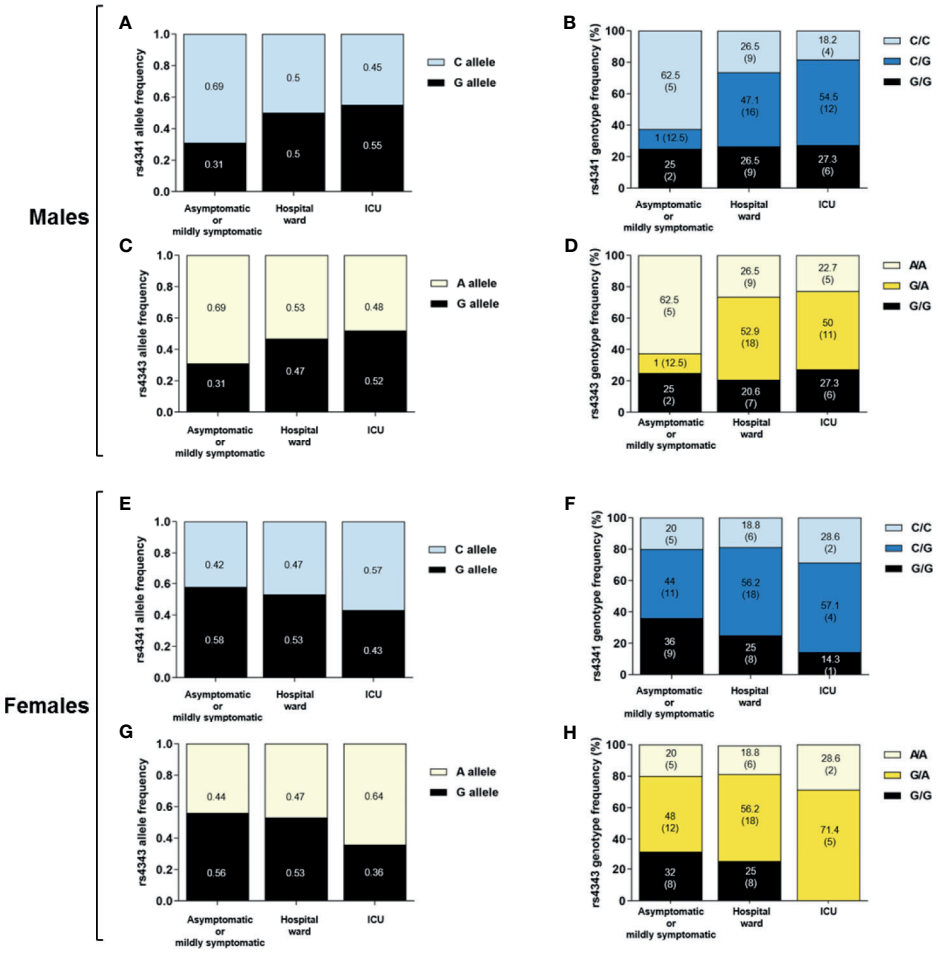

FIGURE 2 | Allele and genotype distribution of the rs4341 and rs4343 polymorphisms among SARS-CoV-2 infected male and female. Allele frequencies of rs4341 and rs4343 polymorphisms among SARS-CoV-2 infected males **(A, C)** and females **(E, G)**. Data are presented as proportions. Genotype frequencies of rs4341 and rs4343 polymorphisms among SARS-CoV-2 infected males **(B, D)** and females **(F, H)**. Data are presented as percentages. The number of patients is indicated in brackets. All data were adjusted for age.

rs4343 polymorphisms of the ACE gene are related to the risk of developing severe COVID-19 (ICU admission) in hypertense, dyslipidemic and diabetic patients. Our study confirms previously reported findings of another ACE polymorphism on COVID-19 patients with hypertension (18), however, it did not corroborate the association with COVID-19 severity found by Gomez et al. (18) and other authors (35) for the ACE I/D polymorphism, regardless of comorbidities. Both studies report higher number of hypertensives in the critically ill group than our study [61% (18) and 73% (35) *vs* 41% in our study], statistically different from the severe group, what could explain the differences observed. Unlike these studies, we also found an additional association of rs4341 and rs4343 with enhanced severity in dyslipidemic and diabetic patients. And interestingly, we further found a higher prevalence of the GG, GC and GA genotypes (all containing the G allele) among deceased ICU-patients, not described so far, confirming the deleterious effect of the rs4341 and rs4343 G allele in COVID-19 outcomes. Precisely, 66% of these patients were hypertensive, thus, the presence of this polymorphism could be involved in the hypertension process *per se* as previously mentioned, but also to be a synergistic risk factor for COVID-19 fatality. It is of great interest to investigate whether the increased risk of severe COVID-19 is due to the potentiation of the metabolic dysfunction by these alleles (acting as synergetic) or just because of such comorbidities. Thus, we performed an additional analysis adjusting for age, sex, BMI and number of comorbidities. Our results show that G alleles remained associated with COVID-19 severity after adjustment for these factors, suggesting that both the metabolic comorbidities and the G allele act synergistically on COVID-19 outcome.

Some studies have reported an association of the GG genotype of rs4343 polymorphism and higher circulating levels and activity of ACE (36, 37), which could explain the higher susceptibility to develop severe forms of the disease in patients with the GG genotype, in addition to hypertension and dyslipidemia. SARS-CoV-2 sequesters ACE2 to invade cells,

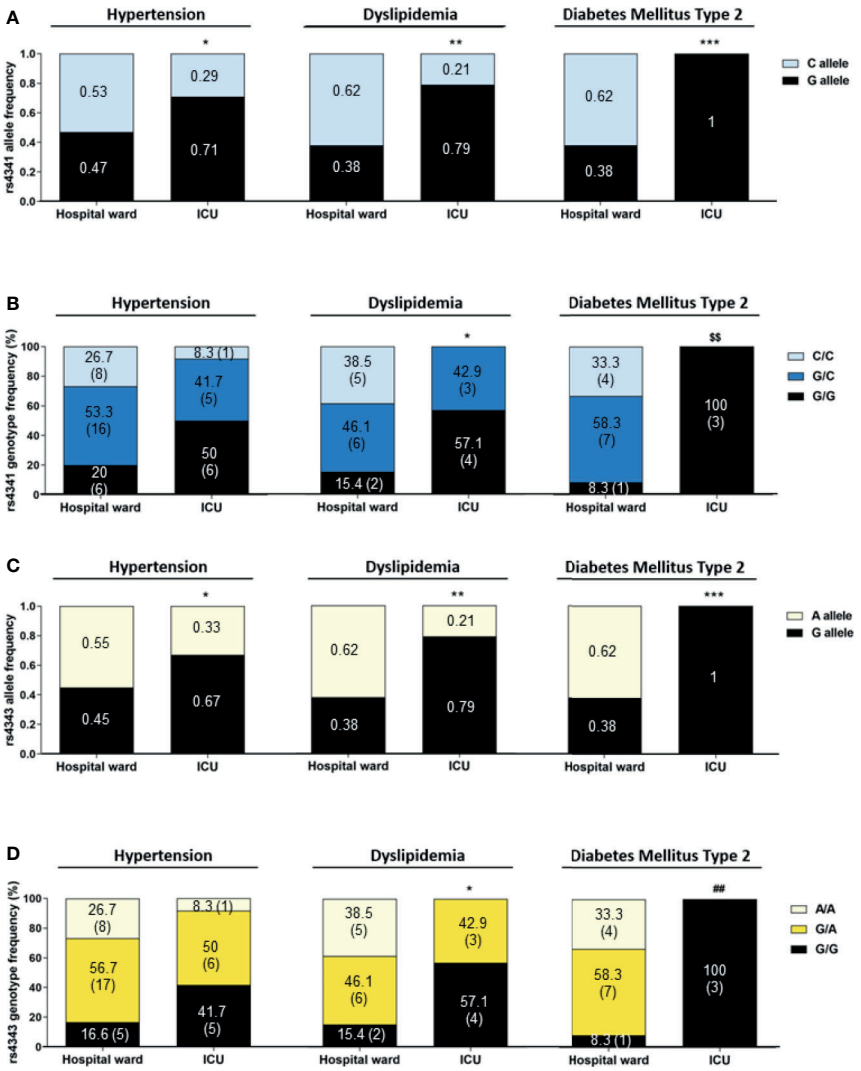

FIGURE 3 | Allelic and genotypic frequencies of rs4341 and rs4343 polymorphisms in COVID-19 patients according to its hypertensive, dyslipidemic or diabetic status. Allele frequencies of rs4341 **(A)** and rs4343 **(C)** polymorphisms. Data are presented as proportions. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs* Hospital ward. Genotype frequencies of rs4341 **(B)** and rs4343 **(D)** polymorphisms. Data are presented as percentages. The number of patients is indicated in brackets. $^{*}p < 0.05$ *vs* Hospital ward group for G carriers (GG+GC *vs* CC and GG+GA *vs* AA). $^{\$\$}p < 0.01$ *vs* Hospital ward between GG and GC and CC, GG+GC and CC, GG and GC+CC. $^{\#\#}p < 0.01$ *vs* Hospital ward between GG and GA and AA, GG+GA and AA, GG and GA+AA. All data were adjusted for age and sex.

decreasing the bioavailability of ACE2 which entails a reduction in the degradation of Ang II and an exacerbation of the damaging effects of Ang II (38). Thus, the lung injury and inflammation caused by the reduced ACE2 levels due to the viral infection, and also by the hypertension, the dyslipidemia and diabetes, may be worsened by ACE genotypes that further increase ACE levels, and hence Ang II levels, such as the GG genotype of rs4341 and rs4343 herein analyzed. In our study we confirmed that ACE levels are associated with the GG genotype of both polymorphisms, which in turn is associated with greater severity of the disease in hypertensive and dyslipidemic patients. Although according to our data we could not affirm a direct association with COVID-19 severity.

There are obviously several limitations in our study, such as the small size of our cohort. Thus, studies with higher number of patients could be of interest to clarify these results. Large-scale GWAS (Genome Wide Association Study) combined with WGS (whole genome sequencing) studies, such as the one being carried out by the SCOURGE consortium (39), will be very interesting to corroborate these results and to explore if they are extrapolable to the whole Spanish cohort or, in contrast, it is specific to our region.

Our pilot results suggest that evaluating the rs4341 and rs4343 genotypes could be a new diagnostic approach to the clinical management of severe COVID-19 risk of SARS-CoV-2 infected patients with hypertension and/or dyslipidemia and diabetes, that can be easily and quickly analyzed. Although knowing the

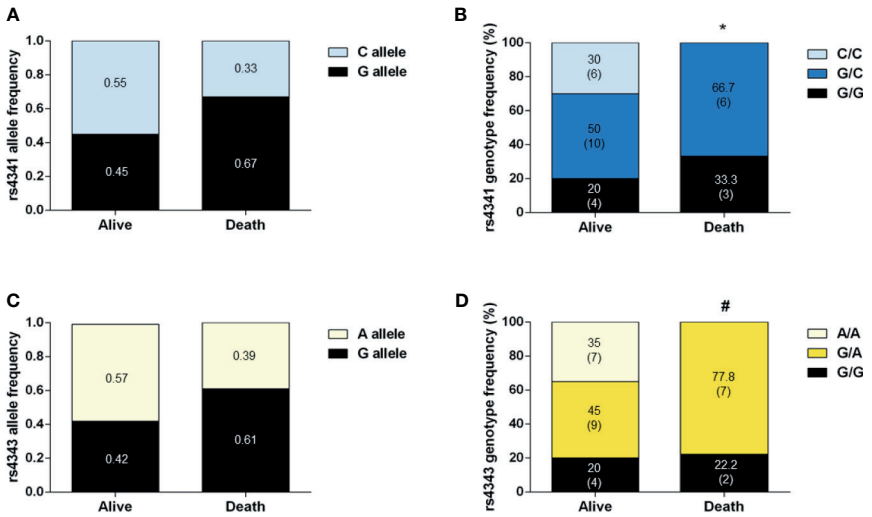

FIGURE 4 | Genotype and allele frequencies of COVID-19 patients in ICU who survived or who died. Allele frequencies of rs4341 **(A)** and rs4343 **(C)** polymorphisms. Data are presented as proportions. Genotype frequencies of rs4341 **(B)** and rs4343 **(D)** polymorphisms. Data are presented as percentages. The number of patients is indicated in brackets. *$p < 0.05$ *vs* survivors for G carriers (GG+GC *vs* CC). #$p < 0.05$ *vs* survivors between GG and GA and AA, GG+GA *vs* AA). All data were adjusted for age and sex.

TABLE 2 | Genotype frequencies of rs4341 and rs4343 polymorphisms in COVID-19 patients according to its hypertensive, dyslipidemic or diabetic status.

		rs4341		
Hypertension	**G/G**	**G/C**	**C/C**	***P*-value**
Hospital ward	6 (20%)	16 (53.3%)	8 (26.7%)	**0.031***
ICU	6 (50%)	5 (41.7%)	1 (8.3%)	**0.03**$
Dyslipidemia	**G/G**	**G/C**	**C/C**	***P*-value**
Hospital ward	2 (15.4%)	6 (46.1%)	5 (38.5%)	**0.043***
ICU	4 (57.1%)	3 (42,9%)	0 (0%)	
Diabetes Mellitus	**G/G**	**G/C**	**C/C**	***P*-value**
Hospital ward	1 (8.3%)	7 (58.3%)	4 (33.3%)	**0.033***
ICU	3 (100%)	0 (0%)	0 (0%)	**0.009**$
		rs4343		
Hypertension	**G/G**	**G/A**	**A/A**	***P*-value**
Hospital ward	15 (16.6%)	17 (56.7%)	8 (26.7%)	**0.03**%
ICU	5 (41.7%)	6 (50%)	1 (8.3%)	
Dyslipidemia	**G/G**	**G/A**	**A/A**	***P*-value**
Hospital ward	2 (15.4%)	6 (46.1%)	5 (38.5%)	**0.043**#
ICU	4 (57.1%)	3 (42,9%)	0 (0%)	
Diabetes Mellitus	**G/G**	**G/A**	**A/A**	***P*-value**
Hospital ward	1 (8.3%)	7 (58.3%)	4 (33.3%)	**0.033**#
ICU	3 (100%)	0 (0%)	0 (0%)	**0.009**%

Data are presented as number of patients and percentages.
**p-value for group difference between GG and GC and CC.*
$p-value for group difference between GG+GC and CC.
#p-value for group difference between GG and GA and AA.
%p-value for group difference between GG+GA and AA.
Significant differences appear in bold case.
All data were adjusted for age, sex, BMI and number of comorbidities.

TABLE 3 | ACE genotype frequencies of COVID-19 patients in ICU who survived or who died.

rs4341	Alive	Death	*P*-value
G/G	4 (20%)	3 (33.3%)	**0.021**$^{\$}$
C/G	10 (50%)	6 (66.7%)	
C/C	6 (30%)	0 (0%)	
rs4343	**Alive**	**Death**	***P*-value**
G/G	4 (20%)	2 (22.2%)	**0.041**$^{\#}$
G/A	9 (45%)	7 (77.8%)	**0.012**$^{\%}$
A/A	7 (35%)	0 (0%)	

Data are presented as number of patients and percentages.
$^{\$}$p-value for group difference between GG+GC and CC.
$^{\#}$p-value for group difference between GG and GA and AA.
$^{\%}$p-value for group difference between GG+GA and AA.
Significant differences appear in bold case.
All data were adjusted for age, sex, BMI and number of comorbidities.

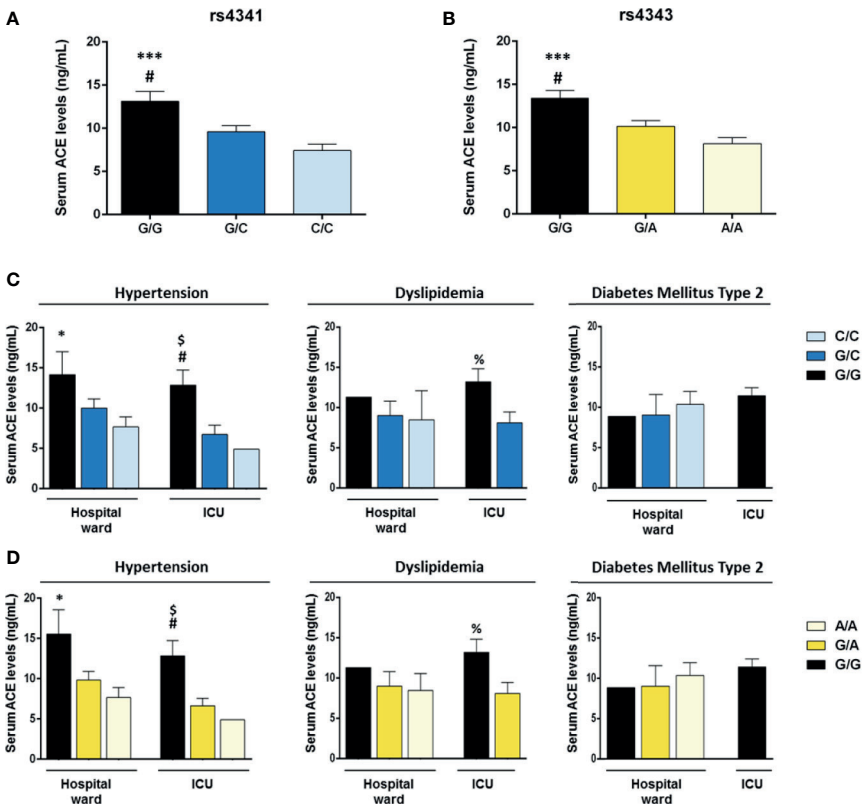

FIGURE 5 | Serum ACE levels of COVID-19 patients according to the genotypes of the rs4341 and rs4343 polymorphisms of ACE. Mean serum ACE levels of COVID-19 patients with different rs4341 **(A)** and rs4343 **(B)** genotypes. $^{\#}p < 0.05$ *vs* G/C group; $^{***}p < 0.001$ *vs* C/C group. **(C)** Mean serum ACE levels according to the genotypes of the rs4341 polymorphism in COVID-19 patients with hypertension, dyslipidemia or diabetes hospitalized in the ward or in the ICU. $^{*}p < 0.05$ *vs* Hospital ward C/C group; $^{\#}p < 0.05$ *vs* ICU G/C group; $^{\$}p = 0.059$ *vs* ICU C/C group; $^{\%}p = 0.07$ *vs* ICU G/C group. **(D)** Mean serum ACE levels according to the genotypes of the rs4343 polymorphism in COVID-19 patients with hypertension, dyslipidemia or diabetes hospitalized in the ward or in the ICU. $^{*}p < 0.05$ *vs* Hospital ward A/A group; $^{\#}p < 0.05$ *vs* ICU G/A group; $^{\$}p = 0.059$ *vs* ICU A/A group, $^{\%}p = 0.07$ *vs* ICU G/A group. Data are presented as mean ± standard deviation.

genotype of these ACE variants does not directly modify the clinical course of these patients, identifying those most at risk of severe COVID-19 will allow better monitoring of these patients to apply all available means to improve their prognosis.

In conclusion, our pilot study suggests that the G-containing genotypes of rs4341 and rs4343 confer an additional risk factor of developing severe forms of COVID-19 in patients with hypertension, dyslipidemia, or possibly with diabetes

independently of gender. These genotypes seem to be also associated with an increased risk of death, mainly in hypertensive patients. Thus, the genotyping of rs4341 and rs4343 in COVID-19 patients could facilitate a more appropriate clinical management at admission.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de Ética de Investigación con medicamentos de La Rioja, CEImLAR, reference number PI-412. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MÍ, PP-M, and JO designed the study, interpreted the data and wrote the manuscript. MÍ, PV-B, ER-F, and PP-M performed the experiments. DE-P, JA and MF-L participated in patient and sample recruitment. All authors contributed to the article and approved the submitted version.

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Corrigendum: ACE Gene Variants Rise the Risk of Severe COVID-19 in Patients With Hypertension, Dyslipidemia or Diabetes: A Spanish Pilot Study

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Keywords: COVID-19, angiotensin converting enzyme, polymorphisms, hypertension, dyslipidemia, diabetes

A Corrigendum on

ACE Gene Variants Rise the Risk of Severe COVID-19 in Patients With Hypertension, Dyslipidemia or Diabetes: A Spanish Pilot Study

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ERROR IN FIGURE/TABLE

In the original article, there was a mistake in **Table 2** as published. Alleles of rs4343 polymorphism were incorrectly written. The corrected **Table 2** appears below.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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TABLE 2 | Genotype frequencies of rs4341 and rs4343 polymorphisms in COVID-19 patients according to its hypertensive, dyslipidemic or diabetic status.

		rs4341		
Hypertension	**G/G**	**G/C**	**C/C**	***P*-value**
Hospital ward	6 (20%)	16 (53.3%)	8 (26.7%)	**0.031***
ICU	6 (50%)	5 (41.7%)	1 (8.3%)	**0.03**$^{\$}$
Dyslipidemia	**G/G**	**G/C**	**C/C**	***P*-value**
Hospital ward	2 (15.4%)	6 (46.1%)	5 (38.5%)	**0.043***
ICU	4 (57.1%)	3 (42,9%)	0 (0%)	
Diabetes Mellitus	**G/G**	**G/C**	**C/C**	***P*-value**
Hospital ward	1 (8.3%)	7 (58.3%)	4 (33.3%)	**0.033***
ICU	3 (100%)	0 (0%)	0 (0%)	**0.009**$^{\$}$
		rs4343		
Hypertension	**G/G**	**G/A**	**A/A**	***P*-value**
Hospital ward	15 (16.6%)	17 (56.7%)	8 (26.7%)	**0.03**$^{\%}$
ICU	5 (41.7%)	6 (50%)	1 (8.3%)	
Dyslipidemia	**G/G**	**G/A**	**A/A**	***P*-value**
Hospital ward	2 (15.4%)	6 (46.1%)	5 (38.5%)	**0.043**$^{\#}$
ICU	4 (57.1%)	3 (42,9%)	0 (0%)	
Diabetes Mellitus	**G/G**	**G/A**	**A/A**	***P*-value**
Hospital ward	1 (8.3%)	7 (58.3%)	4 (33.3%)	**0.033**$^{\#}$
ICU	3 (100%)	0 (0%)	0 (0%)	**0.009**$^{\%}$

Data are presented as number of patients and percentages.
**p-value for group difference between GG and GC and CC.*
$^{\$}$p-value for group difference between GG+GC and CC.
$^{\#}$p-value for group difference between GG+GA and AA.
$^{\%}$p-value for group difference between GG+GA and AA.
Significant differences appear in bold case.
All data were adjusted for age, sex, BMI and number of comorbidities.

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Prognostic Value of Leucocyte to High-Density Lipoprotein-Cholesterol Ratios in COVID-19 Patients and the Diabetes Subgroup

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Background: Blood parameters, such as neutrophil-to-lymphocyte ratio, have been identified as reliable inflammatory markers with diagnostic and predictive value for the coronavirus disease 2019 (COVID-19). However, novel hematological parameters derived from high-density lipoprotein-cholesterol (HDL-C) have rarely been studied as indicators for the risk of poor outcomes in patients with severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) infection. Here, we aimed to assess the prognostic value of these novel biomarkers in COVID-19 patients and the diabetes subgroup.

Methods: We conducted a multicenter retrospective cohort study involving all hospitalized patients with COVID-19 from January to March 2020 in five hospitals in Wuhan, China. Demographics, clinical and laboratory findings, and outcomes were recorded. Neutrophil to HDL-C ratio (NHR), monocyte to HDL-C ratio (MHR), lymphocyte to HDL-C ratio (LHR), and platelet to HDL-C ratio (PHR) were investigated and compared in both the overall population and the subgroup with diabetes. The associations between blood parameters at admission with primary composite end-point events (including mechanical ventilation, admission to the intensive care unit, or death) were analyzed using Cox proportional hazards regression models. Receiver operating characteristic curves were used to compare the utility of different blood parameters.

Results: Of 440 patients with COVID-19, 67 (15.2%) were critically ill. On admission, HDL-C concentration was decreased while NHR was high in patients with critical compared with non-critical COVID-19, and were independently associated with poor outcome as continuous variables in the overall population (HR: 0.213, 95% CI 0.090–0.507; HR: 1.066, 95% CI 1.030–1.103, respectively) after adjusting for confounding

factors. Additionally, when HDL-C and NHR were examined as categorical variables, the HRs and 95% CIs for tertile 3 vs. tertile 1 were 0.280 (0.128–0.612) and 4.458 (1.817–10.938), respectively. Similar results were observed in the diabetes subgroup. ROC curves showed that the NHR had good performance in predicting worse outcomes. The cutoff point of the NHR was 5.50. However, the data in our present study could not confirm the possible predictive effect of LHR, MHR, and PHR on COVID-19 severity.

Conclusion: Lower HDL-C concentrations and higher NHR at admission were observed in patients with critical COVID-19 than in those with noncritical COVID-19, and were significantly associated with a poor prognosis in COVID-19 patients as well as in the diabetes subgroup.

Keywords: cohort study, coronavirus, COVID-19, neutrophil to high-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, prognosis

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has become a serious global public health crisis, severely threatening people worldwide. Although most cases are either asymptomatic or result in only mild symptoms, a few patients with COVID-19 rapidly develop acute respiratory distress syndrome, septic shock, multiple organ failure, and die (1). Thus, it is crucial to identify reliable predictors to stratify the risk for patients with severe COVID-19 and enable timely intervention and treatment to improve prognosis.

The role of inflammation in the progression of various viral pneumonitis, including COVID-19, has received increasing attention (2). Accumulating evidence suggests that patients with severe COVID-19 exhibit a hyperinflammatory response and impaired immune function (3). Therefore, circulating biomarkers related to the inflammatory status of patients are good potential predictors of the prognosis of patients with COVID-19. Indeed, the neutrophil-to-lymphocyte ratio has been confirmed as a well-established biomarker for predicting poor prognosis due to COVID-19 (4). However, there is an urgent need to identify novel biomarkers associated with COVID-19 progression.

In addition to reverse cholesterol transport, high-density lipoprotein cholesterol (HDL-C) also displays pleiotropic protective functions, including anti-infectious, anti-inflammatory, antioxidant, and antithrombotic effects (5). During infections or acute conditions, HDL-C levels decrease very rapidly (5, 6). Low concentrations of HDL-C have been used as prognostic markers in patients with sepsis, community-acquired pneumonia and other infections (7–9). In addition, several studies have shown that the degree of reduction in HDL-C and apolipoprotein A-I can predict mortality in COVID-19 patients (10, 11). Besides, new hematological parameters related to HDL-C, including neutrophil to HDL-C ratio (NHR) (12), monocyte to HDL-C ratio (MHR) (13), lymphocyte to HDL-C ratio (LHR) (14) and platelet to HDL-C ratio (PHR) (15), have been advocated as potential new indicators of inflammation in recent studies. These biomarkers have been reported in some lung diseases such as chronic obstructive pulmonary disease (16), stroke-associated pneumonia (17), and pulmonary embolism (18), despite limited studies on newly proposed biomarkers. However, how these serological biomarkers change in the peripheral blood and their clinical value in COVID-19 has not yet been reported.

Diabetes is a major comorbidity of COVID-19 and sufferers are at a markedly higher risk of death after severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) infection (19–21). On the other hand, patients with diabetes have a chronic inflammatory condition and a reduced level of HDL-C (22). Therefore, it is clinically important to examine the influence of inflammatory markers in diabetic patients (19). Hyperglycemia can affect immune function. Conversely, SARS-CoV-2 infection-induced inflammation increases insulin resistance, potentially aggravating the impairment of glucose metabolism (23). Whether these ratios have similar predictive effects on poor outcomes in patients with type 2 diabetes and COVID-19 remains unclear.

Thus, we aimed to investigate and compare the prognostic impacts of NHR, MHR, LHR, and PHR in the entire COVID-19 patient population and in the diabetes subgroup, as well as to explore the most useful diagnostic biomarkers and optimal cutoff values.

METHODS

Study Design and Participants

In this multicenter, retrospective cohort study, participants with COVID-19 admitted to five hospitals in Wuhan from January 1 to March 17, 2020, were enrolled. All hospitals were designated to treat COVID-19 individuals, including the Department of Endocrinology, the Department of Infectious Disease, and the Department of Oncology of the Union Hospital of Tongji Medical College, Huazhong University of Science and Technology, and the Department of Endocrinology in the following four hospitals: the Fifth Hospital of Wuhan, the Wuhan Wuchang Hospital Affiliated to Wuhan University of

Science and Technology, the General Hospital of the Yangtze River Shipping, and the Wuhan Hankou Hospital. These seven departments were temporarily converted into isolation wards for patients with COVID-19. We only had access to the data of the seven departments that the investigators were in charge of; thus, data from other departments in the five hospitals were not available.

COVID-19 was diagnosed according to the Diagnosis and Treatment Scheme for the Novel Coronavirus Pneumonia released by the National Health Commission of China (**Supplementary Table 1**). We only included cases with positive results for SARS-CoV-2 by real-time RT-PCR assay of nasal and pharyngeal swab specimens, or positive serum specific IgM and IgG antibodies. Patients were excluded using the following criteria: (1) previous malignancy; (2) no definitive outcome as they were transferred to another hospital; (3) no hematological or HDL-C data available within 3 days of admission (for one of the following reasons: [a] patients had measured before admission; [b] patients tested for these results 3 days after admission). All patients were followed up until discharge or in-hospital death.

The study was approved by the Ethics Committee of the Union Hospital, Tongji Medical College, Huazhong University of Science and Technology(2020-S180). Informed consent was waived as this retrospective study used only anonymous clinical data.

Data Collection

We obtained data from the electronic medical records of the relevant departments. The following data were collected: demographics, comorbidities, clinical symptoms and signs, laboratory findings, treatments, and outcomes (discharge or death). Two researchers independently reviewed and verified the data collection forms.

Comorbidities included hypertension, diabetes, coronary heart disease (CHD), chronic lung disease, chronic kidney disease, chronic hepatic disease, cerebrovascular disease, and dyslipidemia, which were diagnosed according to standard criteria. Laboratory results including complete blood count, renal and liver function, lipid profiles, and inflammation markers were recorded within 3 days of admission and before steroid therapy. Medication (e.g., antiviral and antibacterial drugs, systemic corticosteroid, immunoglobulin G, use of statins, and anti-diabetic agents) received during hospitalization were also recorded.

NHR was calculated by dividing neutrophil counts by the HDL-C value. MHR was calculated by dividing monocyte counts by the HDL-C value. LHR was calculated by dividing lymphocyte counts by the HDL-C value. PHR was calculated as the platelet count divided by the HDL-C value.

Patients were discharged when they met the following discharge criteria: (1) body temperature returned to normal, lasting for more than 3 days; (2) respiratory symptoms significantly improved; (3) imaging examinations revealed that acute exudative lesions were significantly improved; and (4) two real-time RT-PCR tests for the presence of SARS-CoV-2 yielded negative results (with two samples of respiratory specimens taken over 24 h apart) (24).

Study Outcomes

In accordance with previous studies (25, 26), the composite of admission to an intensive care unit (ICU), the use of mechanical ventilation, or death were considered as the primary outcome measure to define critical COVID-19. In contrast, patients discharged without the need for ICU admission or mechanical ventilation were classified as having noncritical COVID-19. The date of disease onset was defined as the day when symptoms suggestive of COVID-19 were first observed. Time (days) was calculated from the symptom onset of COVID-19 to the index date of the composite endpoint of inpatients.

Statistical Analysis

Data are presented as median and interquartile range (IQR) for continuous variables or as counts and proportions for categorical variables. Comparisons between the groups of non-critical and critical COVID-19 were analyzed using the Mann–Whitney U-test for continuous variables and the chi-squared test or Fisher's exact test for categorical variables as appropriate. Univariate and multivariate Cox regression analyses were conducted to examine the association between the ratios and primary outcomes. Considering the total number of critical patients (n = 67) in this study and to avoid overfitting in the model, five variables (age, sex, hospital, hypertension, and diabetes) were chosen for multivariable analysis on the basis of previous findings. Regression analysis of the parameters associated with the composite outcome was repeated in a subgroup with diabetes (n = 150). To assess the discrimination ability of each indicator for adverse outcomes, receiver operating characteristic (ROC) curves were calculated, and the optimal cutoff values were determined by maximizing the Youden index. Time to a composite endpoint was investigated using survival analysis by a Kaplan–Meier plot and compared using the log-rank test. All analyses and mapping were performed using IBM SPSS (version 25.0) and GraphPad Prism (version 8.0). Statistical analyses were two-sided, and significance was set at $P < 0.05$.

RESULTS

Baseline Characteristics of Study Samples

By March 17, 2020, 1028 patients with pneumonia were admitted to the seven departments across the five hospitals. After excluding one case diagnosed with pneumonia caused by influenza A, 312 suspected cases without positive SARS-Cov-2 laboratory results, 27 with a history of malignancy, 9 without outcome as they were transferred to other hospitals, and 239 patients without blood cell counts or HDL-C data, a total of 440 individuals with COVID-19 were included in the analysis (**Figure 1**). The characteristics of included and excluded patients are presented in **Supplementary Table 2**.

The median age of the participants was 60 years [interquartile range (IQR), 45–68 years], and 215 (48.9%) were men (**Table 1**).

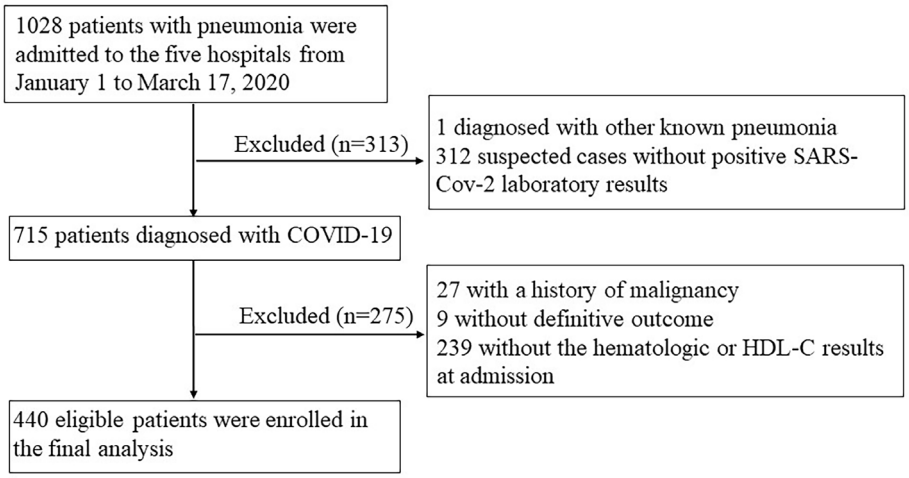

FIGURE 1 | Flow diagram showing the patient selection process.

TABLE 1 | Demographics and baseline characteristics of patients with COVID-19.

	All Patients (n = 440)	Noncritical COVID-19 (n = 373)	Critical COVID-19 (n = 67)	P value
Ages	60 (45-68)	57 (42-67)	69 (60-78)	<0.001
Sex				
Female	225 (51.1%)	206 (55.2%)	19 (28.4%)	<0.001
Male	215 (48.9%)	167 (44.8%)	48 (71.6%)	
Days from illness onset to admission	10 (7-16)	10 (7-20)	10 (5-12)	0.005
Comorbidities				
Hypertension	136 (30.9%)	103 (27.6%)	33 (49.3%)	<0.001
Diabetes	150 (34.1%)	108 (29.0%)	42 (62.7%)	<0.001
Coronary heart disease	44 (10.0%)	34 (9.1%)	10 (14.9%)	0.144
Chronic lung disease	23 (5.2%)	16 (4.3%)	7 (10.4%)	0.074
Chronic liver disease	6 (1.4%)	5 (1.3%)	1 (1.5%)	1
Chronic kidney disease	11 (2.5%)	4 (1.1%)	7 (10.4%)	<0.001
Cerebrovascular disease	21 (4.8%)	11 (2.9%)	10 (14.9%)	<0.001
Dyslipidemia	257 (58.4%)	201 (53.9%)	56 (83.6%)	<0.001
Treatment				
Antiviral therapy	397 (90.2%)	336 (90.1%)	61 (91.0%)	0.807
Antibiotic therapy	241 (54.8%)	202 (54.2%)	39 (58.2%)	0.539
Use of corticosteroid	157 (35.8%)	108 (29.0%)	49 (73.1%)	<0.001
Intravenous immunoglobin	122 (27.8%)	86 (231%)	36 (53.7%)	<0.001
Use of statins	45 (10.2%)	33 (8.8%)	12 (17.9%)	0.024

Data are median (IQR) or n (%). P values comparing critical and noncritical COVID-19 are from χ^2 test, Fisher's exact test or Mann-Whitney U test.

The median time from symptom onset to hospital admission was 10 days (range,7–16 days). Dyslipidemia (58.4%) was the most common comorbidity, followed by diabetes (34.1%) and hypertension (30.9%). At admission, the most prevalent symptoms were fever (79.3%), cough (71.6%), and fatigue (48.4%) (**Supplementary Table 3**).

In this population, 67 (15.2%) had critical COVID-19. Compared with non-critical COVID-19 patients, the critical patients were older, more likely to be male, and more likely to have other comorbidities. Additionally, the critical COVID-19 group was more likely to receive treatment with glucocorticoids [49 (73.1%) *vs*. 108 (29.0%)], intravenous immunoglobulin therapy [36 (53.7%) *vs*. 86 (23.1%)], and statins [12 (17.9%) *vs*. 33 (8.8%)] than the noncritical COVID-19 group (**Table 1**).

Baseline Laboratory Parameters of Patients With COVID-19

The critical COVID-19 group showed significantly increased levels of neutrophil count, but decreased levels of lymphocyte count, platelet count, as well as multiple lipid profiles, such as total cholesterol (TC), HDL-C, and low-density lipoprotein cholesterol (LDL-C), compared with the non-critical COVID-19 group ($P < 0.05$). Regarding inflammation indicators, patients with critical COVID-19 had higher levels of NHR and lower levels of

TABLE 2 | Laboratory findings of patients with COVID-19 on admission to hospital.

	All Patients (n = 440)	Noncritical COVID-19 (n = 373)	Critical COVID-19 (n = 67)	P value
Neutrophils (×10^9 per L)	3.38 (2.46-4.97)	3.20 (2.37-4.42)	5.94 (3.22-8.47)	<0.001
Lymphocytes (×10^9 per L)	1.18 (0.78-1.62)	1.26 (0.91-1.71)	0.66 (0.43-0.98)	<0.001
Monocytes (×10^9 per L)	0.39 (0.29-0.51)	0.40 (0.29-0.52)	0.33 (0.24-0.45)	0.056
Platelets (×109 per L)	192 (144-245)	199 (153-248)	161 (112-233)	<0.001
Total cholesterol (mmol/L)	3.96 (3.38-4.87)	4.02 (3.48-4.92)	3.68 (2.93-4.47)	<0.001
Triglyceride (mmol/L)	1.27 (0.94-1.79)	1.27 (0.95-1.78)	1.33 (0.98-1.88)	0.613
High density lipoprotein cholesterol (mmol/L)	1.04 (0.85-1.30)	1.08 (0.89-1.33)	0.85 (0.71-0.95)	<0.001
Low density lipoprotein cholesterol(mmol/L)	2.30 (1.84-3.00)	2.37 (1.90-3.07)	1.89 (1.56-2.59)	<0.001
Neutrophil to high-density lipoprotein cholesterol ratio (NHR)	3.22 (2.18-5.00)	3.05 (2.02-4.31)	7.71 (4.00-10.96)	<0.001
Monocyte to high-density lipoprotein cholesterol ratio (MHR)	0.38 (0.26-0.51)	0.37 (0.25-0.49)	0.39 (0.27-0.65)	0.118
Lymphocyte to high-density lipoprotein cholesterol ratio (LHR)	1.13 (0.75-1.56)	1.20 (0.81-1.63)	0.82 (0.53-1.11)	<0.001
Platelet to high-density lipoprotein cholesterol ratio (PHR)	175.33 (129.03-250.88)	175.00 (129.36-248.42)	191.74 (115.46-293.83)	0.581

Data are median (IQR). P values comparing critical and noncritical COVID-19 are from Mann-Whitney U test.

LHR than the noncritical group (P < 0.05). No significant differences in monocyte, triglyceride, MHR, and PHR were found (**Table 2**).

Associations of Biomarkers With Outcome in the Whole COVID-19 Patient Population

According to univariate Cox regression analysis, HDL-C, NHR, and LHR levels were associated with adverse outcomes as both continuous and categorical variables (divided by tertiles). Notably, based on multivariate Cox regression analysis, we found that HDL-C (HR: 0.213, 95% CI: 0.090-0.507, P < 0.001) and NHR (HR: 1.066, 95% CI: 1.030-1.103, P < 0.001) were still independently associated with worse outcomes after adjusting for age, sex, hospital, hypertension, and diabetes (**Table 3**), while the association with LHR was attenuated to insignificance (HR: 0.767, 95% CI: 0.501-1.174). Furthermore, patients with the highest tertile of NHR displayed the highest risk for the primary endpoint, while patients with the highest tertile of HDL-C showed the lowest risk (HR: 4.458, 95% CI: 1.817–10.938; HR: 0.280, 95% CI: 0.128–0.612, respectively). Remarkably, ROC analysis revealed that NHR remained valuable for the primary endpoints, with an AUC > 0.80. At a threshold of 5.50, the AUC of the ROC curve of NHR was 0.81 (95% CI: 0.74–0.87, P < 0.001) (**Table 4**). HDL-C and LHR showed weak discrimination of the critical condition (AUC, 0.72; 95% CI: 0.65–0.79, P < 0.001; AUC: 0.70, 95% CI: 0.63–0.77, P < 0.001, respectively). Moreover, the Kaplan–Meier survival curves and log-rank tests demonstrated that patients with higher NHR (> 5.50) had a higher rate of primary endpoints (divided according to the best threshold) (**Figure 2**).

TABLE 3 | Cox proportional hazards regression model for primary composite end point among patients with COVID-19.

Variable	Univariate HR (95% CI)	P value	Adjusted HR* (95% CI)	P value
HDL-C	0.111 (0.047-0.260)	<0.001	0.213 (0.090-0.507)	<0.001
Q1	Ref		Ref	
Q2	0.365 (0.210-0.635)	<0.001	0.495 (0.279-0.879)	0.016
Q3	0.166 (0.078-0.353)	<0.001	0.280 (0.128-0.612)	0.001
NHR	1.082 (1.062-1.102)	<0.001	1.066 (1.030-1.103)	<0.001
Q1	Ref		Ref	
Q2	1.811 (0.679-4.828)	0.235	1.365 (0.496-3.754)	0.547
Q3	7.946 (3.396-18.589)	<0.001	4.458 (1.817-10.938)	0.001
MHR	1.896 (1.208-2.974)	0.005	1.396 (0.879-2.218)	0.157
Q1	Ref		Ref	
Q2	0.688 (0.356-1.330)	0.281	0.759 (0.378-1.523)	0.437
Q3	1.363 (0.776-2.395)	0.281	1.304 (0.696-2.441)	0.407
LHR	0.461 (0.290-0.732)	0.001	0.767 (0.501-1.174)	0.222
Q1	Ref		Ref	
Q2	0.479 (0.281-0.817)	0.007	0.722 (0.409-1.273)	0.26
Q3	0.182 (0.085-0.390)	<0.001	0.331 (0.148-0.743)	0.007
PHR	1.000 (0.998-1.002)	0.862	1.000 (0.998-1.002)	0.789
Q1	Ref		Ref	
Q2	0.993 (0.545-1.811)	0.982	0.850 (0.450-1.606)	0.617
Q3	1.033 (0.574-1.860)	0.913	1.159 (0.629-2.135)	0.636

**Adjusted for age, sex, hospital, hypertension, and diabetes.*

Associations of Biomarkers With Outcome in the Diabetic Subgroup

In our study, 150 individuals had diabetes. The baseline characteristics of the subjects with diabetes are presented in **Table 5**. Similar results were observed in the diabetes subgroup; the critical illness exhibited higher NHR while exhibiting lower HDL-C and LHR compared with those with noncritical illness, whereas MHR and PHR were comparable between the groups. We further evaluated the predictive performance of several hematological ratios for primary endpoints. Univariate Cox regression analysis showed that HDL-C and NHR were associated with poor outcomes as both continuous and categorical variables (divided by tertiles). Multivariate Cox analysis showed that when correcting for age, sex, and hospital, HDL-C (HR: 0.338, 95% CI: 0.118–0.971, P = 0.044) and NHR (HR: 1.076, 95% CI: 1.028–1.126, P = 0.002) were independent predictors of adverse outcomes

TABLE 4 | Diagnostic values of serological indicators in assessment adverse outcome of COVID-19.

	AUC (95% CI)	Best threshold	Sensitivity	Specificity	P
HDL-C	0.72 (0.65-0.79)	0.95	0.67	0.73	<0.001
NHR	0.81 (0.75-0.87)	5.50	0.64	0.88	<0.001
MHR	0.56 (0.48-0.64)	0.56	0.34	0.83	0.119
LHR	0.70 (0.63-0.77)	1.03	0.62	0.75	<0.001
PHR	0.52 (0.44-0.60)	292.21	0.25	0.84	0.581

AUC, Area under ROC Curve.

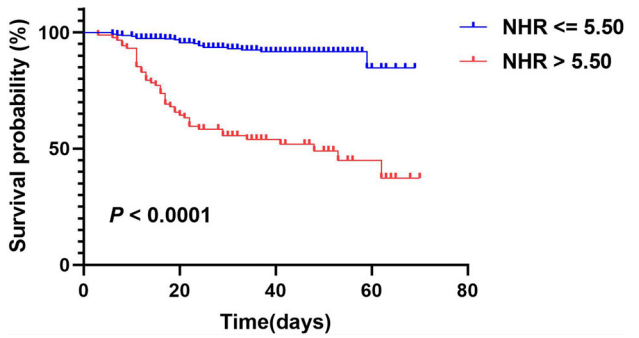

FIGURE 2 | Kaplan-Meier survival curves for primary composite endpoint according to NHR optimal cutoff value. A log-rank test was used to evaluate difference between groups.

(**Table 6**). Among them, 34 (22.7%) patients received metformin therapy and more noncritical patients received metformin compared with critical patients (30.6% *vs.* 2.4%, P < 0.001). Of note, the metformin group had a lower NHR than the non-metformin group, but the difference was not statistically significant (**Supplementary Table 4**).

DISCUSSION

In this multicenter retrospective cohort study, we explored the association between four novel serological indicators and fatal clinical outcomes in patients with COVID-19. Our findings suggested that low HDL-C concentration and high NHR on admission are closely associated with an increased risk of poor clinical outcome not only in all COVID-19 patients but also in the diabetes subgroup. Additionally, ROC curve analysis showed that NHR can effectively forecast the worse outcome of COVID-19.

The COVID-19 outbreak has caused widespread concern, and the progression to severe illness results in high rates of mortality (40%) in patients (27). Hence, it is essential to determine several early markers to make timely assessment as to which cases will likely become clinically severe. The critical cases showed significantly lower HDL-C concentrations than the non-critical cases in this study. Above all, decreased HDL-C concentration was associated with poor prognosis of COVID-19, which was consistent with previous studies (11, 28). More notably, recent studies have focused on the association between HDL-C-related

TABLE 6 | Cox proportional hazards regression model for primary end point among diabetic patients with COVID-19.

Variable	Univariate HR (95% CI)	P value	Adjusted HR* (95% CI)	P value
HDL-C	0.294 (0.109-0.791)	0.015	0.338 (0.118-0.971)	0.044
Q1	Ref		Ref	
Q2	0.737 (0.375-1.448)	0.376	0.499 (0.233-1.068)	0.073
Q3	0.302 (0.126-0.720)	0.007	0.307 (0.125-0.755)	0.01
NHR	1.061 (1.036-1.086)	<0.001	1.076 (1.028-1.126)	0.002
Q1	Ref		Ref	
Q2	0.631 (0.200-1.993)	0.433	0.641 (0.196-2.090)	0.46
Q3	4.991 (2.177-11.444)	<0.001	3.961 (1.604-9.783)	0.003
MHR	1.372 (0.816-2.306)	0.233	1.266 (0.736-2.180)	0.394
Q1	Ref		Ref	
Q2	0.628 (0.290-1.358)	0.237	0.565 (0.245-1.305)	0.182
Q3	0.811 (0.393-1.677)	0.572	0.810 (0.354-1.854)	0.618
LHR	0.580 (0.331-1.019)	0.058	0.825 (0.540-1.261)	0.375
Q1	Ref		Ref	
Q2	0.604 (0.313-1.166)	0.133	0.625 (0.300-1.301)	0.209
Q3	0.124 (0.042-0.365)	<0.001	0.168 (0.055-0.514)	0.002
PHR	0.999 (0.996-1.001)	0.308	0.999 (0.997-1.002)	0.53
Q1	Ref		Ref	
Q2	0.633 (0.301-1.330)	0.227	0.542 (0.240-1.223)	0.14
Q3	0.664 (0.316-1.395)	0.28	0.804 (0.366-1.768)	0.587

**Adjusted for age, sex, and hospital.*

TABLE 5 | Baseline characteristics of diabetic patients with COVID-19 on admission to hospital.

	All Patients (n = 150)	Noncritical COVID-19 (n = 108)	Critical COVID-19 (n = 42)	P value
Ages	64 (57-71)	62 (55-70)	69 (61-75)	0.001
Sex				
Female	67 (44.7%)	54 (50.0%)	13 (31.0%)	0.035
Male	83 (55.3%)	54 (50.0%)	29 (69.0%)	
Days from illness onset to admission	10 (7-15)	10 (7-20)	10 (7-12)	0.126
Metformin	34 (22.7%)	33 (30.6%)	1 (2.4%)	<0.001
Neutrophils ($\times 10^9$ per L)	4.16 (2.82-6.23)	3.58 (2.63-5.14)	6.83 (4.25-9.32)	<0.001
Lymphocytes ($\times 10^9$ per L)	0.97 (0.57-1.33)	1.11 (0.76-1.51)	0.62 (0.35-0.84)	<0.001
Monocytes ($\times 10^9$ per L)	0.36 (0.26-0.51)	0.39 (0.29-0.53)	0.33 (0.21-0.45)	0.112
Platelets (×109 per L)	182 (128-245)	193 (150-264)	152 (103-204)	<0.001
Total cholesterol (mmol/L)	3.83 (3.20-4.66)	3.96 (3.42-4.70)	3.38 (2.92-4.57)	0.02
Triglyceride (mmol/L)	1.42 (1.05-1.90)	1.37 (1.05-1.87)	1.58 (1.04-2.02)	0.734
High density lipoprotein cholesterol (mmol/L)	0.92 (0.79-1.23)	1.03 (0.84-1.27)	0.85 (0.72-0.96)	0.002
Low density lipoprotein cholesterol(mmol/L)	2.20 (1.69-2.78)	2.22 (1.77-2.80)	1.81 (1.44-2.56)	0.062
Neutrophil to high-density lipoprotein cholesterol ratio (NHR)	4.16 (2.73-7.24)	3.66 (2.44-5.07)	8.64 (4.82-12.29)	<0.001
Monocyte to high-density lipoprotein cholesterol ratio (MHR)	0.38 (0.25-0.51)	0.39 (0.25-0.51)	0.38 (0.25-54)	0.844
Lymphocyte to high-density lipoprotein cholesterol ratio (LHR)	0.96 (0.59-1.45)	1.18 (0.73-1.60)	0.73 (0.49-0.99)	<0.001
Platelet to high-density lipoprotein cholesterol ratio (PHR)	186.18 (129.06-262.53)	187.25 (134.44-263.16)	183.20 (113.79-252.57)	0.451

Data are median (IQR) or n (%). P values comparing critical and noncritical COVID-19 are from χ^2 test and Mann-Whitney U test.

biomarkers and COVID-19. For example, an increased triglyceride to HDL-C ratio indicates a greater risk of worse prognosis in patients with COVID-19 (29). Additionally, in a retrospective cohort study, a high C-reactive protein to HDL-C ratio was independently associated with an increase in mortality and poor prognosis (28). In the present study, we investigated the leucocyte-to-HDL-C ratio, and our results suggested that elevated NHR was associated with poor clinical outcomes after adjusting for confounding factors.

Several factors may contribute to the increased risks of severity related to higher NHR. First, excessive inflammation is an important features of COVID-19 patients. SARS-CoV-2 infection induces a series of immune responses and causes changes in peripheral white blood cells, such as neutrophilia (30). Furthermore, proinflammatory cytokines such as CRP and IL-6 can directly inhibit apolipoprotein synthesis enzyme activity, resulting in decreased ApoA-1 and HDL-C production (5–7, 31). In this context, the cytokine storm described in COVID-19 patients might induce immune-mediated dyslipidemia, leading to reduced HDL. Our study also demonstrated that CRP and IL-6 levels were higher in the critical COVID-19 group and correlated positively with NHR (**Supplementary Tables 3** and **5**). Consequently, NHR may reflect the overactive status of the inflammatory response, which can cause multiple organ damage and death. Second, HDL-C has the ability to inhibit neutrophil activation, attachment, diffusion, and migration (32). However, a large number of activated neutrophils can influence HDL-C composition and function by altering the structure and content of a variety of apolipoproteins (33). Moreover, a recent study in COVID-19 patients also reported altered HDL-C composition and function during severe COVID-19. For example, paraoxonase 1 (PON-1) is less abundant on HDL particles isolated from COVID-19 patients, which may be degraded by elastase released from neutrophil activation during COVID-19 (34). Consequently, a vicious cycle may occur in severe COVID-19 patients, with neutrophil overproduction resulting in deficiency in HDL-C and further neutrophil activation, which is detrimental to the prognosis of COVID-19. Third, the NHR has been shown to be associated with many diseases, such as metabolic syndrome (12) and cardiovascular disease (35, 36), which are also known risk factors for severe COVID-19 (37–39).

In our study, 257 (58.4%) patients with COVID-19 had dyslipidemia, and the prevalence of dyslipidemia was much higher than that in other studies (5–32.5%) (40–42). One reason may be that the definition of dyslipidemia itself is rather complicated. Statin is one of the drugs most commonly used by patients with dyslipidemia. Statins have favorable anti-inflammatory effects and have been suggested as adjunct therapy for COVID-19 (43). However, our study found that plasma levels of inflammatory biomarkers were not significantly affected by statin therapy (**Supplementary Table 6**). Of note, critical patients were more likely to use statins than non-critical patients (17.9% *vs.* 8.8%). Although it has been reported that statins may increase the risk of SARS-CoV-2 viral entry by inducing angiotensin-converting enzyme 2 (ACE2) expression (44), it is also worth noting that individuals on statins were older and had a greater incidence of chronic diseases (**Supplementary Table 6**), which may explain the higher proportion of statin use in the critical group. Further studies are urgently needed to explore the efficacy of statins on COVID-19 outcomes.

Diabetes is a common comorbidity in patients with COVID-19 and is associated with greater disease severity and higher mortality of COVID-19 (19–21). This may be attributed to the dysregulated immunological status and the exaggerated pro-inflammatory cytokine response, manifesting as higher ratios of lymphopenia and increased levels of neutrophils, serum CRP, and IL-6 in patients with COVID-19 with pre-existing T2D compared to non-diabetic patients (19, 23). In addition, diabetic patients usually exhibit reduced HDL level (22) and impaired HDL function (45). In view of these findings, the role of these new markers in subsets of diabetes is worth exploring. Similarly, higher neutrophil counts but lower HDL-C levels were observed in critical COVID-19 patients compared with non-critical COVID-19 patients in the diabetic population. Therefore, not surprisingly, the NHR tended to be higher in the noncritical group than in the critical group and was associated with an increased risk of adverse outcomes (HR:1.076, 95% CI:1.028-1.126), after adjusting for age, sex, and hospitals. Generally, patients with diabetes show a higher degree of glycation. Glycated HDL showed much lower antiviral activity against SARS-CoV-2 than native HDL (46). In addition, hyperglycemia in diabetes primes neutrophils to release neutrophil extracellular traps (NETs) (47) which might further contribute to the cytokine storm, systemic inflammatory response syndrome (SIRS), and sepsis in COVID-19. Thus, diabetic patients with high NHR are more likely to develop critical illness with COVID-19 because of uncontrolled inflammation. Similar results were also seen in hypertension patients but not CHD patients (**Supplementary Tables 7–10**). This may be due to the limited sample size and the small observed events, which limits the statistical power of this explorative study.

Acute inflammation caused by viral infection may result in dyslipidemia in patients, and lipid metabolism is known to play an important role in the host immune response (5, 6). In fact, inflammation and lipid abnormalities are considered to be associated with poor outcomes in COVID-19. These comprehensive biomarkers might be more reliable and have a better ability to reflect the inflammatory status and lipid metabolism. Furthermore, they are simply calculated from the leukocyte subsets and HDL-C, which are routinely checked, inexpensive, and readily available biomarkers.

Previous studies have independently assessed the clinical significance of these biomarkers in various patients, but only one preprint report so far has investigated the relationship with COVID-19, and only involving MHR (48), which showed a higher value of MHR in male patients than in female patients. Here, we evaluated the early predictive value of NHR, LHR, MHR, and PHR in SARS-Cov-2 infection. To the best of our knowledge, this is the first time that these markers have been simultaneously investigated in COVID-19. Our study will provide a supplement to the research on COVID-19 pneumonia epidemics and references for clinicians to identify individuals at risk.

However, there were several limitations to our study. First, this was a retrospective study and the sample size was relatively

small; therefore, further prospective studies with larger cohorts are required to verify our conclusions. Second, the time from the onset of symptoms to the time of serum sample collection when patients were admitted to the hospital varied among patients, which may have caused some bias in the analysis of the relationship between blood biomarkers and COVID-19. Third, a proportion of patients were excluded due to missing data, which may have led to selection bias. Fourth, although we adjusted for several known potential confounders, residual and unmeasured confounding factors might not be fully considered. Finally, the makeup of the study population might limit the generalizability of the results to other ethnic groups.

In conclusion, this retrospective cohort study indicates that lower HDL-C concentration and higher NHR on admission were found in patients with critical COVID-19 than in those without critical COVID-19 and were independently associated with adverse events not only in the overall COVID-19 patients but also in the diabetes subgroup after adjusting for confounding factors. However, further prospective cohort studies are required to confirm our findings.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JiZ, LC, and TZ conceived and supervised the overall study. QW, YX, LL, QL, LY, HYW, GL, XZ, KQ, YaL, HW, YW, XS, HLiu, SX, YuL, and ZC collected the epidemiological and clinical data. HYW, KQ, HW, YW, GL, and XZ summarized all data. YW did the analysis. JiZ and YW drafted the manuscript. HLi, WK, JuZ, WX, and HS contributed to the discussion. LC and TZ revised the final manuscript. TZ is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

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SYSTEMATIC REVIEW
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Mortality Risk of Antidiabetic Agents for Type 2 Diabetes With COVID-19: A Systematic Review and Meta-Analysis

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Aims: We conducted a systematic review and meta-analysis to assess various antidiabetic agents' association with mortality in patients with type 2 diabetes (T2DM) who have coronavirus disease 2019 (COVID-19).

Methods: We performed comprehensive literature retrieval from the date of inception until February 2, 2021, in medical databases (PubMed, Web of Science, Embase, and Cochrane Library), regarding mortality outcomes in patients with T2DM who have COVID-19. Pooled OR and 95% CI data were used to assess relationships between antidiabetic agents and mortality.

Results: Eighteen studies with 17,338 patients were included in the meta-analysis. Metformin (pooled OR, 0.69; *P*=0.001) and sulfonylurea (pooled OR, 0.80; *P*=0.016) were associated with lower mortality risk in patients with T2DM who had COVID-19. However, patients with T2DM who had COVID-19 and received insulin exhibited greater mortality (pooled OR, 2.20; *P*=0.002). Mortality did not significantly differ (pooled OR, 0.72; *P*=0.057) between DPP-4 inhibitor users and non-users.

Conclusions: Metformin and sulfonylurea could be associated with reduced mortality risk in patients with T2DM who have COVID-19. Furthermore, insulin use could be associated with greater mortality, while DPP-4 inhibitor use could not be. The effects of antidiabetic agents in patients with T2DM who have COVID-19 require further exploration.

Systematic Review Registration: PROSPERO (identifier, CRD42021242898).

Keywords: COVID-19, diabetes, antidiabetic agents, mortality, type 2 diabetes

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INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2, is a serious global public health problem that has affected more than 100 million people worldwide (as of January 26, 2021) (1). SARS-CoV-2 exhibits high infectivity, causing rapid spread and associated outbreaks; moreover, the mortality of COVID-19 was 61.5% at the onset of the epidemic (2). Although mortality rates have declined with increasing disease awareness, poor outcomes persist, especially among people with chronic diseases (3). Diabetes, characterized by hyperglycemia, is an increasingly common illness worldwide; it is associated with the onset of various complications, including infections. Richardson et al. found that 42% of patients with COVID-19 had diabetes (defined as HbA1c level >6.5%) (4). Patients with diabetes, especially those who have COVID-19, are vulnerable to the onset of severe disease. In a study of 1000 COVID-19 patients, diabetes was present in 16.2% of patients with severe disease; the final outcomes of these patients were mechanical ventilation and/or mortality (5).

Among patients with diabetes or hyperglycemia, the in-hospital mortality is reportedly 29%, compared with 6% among people without diabetes or hyperglycemia (4). This indicates a fourfold increase in COVID-19 mortality among patients with diabetes or hyperglycemia. In a retrospective study of 72,314 COVID-19 patients, the mortality rate (7.3%) was significantly greater in patients with diabetes (6). Crouse et al. (7) found that increased mortality persisted in patients with diabetes who had COVID-19, despite adjustment for covariates such as age, ethnicity, obesity, and hypertension. Sourij H et al. (8) also found that in-hospital mortality for COVID-19 was higher in patients with diabetes.

Glycemic control is critical in diabetes mellitus patients with COVID-19 because hyperglycemia can increase mortality in these patients. Type 2 diabetes mellitus (T2DM), which is present in most individuals with diabetes, is characterized by insulin hyposecretion and insulin resistance. The primary treatment involves administration of oral antihyperglycemic drugs or insulin. Several reports have investigated mortality among patients with T2DM who have COVID-19 that receive treatment with antihyperglycemic therapy. Previous meta-analysis has done the use of metformin in diabetes since the early pandemic time (9). However, this study is still limited in the included study. In addition, due to variations in experimental design, final research outcomes, and patient populations, results are inconsistent among studies. Accordingly, we conducted a systemic review and meta-analysis to investigate the associations of antidiabetic agents with mortality in patients with T2DM who have COVID-19.

METHODS

Search Strategy

We performed a comprehensive literature search from the date of inception until February 2, 2021 in four databases: PubMed, Web of Science, Embase, and the Cochrane Library. The search strategy used the following specific keywords: "COVID-19" OR "coronavirus" OR "SARS-CoV-2 infection" OR "2019 novel coronavirus disease" AND "diabetes mellitus, type 2" OR "diabetes, type 2" OR "type 2 diabetes" AND "antidiabetic agents" OR "metformin" OR "sulfonylurea compounds" OR "glucagon-like peptide 1" OR "dipeptidyl-peptidase IV inhibitors" OR "sodium-glucose transporter 2 inhibitors" OR "glycoside hydrolase inhibitors" OR "Insulin". After screening to identify relevant abstracts, we searched the reference lists of retrieved articles to discover additional potentially eligible studies. This study was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (10). Two reviewers independently evaluated all articles that met the requirements (described in Section 2.2) and extracted data, then compared their findings. Any differences were resolved through discussion.

Inclusion and Exclusion Criteria

The search strategy was based on a Population, Intervention, Comparison, and Outcome (PICO) framework. Population: patients with T2DM who had COVID-19; Intervention: any anti-diabetic agent; Comparison: without anti-diabetic agent usage; Outcome: study type, mortality.

Inclusion criteria were as follows: study type, published studies regarding associations between antidiabetic agents and patients with T2DM who had COVID-19; exposure intervention, patients with T2DM who had COVID-19 received antidiabetic agents (e.g., metformin, sulfonylurea, glucagon-like peptide 1 [GLP-1] receptor agonists, dipeptidyl peptidase 4 [DPP-4] inhibitors, sodium-glucose cotransporter 2 [SGLT2] inhibitors, α-glucosidase inhibitors, and/or insulin); and outcome indicator, quantitative assessment of associations between antidiabetic agent use and patient mortality, including odds ratios (ORs) and 95% confidence intervals (CIs).

Exclusion criteria were as follows: study type, non-research/original articles (e.g., review articles, case reports, case series, summaries of meetings or discussions, or letters to the editor); incomplete patient information; duplicate/overlapping data; and absence of mortality outcomes.

Data Extraction and Quality Assessment

Following application of the inclusion and exclusion criteria, two investigators (Kan CX and Hou NN) independently collected data from the 18 identified studies, using a standardized method. The following data were extracted from enrolled studies: first author surname, type of study, country of origin, and sample size; clinical data including age and nationality; and statistical data (e.g., OR or HR and corresponding 95% CI values). When both univariate and multivariate analyses were reported, multivariate data were included in the analysis. If the OR was not reported directly, relevant data were used to calculate the OR.

Article quality was evaluated using the Newcastle-Ottawa Scale tool (11). A Newcastle-Ottawa Scale score ≥7 was considered high-quality; higher Newcastle-Ottawa Scale scores were presumed to indicate higher literature quality.

Statistical Analysis

Meta-analysis was carried out using Stata software, version 16.0. Pooled OR and 95% CI values were used to estimate relationships between antidiabetic agents and mortality. Heterogeneity was evaluated using the Cochran Q statistic and the I^2 statistic. A random-effects model ($P<0.1$ or $I^2 \geq 50\%$) and a fixed-effects model ($P>0.1$ and $I^2<50\%$) were used to assess heterogeneity. If >5 studies were included, Egger's test and Begg's test were used to evaluate publication bias (12). $P>0.05$ was considered to indicate the absence of publication bias.

RESULTS

Study Selection

We retrieved 676 studies from PubMed, Web of Science, Embase, and the Cochrane Library database. After the removal of duplicates, 318 articles were included in title and abstract screening; 283 articles were excluded on the basis of abstract or title. The remaining 35 articles were included in the full-text review; 17 studies were then excluded (eight reviews, five studies that did not report mortality, two meta-analyses, one case report, and one study that included outpatients). The remaining 18 eligible studies were included in the analysis (**Figure 1**).

Study Characteristics and Quality Assessment

We investigated 18 studies that included 17,338 patients with T2DM who had COVID-19. Among these 18 studies, seven were performed in China, two were performed in the United States, one was performed in South Korea, two were performed in the United Kingdom, three were performed in Italy, and three were performed in France. All Newcastle-Ottawa Scale scores were ≥7 (**Table 1**).

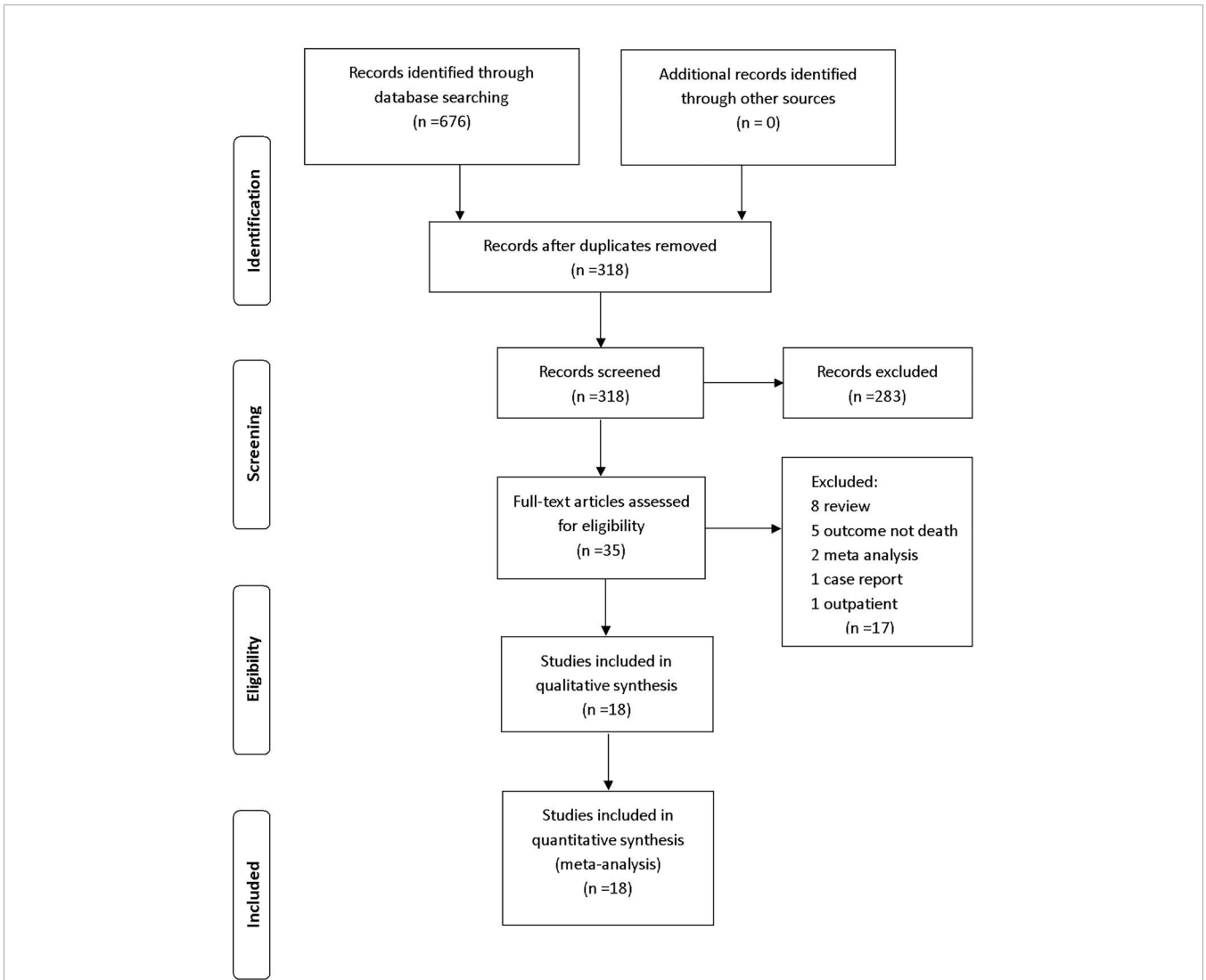

FIGURE 1 | PRISMA study.

TABLE 1 | The characteristics of the included studies in meta-analysis.

Study	Study type	Country	All Subjects	Patients (n)	Ages (years) Mean±SD/median (IQR)	Mortality	Odds ratio/Hazard ratio (95% CI)	NOS
Metformin users/non users								
Chen et al. (13)	Retrospective	China	120	43/77	62.0 (56.0-69.0) *vs* 67.0 (57.5-73.0)	4 (9.3%) *vs* 15 (19.5%)	0.62 (0.17-2.20)	8
Bramante et al. (14)	Retrospective	USA	6256	2333/3923	73 *vs* 76	394 (16.9%) *vs* 791 (20.2%)	Female subgroup 0.74 (0.56- 0.98)	8
Luo et al. (15)	Retrospective	China	283	104/179	63.0 (55.8-68.3) *vs* 65.0 (57.5-71.0)	3 (2.9%) *vs* 22 (12.3%)	4.36 (1.22-15.59)	9
Crouse et al. (7)	Retrospective	USA	220	76/144	N/A	8 (19.1%) *vs* 34 (81.0%)	0.33 (0.13-0.84)	8
Kim et al. (16)	Retrospective	South Korea	235	113/122	68.3±11.9 (all group)	N/A	0.36 (0.10-1.23)	9
Philipose et al. (17)	Retrospective	UK	159	100/59	N/A	N/A	1.39 (0.84-2.16)	8
Cheng et al. (18)	Retrospective	China	1213	678/535	62.0 (55.0-68.0) *vs* 64.0 (58.0-70.0)	N/A	HR:1.65 (0.71-3.86)	9
Abu-Jamous et al. (19)	Retrospective	UK	191	23/168	N/A	4 (17.4%) *vs* 94 (56.0%)	0.19 (0.05-0.70)	9
Jiang et al. (20)	Retrospective	China	328	100/228	64.0 (56.5, 70.0) *vs* 67.0 (60.0, 76.0)	3 (3.0%) *vs* 25 (11.0%)	0.48 (0.13-1.74)	8
Mirani et al. (21)	Retrospective	Italy	90	69/21	69±13 *vs* 75±8	25 (36.2%) *vs* 13 (61.9%)	0.43 (0.21-0.85)	7
Li et al. (22)	Retrospective	China	131	37/94	64.6 ± 11.2 *vs* 67.7 ± 11.7	2 (5.4%) *vs* 21 (22.3%)	0.20 (0.04-0.90)	8
Lalau et al. (23)	Prospective	France	2449	1496/953	68.5 ± 11.9 *vs* 74.6 ± 12.5	Day 7:122 (8.2%) *vs* 153 (16.1%) Day 28:239 (16.0%)*vs* 273 (28.6%)	Day 28 0.71 (0.54-0.94)	9
Cariou et al. (24)	Prospective	France	1317	746/571	69.8±13.0 (all group)	N/A	0.59 (0.42-0.84)	8
Wargny et al. (25)	Prospective	France	2794	1553/1241	68.9 ± 13.2 (all group)	N/A	0.63 (0.52-0.77)	8
Sulfonylurea users/non users								
Chen et al. (13)	Retrospective	China	120	53/67	66.0 (60.0-72.5) *vs* 64.0 (55.0-73.0)	7 (13.20%) *vs* 12 (17.91%)	0.68 (0.21-2.16)	8
Kim et al. (16)	Retrospective	South Korea	235	60/175	68.3±11.9 (all group)	N/A	0.84 (0.23-3.09)	9
Mirani et al. (21)	Retrospective	Italy	90	Oct-80	75±8 *vs* 70±12	3 (30%) *vs* 35 (43.8%)	0.34 (0.08-1.42)	7
Cariou et al. (24)	Prospective	France	1317	367/950	69.8±13.0 (all group)	N/A	0.74 (0.49-1.13)	8
Wargny et al. (25)	Prospective	France	2794	782/2012	68.9 ± 13.2 (all group)	N/A	0.83 (0.67-1.03)	8
DPP-4 inhibitors users/non users								
Chen et al. (13)	Retrospective	China	120	20/100	66.0 (56.0-73.0) *vs* 65.0 (57.0-72.0)	5 (25.00%) *vs* 14 (14.00%)	1.48 (0.40-5.53)	8
Kim et al. (16)	Retrospective	South Korea	235	85/150	68.3±11.9 (all group)	N/A	1.47 (0.45-4.78)	9
Mirani et al. (21)	Retrospective	Italy	90	Nov-79	70±13 *vs* 71±12	1 (9.1%) *vs* 37 (46.8%)	0.13 (0.02–0.92)	7
Solerte et al. (26)	Retrospective	Italy	338	169/169	69.0 ± 0.9 *vs* 69.0 ± 1.0	31 (18%) *vs* 63 (37%)	0.44 (0.29-0.66)	8
Fadini et al. (27)	Retrospective	Italy	81	Sep-72	72.2 (12.8) *vs* 70.1 (13.3)	1 (11.1%) *vs* 10 (13.9%)	0.77 (0.09-6.88)	8
Zhou et al. (28)	Retrospective	China	444	111/333	63 (55-67) *vs* 64 (56.5-69)	2 (1.8%) *vs* 11 (3.3%)	0.58 (0.12-2.68)	8
Cariou et al. (24)	Prospective	France	1317	285/1032	69.8±13.0 (all group)	N/A	0.85 (0.55-1.32)	8
Wargny et al. (25)	Prospective	France	2794	615/2179	68.9 ± 13.2 (all group)	N/A	0.83 (0.65-1.05)	8
GLP-1 analogs users/non users								
Cariou et al. (24)	Prospective	France	1317	123/1194	69.8±13.0 (all group)	N/A	0.64 (0.32-1.29)	8
Wargny et al. (25)	Prospective	France	2794	254/2540	68.9 ± 13.2 (all group)	N/A	0.78 (0.53-1.15)	8
SGLT-2 inhibitors users/non users								
Kim et al. (16)	Retrospective	South Korea	235	8/227	68.3±11.9 (all group)	N/A	5.05 (0.48-53.26)	9
α-Glucosidase inhibitors users/non users								
Chen et al. (13)	Retrospective	China	120	69/51	66.0 (56.5-73.0) *vs* 65.0 (56.0-72.0)	11 (15.94%) *vs* 8 (15.69%)	0.99 (0.31-3.14)	8
Insulin users/non users								
Chen et al. (13)	Retrospective	China	120	71/49	65.0 (57.0-72.0) *vs* 65.0 (56.0-73.0)	16 (22.5%) *vs* 3 (6.12%)	2.99 (0.67-13.30)	8
Crouse et al. (7)	Retrospective	USA	220	87/133	N/A	15 (35.7%) *vs* 27 (64.3%)	0.97 (0.42-2.23)	8
Kim et al. (16)	Retrospective	South Korea	235	19/216	68.3±11.9 (all group)	N/A	0.26 (0.03-2.63)	9
Mirani et al. (21)	Retrospective	Italy	90	29/61	72 ±10 *vs* 70 ±13	19 (65.5%) *vs* 19 (31.2%)	3.34 (1.74-6.41)	7
Yu et al. (29)	Retrospective	China	689	346/343	67 (58-75) *vs* 65 (56-71)	94 (27.2%) *vs* 12 (3.5%)	7.70 (4.22-14.05)	8
Cariou et al. (24)	Prospective	France	1317	504/813	69.8±13.0 (all group)	N/A	1.71 (1.20-2.43)	8
Wargny et al. (25)	Prospective	France	2796	1039/1757	68.9 ± 13.2 (all group)	N/A	1.72 (1.41-2.08)	8

NA means Not Applicable.

Relationships of Antidiabetic Agents With Mortality Risk

The relationships between hypoglycemic drugs and mortality are shown in **Figures 2–5**. Because only two studies examined GLP-1 receptor agonists (24, 25), one study examined α-glucosidase inhibitors (13), and one study examined SGLT2 inhibitors (16), the OR values of these studies could not be combined. Significant associations were identified between antidiabetic agent use (i.e., metformin, sulfonylurea, and insulin) and mortality (all $P<0.05$). There was no significant association between DPP-4 inhibitor use and mortality ($P>0.05$).

Effects of Metformin, Sulfonylurea, DPP-4 Inhibitors, and Insulin on Mortality

Fourteen studies (7, 13–25) focused on metformin users in the meta-analysis. Pooled OR analysis revealed signifi0063antly lower mortality in metformin users than in metformin non-users (**Figure 2**; pooled OR, 0.69; 95% CI, 0.55–0.86; $P=0.001$). Five studies (13, 16, 21, 24, 25) showed lower mortality in sulfonylurea users than in sulfonylurea non-users (**Figure 3**; pooled OR, 0.80; 95% CI, 0.66–0.96; $P=0.016$). Eight studies (13, 16, 21, 24–28) revealed no significant difference in mortality between DPP-4 inhibitors users and non-users (**Figure 4**; pooled OR, 0.72; 95% CI, 0.51–1.01; $P=0.057$). Finally, seven studies (7, 13, 16, 21, 24, 25, 29)showed greater mortality in insulin users than in insulin non-users (**Figure 5**; pooled OR, 2.20; 95% CI, 1.34–3.60; $P=0.002$).

Publication Bias

Begg's test and Egger's test showed no significant publication bias among the included studies (**Table 2**).

DISCUSSION

This comprehensive meta-analysis included 18 studies with 17,338 subjects. Overall, metformin and sulfonylurea users exhibited lower mortality risk, compared with the respective non-users, among patients with T2DM who had COVID-19. However, patients who received insulin treatment exhibited greater mortality risk, while DPP-4 inhibitor use was not associated with mortality. This is the first study to evaluate the mortality risk associated with the use of various antidiabetic agents among T2DM patients with COVID-19.

Diabetes is associated with a poor outcome that involves Acute Respiratory Distress Syndrome (ARDS), serious clinical manifestations, and mortality (30). Meta-regression analysis showed that, as a single risk factor, diabetes was a stronger influence among younger and non-hypertensive patients; however, this finding requires further investigation. Patients with diabetes are more susceptible to infection, as indicated by Pearson-Stuttard et al. (31). Given the high incidence rate of diabetes worldwide, these people comprise a considerable proportion of individuals with COVID-19. Research regarding influenza A virus (H1N1) (32) in 2009, SARS-CoV (33) in 2002,

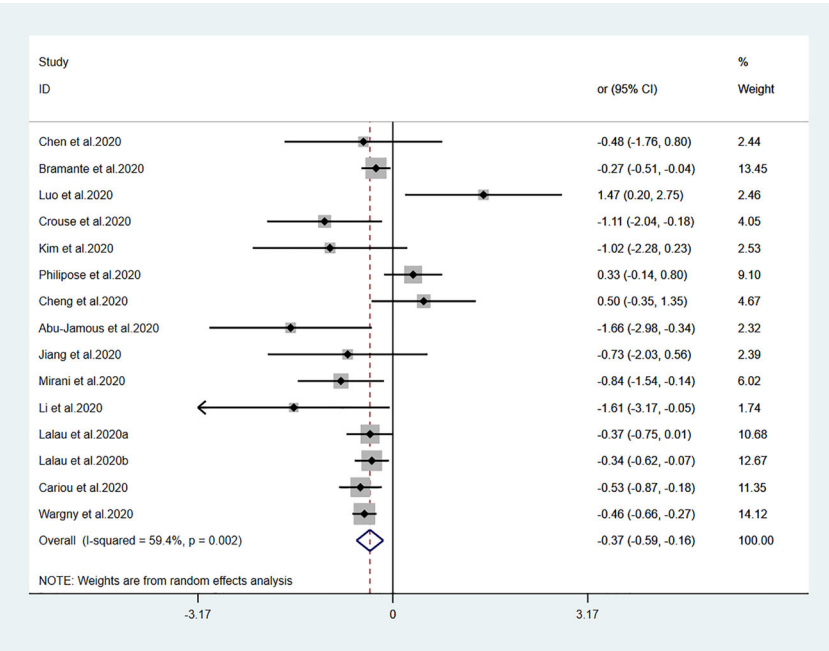

FIGURE 2 | Forest plots of meta-analysis of the relationship between metformin therapy and the risk of mortality in patients with type 2 diabetes who have COVID-19. The diamonds and horizontal lines indicate the corresponding odds ratio and 95% confidence interval. The size of the gray area represents the specific statistical weight of the study. The vertical solid line represents the OR of 1, and the vertical red dotted line shows the combination effect estimation. The suffix "a" or "b" after the studies indicates results of the same study at different times.

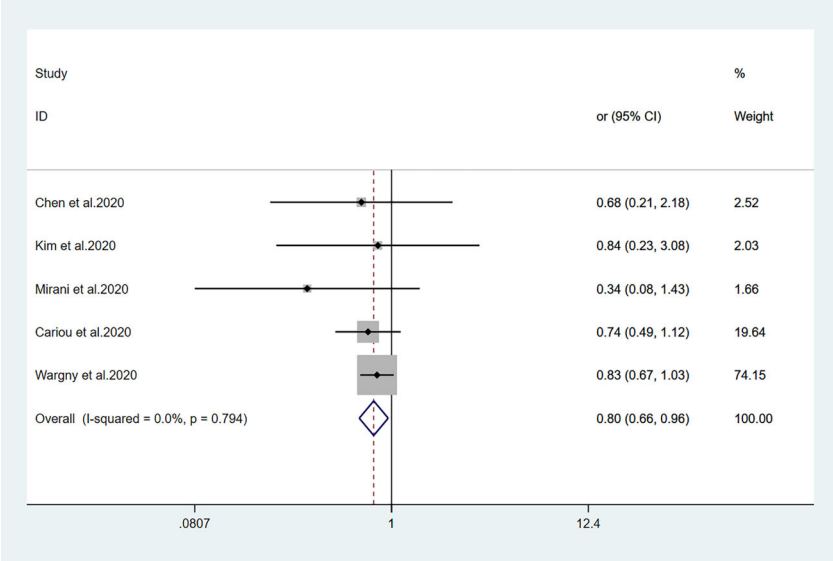

FIGURE 3 | Forest plots of meta-analysis of the relationship between sulfonylurea therapy and the risk of mortality in patients with type 2 diabetes who have COVID-19.

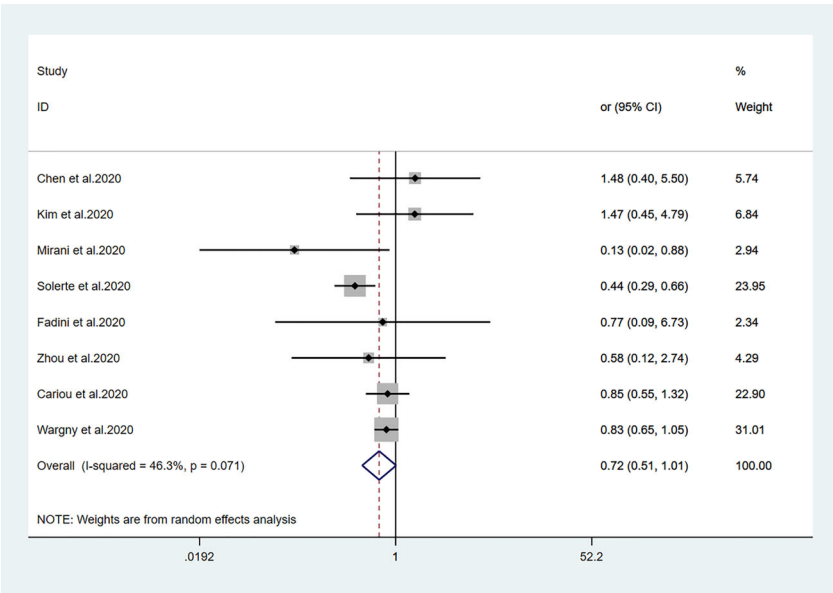

FIGURE 4 | Forest plots of meta-analysis of the relationship between DPP4 inhibitors therapy and the risk of mortality in patients with type 2 diabetes who have COVID-19.

and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) (34) in 2012 showed that hyperglycemia was an essential predictor for severity and mortality. Researches regarding SARS-CoV-2 pandemic have found that patients with T2DM are at greater risk of mortality from COVID-19 (5, 6, 35). Patients with a more severe course of diabetes have a poorer prognosis of COVID-19 (36). Thus far, there have been multiple studies regarding antihyperglycemic drugs and mortality in T2DM patients with COVID-19. However, the mortality risks associated with the use of each antihyperglycemic drug in these patients remain unclear. The present meta-analysis aimed to assess mortality in those patients.

Compared with metformin non-users, metformin users exhibited lower COVID-19-related mortality in our

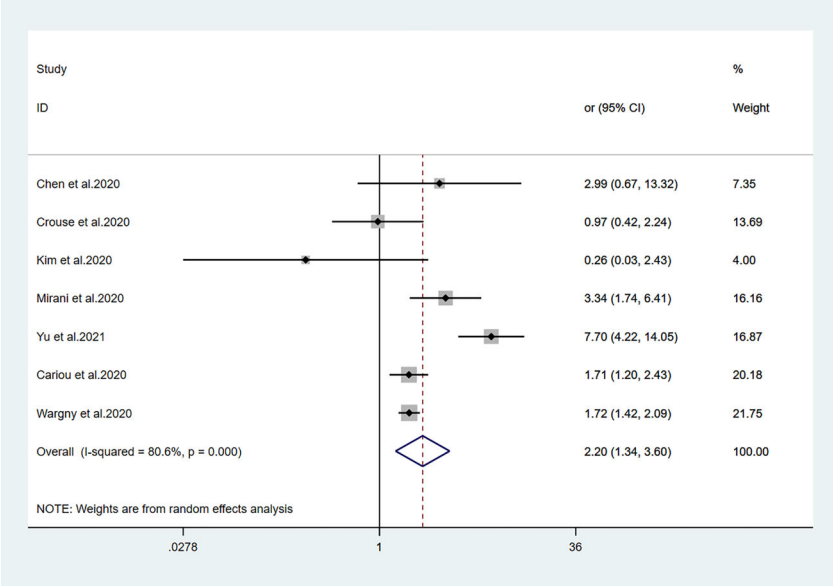

FIGURE 5 | Forest plots of meta-analysis of the relationship between insulin therapy and the risk of mortality in patients with type 2 diabetes who have COVID-19.

TABLE 2 | The result of publication bias.

Death events	No. of studies	OR (95% CI)	P-value	Heterogeneity		Model used	Begger's test	Egger's test
				I2	P-value			
Metformin	14	0.69 (0.55-0.86)	0.001	59.4%	0.002	Random	0.198	0.745
Sulfonylurea	5	0.80 (0.66-0.96)	0.016	0	0.794	Fixed	0.462	0.180
DPP-4 inhibitors	8	0.72 (0.51-1.01)	0.057	46.3%	0.071	Random	0.386	0.777
Insulin	7	2.20 (1.34-3.60)	0.002	80.6%	0.000	Random	1.000	0.706

meta-analysis. Several previous meta-analyses have shown that metformin was associated with lower mortality; these include the studies by Kow et al. (37) (OR, 0.62; 95% CI, 0.43–0.89), Scheen et al. (38) (OR, 0.75; 95% CI, 0.67–0.85), and Lukito et al. (39) (OR, 0.64; 95% CI, 0.43–0.97) and Hariyanto et al. (9) (RR, 0.54; 95% CI, 0.32–0.90). Our meta-analysis revealed that metformin use was associated with lower mortality risk in T2DM patients with COVID-19, similar to previous findings. We presume that metformin reduces the mortality risk through two potential mechanisms. First, metformin can mediate an anti-inflammatory effect (40, 41). In particular, metformin can reverse pulmonary fibrosis, delay ARDS progression, improve patient prognosis, and reduce mortality (42). There is increasing evidence that the Interleukin-6 (IL-6) signaling pathway can be inhibited by metformin; specifically, metformin substantially reduces the expression of IL-6 receptors and promotes myeloma cell apoptosis in patients with primary myeloma (43). Polycystic ovary syndrome patients reportedly exhibit lower serum IL-6 levels and improved chronic inflammation during early use of metformin (44). Metformin can also reduce the secretion of IL-6 by alveolar macrophages, thereby reducing pulmonary thrombosis in mice (45). Therefore, the use of metformin is beneficial in patients with T2DM who have COVID-19; we suggest the use of metformin for hypoglycemic treatment in those patients.

Sulfonylurea is a well-established oral hypoglycemic drug. Because of its precise hypoglycemic effect, good quality, and low price, it is both convenient and practical for many patients with diabetes. Five studies have reported the risk of mortality during sulfonylurea treatment in patients with COVID-19. Kim et al. (16) reported a 1:1 propensity cohort study, which revealed no difference in mortality (OR, 0.84; 95% CI, 0.23–3.09) between sulfonylurea users and non-users. Similarly, Wargny et al. (25) reported no difference in mortality (OR, 0.83; 95% CI, 0.67–1.03) between sulfonylurea users and non-users over a 28-day interval. Our findings are surprising because in-hospital use of sulfonylurea has been associated with lower mortality. The specific underlying mechanism is unclear. If islet function is acceptable, sulfonylurea drugs can be considered for hypoglycemic treatment in patients with T2DM who have COVID-19. However, sulfonylurea drugs can easily cause hypoglycemia; therefore, the use of sulfonylurea drugs in patients with severe COVID-19 requires careful blood glucose monitoring.

Our meta-analysis did not show any relationship between DPP-4 inhibitor use and mortality outcomes in patients with T2DM who

had COVID-19. This is consistent with the previous comprehensive meta-analysis which showed that DPP-4 inhibitor did not alter the mortality from COVID-19 (46). Fadini et al. (27) reported an Italian case-control study involving 85 T2DM patients hospitalized with COVID-19; in that study, DPP-4 inhibitor treatment in nine patients was not associated with COVID-19 mortality. However, the small number of samples was an important limitation of that study deficiency. In the CORONADO study, a large-scale, nationwide observational study of 1317 T2DM patients with COVID-19, no differences in 7-day or 28-day mortality were observed between DDP-4 inhibitor users and non-users (24, 25). Similarly, in a study of 3818 patients with COVID-19, Strollo et al. (47) suggested that the pharmacological effects of DPP-4 inhibitors might not influence SARS-CoV-2 infection and COVID-19 progression. However, Mirani et al. (21) reported a decline in fatalities among DPP-4 inhibitor users, compared with non-users. A retrospective case-control study by Solerte et al. (26) showed that, compared with insulin therapy alone, patients who received sitagliptin plus insulin showed a >50% relative mortality reduction. However, Nauck et al. (48) reported some limitations regarding the studies by Mirani et al. and Solerte et al. In conclusion, DPP-4 inhibitors may slow the progression of acute respiratory complications in T2DM patients with COVID-19 (49). Although DPP-4 inhibitors are presumed to interact with the MERS-CoV receptor (49), which has characteristics similar to the SARS-CoV-2 receptor, these drugs have not been confirmed to interact with the SARS-CoV-2 receptor.

Viral infection can worsen hyperglycemia in patients with diabetes; it may induce acute diabetes complications. Hyperglycemia can cause immune abnormalities that aggravate infection, hypoxia, and overall COVID-19. Insulin is consistently the first choice for emergency treatment. Therefore, many experts recommend insulin for T2DM patients with SARS-CoV-2 infections (50, 51). Previous clinical studies regarding patients with diabetes who had sepsis, the 30-day mortality rate was higher among those receiving insulin treatment than among those receiving oral antidiabetic agents (52). Another study showed that insulin use led to greater mortality in 6104 intensive care unit patients (53). These findings suggest that glycemic control with insulin therapy leads to greater mortality risk in critically ill patients, possibly by promoting inflammation. Similarly, a study by Yu et al. (29) suggested that mortality was significantly greater in patients with T2DM who had COVID-19 and received insulin, compared with those who did not receive insulin (HR, 7.70; 95% CI, 4.22–14.05). The conclusion was still valid when analyzing the subgroups established by propensity score matching and different baseline characteristics or severity stratification. As shown in **Figure 5**, insulin use tended to be associated with greater mortality in patients with COVID-19. Although the underlying mechanism is unclear, our meta-analysis results suggest the need for careful assessment of the benefits and potential adverse effects of insulin therapy for patients with COVID-19.

To the best of our knowledge, this study is the first to compare mortality risks among various antidiabetic agents for the treatment of patients with T2DM who have COVID-19. We found that metformin and sulfonylurea treatments led to reduced mortality risk, while insulin was significantly associated with greater mortality. However, DPP-4 inhibitors showed no relationship with mortality risk in patients with T2DM who had COVID-19.

However, this study had some limitations. First, since all studies are observational, this meta-analysis is only hypothesis-generating. Prospective randomized studies are required to draw conclusions and to give treatment advice for patients with T2DM who have COVID-19. Second, although the included study findings were adjusted to control for major potential confounding factors, the various degrees of residual confounding bias, including the different severity of diabetes itself, could not be fully excluded. Additionally, comorbidity such as dementia or Parkinson's disease may be a confounder for elderly patients related to mortality (54, 55). Finally, bias may have been present because of variable research quality among the included studies.

CONCLUSION

Metformin and sulfonylurea treatments could be associated with reduced mortality risk, while insulin treatment could be associated with enhanced mortality risk, in patients with T2DM who had COVID-19. However, DPP-4 inhibitor treatment could not be associated with mortality risk in these patients. The results of this meta-analysis should be interpreted carefully because of the limitations of included studies, although the effects of sulfonylurea and DPP-4 inhibitors should be more fully evaluated in subsequent studies. Further larger trials should also be done to confirm these results and especially other diabetes drugs including SGLT2 inhibitors and DPP-4 inhibitors.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

CK, YZ, and FH: Conceptualization, methodology, and writing – original draft preparation. TY and QX: Data curation and investigation. XS and NH: Supervision, writing – reviewing and editing, and funding acquisition. All authors contributed to the article and approved the submitted version.

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GENERAL COMMENTARY
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Commentary: Mortality Risk of Antidiabetic Agents for Type 2 Diabetes With COVID-19: A Systematic Review and Meta-Analysis

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A Commentary on

Mortality Risk of Antidiabetic Agents for Type 2 Diabetes With COVID-19: A Systematic Review and Meta-Analysis

By Kan C, Zhang Y, Han F, Xu Q, Ye T, Hou N, et al. Mortality Risk of Antidiabetic Agents for Type 2 Diabetes With COVID-19: A Systematic Review and Meta-Analysis. Front Endocrinol (Lausanne) (2021) 12:708494. doi: 10.3389/fendo.2021.708494

INTRODUCTION

In a recent article (1), Kan and colleagues assessed the association between use of four kinds of hypoglycemic agents (i.e., metformin, sulfonylurea, dipeptidyl peptidase-4 inhibitors [DPP4i], and insulin) and risk of COVID-19 mortality in patients with type 2 diabetes mellitus (T2DM), by performing meta-analysis based on eligible studies. However, the authors did not address the association of two novel classes of hypoglycemic agents (i.e., sodium-glucose cotransporter-2 inhibitors [SGLT2i] and glucagon-like peptide-1 receptor agonists [GLP1RA]) with COVID-19 mortality, due to the limited number of included studies regarding SGLT2i and GLP1RA. Nowadays, more and more studies have been published, mainly targeting the relationship between SGLT2i or GLP1RA use and COVID-19 mortality risk (2–10). Therefore, we intended to include these recent studies regarding SGLT2i and GLP1RA to conduct an updated meta-analysis to address their association with COVID-19 mortality in T2DM patients. Moreover, several new cohort studies (2–4, 8, 11–14) targeting the relationship of DPP4i use with COVID-19 death risk

have been published after Kan et al.'s meta-analysis (1). Thus, in this updated meta-analysis we would also include these new DPP4i studies to reassess the association between use of DPP4i and risk of COVID-19 death in T2DM patients.

METHODS

Eligible studies for inclusion in this updated meta-analysis were cohort studies that enrolled T2DM patients with COVID-19 and estimated the COVID-19 mortality risk of use of DPP4i, SGLT2i, or GLP1RA, compared to no use of DPP4i, SGLT2i, or GLP1RA, respectively. The comparison of SGLT2i/GLP1RA use versus no SGLT2i/GLP1RA use included that of SGLT2i/GLP1RA use versus DPP4i use, whereas the comparison of DPP4i use versus no DPP4i use did not include that of DPP4i use versus SGLT2i/GLP1RA use. Outcome of interest was COVID-19 death, and we preferred to use the most long-term outcome data. For example, if 7-day death (i.e., death within 7 days following hospital admission due to COVID-19) and 30-day death were both reported in included studies, we would use 30-day death. We searched PubMed, Cochrane Library, and Embase from the inception date of databases to November 21, 2021, using the main search terms: "COVID-19", "SARS-CoV-2", "type 2 diabetes mellitus", "DPP4i", "SGLT2i", "GLP1RA", "death", and "mortality". Two authors independently performed study selection, quality assessment, and data extraction; and a third author addressed their divergences when necessary. The quality of included studies was assessed using the Newcastle-Ottawa Scale (15), according to which a quality score for included studies ranged from 0 to 9, with a score of ≥7 being regarded as "high quality".

We extracted the estimators of relative risk (RR) and 95% confidence interval (CI) from included studies to perform meta-analysis. We preferentially extracted hazard ratio as RR, followed by risk ratio and odds ratio. Meta-analysis was conducted based on the comparisons of DPP4i versus Non-DPP4i, SGLT2i versus Non-SGLT2i, and GLP1RA versus Non-GLP1RA, respectively. Meta-analysis was done using a random-effects model when I^2 ≥50%, or using a fixed-effects model when I^2 <50%. We drew funnel plots and performed Egger's test to judge publication bias. Sensitivity analysis was conducted *via* the leave-one-out method (omitting one study each time), to evaluate the robustness of meta-analysis results. All statistical analyses were completed in Stata/MP 16.0.

RESULTS

We finally included 18 cohort studies (2–14, 16–20) in this meta-analysis. All of them were evaluated as "high quality". The detailed features of included studies and the outcome data extracted from them are presented in **Table S1**. Meta-analysis incorporating 16 studies (2–4, 7–14, 16–20) involving 549817 participants showed that compared to Non-DPP4i use, DPP4i use was significantly associated with a 17% reduction in COVID-19 mortality risk (RR 0.83, 95% CI 0.71-0.98, P =0.023; **Figure 1.1**). Meta-analysis incorporating 6 studies (2–7) involving 275468 participants showed that compared to Non-SGLT2i use, SGLT2i use was significantly associated with a 22% reduction in COVID-19 mortality risk (RR 0.78, 95% CI 0.61-0.98, P =0.035; **Figure 1.2**). Meta-analysis incorporating 6 studies (2, 3, 6, 8–10) involving 140859 participants showed that compared to Non-GLP1RA use, GLP1RA use was significantly associated with a 25% reduction in COVID-19 mortality risk (RR 0.75, 95% CI 0.60-0.94, P =0.013; **Figure 1.3**).

Publication bias was observed in the meta-analysis of DPP4i (P_{Egger} =0.012; **Figure S1**), and was not observed in the meta-analyses of SGLT2i (P_{Egger} =0.885; **Figure S2**) and GLP1RA (P_{Egger} =0.092; **Figure S3**). The results of sensitivity analysis for DPP4i (**Figure S4**), SGLT2i (**Figure S5**), and GLP1RA (**Figure S6**) were similar with primary analysis results, which suggested the robustness of meta-analysis results.

DISCUSSION

In Kan and colleagues' article (1) the authors did not perform meta-analysis to assess the association between use of SGLT2i and GLP1RA and risk of COVID-19 mortality since they included only one SGLT2i study (7) and two GLP1RA studies (9, 10). On the contrary, we conducted meta-analysis respectively based on 6 SGLT2i studies (2–7) and 6 GLP1RA studies (2, 3, 6, 8–10), and accordingly revealed the significant association of SGLT2i use with a 22% reduction in COVID-19 mortality risk, and of GLP1RA use with a 25% reduction in that risk. Moreover, Kan et al.'s meta-analysis (1) did not include 8 recently-published DPP4i studies (2–4, 8, 11–14), which limited its statistical power. Accordingly, a statistically significant difference between DPP4i and Non-DPP4i was not observed in that meta-analysis (1). In contrast, this updated meta-analysis included 16 DPP4i studies (2–4, 7–14, 16–20) including the 8 recent ones (2–4, 8, 11–14), and therefore with sufficient statistical power identified that DPP4i use versus Non-DPP4i use was significantly associated with a 17% reduction in COVID-19 mortality risk.

One strength of this study is that sensitivity analyses suggested the robustness of meta-analysis results although possible publication bias was observed in DPP4i's meta-analysis. Oppositely, this study has two main weaknesses. First, we did meta-analyses based on cohort studies. Although we extracted the adjusted RRs and 95% CIs from included studies to perform meta-analyses, there must be certain confounding factors unadjusted. Thus, relevant randomized trials are needed to verify our findings. Second, we failed to compare various hypoglycemic agents in COVID-19 death risk, and future studies comparing them are clinically meaningful.

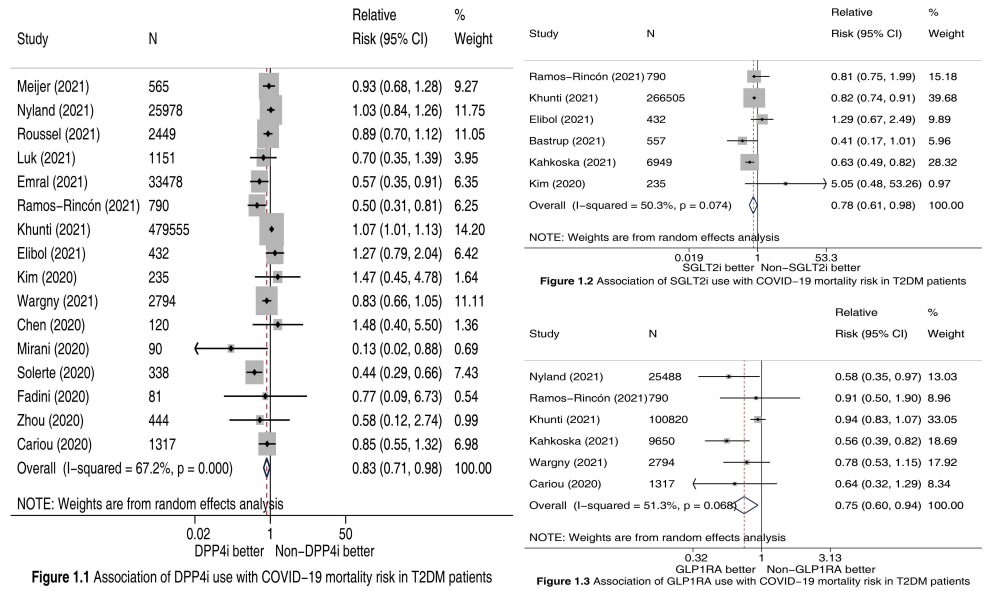

FIGURE 1 | Forest plots illustrating the association of DPP4i **(Figure 1.1)**, SGLT2i **(Figure 1.2)**, and GLP1RA **(Figure 1.3)** use with COVID-19 mortality risk in T2DM patients. DPP4i, dipeptidyl peptidase-4 inhibitors; SGLT2i, sodium-glucose cotransporter-2 inhibitors; GLP1RA, glucagon-like peptide-1 receptor agonists; T2DM, type 2 diabetes mellitus; CI, confidence interval.

Three new classes of hypoglycemic agents (i.e., SGLT2i, GLP1RA, and DPP4i) were significantly associated with reduced risk of COVID-19 death in T2DM patients with COVID-19, which highlights the potential of these new drugs used to prevent COVID-19 death in T2DM patients with COVID-19. Further studies are needed to assess whether there are significant differences in the risk of COVID-19 death among various kinds of hypoglycemic agents.

AUTHOR CONTRIBUTIONS

Design: MQ. Conduct/data collection: L-MZ, X-HC, and MQ. Analysis: L-MZ, and X-HC. Writing manuscript: L-MZ. Reviewing and editing manuscript: X-HC, and MQ. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.825100/full#supplementary-material

Supplementary Figure 1 | Test of publication bias for the meta-analysis of DPP4i versus Non-DPP4i.

Supplementary Figure 2 | Test of publication bias for the meta-analysis of SGLT2i versus Non-SGLT2i.

Supplementary Figure 3 | Test of publication bias for the meta-analysis of GLP1RA versus Non-GLP1RA.

Supplementary Figure 4 | Sensitivity analysis of DPP4i versus Non-DPP4i.

Supplementary Figure 5 | Sensitivity analysis of SGLT2i versus Non-SGLT2i.

Supplementary Figure 6 | Sensitivity analysis of GLP1RA versus Non-GLP1RA.

Supplementary Table 1 | Features of included studies and outcome data extracted from them.

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Interaction of Severe Acute Respiratory Syndrome Coronavirus 2 and Diabetes

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is causing a worldwide epidemic. It spreads very fast and hits people of all ages, especially patients with underlying diseases such as diabetes. In this review, we focus on the influences of diabetes on the outcome of SARS-CoV-2 infection and the involved mechanisms including lung dysfunction, immune disorder, abnormal expression of angiotensin-converting enzyme 2 (ACE2), overactivation of mechanistic target of rapamycin (mTOR) signaling pathway, and increased furin level. On the other hand, SARS-CoV-2 may trigger the development of diabetes. It causes the damage of pancreatic β cells, which is probably mediated by ACE2 protein in the islets. Furthermore, SARS-CoV-2 may aggravate insulin resistance through attacking other metabolic organs. Of note, certain anti-diabetic drugs (OADs), such as peroxisome proliferator-activated receptor γ (PPARγ) activator and glucagon-like peptide 1 receptor (GLP-1R) agonist, have been shown to upregulate ACE2 in animal models, which may increase the risk of SARS-CoV-2 infection. However, Metformin, as a first-line medicine for the treatment of type 2 diabetes mellitus (T2DM), may be a potential drug benefiting diabetic patients with SARS-CoV-2 infection, probably *via* a suppression of mTOR signaling together with its anti-inflammatory and anti-fibrosis function in lung. Remarkably, another kind of OADs, dipeptidyl Peptidase 4 (DPP4) inhibitor, may also exert beneficial effects in this respect, probably *via* a prevention of SARS-CoV-2 binding to cells. Thus, it is of significant to identify appropriate OADs for the treatment of diabetes in the context of SARS-CoV-2 infections.

Keywords: SARS-CoV-2 (2019-nCoV), COVID – 19, diabetes - quality of life, OADs, immunocellular response

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1 INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-a novel β-coronavirus, has influenced over 100 million people. This SARS-CoV-2 strain has become the third most lethal pathogenic human coronavirus since SARS-CoV-1, which was responsible for severe acute respiratory syndrome (SARS) in 2002 (1), and Middle-East respiratory syndrome coronavirus (MERS-CoV), which was responsible for MERS in 2012 (2).

The coronavirus belongs to a large family of single-stranded, enveloped RNA viruses, which can be divided into four genera: α-, β-, γ- and δ- coronavirus (3). All of the above 3 viruses belong to the β-genus (1, 2, 4), sharing a structural analogy (4, 5). Although the pathophysiological mechanisms and clinical invasiveness of SARS-CoV-2 have not yet been fully investigated, it probably, at least in part resembles SARS-CoV-1 and MERS-CoV.

There are more and more studies demonstrating the association between COVID-19 and diabetes, disclosing that these two diseases appear to be bi-directional. Diabetes may magnify the pathogenicity of SARS-CoV-2 because part of pathological mechanisms of diabetes overlaps with COVID-19, resulting in an increase in susceptibility and severity of COVID-19 among diabetic patients. Investigation of the underlying mechanisms would contribute to improve the clinic outcomes of these patients. On the other hand, SARS-CoV-2 infection may induce new onset diabetes, which could be possibly overlooked by nonendocrinologists. Thus, this review focuses on the bi-directional interaction between SARS-CoV-2 and diabetes and the possible mechanisms. Moreover, some glucose lowering agents may provide extra benefits for COVID-19 treatment, which may disclose some new clues for the treatment of non-diabetic patients with SARS-CoV-2 infection.

2 IMPACT OF PRE-EXISTING DIABETES ON COVID-19 PROGRESSION

Emerging data indicates that patients with diabetes are at a higher risk of SARS-CoV-2 infection. In one SARS-CoV-2 study, which includes 1,099 patients from 552 hospitals in China, the rate of diabetes was around 15%, including children and adults (6). Diabetes was reported in 34.6% of the subjects with a composite endpoint including intensive care unit admission, requirement for ventilation, and death (7). Moreover, a study with 5700 patients in New York City, USA, identified that one of the most common comorbidities caused by SARS-CoV-2 was diabetes, with a rate of 33.8% (8). Stokes et al. reported that, as of May 30, 2020, in a population of about 1.3 million patients with SARS-CoV-2 infection in the USA, around 30% of whom have been suffering from diabetes (9). Of note, the exact rates of type 2 diabetes mellitus (T2DM) in patients with SARS-CoV-2 infection vary. The inconsistent rate can be attributed to the diagnosis of both conditions, which depends on the testing methods of SARS-CoV-2 (nasal test or pharyngeal test, nucleic acids or antibodies), enrolled population (out-patient or hospitalized patients), the severity of illness (mild or severe symptom), sample sizes, and different locations.

In addition, diabetic patients are associated with a more severe course of COVID-19, who account for a higher proportion of intensive care unit (ICU)-admitted cases. A retrospective cohort study of 7,337 patients with SARS-CoV-2 infection in China showed that subjects with T2DM had a significantly higher mortality and morbidity of multiple organ injury than the non-diabetic individuals (10). The authors further demonstrated that the in-hospital death rate was significantly lower in the well-controlled blood glucose group relative to the poorly controlled group (10). An analysis of a randomly selected subset of fatal SARS-CoV-2 cases in Italy revealed a high prevalence of diabetes, with a rate as high as 35.5% (11). A COVID-19 study in Pisa, Italy identified that the mortality of SARS-CoV-2 patients was greater in hyperglycemia subjects (about 39.4%) than in patients with normal glycemia (about 16.8%) (12). The authors concluded that hyperglycemia acts as an independent factor associated with severe prognosis in hospitalized patients with SARS-CoV-2 infection (12). Lately, a large study relevant to diabetic patients from the Chinese Center for Disease Control and Prevention with 72,314 SARS-CoV-2 cases across the Mainland China also showed a higher fatality rate in diabetic patients (7.3% vs. 2.3% overall) than regular patients, second only to patients with cardiovascular diseases (13). A meta-analysis based on six published clinical studies further confirmed the negative influence of diabetes on SARS-CoV-2 infection, that diabetic patients displayed 2.95-fold higher risk of fatality after SARS-CoV-2 infection, compared to those without diabetes (14). However, most of these studies did not indicate whether the included patients were type 1 diabetes mellitus (T1DM) or T2DM. In addition, stratified analyses according to Hemoglobin A1c (HbA1c), insulin sensitivity, β cell function, and c peptide level were limited.

Likewise, patients with SARS-CoV-2 infection display increased risk of ketosis and hyperglycaemic emergencies. One observation retrieved from the Chinese population described that 42 (6.4%) out of 658 patients with SARS-CoV-2 infection presented with ketosis on admission with no obvious fever or diarrhea, and 3 (20.0%) out of the 15 COVID-19 cases with diabetic ketosis developed acidosis (15). Of note, there was only one T1DM among these included patients, indicating that it was T2DM rather than T1DM tended to develop ketosis under the context of COVID-19. A recent retrospective analysis characterized 35 T1DM and T2DM patients with hyperglycaemic emergencies including diabetic ketoacidosis (DKA), hyperosmolar hyperglycaemic state (HHS), and hyperglycaemic ketosis in the context of SARS-CoV-2 from three hospitals in north London in UK. They concluded that SARS-CoV-2 is associated with the occurrence of hyperglycemia emergencies with overrepresentation of T2DM in patients presenting with DKA and long-lasting ketosis (16). Accordingly, ketone monitoring becomes very important among diabetic patients in the COVID-19 epidemics, especially in T2DM patients. Howbeit, the sample size of these studies was relatively small. Larger study population and longer observation were needed.

3 POTENTIAL MECHANISMS OF DIABETES ON COVID-19 PROGRESSION

The reasons for increased risk of coronaviral infection in diabetic patients are not fully identified. Multifarious mechanisms are possibly involved in the disease susceptibility and progression. Particularly, previous investigations on SARS-CoV-1 and MERS-CoV may provide evidence on the pathophysiology of SARS-CoV-2 infection in patients with diabetes.

3.1 Diabetes and Lung Dysfunction

Diabetes is associated with a change in lung physiology and structure. It has been reported an increase of extracellular matrix synthesis in the pulmonary alveolar wall in diabetic rat model, resulting in a reduction of lung elasticity and a decrease in alveolar space (17). Furthermore, Weynand et al. identified that alveolar epithelial basal lamina in diabetic patients was significantly thicker than that in healthy subjects. This result reveals that hyperglycemia leads to the thickening of the pulmonary microvascular wall (18), which potentially participates in the damage of alveolar-capillary membrane, and in turn, reduces the gas diffusion rate in diabetic patients (19).

The above structure changes in lung result in abnormalities of pulmonary function including lung volume, pulmonary diffusing capacity, pulmonary ventilation, bronchomotor tone, and neuroadrenergic bronchial innervation (20). A prospective study of 125 patients with T2DM revealed that absolute and percentage-predicted lung function measures including forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), vital capacity (VC), and peak expiratory flow (PEF) were significantly reduced after 7-year follow-up (21).

Accordingly, the impaired pulmonary function in patients with pre-existing diabetes may partly explain the susceptibility and poor outcomes of diabetic patients after SARS-CoV-2 infection (**Figure 1**).

3.2 Diabetes and Immune Dysfunction

Diabetes, even a short-term hyperglycemia, has been shown to impair the balance of immune system (22). Although the immunologic mechanisms induced by SARS-CoV-2 are not fully elucidated, existing data derived from the close counterparts SARS-CoV and MERS-CoV may bring some enlightenment. Part of mechanisms of immune dysfunction between diabetes and COVID-19 may cross, including impaired neutrophil function, pro-inflammatory inclination and T cell imbalance under hyperglycemia.

3.2.1 Diabetes and Impaired Neutrophil Function

In the innate immune response, neutrophils play a crucial role in chemotaxis and phagocytosis. Under hyperglycemia, neutrophil dysfunction has been observed in both humans and rodents (23). Possibly, acute hyperglycemia impairs the respiratory burst capacity of neutrophils, the process by which immune cells release toxic chemicals and kill pathogensr (24). Moreover, hyperglycemia reduced neutrophil degranulation (25) and impaired superoxide production from activated neutrophils (26).

Thus, impaired neutrophil function leads to an increase in risk and severity of infections (**Figure 1**), suggesting that glucose homeostasis in patients with SARS-CoV-2 infection may help to stabilize the ability of neutrophils for a proper innate immune response. Interestingly, a significant increase in neutrophil counts was observed in patients with both diabetes and SARS-CoV-2 infection (10, 12). Howbeit, few literature have described the function of neutrophils under the context of SARS-CoV-2 infection, which requires further investigation.

3.2.2 Diabetes and Pro-Inflammatory State

It has been known that both diabetes and obesity cause a low grade pro-inflammatory state in the body with increased secretion of cytokines including interleukin (IL)-1, IL-6, IL-8 and tumor necrosis factor-α (TNF-α) (23). In diabetic and obese patients, excess cytokines in circulation keep the immune system in "threat" mode.

This hypercytokinemia seems to play a crucial role in the development of pulmonary fibrosis (27, 28). Evidences show that cytokine storm, also known as cytokine release syndrome (CRS),

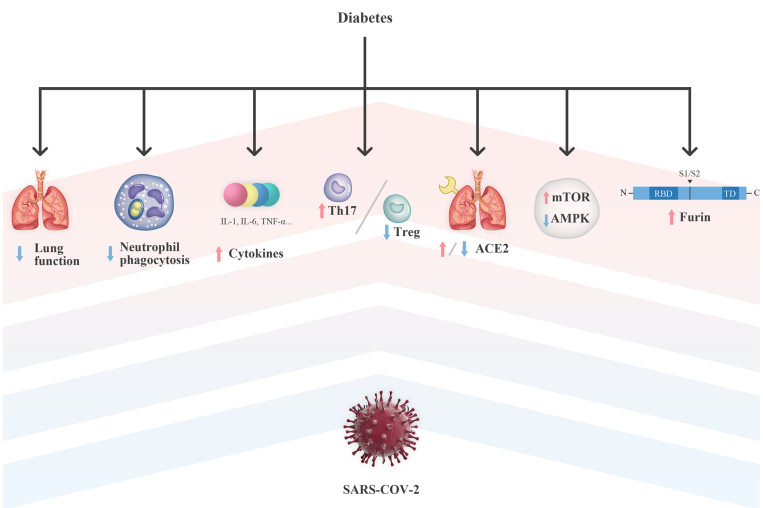

FIGURE 1 | Potential mechanism of diabetes on the susceptibility and severity of COVID-19. ACE2, Angiotensin-converting enzyme 2; AMPK, Adenosine 5'-monophosphate-activated protein kinase; IL, interleukin; mTOR, mechanistic target of rapamycin; RBD, receptor binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TD, transmembrane domain; Th17, T helper 17; TNF-α, tumor necrosis factor-α; Treg, regulatory T.

might be involved in lung injury and increased mortality, thus playing a major role in severe COVID-19 cases. Inflammatory cytokines and chemokines, including IL-1β, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-17, TNF-α, interferon- γ (IFNγ), interferon gamma-induced protein 10 (IP10) and monocyte chemoattractant protein-1 (MCP-1), were significantly elevated in COVID-19 patients (29–34), some of which overlap with the cytokine panel in diabetes.

Among those overlapped cytokines, IL-6 is of particular interest and appears to be closely related to the occurrence of severe SARS-CoV-2 infection. It is an important pleiotropic cytokine, which is involved in acute inflammatory response and lung injury (35). In addition, elevated levels of IL-6 were observed in patients with SARS in 2004 and, in particular, were positively correlated to disease severity (36). A retrospective, multicentre cohort study in Wuhan in this epidemic found that IL-6 levels were closely associated with the mortality in patients with SARS-CoV-2 infection (34). Likewise, ICU patients with severe pneumonia in another study also showed a significant elevation in plasma level of IL-6 (37).

Importantly, serum levels of IL-6 in patients with diabetes and SARS-CoV-2 infection were significantly higher than those in non-diabetic patients (10, 38). This might be explained by that the increased cytokine baseline level and pro-inflammatory state observed in diabetic patients are further amplified by SARS-CoV-2 infection (**Figure 1**), resulting in an aggressive inflammatory response, even CRS.

3.2.3 Diabetes and T Cell Imbalance

It has been demonstrated that in patients with either T1DM or T2DM, some "helpful" immune cells including certain subsets of $CD4^+$ and $CD8^+$ T cells are decreased, while some proinflammatory immune cells, such as T helper 17 (Th17) cells, are increased (39). Data supports the concept that Th17/regulatory T (Treg) cell mediated immune responses are involved in the pathogenesis in obesity-related T2DM (39).

A previous evidence demonstrated that acute intracellular bacterial infection in the diabetic host was associated with the Th17/Treg-mediated immune imbalance, resulting in exaggerated inflammatory cascades (40). In 2019, Kulcsar et al. created a diabetic mouse model with the expression of human dipeptidyl peptidase 4 (DPP4), which is identified as the cellular binding site for MERS-CoV (41). These mice developed severe infection and displayed a more prominent Th17 response with increased levels of IL-17. Due to the viral homology between SARS-CoV-2 and MERS-CoV, it is speculated that Th17/Treg-mediated immune responses may play an essential role in the aggravation of SARS-CoV-2 infection in diabetic patients.

It has been revealed that the pathological characteristics of COVID-19 observed by postmortem pathology greatly resemble those observed in SARS-CoV-1 and MERS-CoV infection (42). In this SARS-CoV-2 case, pro-inflammatory T cells were overactivated, manifested by increase of $CCR4^+CCR6^+$ Th17 cells and high cytotoxicity of $CD8^+$ T cells (42). Notably, an extensive multi-omics dataset demonstrated that the frequencies of $CD4^+$ T cells that can secrete Th17 cytokines, including IL17-A and IL17-F, are increased in SARS-CoV-2 infected patients (43). Moreover, Pacha et al. indicated that the severity of disease is positively correlated with plasma levels of IL-17 and other Th17 cell-related cytokines (33). Specifically, Qin et al. identified that patients with SARS-CoV-2 infection also show lower levels of Treg cells, which are even lower in severe cases (44).

Thus, SARS-CoV-2 may affect circulating immune cells and exacerbate the uncoordination of the innate immune system, which are previously present in diabetic patients, and therefore, resulting in a deluge of inflammatory cytokines and a further damage of the organs (**Figure 1**).

3.3 Diabetes and mTOR Signaling

During the progression of T2DM, Adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) is inactivated, leading to a chronic overactivation of mechanistic target of rapamycin (mTOR) C1 (45), which has been associated with insulin resistance and progression of diabetes-induced complications (46).

Increasing evidences also highlight mTOR as a key factor in regulating the replication of viruses. Rapamycin, which is a mTOR inhibitor, was found to be a potent inhibitor for the RNA replication of hepatitis C virus (HCV) (47) and MERS-CoV (48). In patients with severe H1N1 pneumonia, early adjuvant treatment with corticosteroids and mTOR inhibitor effectively blocked the expression of viral protein and the release of virion, and therefore, significantly improved the prognosis of disease (49). These findings disclose a potential anti-viral treatment for the patients with SARS-CoV-2 infection through an inhibition of the metabolic sensor mTOR.

It is known that stress-induced Regulated in Development and DNA Damage Responses 1 (REDD1) negatively regulates mTOR activity (50). Interestingly, IL-6, which is closely related to the progression and severity of COVID-19, directly enhances the activation of mTOR by reducing the expression of REDD1 (50). On the contrary, IL-37 performs its immunosuppressive activity by targeting mTOR as well, suggesting that IL-37 administration may be a possible therapeutic strategy for SARS-CoV-2 treatment (51). More importantly, a recent study tested 16 repurposable anti-human coronavirus (HCoV) drugs, that may provide a synergistic effect for the treatment of SARS-CoV-2, by analyzing their drug-gene signatures and HCoV-induced transcriptomics in human cell lines. Among these candidates, Sirolimus, which consists of an mTOR inhibitor and dactinomycin, may be a potential drug for COVID-19 treatment (52).

Thus, these findings suggest that the dysregulation of AMPK/mTOR signaling in the setting of T2DM may be a plausible explanation for the increased susceptibility of diabetic patients to COVID-19 (**Figure 1**).

3.4 Diabetes and ACE2

Angiotensin-converting enzyme 2 (ACE2) is a transmembrane glycoprotein which is expressed in organs throughout the whole human body, mainly in lung, intestine, kidney, blood vessels, and pancreas. The renin-angiotensin system (RAS) signaling pathway comprises both ACE, which metabolizes angiotensin I (Ang I) into angiotensin II (Ang II), and ACE2, which degrades Ang II to Ang 1-7 peptide (53). It has been reported that both

SARS-CoV-1 and SARS-CoV-2 bind and gain entry to infected cells through ACE2 (4, 54), so that increased expression of ACE2 may contribute to increased chances of SARS-CoV-2 infection.

However, the role of ACE2 in the crosstalk between COVID-19 and diabetes is still a matter of debate, which may be largely attributed to the inconsistent expression of ACE2 in different tissues and stages of diabetes (**Figure 1**). The effect of hyperglycemia on the expression of ACE2 in different organs was investigated in non-obese diabetes (NOD) mouse models with the increased expression of ACE2 in the serum, liver, and pancreas (55). In rats with streptozotocin-induced diabetes, there also showed an upregulation of ACE2 in isolated jejunal enterocytes (56). In addition, in adipocytes from obese and diabetic patients, an increased expression of ACE2 was also observed, suggesting that adipose tissue is a viral reservoir and could be a potential target for antiviral treatment (57). Recently, a phenome-wide Mendelian randomization study has explored and identified T2DM as a disease causally associated with increased expression of ACE2 (58).

Surprisingly, Roca-Ho et al. has reported that the activity ratio of ACE2/ACE in the lung was significantly decreased in the late-stage of diabetes in NOD Mouse (55). Although a study from Wysocki et al. has concluded that ACE2 expression was increased at the posttranscriptional level in the renal cortex in diabetic mice (59), ACE2 expression was shown to be decreased in the tubules in individuals with overt diabetic nephropathy (60).

Remarkably, it remains controversial whether an increased expression of ACE2 is responsible for the increased infectivity and severity of COVID-19. Some authors even consider that ACE2 plays a beneficial role in patients with COVID-19 (61, 62). It was demonstrated that ACE2 protects murine lungs from acute respiratory distress syndrome (ARDS) by decreasing inflammation and vascular permeability (63, 64). Previous studies have shown that the transmembrane spike (S) glycoprotein of SARS-CoV-1 downregulates ACE2 by shedding its ectodomain, an enzymatically active domain, and transforming it to soluble ACE2 (sACE2) (65–68). The biological function of sACE2 remains poorly investigated. However, it is assumed that sACE2 may act as a competitive interceptor for SARS-CoV-2 by inhibiting the binding of the S protein to ACE2 (69). A recent study has shown that treatment with clinical-grade human recombinant soluble ACE2 (hrsACE2) *in vitro* significantly inhibited the growth of SARS-CoV-2 in the monkey kidney cells (70), indicating that sACE2 potentially prevents SARS-CoV-2 infection. Howbeit, whether sACE2 plays a role in the disease progress in diabetic patients with SARS-CoV-2 infection remains unknown.

Taken together, ACE2 not only serves as a portal entry for SARS-CoV-2, but also plays a protective role against lung injury in its soluble form. The contradictory function of ACE2, especially, its role in the state with both diabetes and SARS-CoV-2 requires further investigations.

3.5 Diabetes and Furin

Afore mentioned transmembrane S protein of coronavirus is composed of two functional subunits: S1 region, which is responsible for its binding to the host cell ACE2 receptor, and S2 region, which is responsible for fusion of the viral RNA and cellular membranes. The S1 region could be cut and released by proteases, following by the entry of virus into the cells (71). It was previously discovered that MERS-CoV S protein can be activated by furin, a common protease responsible for membrane fusion (72). Likewise, it has been identified a furin cleavage site at the S1/S2 boundary of SARS-CoV-2 (73), indicating that furin could also cleave SARS-CoV-2 S protein (74) and possibly promote the viral entry.

Interestingly, diabetic patients show increased levels of furin (75). It may be driven by osteopontin, which is a cytokine-like matrix-associated phosphoglycoprotein and is shown to be elevated in diabetes (76). In addition, both T1DM and T2DM are associated with higher levels of plasmin(ogen), a protease enzyme that can cleave S protein of SARS-CoV-2 at furin site (77, 78). Thus, increased levels of furin and plasmin may aggravate the viral infection through assisting their entry, fusion, duplication, and release in cells.

4 COVID-19 MAY TRIGGER/WORSEN THE DEVELOPMENT OF DIABETES

As described above, diabetes worsens SARS-CoV-2 infection through mechanisms including impairing the pulmonary structure and function, disturbing the immune function, enhancing the expression of ACE2, overactivating the mTOR signaling, and inducing the furin levels. However, interplay between diabetes and COVID-19 appears to be bi-directional, as new onset diabetes has been observed in patients with SARS-CoV-2 infection (79). Multicenter regional data from North West London reported that thirty children, aged 23 months to 16.8 years, displayed new onset T1DM in this season. In comparison with the data from a typical year, it represents an additional 12-15 new T1DM cases with an increase rate of 80% during the COVID-19 pandemic (80).

These observations revealed a potential diabetogenic effect of COVID-19. The so induced diabetes may be different from the well-recognized notion of hyperglycemia induced by severe illness-associated stress response. To address the issues about the frequency, phenotype, and pathophysiology of SARS-CoV-2-induced new onset diabetes, an international group of leading diabetes researchers participating in the CoviDIAB Project have established a global registry of patients with SARS-CoV-2-related diabetes (covidiab.e-dendrite.com) (81).

The theoretical basis of new onset diabetes induced by SARS-CoV-2 could be supported by the findings of ACE2 expression in both exocrine and endocrine pancreas (islet cells) (82). In addition to lung, SARS-CoV-2 also attacks other organs and tissues including liver, brain, cardiovascular system, gastrointestinal system and pancreas, largely because of the wide distribution of ACE2 in those organs. Interestingly, the expression of ACE2 on the mRNA level in pancreas is slightly higher than that in the lung (82). Thus, SARS-CoV-2 probably causes the damage of pancreatic islets *via* ACE2 in pancreas (**Table 1**).

SARS-CoV-2 infection may not only worsen the preexisting diabetes but also cause new cases of diabetes in non-diabetic

subjects through a direct pancreatic damage and a resultant impairment of insulin secretion from β-cells. This can be partly proved by the study from Wang and colleagues (83). It was found that 9 out of 52 hospitalized patients with SARS-CoV-2-associated pneumonia in China displayed a pancreatic injury, which was determined by the plasma levels of amylase and lipase. Of note, 6/9 of these subjects exhibited moderate increases in plasma glucose (83). This phenomenon is in accordance with what is observed in patients after SARS-CoV-1 infection at the beginning of this century. Yang et al. reported that patients with SARS-CoV-1 infection, who had never received glucocorticoids, presented significantly higher levels of fasting plasma glucose compared to patients with non-SARS pneumonia (87). They supposed that SARS-CoV-1 may mediate the damage of pancreatic β-cells and resulted in a development of 'acute diabetes' in patients with SARS (87). In fact, SARS-CoV-1 has been identified in the pancreas of the patients who died of SARS by means of immunohistochemistry and *in situ* hybridization (88). Thus, coronavirus, SARS-CoV-1 as well as SARS-CoV-2, could be one of the pathogens and trigger the development of diabetes.

Apart from direct pancreatic β-cell damage, abnormal immune response such as inflammatory storm and T cell imbalance could also be another explanation for the new onset diabetes caused by SARS-CoV-2 (**Table 1**). As described previously, cytokines including IL-1β, IL-6, IL-17, TNF-α, and IFNγ were significantly elevated in patients with SARS-CoV-2 infection (29–34). Interestingly, the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study revealed that a combined elevation of IL-1β and IL-6 has been shown to independently increase the risk of the development of T2DM (84). Th17 cytokine profile, which plays a major role in the regulation of inflammation and hyperglycemia, could mathematically predict T2DM in obese people (89). These findings further suggest that SARS-CoV-2 might trigger the development of new onset diabetes through an overactivation of the immune system.

Moreover, SARS-CoV-2 could worsen the insulin resistance in patients with pre-existing T2DM. As ACE2 expression is particularly amplified in key metabolic organs such as the liver, adipose tissue, and the small intestine (55–57), SARS-CoV-2 may attack these organs, resulting in insulin insensitivity and an exacerbation of hyperglycemia. In addition, it has been reported that acute viral infection by murine cytomegalovirus (MCMV) could induce a rapid development of transient insulin resistance through a specifical downregulation of the insulin receptors in skeletal muscle in rodents (90). Whether SARS-CoV-2 enhances the progression of systemic insulin resistance and its mechanisms still require further investigations.

Notably, glucocorticoid, which is an essential medication to control CRS in COVID-19, impairs insulin sensitivity and result in hyperglycemia too. Human immunodeficiency virus (HIV) protease inhibitors (PIs), such as Lopinavir-ritonavir, are used for the treatment of COVID-19. However, it is reported that HIV PIs may acutely and reversibly inhibit the insulin-responsive glucose transporter 4, leading to peripheral insulin resistance and impaired glucose tolerance (85). In a cross-sectional study recruited 710 HIV-infected patients, lopinavir/ritonavir was found to be significantly associated with some metabolic syndromes after adjustment for age and BMI (86). Thus, some medications, such as glucocorticoid and HIV PIs, which are used for SARS-CoV-2 treatment, should be further evaluated, particularly in SARS-CoV-2 infected patients with pre-existing diabetes or at high risk of diabetes (**Table 1**).

5 TREATMENT OF COVID-19 IN PATIENTS WITH DIABETES

In concerning to an optimized treatment of patients with coexistent diabetes and COVID-19, a glycemic control seems to be very urgent (10, 91). However, there are still several cautions need to be taken to achieve the appropriate therapeutic strategies, especially in T2DM patients.

5.1 Metformin

As mentioned above, SARS-CoV-2 infection might worsen the prognosis of diabetes by dysregulating the AMPK/mTOR signaling (**Figure 1**) (45, 46, 51, 52). Observations suggest that activating AMPK and/or inhibiting mTOR-mediated signaling could be novel therapeutic intervention strategies for COVID-19 treatment (51, 52). Of note, dimethylbiguanide metformin is so far a first-line medication for the treatment of T2DM, which works as an AMPK activator (92). To a certain extent, this molecular mechanism makes the classical medication possible as antivirals in patients with COVID-19.

Interestingly, although a hypoglycemic effect of metformin was discovered in the 1920s, it was disregarded until 1940s when biguanide was reinvestigated in the search for antimalarial agents and repurposed to treat influenza (93). Another biguanide, which is so-called flumamine, is still in use as an anti-influenza and anti-malarial medication in the Philippines (94). As

TABLE 1 | Potential mechanisms of SARS-CoV-2 that triggers and worsens the development of diabetes.

Potential mechanism	Reference
SARS-CoV-2 causes the direct damage of pancreatic islets, partly *via* ACE2 in pancreas	(82, 83)
SARS-CoV-2 causes abnormal immune response such as inflammatory storm and T cell imbalance	(84)
SARS-CoV-2 worsens the insulin resistance through attacking key metabolic organs such as the liver, adipose tissue, and the small intestine	(55–57)
Medications for SARS-CoV-2 treatment, such as glucocorticoid and HIV PIs, impairs insulin sensitivity	(85, 86)

ACE2, Angiotensin-converting enzyme 2; HIV, Human immunodeficiency virus; PI, protease inhibitor; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; T2DM, type 2 diabetes mellitus.

antivirals, biguanides do not cause lung toxicity. Thus, when inhaled, it may deliver more predictable amount of biguanide to the lung than oral dosing and limit the risk of systemic side-effects (95).

Moreover, metformin exerts anti-inflammatory actions by decreasing the circulating biomarkers of inflammation in people with or without T2DM (96). Metformin can also modulate the immune response and restore immune homeostasis in T cells (97), and more in particular, a reciprocal balance between Th17 and Treg cells *in vitro* and *in vivo* (98). In a cohort of 1,213 hospitalized patients with COVID-19 and pre-existing T2DM, Cheng et al. show that the dynamic trajectories of serum inflammatory factors, including IL-6, IL-2, and TNF-a, all showed lower degrees of elevation in the metformin group than in the non-metformin group, particularly compared to the subgroup of individuals with severe COVID-19 (99). This consists with the findings from Chen et al. that metformin applicants showed overall lower levels of IL-6 on admission compared to ordinary patients (100).

Lately, it was identified that metformin exerts potent antifibrotic effects in the lung by inhibiting transforming growth factor (TGF)-β1 action, suppressing collagen formation, activating peroxisome proliferator-activated receptor γ (PPARγ) signaling and inducing lipogenic differentiation in lung fibroblasts (101). However, there is scant information about the antifibrotic actions of metformin in the context of coronavirus infection.

In this respect, metformin may have the potential to benefit diabetic patients with SARS-CoV-2 infection in some ways (**Figure 2**). Interestingly, two observational studies also identified its application resulted in a decrease in the mortality of patients with COVID-19 and T2DM (102, 103). More extensive studies are necessitated to estimate the effectiveness of metformin in COVID-19. Howbeit, metformin-associated acidosis in these patients, particularly in cases with severe COVID19 needs to be noted (99). Regular monitoring of lactic acid levels is recommended after metformin administration in certain patients.

5.2 DPP4 Inhibitor

DPP4 inhibitors act selectively to inhibit the catalytic activity of DPP4, and are widely used in clinic for the treatment of T2DM (104). A recent retrospective study identified that the use of DPP4 inhibitors was significantly and independently associated with a lower mortality (Hazard ratio 0.13) and a less severe pneumonia among diabetic patients with SARS-CoV-2 infection (105). Consistent results were reported by Solerte and colleagues, that treatment with sitagliptin, a highly selective DPP4 inhibitor, at the time of hospitalization resulted in an improved clinical outcomes and a greater number of hospital discharges (106).

Interestingly, membrane-associated human DPP4 acts as a functional coronavirus receptor (107). Transgenic mice engineered to express human DPP4 (hDPP4) became susceptible to MERS-CoV (108). Moreover, DPP4 also acts as a functional receptor for hCoV-Erasmus Medical Center (hCoV-EMC), another member of coronavirus family, which was identified to cause severe and sometimes lethal lower respiratory tract infection (107). Furthermore, it was identified that the S1 domain of SARS-CoV-2 spike glycoprotein potentially interacts with the human DPP4 by means of a computational model based selective docking (109).

Although above findings revealed that DPP4 serves as a potential co-receptor for coronaviruses, few data have been shown whether DPP4 inhibitors interfere the binding of SARS-CoV-2 to DPP4. Available evidences were mostly derived from studies on MERS-CoV. Anti-DPP4 antibodies blocked acute MERS-CoV infection in susceptible bat cells in a dose-dependent manner (110). Administration of two human anti-DPP4 antibodies (REGN3051 and REGN3048) could interrupt the interaction between MERS-CoV spike protein and hDPP4, and lead to a mitigated lung pathology in mice with experimental MERS-CoV infection (111). Similarly, Tang et al. also reported that human neutralizing antibodies directed against the receptor-binding domain (RBD) of the MERS-CoV spike protein, which blocked the viral binding to hDPP4 (112).

Our previous review has proposed that DPP4 inhibition also presents potential modulatory functions in the immune system (113), which may contributes to a reduction in inflammatory response in patients with SARS-CoV-2 infection (**Figure 2**). DPP4, originally known as the lymphocyte cell surface protein CD26, is widely expressed in many types of immune cells including $CD4^+$ and $CD8^+$ T cells, B cells, natural killer (NK) cells, dendritic cells, and macrophages; and plays a role in the regulation of cell function (113). Enzymatic activity inhibition of DPP4 leads to an upregulation of Treg lymphocytes, downregulation of Th17 cells, and suppression on the secretion of pro-inflammatory cytokines, such as IL-1, 6 and 10 (113). Thus, when applied in T2DM patients with SARS-CoV-2 infection, it would be significant to monitor DPP4 inhibitor-induced alterations in immune indexes.

5.3 PPARγ Activator and GLP-1R Agonist

Certain anti-diabetic drugs like PPARγ activators have been shown to upregulate ACE2 in animal models (114). Treatment of diabetic rats with liraglutide, a glucagon-like peptide 1 receptor (GLP-1R) agonist, also provoked a strong elevation in pulmonary ACE and ACE2 expression on mRNA levels (115). As such, it is speculated that treatment for diabetes with pioglitazone/liraglutide may increase the risk of severe and fatal COVID-19 development (**Figure 2**).

On the other hand, pulmonary lipofibroblasts located in the alveolar interstitium displayed an ability to differentiate into myofibroblasts, which play an integral role in pulmonary fibrosis (57) and are potentially involved in the exacerbation of pneumonia in patients with SARS-CoV-2 infection. Yet, PPARγ activators are able to stabilize lipofibroblasts in their "inactive" state, preventing the transition of the cells into myofibroblasts, so that they inhibit the development of pulmonary fibrosis (116). The therapeutic effect of rosiglitazone in the murine models of bleomycin-induced pulmonary fibrosis is well determined (117, 118). However, the putative pathophysiological significance of these findings in the context of experimental coronavirus infection has not been fully explored.

GLP-1R agonists exert broad anti-inflammatory actions in humans with T2DM as well as in obese individuals (119). It was

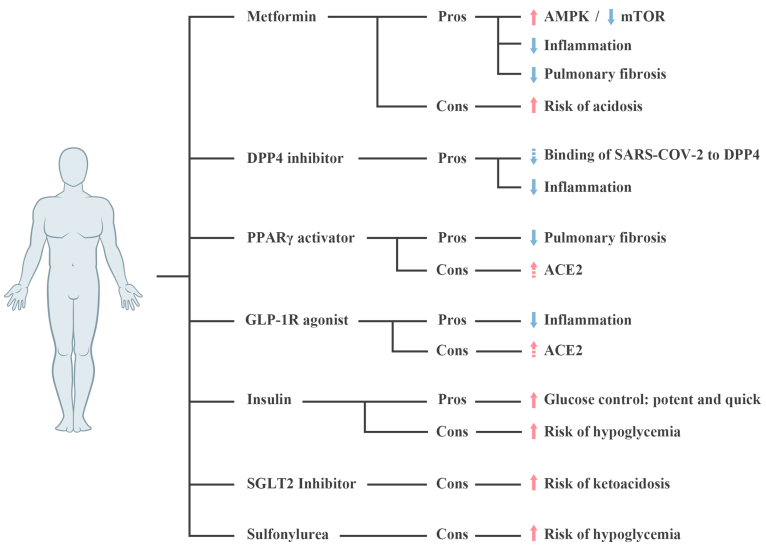

FIGURE 2 | Pros and cons of glycemic lowering agents in diabetic patients with SARS-CoV-2 infection. Dashed arrow represents the assumption requiring further verification. ACE2, Angiotensin-converting enzyme 2; AMPK, Adenosine 5'-monophosphate-activated protein kinase; mTOR, mechanistic target of rapamycin; DPP4, dipeptidyl Peptidase 4; GLP-1R, glucagon-like peptide 1 receptor; PPARγ, peroxisome proliferator-activated receptor γ; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SGLT2, sodium-glucose cotransporter 2.

revealed that GLP-1 agonists contribute to a reduction in plasma levels of pro-inflammatory cytokines including TNF-α, IL-1β and IL-6, and an increase in plasma levels of adiponectin, which belongs to the anti-inflammatory adipokines, in T2DM patients (120). Moreover, multiple preclinical studies have demonstrated that GLP-1R agonists attenuate pulmonary inflammation and even preserve lung function in rodent models with experimental lung injury (121–124).

So far, the safety about continuous administration of PPARγ activators and GLP-1R agonists in patients with SARS-CoV-2 infection are not stated. Further studies are needed to clarify whether the administration of these two medicines is suitable for diabetic patients with SARS-CoV-2 infection.

5.4 Insulin, SGLT2 Inhibitor, and Sulfonylurea

Insulin has been extensively used for decades to control blood glucose in critically ill patients with diabetes (125, 126). Accordingly, insulin therapy has been recommended by many experts for the treatment of diabetic patients with SARS-CoV-2 infection (127, 128). However, in a study with 120 patients with COVID-19 and T2DM by Chen et al., the insulin and non-insulin groups showed no significant difference in the percentages of severe and critical illness on admission (100). Surprisingly, a retrospective analysis of 689 patients with COVID-19 and T2DM revealed a markedly increased mortality in patients with insulin treatment (129). Hypoglycemia may be one of the important drivers causing insulin-associated higher mortality. Accordingly, frequent glucose monitoring, even the application of continuous glucose monitoring may lower the rates of hypoglycemia emergence and improve the clinical outcomes.

Sodium-glucose cotransporter 2 (SGLT2) inhibitors are a novel class of anti-diabetic drugs (OADs) which inhibit the reabsorption of sodium and glucose in urine (130). It has been reported that this kind of OAD could increase ketone accumulation and may induce euglycemic ketoacidosis (euDKA) (130). Besides, SARS-CoV-2 infection may be associated with anorexia, dehydration, and rapid deterioration in clinical status. Although SGLT2 inhibitors are generally well tolerated in the outpatient setting, it may still increase the risk of volume depletion and euDKA in symptomatic individuals with T2DM and acute SARS-CoV-2 infection (131). Accordingly, the use of SGLT2 inhibitors should be cautiously re-evaluated in patients with severe SARS-CoV-2 infection, particularly in patients suffering from dehydration. Furthermore, sulfonylureas should be avoided to use in T2DM patients with gastrointestinal symptoms such as diarrhea and nausea, owing to its hypoglycemia side effect.

5.5 Non-Hypoglycemic Agents

ACE inhibitors (ACEIs) and angiotensin II type-I receptor blockers (ARBs) are frequently taken by individuals with diabetes. However, it was found that ACEI and ARB treatment in T1DM and T2DM cause a substantially raise of ACE2 expression in the renal, duodenal, and cardiovascular systems (132–134). Yet, an elevation of ACE2 levels in the respiratory system after ACE/ARBs application has not been reported. Clinical outcomes of patients with SARS-CoV-2 infection who took RAS inhibitors were comparable with those of patients without ACEI/ARB administration (100, 135). Nevertheless, whether these medicines are safe for the treatment of patients with SARS-CoV-2 infection requires more experimental and clinical evidences.

6 CONCLUSIONS

In this review, the relationship of diabetes and COVID-19 were discussed. However, most of the existing studies failed to distinguish the potential difference of type 1 and type 2 diabetes among COVID-19 subjects. Furthermore, we describe the potential mechanisms which are involved in the regulation of an increased susceptibility and illness severity of diabetic patients to COVID-19. On the other hand, SARS-CoV-2 infection may also trigger the new onset diabetes. The rapid increase in new experimental information stemming from the SARS-CoV-2 epidemic requires careful evaluation to help understand the pathology of COVID-19, especially for the treatment of patients with both diabetes and COVID-19. All medications used in diabetes displayed advantages and disadvantages. Physicians should estimate the status of patients individually, especially the risk of hypoglycemia, acidosis and gastrointestinal symptoms, to optimize the therapeutic strategies for glycemic control in patients with SARS-CoV-2 infection.

AUTHOR CONTRIBUTIONS

SS and YC wrote the manuscript. RP and QY edited the manuscript. XY edited the revised manuscript. All authors contributed to the article and approved the submited version.

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Blood Hemoglobin Substantially Modulates the Impact of Gender, Morbid Obesity, and Hyperglycemia on COVID-19 Death Risk: A Multicenter Study in Italy and Spain

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Background: Hyperglycemia and obesity are associated with a worse prognosis in subjects with COVID-19 independently. Their interaction as well as the potential modulating effects of additional confounding factors is poorly known. Therefore, we aimed to identify and evaluate confounding factors affecting the prognostic value of

obesity and hyperglycemia in relation to mortality and admission to the intensive care unit (ICU) due to COVID-19.

Methods: Consecutive patients admitted in two Hospitals from Italy (Bologna and Rome) and three from Spain (Barcelona and Girona) as well as subjects from Primary Health Care centers. Mortality from COVID-19 and risk for ICU admission were evaluated using logistic regression analyses and machine learning (ML) algorithms.

Results: As expected, among 3,065 consecutive patients, both obesity and hyperglycemia were independent predictors of ICU admission. A ML variable selection strategy confirmed these results and identified hyperglycemia, blood hemoglobin and serum bilirubin associated with increased mortality risk. In subjects with blood hemoglobin levels above the median, hyperglycemic and morbidly obese subjects had increased mortality risk than normoglycemic individuals or non-obese subjects. However, no differences were observed among individuals with hemoglobin levels below the median. This was particularly evident in men: those with severe hyperglycemia and hemoglobin concentrations above the median had 30 times increased mortality risk compared with men without hyperglycemia. Importantly, the protective effect of female sex was lost in subjects with increased hemoglobin levels.

Conclusions: Blood hemoglobin substantially modulates the influence of hyperglycemia on increased mortality risk in patients with COVID-19. Monitoring hemoglobin concentrations seem of utmost importance in the clinical settings to help clinicians in the identification of patients at increased death risk.

Keywords: COVID-19, hemoglobin, hyperglycemia, obesity, epidemiology, mortality, machine learning

1 INTRODUCTION

Since the first reports from China at the beginning of the COVID-19 pandemic, age, male sex, obesity, type 2 diabetes mellitus (T2D), arterial hypertension (AHT) and cardiovascular disease have been identified as established risk factors for a poor prognosis in patients with SARS-CoV-2 infection (1). Initial multivariate analysis confirmed that individuals with BMI > 40 kg/m^2 had 2.5 (1.8-3.4) (odds ratio and 95% confidence interval) times more risk for hospital admission, and those with grade II obesity had 7.36 (1.6-33) times more requirement of invasive mechanical ventilation, compared with normal-weight COVID-19 patients (1–3).

Different systematic reviews have substantiated that subjects with obesity are at higher risk for hospitalization, ICU admission and mortality (4, 5). However, these studies did not mention the detailed comorbidities of patients with obesity, which may confound the role of obesity as an independent risk factor in COVID-19 (6). For instance, in a community-based cohort study, both obesity and central obesity had an upward linear trend with the COVID-19 hospitalization that was attenuated after adjustment for confounding factors. For example, HbA1c and HDL cholesterol attenuated the association by 33% and 46%, respectively (7). In addition, the different results across the studies can be simply justified by the fact that many of them did not analyze laboratory parameters, but just the demographic characteristics of patients.

On the other hand, the prevalence of diabetes among patients with COVID-19 varied from 6% to more than 22% in subjects with mild disease or severe forms, respectively, and raised to more than 30% among subjects requiring ICU admission (1, 8). In a retrospective, multi-centered study of 7,337 cases in HubeiT2D patients required more medical interventions, had a significantly higher mortality (7.8% versus 2.7%; adjusted hazard ratio (HR), 1.49), and multiple organ injury than non-diabetic individuals. Patients with well-controlled blood glucose concentration (glycemic variability within 3.9 to 10 mmol/L) had markedly lower mortality compared to individuals with poorly controlled blood glucose (upper limit of glycemic variability exceeding 10.0 mmol/L) (adjusted HR=0.14) during hospitalization. However, the results were not adjusted by the potential confounding effects of adiposity (9). Other studies have found diverging effects of impaired fasting glucose and T2D diagnosis on COVID-19 prognosis (10). Hyperglycemia in the acute phase also predicted worse outcomes in hospitalized patients with COVID-19. For instance, a study of 184 hospitalized patients showed that severe COVID-19 occurred in the presence of both impaired glucose metabolism and obesity (11). However, most of the studies reporting an association between hyperglycemia and a poorer prognosis did not take

into account the confounding effects of obesity status, whereas studies assessing obesity status on COVID-19 did not include comorbidities such as diabetes (12–16).

Besides considering the joint role of obesity and diabetes in modifying COVID-19 outcomes, it is vital to identify those factors that modulate the impact of both obesity and hyperglycemia on COVID-19 prognosis. For example, the levels of albumin, hemoglobin, the mean platelet volume, and inflammatory markers (monocyte to eosinophil or neutrophil to lymphocyte ratios) are prognostic markers in patients with COVID-19 (17–19). In particular, anemia has been shown to be an independent predictor of mortality in COVID-19 patients (20–22). Therefore, we hypothesized that the inclusion of inflammatory and hematological markers would modulate the associations of obesity and hyperglycemia with death and ICU admission. We first aimed to evaluate the joint role of obesity and diabetes in modifying COVID-19 outcomes. Then, we applied a machine learning algorithm to identify additional confounding factors with potential impact on modulating the obesity and hyperglycemia prognostic values.

2 MATERIALS AND METHODS

2.1 Outcomes

The primary outcomes were all-cause mortality due to COVID-19 and admission to an ICU. For ICU and mortality risk, only hospital-admitted patients were evaluated. Consecutive unselected patients who were admitted to the hospital and diagnosed with pneumonia by SARS-CoV-2 were included. In Primary Health care centers, all consecutive patients fulfilling inclusion criteria were also analyzed. Secondary outcomes included clinical routine variables such as age, sex, glucose, BMI, SBP and creatinine. Finally, tertiary outcomes included hemoglobin, bilirubin, platelet counts, sodium levels, and hs-CRP.

2.2 Inclusion Criteria of the Different Hospitals and Centers

A description of the study design and the inclusion criteria can be found in **Figure 1**. We had available data from patients of five different hospitals and participants from one primary care center with a diagnosis of COVID-19 (n=5,345). From these patient, n=3,065 had available data on both the primary and secondary outcomes. In addition, a subset of these patients (n=1,114) had also available data about the tertiary outcomes, including hemoglobin (n=1445), platelet counts, sodium levels, bilirubin or hs-CRP.

2.2.1 Catholic University Hospital of Rome

All patients admitted to the hospital between March 1st and May 1st, 2020 were included. Inclusion criteria for admission at the Fondazione Policlinico Gemelli IRCCS in Rome, Italy, were diagnosis of pneumonia from SARS-CoV-2, confirmed by two consecutive real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) assays of both nasal and pharyngeal swab specimens and computed tomography (CT) scan.

2.2.2 Hospital of Bologna

Consecutive patients admitted to the hospital between March 1 and April 20, 2020were included. The last follow-up date was April 27, 2020. Inclusion criteria: patients who had a confirmed COVID-19 diagnosis using a positive RT-PCR (Reverse transcription polymerase chain reaction) test on nasopharyngeal swabs and available BMI.

2.2.3 Bellvitge University Hospital

Patients consecutively admitted to Bellvitge University Hospital with laboratory-confirmed COVID-19 infection by RT-PCR assay for SARS-CoV-2 between March and May, 2020 were included. The inclusion criteria were the presence of sore throat, congestion, cough, dyspnoea, new loss of taste and/or smell, as well as uni- or bilateral interstitial infiltrates on chest X-ray, and availability of anthropometric data.

2.2.4 Hospital Clínic, Barcelona

All patients admitted with COVID-19 for ≥48 hours between 28 February and 22 April 2020 were included. All patients had a diagnosis of COVID-19 confirmed by real-time reverse transcription PCR (RT-PCR) testing performed on nasopharyngeal throat swab specimens, and/or by fulfilling clinical diagnostic criteria provided during the pandemic peak for SARS-CoV-2. These criteria comprised the presence of any of the following respiratory symptoms: sore throat, congestion, cough, dyspnoea, new loss of taste and/or smell as well as uni- or bilateral interstitial infiltrates on chest X-ray, and, have available anthropometric data registered up to one year before admission.

2.2.5 Hospital of Girona

Consecutive adult patients hospitalized between March 14, and June 30, 2020 with confirmed COVID-19 pneumonia defined as a positive result on real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) of nasal or pharyngeal swab specimens assays and also by a chest-X- ray or lung CT showing interstitial alterations and/or consolidation were included. The criterion for patients' admission to ICU was the failure to conventional oxygen therapy delivered through a non-rebreathing mask requiring a most intensive form of ventilatory support.

2.2.6 Girona Primary Care participants

All the individuals with confirmed COVID-19 (defined as having a positive RT-PCR test for SARS-CoV-2 virus) and attended in the general practices in Girona patients in whom management did not require oxygen therapy or hospital admission.

2.3 Study Variables

The data gathered for all cohorts were obtained from the electronic clinical health records, registered previously to the COVID19 diagnosis (in primary health care centers) or during hospital admission. Patient confidentiality was protected by assigning an anonymous identification code, anonymously discharged, and included the following: age, sex, systolic and diastolic blood pressure, body mass index, vascular risk factors (diabetes mellitus, hypertension, obesity, hypercholesterolemia, smoking) and laboratory tests (blood hemoglobin, serum

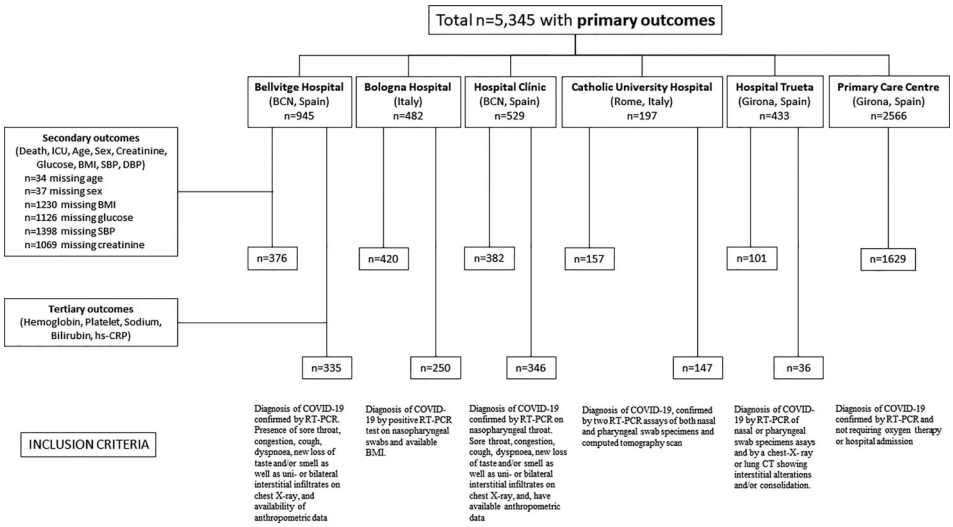

FIGURE 1 | Flow chart of study design and inclusion criteria.

bilirubin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, creatinine, and fasting glucose, among others). In all cases, glycemia and other analytes were measured using routine laboratory analyses in fasting conditions within 24 hours of hospital admission. All patients were followed until discharge or until death.

2.4 Ethics Committee' Approvals

The protocol was approved by the Ethical Committee of the Fondazione Policlinico Universitario A. Gemelli IRCCS, Catholic University, Rome, Italy with Approval Number: 0014355/20. Before enrolment, each subject gave informed consent. ClinicalTrials.gov ID: NCT04324684. The protocol was also independently approved by the ethics committee of the Hospital of Bologna, Hospital of Bellvitge, Hospital Clínic, Hospital of Girona and the Primary Care Ethics Committee.

2.5 Statistical Analysis

We examined the associations among potential prognostic variables and the main outcomes using multivariate logistic regression models. We used the glm function in R with a binomial family, a logit link function, and the Wald test to estimate the p-values of the model parameters. Odds ratios were then obtained as the exponentials of the parameters estimates and 95% confidence intervals (CI) were computed using the "confint" function from the MASS package. Age was categorized in three groups [<50 (50,70), and >70 years], SBP was dichotomized [(0,130), ≥130 mmHg], obesity was categorized according to the BMI [underweight, <18.5; normal range, (18.5,25); overweight, (25,30); obese class I, (30,35), obese class II, (35,40), obese class III, ≥40 kg/m2] and glycemia status at hospital admission (generally in the first 24 hours after overnight fasting) or in fasting conditions in primary health care centers. It was categorized based on fasting blood glucose levels [hypoglycemia, <70; normal, (70,100); moderate hyperglycemia, (100,126); severe hyperglycemia, (126,200); extreme hyperglycemia, ≥200]. Creatinine was categorized as below or above 1.3 mg/dl. For the subset of patients with complete data on all clinical variables (n=1,114), we further analyzed the data dichotomizing iron-related parameters (hemoglobin and bilirubin) based on the median for each gender and building logistic regression models for each group and gender. The performance of these models in classifying patients (predictive ability) was evaluated using the area under the receiver operating characteristics (AUCROC) curve. In addition, we further validated the classification prediction accuracy of these models by external validation. A total of n=1,445 patients had available data on age, sex, SBP, BMI, glucose and hemoglobin levels. Therefore, we used the n=331 samples not used in training the models as a test set and predicted the main outcome (death or survival) in this test set using the models for both low and high hemoglobin levels. We used a probability threshold of 0.5 to classify samples in one group (death) or another (survival) and we assessed the model accuracy as the proportion of samples that had been correctly classified.

2.6 Machine Learning

In addition to using multivariate logistic regression models, we also applied a machine learning algorithm (Boruta) to the subset of samples with complete (primary, secondary and tertiary outcomes) clinical data (n=1,114) to identify the most relevant clinical variables related to both death and ICU admission. The Boruta algorithm is a wrapper algorithm that performs feature selection based on the learning performance of the model. It has been recently proposed as one of the two best-performing variable selection methods making use of random forests (23). It performs variables selection in four steps: a) Randomization. To create a duplicate copy of the original features randomly permutate across the observations (the so-called shadow

features) to remove their correlation with the response; b) Model building. To add the shadow feature to the original predictor feature data set, built a RF with the extended data set, and compute the normalized permutation importance (Z) scores for each predictor and shadow feature; c) Statistical testing. To find the maximum normalized importance among the shadow attributes (MZSA) and compare it with each original predictor feature using a Bonferroni corrected two-tailed binomial test. Predictor features with significantly higher, significantly lower, or non-significantly different Z scores than expected at random compared to the MZSA are deemed important, unimportant, or tentative, respectively. d) Iteration. Unimportant and shadow features are removed and the previous steps are repeated until the status of all features is decided or a predefined number of iterations has been performed.

We run the Boruta algorithm with 1000 iterations, a confidence level cut-off of 0.005 for the Bonferroni adjusted p-values, 2000 trees to grow the forest (ntree), and several features randomly sampled at each split given by the square root of the number of features (the mtry recommended for discriminant analysis).

3 RESULTS

Out of 5,345 patients included in the database with available data on the primary outcomes, 3,065 had all data available for the secondary outcomes for further analyses (**Table 1**). Within subjects admitted to the hospital, both obesity and hyperglycemia were independent predictors of admission in the ICU (**Figure 2A**). Hence, while subjects with obesity were 2-3 times more likely to be admitted to the ICU compared to normal weight individuals, subjects with hyperglycemia were 3.5-5 times more likely to enter the ICU than normoglycemic individuals. In line with the death incidence, men were about 63% more likely to be admitted to the ICU than women.

Results from multivariate logistic regression analysis among prognostic variables and the incidence of mortality from COVID-19 are shown in **Figure 2B**. We identified age as the main independent predictor of mortality, with subjects over 70 years having a 29 times higher likelihood of presenting death due to COVID-19 than subjects younger than 50. Sex, SBP, glucose and creatinine were also independent predictors of death. Hence, men were about 64% more likely to present death due to COVID-19 than women. A unit increase in the standard deviation of plasma creatinine levels was associated with a 26% increase in mortality due to COVID-19. Remarkably, while subjects with glycemia>100 (moderate hyperglycemia) and >126 mg/dl (severe hyperglycemia) had 3.5 and 5.5 times higher mortality risk than normoglycemic subjects, no differences were found in COVID-19 mortality by weight categories. Only underweight, BMI < 18.5 kg/m^2, was associated with a higher mortality risk. We also considered the potential interaction between some of the predictor variables in the models. However, we did not find any significant interaction between age and sex (P=0.274), age and SBP (P=0.710), age and BMI (P=0.131), age and glucose (P=0.774), glucose and SBP (P=0.567), glucose and BMI (P=0.599), or BMI and SBP (P=0.309).

These results were replicated considering only patients admitted to the hospital in each country independently (**Supplementary Figures 1A, B** for mortality and ICU in Spain and **Supplementary Figures 3C, D** for mortality and ICU in Italy, respectively). The addition of previously known T2D to the models did not significantly change any of the previous results.

Finally, when the results were compared between patients recruited in primary care and those admitted to hospitals, there were no appreciable differences in the magnitude of the associations.

3.1 Machine Learning Results

We further analyzed the data applying a machine learning variable selection strategy based on multiple random forest as implemented in the Boruta algorithm (24) to identify the most important outcome predictive variables. In line with the previous analysis, age was the strongest predictor of both mortality (**Figure 3A**) and ICU admission (**Figure 3B**). Notably, BMI discriminated those subjects admitted to the ICU from subjects who did not need admission, but was unable to discriminate between individuals who died due to COVID-19 from those who survived, thereby corroborating the results from the logistic regression models. In the case of hospital patients, we were

TABLE 1 | Characteristics of the patients included in each Institution.

	Bellvitge (N=376)	Bologna (N=420)	Clinic (N=382)	Trueta (N=101)	NIKE (N=157)	Primary Care (N=1629)	Total (N=3065)
Death	88 (23.4%)	83 (19.8%)	60 (15.7%)	9 (8.9%)	26 (16.6%)	0 (0.0%)	266 (8.7%)
ICU	48 (12.8%)	51 (12.1%)	154 (40.3%)	20 (19.8%)	17 (10.8%)	0 (0.0%)	290 (9.5%)
Age (years)	68.0 (56.0,76.0)	68.0 (55.0,80.0)	64.0 (54.0,73.0)	62.0 (48.0,73.0)	65.0 (54.0,76.0)	61.9 (43.8,84.6)	65.0 (49.4,80.0)
Sex	151 (40.2%)	160 (38.1%)	148 (38.7%)	44 (43.6%)	59 (37.6%)	1103 (67.7%)	1665 (54.3%)
Creatinine (mg/dL)	0.9 (0.7, 1.2)	0.9 (0.8, 1.1)	0.9 (0.8, 1.1)	0.7 (0.6, 1.0)	0.9 (0.8, 1.2)	0.8 (0.7, 1.0)	0.8 (0.7, 1.0)
Glucose (mg/dL)	113.5 (100.9,140.6)	111.0 (99.0,132.2)	110.0 (97.0,131.8)	117.0 (105.0,140.0)	110.0 (98.0,128.0)	90.0 (81.0,101.0)	99.1 (86.5,119.0)
BMI (kg/m^2)	28.9 (26.0, 31.6)	25.1 (23.0,28.4)	27.8 (24.9,31.2)	31.2 (27.0, 38.0)	25.4 (24.1,27.3)	26.2 (23.0,29.9)	26.7 (23.7,30.3)
SBP (mmHg)	130.5 (117.0, 43.0)	125.0 (111.5,135.0)	124.0 (112.0,140.0)	131.0 (119.0, 144.0)	125.0 (115.0,140.0)	127.0 (116.0,136.0)	127.0 (115.0, 38.0)
DBP (mmHg)	72.0 (64.0, 81.0)	70.0 (70.0, 80.0)	72.0 (65.0, 81.0)	74.0 (65.0, 85.0)	79.0 (70.0, 85.0)	74.0 (67.0, 80.0)	73.0 (66.0, 80.0)

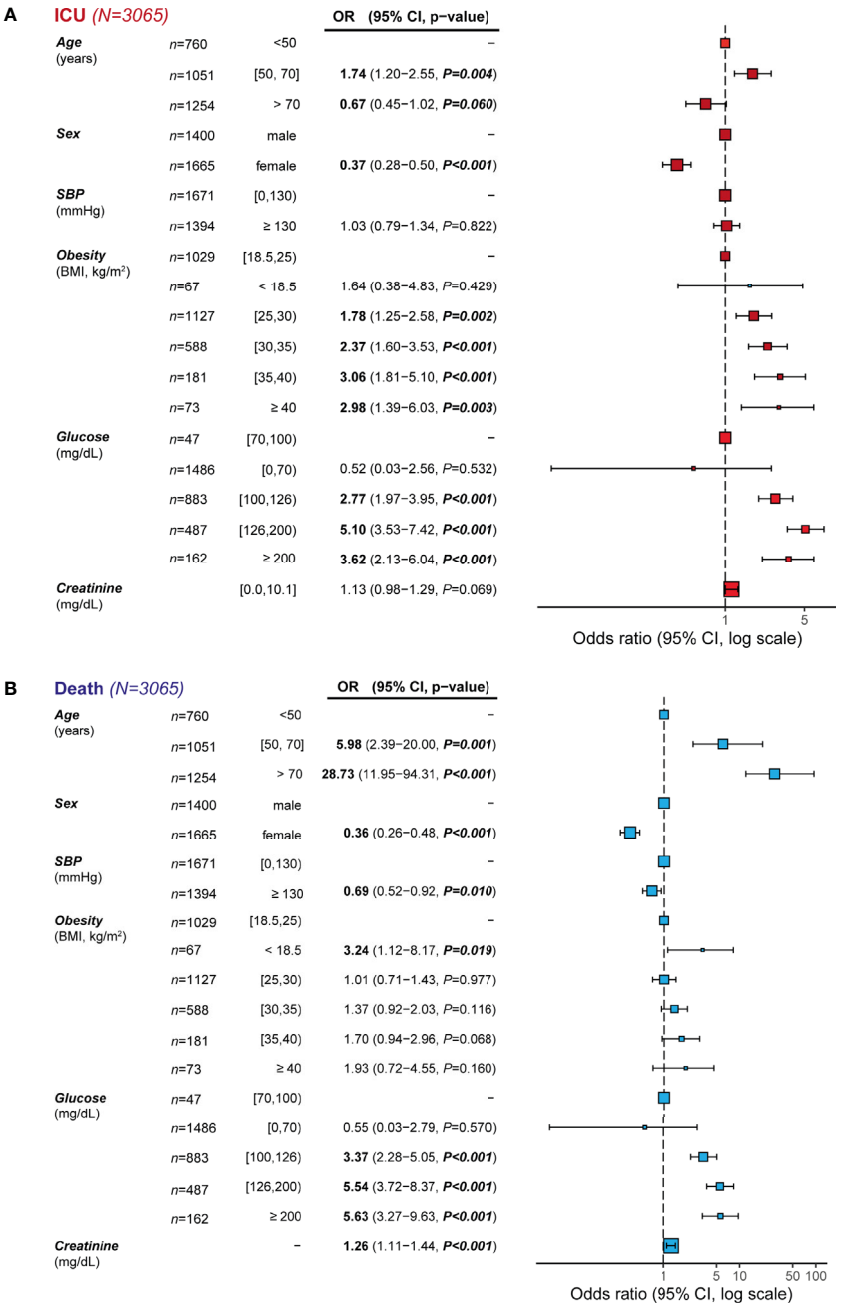

FIGURE 2 | Incidence odds ratio of **(A)** admission to the ICU and **(B)** mortality from COVID-19 by potential prognostic variables. Data are presented as odds ratio (OR) and 95% confidence intervals (CI). The reference groups (OR=1) for each variable are shown as "-".: The odds ratios shown are adjusted through a logistic regression model which includes all variables listed. Data are also represented as a forest plot.

able to include additional variables to the machine learning algorithms. From the total of 1,446 admitted to the hospital, 1,114 also had measurements of plasma C-Reactive Protein (CRP), bilirubin, sodium, platelet, and hemoglobin levels. Once again, age was the most differentiating variable in the case of death (**Figure 3C**) and ICU (**Figure 3D**) admission outcomes, whereas BMI was only able to discriminate among ICU patients (**Figure 3D**). It is worth noting that bilirubin, CRP, and creatinine had a strong association with death, but only bilirubin and CRP were also associated with ICU admission. Hyperglycemia was independently associated with a poorer prognosis.

The machine learning strategy identified two iron-related parameters (hemoglobin and bilirubin) as significant predictors of death. Therefore, we built new multivariate logistic regression models including hemoglobin and bilirubin dichotomized based

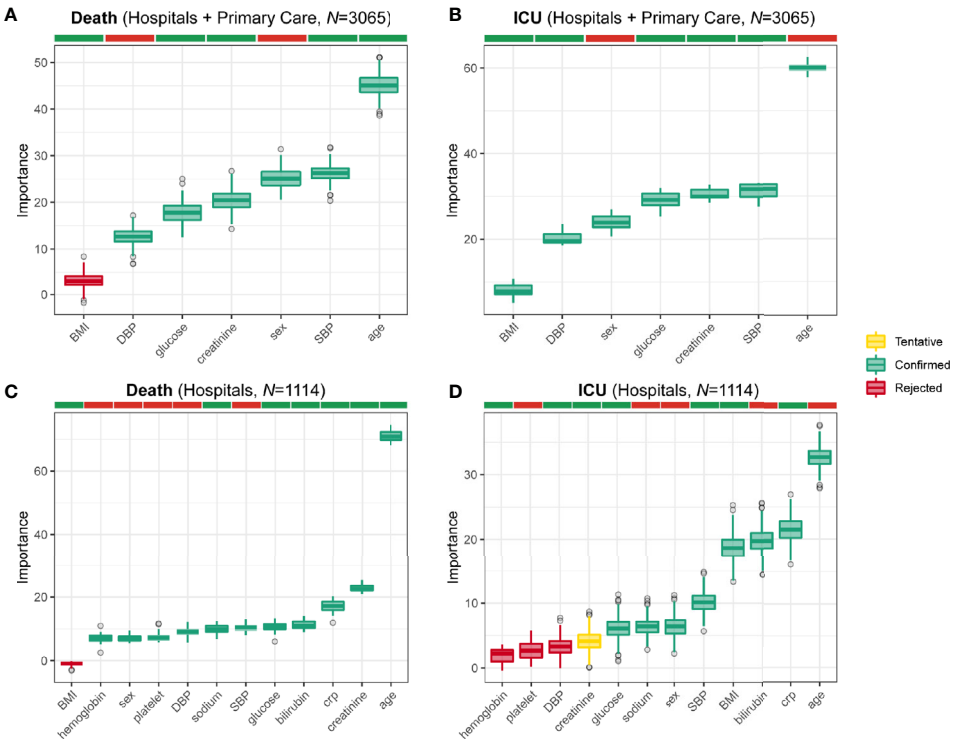

FIGURE 3 | Mortality and ICU admission prognostic variables identified from machine learning. Boxplots of the normalized permutation importance obtained from the Boruta algorithm for the potential prognostic variables associated to the **(A)** mortality from COVID-19 in all subjects (*n*=3065), **(B)** admission to the ICU in all subjects (*n*=3065), **(C)** mortality from COVID-19 in hospital patients (*n*=1114), and **(D)** admission to the ICU in hospital patients (*n*=1114).

on their median values. As both parameters are strongly dependent on gender, separate analyses were also performed for men and women. We found that hemoglobin and bilirubin were not predictors of mortality in women. However, hemoglobin had a significant prognosis effect in the case of men (**Figure 4**). Men having hemoglobin values above the median were less likely to die due to COVID-19 than men with concentrations below the median. Remarkably, glucose was a strong predictor of mortality in men with hemoglobin or bilirubin values above the median, but not in those with concentrations below the median (**Supplementary Figure 2**). Therefore, men with moderate or severe hyperglycemia that also had blood hemoglobin concentrations over the median had about 17 and 30 times increased mortality risk compared to men without hyperglycemia, respectively (**Supplementary Figure 2B**), whereas there were no significant differences in mortality among men with low hemoglobin values (**Supplementary Figure 2A**). Similarly, morbidly obese men also had a higher mortality risk than normo-weight individuals only in those individuals with hemoglobin levels above the median (**Supplementary Figure 2**). Likewise, glucose concentration was not a prognosis predictor of mortality in men with bilirubin values below the median (**Supplementary Figure 2C**), but had a strong impact in men with bilirubin concentrations above the median (**Supplementary Figure 2D**). Women with hyperglycemia and increased blood hemoglobin also had a trend towards mortality risk compared with women without hyperglycemia (OR=3.17, *P*=0.096).

Considering that, apart from age, glucose was the strongest mortality predictor in our logistic regression models and the strong impact of hemoglobin on glucose modulating effects on prognosis, we built two final predictive models using the subset of patients with information about all clinical variables (*n*=1,114, one for hemoglobin concentrations below- and another one for above the median; cut-off =13.6 g/dL) (**Figure 5**). In the model for hemoglobin values above the median, glucose was a significant predictor of COVID-19 mortality (**Figure 5A**), whereas in the hemoglobin model for concentrations below the median, glucose concentrations had no prognostic value (**Figure 5B**). Notably, in subjects with hemoglobin levels above the median, women had the same likelihood of COVID-19 mortality than men. Thus, female sex was not a protective factor in individuals with hemoglobin concentrations above the median. Again, morbidly obese patients had a higher mortality risk than non-obese subjects only when hemoglobin concentrations were above the median. These mortality prediction models had an AUCROC of 81.6% and 83.3%, respectively (**Figures 5C, D**). We also checked for potential interactions between some predictors in these models and found no significant interactions. For the model with hemoglobin levels below the median, we did not find any interaction between age and sex (P=0.262), age and SBP

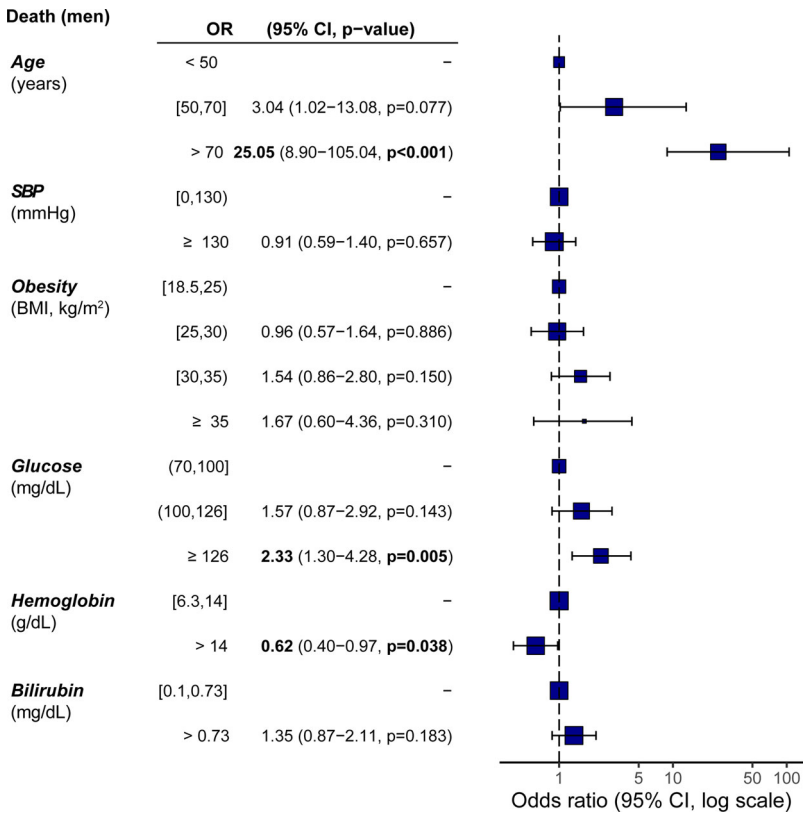

FIGURE 4 | Incidence odds ratio of mortality from COVID-19 in men including iron-related parameters as prognostic variables. Data are presented as odds ratio (OR) and 95% confidence intervals (CI). The reference groups (OR=1) for each variable are shown as "-".: The odds ratios shown are adjusted through a logistic regression model which includes all variables listed. Data are also represented as a forest plot. Hemoglobin and bilirubin were dichotomized based on the median values.

(P=0.286), age and BMI (P=0.880), age and glucose (P=0.740), glucose and SBP (P=0.413), glucose and BMI (P=0.722), or BMI and SBP (P=0.942). Similarly, no interactions were found for the model with hemoglobin levels above the median for age and sex (P=0.155), age and SBP (P=0.268), age and BMI (P=0.822), age and glucose (P=0.433, glucose and SBP (P=0.825), glucose and BMI (P=0.876), or BMI and SBP (P=0.076).

We finally performed an external validation of these models using a test set of *n*=331 not used in training the final models. These patients had available data on hemoglobin levels but not on other parameters included in the machine learning analyses such as bilirubin, hs-CRP, sodium or platelets. In the case of the model for low hemoglobin, we obtained a classification prediction accuracy of 78%, whereas the prediction accuracy for the high hemoglobin model was 81%. The corresponding confusion matrices are shown in **Tables 2** and **3**. We also checked whether the results of these models could be affected by potential confounding factors known to alter the hemoglobin levels (smoking, COPD or dehydration based on hematocrit) or influencing liver steatosis (measured by alanine aminotransferase, ALT). The inclusion of these variables in the final models did not change the results (**Supplementary Figures 3** and **4**). Thus, in subjects with hemoglobin levels above the median, hyperglycemia was a strong independent predictor of mortality associated with COVID-19, while female sex lost its protective effect. Conversely, in subjects with hemoglobin levels below the median, women were less likely to die because of COVID-19 but hyperglycemia had no prognostic value.

In order to present the final models in a useful and user-friendly format for the clinical practice, we implemented the models as an interactive web application using html/CSS and Java Script at http://isvgirona.net/sarscov2/20201110/. The clinician only has to introduce the patient's age, sex, SBP, BMI, glucose, and hemoglobin values and it internally categorizes the variables and uses the model corresponding to hemoglobin concentrations below or above the median to predict the probability of mortality.

4 DISCUSSION

The results from this multi-center study of five different hospitals and Primary Health Care centers from Italy and Spainconfirm previous observations that both elevated blood glucose levels and higher BMI are associated with worse COVID-19 related outcomes. However, most of these studies have not taken into account the interaction of both parameters at the same time. Thus, several studies assessing obesity effects did not include comorbidities, such as diabetes, while studies that found

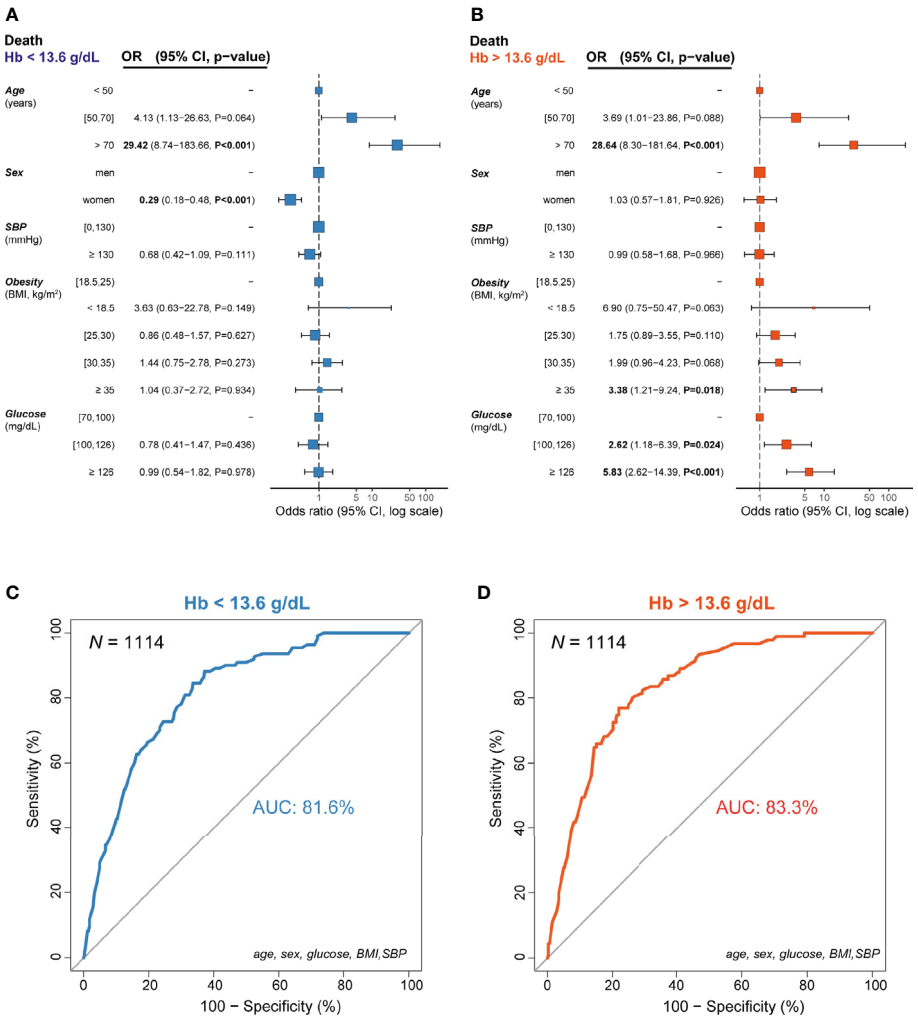

FIGURE 5 | Incidence odds ratio of mortality from COVID-19 for the final models (*n*=1,114) according to the median hemoglobin concentrations (13.6 g/dL). **(A)** In individuals with hemoglobin levels below the median, **(B)** In individuals with hemoglobin levels above the median, **(C)** Receiver operating characteristic curve for the logistic regression model in individuals with hemoglobin levels below the median, **(D)** Receiver operating characteristic curve for the logistic regression model in individuals with hemoglobin levels above the median. Data are presented as odds ratio (OR) and 95% confidence intervals (CI). The reference groups (OR=1) for each variable are shown as "-".: The odds ratios shown are adjusted through a logistic regression model which includes all variables listed. Data are also represented as a forest plot. AUC, area under the curve.

associations with hyperglycemia did not consider obesity as a confounding factor (12–16). A recent review about prediction models for the diagnosis and prognosis of COVID-19 identified 23 models for the estimation of mortality risk (25). Several of these models included comorbidities. However, only one of these 23 models considered the joint role of obesity and diabetes in modifying COVID-19 outcomes (26). Thus, Bello-Chavolla et al. found that, in a Mexican population, early-onset diabetes

TABLE 2 | Confusion matrix for the model with hemoglobin levels below the median (<13.6 mg/dL).

		Actual class		Total	
		Death	**Survival**		
Predicted class	**Death**	TP=122	FP=30	152	PPV=80%
	Survival	FN=7	TN=9	16	NPV=56%
	Total	129	39	168	
		TPR=94%	TNR=23%		**Accuracy** 78%

TP, true positive; FP, false positive; FN, false negative; TN, true negative; PPV, Positive Predictive Value Precision; TPR, True Positive Rate Sensitivity; TNR, True Negative Rate, Specificity; NPV, Negative Predictive Value.

TABLE 3 | Confusion matrix for the model with hemoglobin levels above the median (>13.6 mg/dL).

		Actual class		Total	
		Death	**Survival**		
Predicted class	**Death**	TP=120	FP=16	136	PPV=88%
	Survival	FN=15	TN=11	26	NPV=42%
	Total	135	27	162	
		TPR=89%	TNR=41%		**Accuracy** 81%

TP, true positive; FP, false positive; FN, false negative; TN, true negative; PPV, Positive Predictive Value Precision; TPR, True Positive Rate Sensitivity; TNR, True Negative Rate, Specificity; NPV, Negative Predictive Value.

increased the risk of COVID-19-related mortality, while obesity mediated 50% of the effects of diabetes on COVID-19 lethality and conferred an increased risk for ICU admission. Here, we have found that, when both obesity and hyperglycemia are considered together, although subjects with obesity and hyperglycemia had an increased likelihood of admission to the ICU, only the latter was an independent predictor of mortality.Notably, these results were replicated indepenently in two different countries. We also observed that both extremes of BMI were associated with a poorer prognosis. This is in line with a recent population study in England in which BMI had a U shape relation to mortality in patients with type 1 and type 2 diabetes (27).

Modern machine learning methods, such as random forest, are promising computational approaches for feature selection in predictive modeling that are increasingly applied to clinical problems. Machine Learning tree-based algorithms are particularly well-suited to this aim. In contrast to classical linear model-based statistical methods, they are fully non-parametric model-free methods that can capture complex dependency patterns within the datasets affecting the phenotype. They provide variable importance measures that can be used to identify relevant features. In addition, they are invariant to monotonic transformations, have a good performance in non-linear datasets and can auto-correct dependencies among variables, thereby rendering them as particularly suitable to deal with complex datasets. Notably, tree-based variable selection methods have shown to perform better than classic regression-based methods in large, multicenter datasets (28). Therefore, we also performed machine learning analyses including clinical parameters such as plasma CRP, creatinine, platelets levels, and iron-related parameters (hemoglobin and bilirubin).

The most frequently used variables in mortality risk models included comorbidities, age, sex, lymphocyte counts, CRP and creatinine (25). Renal impairment (9, 16, 27, 29), plasma CRP concentration (9, 27, 29) and blood platelet count have been previously reported to be associated with a poorer prognosis in patients with COVID-19 and we here confirm their independent associations in a Machine Learning model. Both hemoglobin and bilirubin were also associated with COVID-19 mortality. In a recent meta-analysis that identified more than 30 risk factors associated with a higher risk of severe COVID-19, lower levels of hemoglobin but increased serum ferritin, among others, were associated with severe COVID-19 (29). These results were confirmed in an independent meta-analysis (30). Compared to moderate cases, severe COVID-19 cases had lower hemoglobin [weighted mean difference (WMD), - 4.08 g/L (95% CI - 5.12; - 3.05)] and higher ferritin [WMD, - 473.25 ng/mL (95% CI 382.52; 563.98)]. A significant difference in mean ferritin levels of 606.37 ng/mL (95% CI 461.86; 750.88) was found between survivors and non-survivors, but not in hemoglobin levels (30). Similarly, low levels of hemoglobin have been recently shown to be predictive factors for the diagnosis and prediction of patients with COVID-19 (17, 18). Hemoglobin levels have also shown to predict the severity of COVID-19, with a gradual decrease with disease progression (19). In addition, anemia has been associated with severe COVID-19 (21)and has also been identified as a single independent predictor of mortality in COVID-19 patients (20, 22). Finally, in a recent meta-analysis of 83 studies, total bilirubin (mean difference 2.08 mmol/L, 95% CI 1.36-2.80 mmol/L; P<0.001) was also observed to be increased in patients with severe compared to non-severe COVID-19 patients (31). In agreement with these previous results, we also found that hemoglobin levels below the median were associated with a higher risk of mortality due to COVID-19 in men. However, we did not find significant differences in women.

Intriguingly, we found that the glucose predictive value for COVID-19 mortality was strongly modulated by hemoglobin levels. Despite anemia is considered to be an independent predictor of mortality due to COVID-19 (20)and hemoglobin was negatively associated with COVID-19 mortality in men in our study, we found that high hemoglobin levels had a strong impact on modulating the prognostic value of hyperglycemia in relation to COVID-19 mortality. Hence, men with moderate and severe hyperglycemia had a strikingly increased mortality risk due to COVID-19 compared to normoglycemic individuals only when they also had increased hemoglobin levels. Women with moderate and severe hyperglycemia and increased hemoglobin concentrations also showed a trend towards a higher mortality risk compared to women with normal glucose concentrations. As the number of women with hemoglobin levels above the median was substantially smaller than that of men, it is likely that increasing the number of women would also result in significant associations. In any case, when both men and women were included, we also found that glucose was a significant predictor of COVID-19 mortality in subjects with increased hemoglobin levels. Remarkably, gender had no effect

on mortality in these subjects. Thus, women were not more protected from COVID-19 mortality than men when hemoglobin concentrations were above the median. Our results, are in line with a recent study including 9,467 hospitalized COVID-19 patients showing that patients with hemoglobin ≥ 16 g/dL had significantly higher adjusted in-hospital mortality compared to patients with hemoglobin between 12 and 14 g/dL [OR (95% CI): 1.62 (1.15-2.27), *P*=0.005]. As SARS-CoV-2 infection increases coagulopathy (32), high hemoglobin levels may be associated with and hypercoagulable state leading to systemic thrombosis.

A viral interaction with hemoglobin molecule, through ACE2, CD147, CD26 and other receptors located on erythrocytes and/or blood cell precursors, has been highlighted (33, 34). Certain viral proteins could attack the 1-beta chain of hemoglobin to dissociate iron, generating dysfunctional hemoglobin with reduced oxygen transport capacity (35). SARSCoV-2 has been suggested to invade host cells *via* CD147-spike protein interaction (36). As there are about 3000 molecules of CD147 per erythrocyte, the entry of SARS-CoV-2 through this pathway has been considered a possible basic pathogenic mechanism. In fact, SARS-CoV was previously shown to interfere with hemoglobin at erythrocyte and bone marrow level (34). In a recent study, Lancman *et al.* found that from 38 hospitalized COVID-19 patients, 80% had elevated plasma-free hemoglobin levels (> 5 mg/dL), a marker of hemolysis. Viral spike protein interaction with CD147 on red blood cells could be a potential mechanism of this intravascular hemolysis.

Regarding the possible pathophysiology mediating all these effects, hyperglycemia itself may up-regulate the expression of ACE2, facilitating the entry of the virus into cells (37) leading to a poorer prognosis. It could be possible that the co-existence of hyperglycemia and increased hemoglobin levels amplify the entry of SARS-CoV-2 in blood cells, facilitating its intracellular replication. This could partly explain the strong impact of glucose on COVID-19 mortality that we observed in men with hemoglobin concentrations above the median. Importantly, none of the 23 models published so far to predict COVID-19 mortality has included hemoglobin levels.

4.1 Strengths and Limitations

This study has several strengths. The findings in this multicenter and diverse study in five different hospitals and the Primary Health Care system have been homogenous. This allowed the replication of the importance of hyperglycemia across the different centers. This study also has potential limitations. There is missingness for some variables, but this is randomly distributed across the centers. Further, we may have missed deaths that occurred outside the hospital. There is also a relative over-representation of in-hospital patients (36% of all subjects) while the number of subjects requiring hospitalization is far lower when considering asymptomatic patients, so the finding may be only applicable to the most severe patients. However, even taking into account this limitation, the targeting of glycemia and hemoglobin concentrations in these severe patients seems of utmost importance.

In summary, hemoglobin levels had a strong impact on hyperglycemia and morbid obesity prognostic value. Only morbidly obese subjects or subjects with hyperglycemia and hemoglobin concentrations above the median were at increased mortality risk. Considering the strong impact of glucose and morbid obesity on COVID-19 mortality, monitoring hemoglobin levels in subjects with hyperglycemia is thus of utmost importance. Low hemoglobin levels have already been identified as an independent predictor of mortality due to COVID-19. In the present study, we add additional valuable information regarding the role of hemoglobin in COVID-19 and extend these findings to high hemoglobin levels. We provide an online calculator that may have utility in the clinical settings to help clinicians identify and prioritize those patients at higher risk of death. To our knowledge, this is the first study to analyze different independent samples from different institutions from two countries and to use machine learning tools for the analysis.

OBESITY AND TYPE 2 DIABETES COVID-19 STUDY GROUP

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DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The protocol was approved by the Ethical Committee of the Fondazione Policlinico Universitario A. Gemelli IRCCS, Catholic University, Rome, Italy with Approval Number: 0014355/20. Before enrolment, each subject gave informed consent. ClinicalTrials.gov ID: NCT04324684. The protocol was also independently approved by the ethics committee of the Hospital of Bologna, Hospital of Bellvitge, Hospital Clínic, Hospital of Girona and the Primary Care Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JM-P analysed the data. JMP-P and JF-R wrote the manuscript. All authors provided data and contributed to the discussion. JF-R takes the responsibility for the contents of the article. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.741248/full#supplementary-material

Supplementary Figure 1 | Incidence odds ratio of mortality and ICU admission from COVID-19 by potential prognostic variables in hospital patients by country. **(A)** Mortality in Spain, **(B)** ICU admission in Spain, **(C)** Mortality in Italy, **(D)** ICU admission in Italy. Data are presented as odds ratio (OR) and 95% confidence intervals (CI). The reference groups (OR=1) for each variable are shown as "-".The odds ratios shown are adjusted through a logistic regression model which includes all variables listed. Data are also represented as a forest plot.

Supplementary Figure 2 | Incidence odds ratio of mortality from COVID-19 by potential prognostic variables in men according to iron-related parameters. **(A)** men with hemoglobin concentrations below the median, **(B)** men with hemoglobin concentrations above the median, **(C)** men with bilirubin concentrations below the median, **(D)** men with bilirubin concentrations above the median. Data are presented as odds ratio (OR) and 95% confidence intervals (CI). The reference groups (OR=1) for each variable are shown as "-".: The odds ratios shown are adjusted through a logistic regression model which includes all variables listed. Data are also represented as a forest plot. Iron parameters were dichotomized based on the median.

Supplementary Figure 3 | Incidence odds ratio of mortality from COVID-19 including smoker status and alanine aminotransferase (ALT) as potential confounder variables in the final models according to the median hemoglobin concentration (13.6 g/dL). **(A, B)** Models including smoker status (*n*=878) as a confounding factor in subjects with hemoglobin levels below and above the median, respectively. **(C, D)** Models including liver function assessed by alanine aminotransferase (ALT, *n*=1333) as a confounding factor in subjects with hemoglobin levels below and above the median, respectively.

Supplementary Figure 4 | Incidence odds ratio of mortality from COVID-19 including chronic obstructive pulmonary disease (COPD) and hematocrit as potential confounder variables in the final models according to the median hemoglobin concentration (13.6 g/dL). **(A, B)** Models including chronic obstructive pulmonary disease (COPD, *n*=422) as a confounding factor in subjects with hemoglobin levels below and above the median, respectively. **(C, D)** Models including hematocrit (*n*=926) as a confounding factor in subjects with hemoglobin levels below and above the median, respectively.

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REVIEW
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COVID-19 and Diabetes: A Comprehensive Review of Angiotensin Converting Enzyme 2, Mutual Effects and Pharmacotherapy

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The potential relationship between diabetes and COVID-19 has been evaluated. However, new knowledge is rapidly emerging. In this study, we systematically reviewed the relationship between viral cell surface receptors (ACE2, AXL, CD147, DC-SIGN, L-SIGN and DPP4) and SARS-CoV-2 infection risk, and emphasized the implications of ACE2 on SARS-CoV-2 infection and COVID-19 pathogenesis. Besides, we updated on the two-way interactions between diabetes and COVID-19, as well as the treatment options for COVID-19 comorbid patients from the perspective of ACE2. The efficacies of various clinical chemotherapeutic options, including anti-diabetic drugs, renin-angiotensin-aldosterone system inhibitors, lipid-lowering drugs, anticoagulants, and glucocorticoids for COVID-19 positive diabetic patients were discussed. Moreover, we reviewed the significance of two different forms of ACE2 (mACE2 and sACE2) and gender on COVID-19 susceptibility and severity. This review summarizes COVID-19 pathophysiology and the best strategies for clinical management of diabetes patients with COVID-19.

Keywords: diabetes mellitus, ACE2 (angiotensin converting enzyme 2), COVID - 19, SARS- CoV-2, therapeutic management, receptor

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INTRODUCTION

The outbreak of the novel coronavirus disease (COVID-19) in 2019 posed a serious and continuing challenge to the global public health system. This disease is caused by a novel sense, single-stranded, enveloped RNA β-coronavirus referred to as the Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) (1). As of 31st August 2021, the World Health Organization had reported a total of 216867420 globally diagnosed COVID-19 cases, including 4507837 COVID-19 associated mortalities. The pathophysiology of COVID-19 interacts with that of diabetes, which is a chronic disease. SARS-CoV-2 infection can lead to a new-onset of diabetes and severe metabolic complications in previous diabetes, including diabetic ketoacidosis (DKA) and hyperosmolar hyperglycemic state (HHS). Besides, diabetes is associated with an increased risk of severe COVID-19. Therefore, understanding the clinical processes of SARS-CoV-2 infections and therapeutic efficacies of commonly used drugs in diabetic patients is key in COVID-19 management.

SARS-CoV-2 gains entry into target cells by attaching to angiotensin-converting enzyme 2 (ACE2) receptor. During complex virus-host cell fusion processes, the receptor-binding domain (RBD) of SARS-CoV-2 spike glycoprotein (S1) binds ACE2 (2). Then, proximal serine proteases [such as transmembrane serine protease 2 (TMPRSS2)] cleave the SARS-CoV-2 spike protein and ACE2, thereby promoting viral entry (3).

BIOLOGICAL CHARACTERISTICS OF ACE2 AND ACE

The two enzymes of the renin-angiotensin system (RAS), angiotensin-converting enzyme (ACE) and ACE2, have about 60% homology and play opposite roles. In RAS, renin cleaves angiotensinogen into angiotensin I (Ang I), which is then converted into angiotensin II (Ang II) by ACE. Ang II acts on type 1 angiotensin receptor (AT1R), which increases blood pressure levels by inducing vasoconstriction and by increasing renal reabsorption of sodium as well as water. Moreover, Ang II promotes oxidative stress, inflammation and fibrosis (4). In addition to causing pulmonary edema and lung injury, it can also lead to insulin resistance, endothelial dysfunction and proteinuria. These effects of ACE-Ang II are directly opposite to those of ACE2-Ang- (1-7) signal transduction. ACE2 converts Ang II to Ang 1-7, which acts on Mas receptors and lowers blood pressure levels by mediating vasodilation, promoting renal sodium and water excretion. It suppresses inflammatory response and oxidative stress levels by producing nitric oxide (5). It also has anti-fibrosis, anti-proliferation and anti-thrombosis effects. Therefore, the classical ACE-Ang II-AT1R regulatory axis and ACE2-Ang1-7-MAS anti-regulatory axis can maintain homeostasis *in vivo* **(Figure 1).**

ACE2 is essential in regulation of lung homeostasis and lung injury prevention. After SARS-CoV-2 binds ACE2 receptors on the surface of alveolar epithelial cells, expressions of ACE2 in alveolar epithelial cells are down-regulated by internalization, shedding, viral replication and other mechanisms (6). Subsequently, elevated Ang II levels trigger inflammatory responses, leading to neutrophil, macrophage and fibrin exudation, thereby aggravating vascular permeability and pulmonary edema. These effects lead to the loss of pulmonary ventilation, persistent oxygenation difficulties, and increased risk of acute respiratory distress syndrome (ARDS) (7). Therefore, during SARS-CoV-2 pathogenesis, ACE2 is involved in viral entry and in lung protection against injury.

MAIN RECEPTOR ACE2

There are two forms of ACE2: structural transmembrane protein ACE2 (mACE2) with the extracellular domain, which can act as the receptor for SARS-CoV-2 spike protein, while soluble form represents cyclic ACE2 (sACE2). Under normal circumstances, the functions of ACE2 in the lungs are limited, however, they may be up-regulated in some clinical conditions. Importantly, plasma levels of ACE2 (sACE2) cannot be used as reliable indicators for complete membrane-bound ACE2 (mACE2) activity. This is partly due to ACE2 shedding from the membrane, which seems to be endogenously regulated. In addition to expression levels, biological roles of ACE2 may vary among tissues as well as clinical status.

mACE2 is highly expressed in the heart, airway, kidney and liver tissues, while sACE2 is produced by mACE2 shedding in response to inflammatory signals and diseases. A-disintegrin and metalloproteinase (ADAM) 17 cleaves mACE2 to release soluble ACE2 into plasma. Under elevated Ang II conditions, the extracellular catalytic domain of mACE2 is released by ADAM-17 as a feedback mechanism. sACE2, which retains enzyme activity and complete SARS-CoV-2 interaction sites, allows easy access to Ang II to counteract its effects and to bind extracellular floating viral S proteins in a manner similar to mACE2 **(Figure 2)** (8–10). However, the precise roles of sACE2 during the course of COVID-19 infection are still unclear.

Membrane Binding Form mACE2

As a COVID-19 receptor, differential expressions of mACE2 may lead to different physiological responses to SARS-CoV-2 infection. Both SARS-CoV-1 and SARS-CoV-2 gain entry into lung cells by binding mACE2. After SARS-CoV-1 infection, ADAM-17 induces mACE2 shedding and enhances the secretion of tumor necrosis factor by cells (11), resulting in elevated Ang II levels and imbalanced RAS signaling (12). In COVID-19, Ang II levels are correlated with lung injury. Therefore, SARS-CoV-2 infected individuals with previous mACE2 deficiencies may be highly predisposed to severe mACE2 deficiency in the lungs, which may increase the risk of acute lung injury and death (13).

The elderly, male gender, complications associated with hypertension, cardiovascular disease, obesity and diabetes mellitus have been identified as important predisposing factors for the development of severe COVID-19 and COVID-19-associated mortality (14–16). Even though the underlying mechanisms of these associations have not been clearly established, the increased risk may be attributed to the relative lack of mACE2 and the corresponding increased expressions of sACE2. mACE2 deficiencies are associated with old age, male gender, cardiovascular disease and diabetes mellitus (13, 17–19). Meanwhile, sACE2 levels have been found to be elevated in elderly individuals, male gender, as well as during cardiovascular and inflammatory diseases (20, 21).

Soluble Form sACE2

In COVID-19

Lung inflammation during COVID-19 is coordinated by Ang II and sACE2 levels. SARS-CoV-2 gains entry into cells by binding mACE2, whose level reduces while that of Ang II, as an early response to SARS-CoV-2 infection, reaches its peak, thereby triggering anti-viral inflammatory responses and neutrophil infiltration into lungs. However, sACE2 levels in the lungs begin to increase with viral entry. At its peak, Ang II increases ADAM-17 levels through AT1R activation, resulting in elevated sACE2 levels, which facilitates the Ang 1-7/MasR pathway, regulates inflammation and prevents further tissue injury (19, 22, 23).

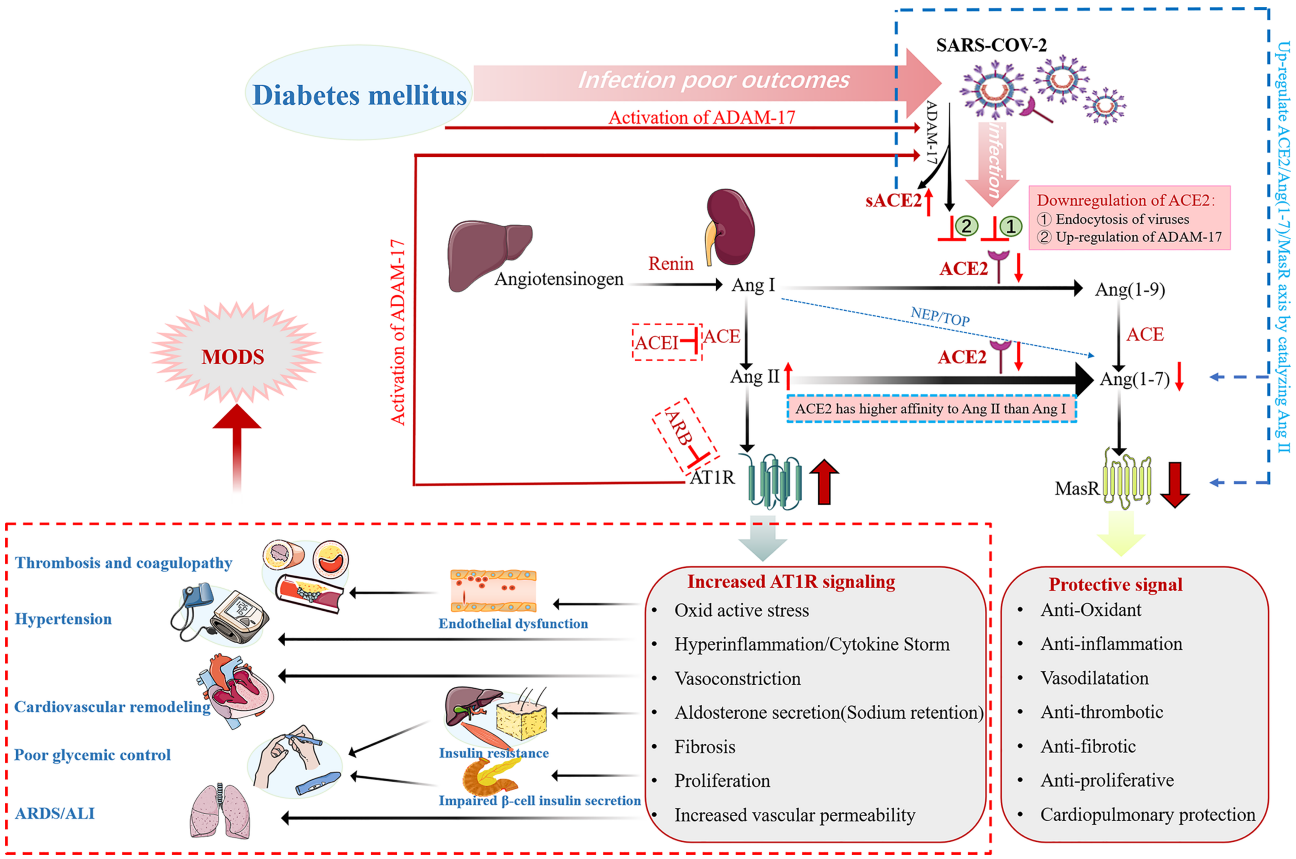

FIGURE 1 | Schematic representation of the alterations of the renin angiotensin aldosterone system (RAAS) in diabetic patients with COVID-19. ADAM-17 is activated in diabetic patients, therefore both DM and SARS-CoV-2 infection downregulate membrane-bound ACE2 in diabetes mellitus patients with COVID-19. ACE2 has a higher affinity for Ang II than Ang I. Therefore, down-regulation of ACE2 leads to Ang II accumulation and decreased Ang (1-7) levels. In turn, accumulation of Ang II activates ADAM-17, resulting in increased sACE2 shedding and a further decrease in membrane-bound ACE2. Thus, the protective signal mediated by ACE2/Ang (1-7) axis is down-regulated, and the detrimental signal mediated by Ang II/AT1R axis is up-regulated, resulting in multiple organ damage effects and even multiple organ failure.

The transmission of SARS-CoV-2 between cells has been reported to occur *via* macropinocytosis, an endocytosis process mediated by receptor-independent filamentous pseudopodia. In this case, the virus-sACE2 complex may enter host cells through endocytosis (24). *In vitro* studies have shown that endogenous sACE2 interacts with SARS-CoV-2 spike proteins in the extracellular compartment. The resulting sACE2-S complex binds AT1 surface receptors to enter cells *via* receptor-mediated endocytosis (25). In addition, the S protein of SARS-CoV-2 interacts with vasopressin to form a sACE2-S-vasopressin complex, which facilitates cellular entry through another vasopressin receptor AVPR1B (25). These new mechanisms allow the virus to enter tissues that poorly express mACE2 (**Figure 3A**). This explains the result that cells from various organs may be susceptible to SARS-CoV-2 after treatment with recombinant ACE2 (rACE2) (25). A study by Haga et al. (11) showed that cells may be sensitive to SARS-CoV by inducing ADAM17 activity to increase the sACE2 production. The authors reported that SARS-CoV S induced ADAM17 activity, resulting in increased ACE2 shedding, which was positively correlated with SARS-CoV infection.

The virus-sACE2 complex, which is formed by sACE2 binding the SARS-CoV-2 S protein in the extracellular compartment, may have two major adverse effects (**Figure 3A**). i. The sACE2 coating on the virus does not interfere with host immune cells, making it easy to spread from the primary site of infection to distant organs to avoid immune attacks, especially in patients with complications, which mediates multiple organ failure. ii. This complex can be internalized by endocytosis. With a large number of sACE2 bound to the complex being internalized into host cells, the rapid decrease in mACE2 and extracellular sACE2 levels as well as the accumulation of Ang II may lead to a serious imbalance in host RAS homeostasis and severe COVID-19.

In Exogenous rACE2 Therapy and Comorbid Patients

Kornilov et al. reported that plasma sACE2 levels are higher in men than in women (26), consistent with Sama et al. (20). As

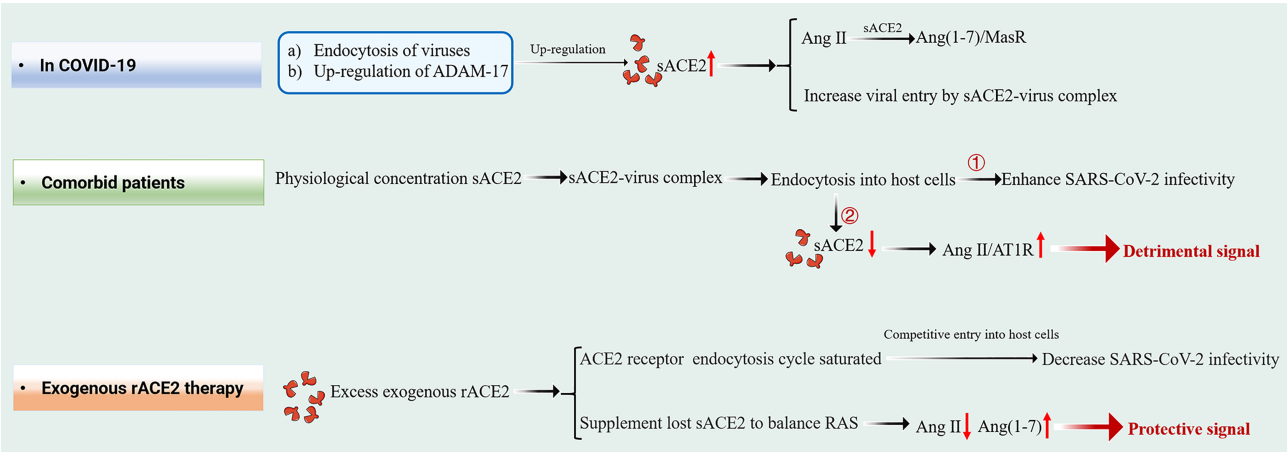

FIGURE 2 | Schematic diagram of the effect of sACE2 in circulation under three different states.

sACE2 levels increase with age, adult males have higher sACE2 levels than adult females and children (21). sACE2 levels are elevated in individuals with higher body mass indices (BMI) and metabolic syndromes while the correlation between sACE2 levels and metabolic syndromes is stronger in males (26).

In a global study involving 10 753 participants, elevated plasma ACE2 levels were correlated with an increased risk of major cardiovascular events (including death, myocardial infarction, stroke, heart failure and diabetes) (27). A recent large-scale study reported that sACE2 levels in patients with severe COVID-19 were higher than in patients with non-severe COVID-19 (28). However, as a potential risk marker for severe COVID-19, the roles of sACE2 have not been fully evaluated. Therapeutic human recombinant soluble ACE2 (hrsACE2) or engineered sACE2 have been shown to reduce Ang II levels, substantially suppress the expressions of key cytokines associated with COVID-19 pathogenesis and prevent viral entry in patients receiving such treatments and in cell experiments (29, 30). Plasma ACE2 (sACE2) needs special considerations. We cannot yet adequately consent with the claim that it decreases viral entry.

Exogenous rACE2 Therapy

With regards to inhibition of viral entry, therapeutic sACE2 exhibited dual effects in SARS-CoV-2 infection **(Figure 2)**. In *in vitro* models, very high concentrations of rACE2 [10-200 μg/mL of ACE2, which are much higher than its physiological concentration (20, 31)] inhibited SARS-CoV-2 infection. These findings are consistent with the results of Yeung et al. who showed that 25 and 100 μg/mL rACE2 can inhibit SARS-CoV-2 infection. We postulated that excess administration of exogenous rACE2 (therapeutic sACE2, at μg/mL level) might saturate the endocytosis cycle of ACE2 receptor, compete with SARS-CoV-2-ACE2 complex to enter host cells, resulting in reduced infectivity of SARS-CoV-2. In contrast, rACE2 at near-physiological concentrations (that is, ηg/mL levels) can enhance SARS-CoV-2 infectivity (25). Interestingly, a similar phenomenon was reported in a recent study (29), which showed that after the administration of rACE2, the viral loads were detected substantially and stably increased in nasopharynx swabs (from ~10^4 copies/mL at day 0 to ~10^5 copies/mL at day 5) and tracheal aspirates (from ~10^3 copies/mL at day 0 to ~10^5 copies/mL at day 2), respectively. Although patients eventually recovered after development of neutralizing antibodies, this *in vivo* data and results from the study by Yeung et al. suggest that the role of sACE2 in SARS-CoV-2 infection should be carefully considered.

Natural and therapeutic sACE2 retain Ang II catalytic activities, in addition to binding viral RBD. Additional infusions of sACE2 supplements lost sACE2 and is important in temporarily balancing RAS during exogenous rACE2 therapy. Continuous transformation of Ang II into Ang1-7 protects Ang II-mediated severe pathological processes. Therefore, exogenous hrsACE2 treatment suppresses excess RAS activation and the increase in Ang II concentrations, thereby reducing damage to multiple organs, including the lungs, kidneys and heart (12, 32, 33).

Patients With Complications

Although comorbid patients have more sACE2, the opposite phenomenon is observed, and COVID-19 patients with comorbidities suffer more serious consequences. We believe that the half-life of natural sACE2 in circulation is short and it occurs in very low levels. It gains entry into host cells in form of virus-sACE2 complex, which may be the reason as to why comorbid patients fail to obtain protection. We propose a viral entry mechanism for comorbid patients. SARS-CoV-2 in the extracellular compartment can bind a large number of sACE2 molecules to form sACE2-viral complexes, and sACE2 levels in circulation decrease dramatically after endocytosis. The resulting rapid rise in Ang II levels may lead to cytokine storms and other pathological complications through the Ang II-AT1R axis, aggravating disease severity in comorbid patients **(Figure 2)**.

As for the effect of soluble ACE2 in SARS-CoV-2 infection, these considerations need to be examined and confirmed

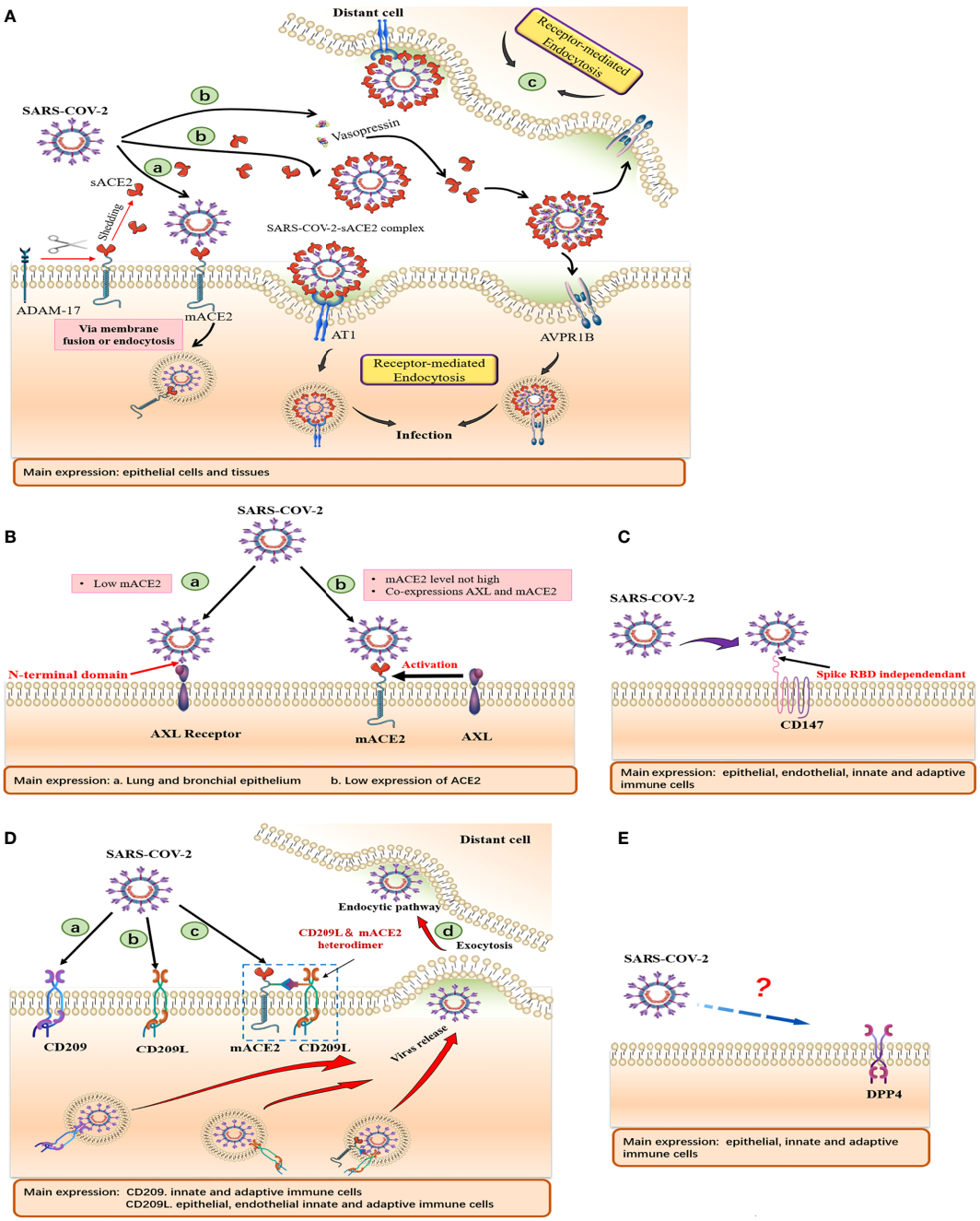

FIGURE 3 | Schematic of cell surface receptors mediated entry of SARS-CoV-2. Summary of tissue and cellular expressions, and models of **(A)** ACE2, **(B)** AXL, **(C)** CD147, **(D)** CD209, CD209L and **(E)** CD26. **(A) (a)** Virus gains entry into host cells *via* mACE2. **(b)** SARS-CoV-2 binds sACE2 to form an sACE2-virus complex, which subsequently enters host cells through AT1 and AVPR1B receptors mediated endocytosis. **(B) (a)** AXL acts as a membrane receptor and its NTD binds SARS-CoV-2 to mediate viral entry. **(b)** AXL, as a host factor facilitating viral entry, cooperates with ACE2 to promote viral entry at low ACE2 expression levels. **(D) (a, b).** CD209 and CD 209L acts as membrane receptors that mediate viral entry. **(c)** CD209L can form heterodimerization complexes with ACE2 to mediate viral entry. **(d)** Cells expressing DC-SIGN and L-SIGN can transfer SARS-CoV-2 to susceptible target cells, release virus through exocytosis and mediate distant cell infection.

experimentally to clarify the precise role of sACE2 *in vitro* and *in vivo*. Biological mechanisms of elevated sACE2 levels are still an active research field, which is likely to be a common result of complex interactions between impaired cell expressions, enzymatic digestion and impaired plasma clearance. However, our biological understanding of the roles of sACE2 is limited and we cannot infer their functions at the biological tissue level.

OTHER MEMBRANE RECEPTORS THAT MEDIATE SARS-CoV-2 INFECTION

ACE2 is expressed in different epithelial cells, including those of the lungs, kidneys, intestines, heart, brain neurons, immune cells, pancreas and blood vessels. Its tissue distribution is organ-specific. Elevated expressions have been found in the kidneys, cardiovascular and gastrointestinal systems (17, 34, 35). Expression levels of ACE2 in the lungs are very low, and they are only expressed in a small part of lung epithelial cells (36), moreover, they may even be undetectable in endothelial cells of lung tissues. ACE2 is rarely expressed in immune cells, but they can be infected by SARS-CoV-2, suggesting that SARS-CoV-2 may enter and infect some human cells by attaching to other receptors, or to combinations of multiple receptors and/or enhancers. These may be the key to infection of tissues with low or without ACE2 expression.

RECEPTOR: AXL

The TAM phosphatidylserine receptor family, AXL, is expressed in almost all human organs, especially in the lungs, bronchial epithelial tissues and cells, where AXL levels are much higher than those of ACE2. ACE2 is rarely expressed in the lungs and trachea (37).

Overexpressed AXL in AXL/ACE2 double KO HEK293T cells promote viral infection, reaching levels that are comparable to those of HEK293T cells overexpressing AXL. AXL down-regulation in ACE2-KO H1299 cells was shown to significantly reduce SARS-CoV-2 pseudotyped infections (38). Human recombinant soluble AXL (rather than ACE2) prevents SARS-CoV-2 infection in cells with elevated AXL expressions. Besides, expression levels of AXL are closely correlated with SARS-CoV-2 S protein levels in bronchoalveolar lavage fluid cells from COVID-19 patients (38). These findings suggest that AXL may be a novel host receptor that mediates SARS-CoV-2 entry and infection. Since AXL is not co-expressed with TMPRSS2 or ACE2 in human lung and tracheal cells, sACE2 fails to prevent cells overexpressing AXL from SARS-CoV-2 infection, and vice versa, implying that AXL is independent of ACE2-mediated viral entry (**Figure 3B**).

AXL and SARS-CoV-2 S are mainly co-localized in the cell membrane. Contrary to the ACE2 receptor, AXL directly interacts with the SARS-CoV-2 spike protein N-terminal domain (NTD) rather than RBD (38). Evidence obtained by measuring viral RNA levels adsorbed on cell surfaces and internalized viral RNA in cells revealed that AXL enhances SARS-CoV-2 infection by promoting viral adsorption.

These data are in contrast to the conclusions of another report, which points out that AXL does not interact with SARS-CoV-2 spike proteins, and neither does it mediate SARS-CoV-2 entry unilaterally, however, it is a host factor promoting SARS-CoV-2 entry (39). Under synergistic effects of low-level ACE2, AXL interacted with viral particle-related phosphatidylserine (PS) to enhance SARS-CoV-2 infection, while the effects of AXL were no longer observed at high ACE2 levels (39). Since expression levels of ACE2 in the lungs are low, AXL may be highly correlated with SARS-CoV-2 infections.

RECEPTOR: CD147

CD147, also known as basigin (encoded by BSG), is a transmembrane receptor. Wang et al. (40) for the first time, reported on the direct interactions between CD147 and SARS-CoV-2 spike proteins, which mediate host cell infection. SARS-CoV-2 viral loads were detected in the lungs of hCD147 mice infected with SARS-CoV-2, but not in the lungs of wild-type mice infected with SARS-CoV-2. In another study, SARS-CoV-2 pseudovirus was shown to infect ACE2 deficient T cells in a dose-dependent manner, and this infection was specifically inhibited by a humanized anti-CD147 antibody (Meplazumab), suggesting that overexpression of CD147 promotes viral infection (40). This result provides a possible explanation for lymphocyte reduction in COVID-19 patients. In addition, Meplazumab can effectively promote the rehabilitation of patients with SARS-CoV-2 pneumonia (41).

mAb, which targets spike RBD, does not prevent viral entry in SW480 and A549 cells (these cells express CD147 but have a low abundance of hACE2). However, anti-CD147 mAb inhibits viral infections in A549 cells (42), indicating that RBD does not interact with CD147 and is not directly involved in CD147-mediated infections in these cells. Besides, CD147 knockdown in Calu-3 cells expressing high levels of hACE2 did not affect susceptibility to SARS-CoV-2 (42), indicating that CD147 seems to act as an alternative receptor in cells without or with a low abundance of hACE2.

Urszula et al. reported elevated CD147 levels in epithelial tissues, congenital and adaptive immune cells, which acted as SARS-CoV-2 receptors (43). Potentially, these cells can be infected in the lungs or can carry SARS-CoV-2 from infected cells through CD147 to participate in local and systemic transmissions of the virus, thereby enhancing the exaggerated immune responses (44).

Interestingly, immunofluorescence assay did not show co-localizations of CD147 and ACE2 in lung tissues of COVID-19 patients, and neither did it show interactions in detected cells (**Figure 3C**). Furthermore, expressions of CD147 and ACE2 in a single lung cell were found to be completely independent, indicating that CD147 and ACE2 may be two complementary receptors mediating SARS-CoV-2 infection (40).

Hyperglycemia and RAGE activation can upregulate expressions of the CD147 glycoprotein while advanced glycation end products (AGEs) have been shown to significantly elevate the expression levels of the CD147 protein through RAGE-dependent mechanisms (45). Due to elevated AGEs expressions in diabetic patients, corresponding CD147 protein expression levels increased, which may increase the accessibility of SARS-CoV-2 to diabetic tissues. Relative to healthy controls, plasma CD147 levels have been reported to be elevated in diabetic patients and can be used to predict 10-year mortality rates (46). CD147 overexpressing in host cells may

promote more viral entry, which may partially explain the high mortality rate in diabetic patients with COVID-19.

RECEPTORS: DC-SIGN AND L-SIGN

Endothelial cells lacking ACE2 receptors can also be infected by SARS-CoV-2. Suppression of CD209L expressions or functions in endothelial cells by soluble CD209L or shRNA inhibited SARS-CoV-2 entry. In addition, ectopic expressions of CD209 and CD209L in HEK-293 cells enhanced SARS-CoV-2 entry (47). Amraei et al. reported that the RBD of SARS-CoV-2 S protein binds CD209L and CD209 to mediate viral entry (47). Kondo et al. documented that L-SIGN mediates viral entry by interacting with high-mannose–type N-glycans on SARS-CoV-2 spike proteins. Blockade of L-SIGN functions can significantly reduce SARS-CoV-2 infections (48). These findings indicate that in addition to ACE2, CD209L/L-SIGN and CD209/DC-SIGN can act as substitute receptors for mediating SARS-CoV-2 entry into host cells **(Figure 3D)**.

CD209L is highly expressed in renal proximal epithelial cells, alveolar type II epithelial cells, as well as in endothelial cells of blood vessels, lungs, liver and lymph nodes (47, 49, 50), CD209 is mainly expressed in tissue-resident macrophages, dendritic cells and B cells (51, 52). These findings suggest that SARS-CoV-2 can invade these cell types by binding CD209L and CD209. Through single-cell RNA sequencing analysis, Chao et al. found that CD209/DC-SIGN is highly expressed in innate immune cells and lymphoid organs (53). They also observed that DC-SIGN can directly bind the SARS-CoV-2 S glycoprotein with a picomolar affinity, trigger S internalization by 3T3DC-SIGN + cells, which might provide a pathway for SARS-CoV-2 to enter macrophages and dendritic cells. These newly discovered SARS-CoV-2 receptors on innate immune cells may aggravate cytokine release syndrome and severe pathological inflammation.

In addition, the vascular system is the main attack site of SARS-CoV-2. Patients with COVID-19 exhibit endothelial cell injuries (namely endodermis), angiogenesis changes and extensive microvascular thrombosis (54, 55). SARS-CoV-2 gains entry into endothelial cells using CD209L as the receptor. This leads to suppressions of CD209L functions in endothelial cells and alterations in angiogenesis as well as endothelial cell damage (47).

CD209L and CD209 have been shown to directly physically interact with the SARS-CoV-2 S-RBD protein. Consistent with the findings of Amraei et al., van Kooyk et al. reported that CD209L binds the S protein of SARS-CoV-2 in an ACE2-independent manner (56). It has also been reported that the S protein of SARS-CoV-2 binds CD209 in a glycosylation-dependent manner (53). Co-immunoprecipitation assays showed that CD209L can form heterodimerized complexes with ACE2, which may play an important role in infection of cell types expressing both CD209L and ACE2 (47). These findings indicate that CD209L and ACE2 are co-receptors for SARS-CoV-2 infections. Taken together, CD209L promotes SARS-CoV-2 entry into host cells in ACE2-dependent and non-dependent ways.

Cells expressing DC-SIGN and L-SIGN can transfer SARS-CoV to susceptible target cells (57, 58). Therefore, it is postulated that SARS-CoV-2 binds CD209L and CD209 receptors on innate immune cells, which then transfer and redistribute them to target tissues, where they cause more damage after viral internalization (59). This hypothesis provides a possible explanation for how SARS-CoV-2 spreads to extrapulmonary tissues in the host.

RECEPTOR: DPP4

Another potential receptor that may explain the association between COVID-19 and diabetes involves the dipeptidyl peptidase-4 (DPP4 or CD26) enzyme **(Figure 3E)**, which is widely expressed in many tissues (such as kidneys, lungs, intestines and immune cells) and plays a key role in inflammation as well as glucose homeostasis. DPP4, a common pharmacological target of type 2 diabetes (T2DM) and a functional coronavirus receptor, can enhance sensitivity to coronavirus infections. In MERS-CoV infections, MERS-CoV S glycoprotein RBD binds human receptor dipeptidyl peptidase 4 (hDPP4) to mediate viral entry (60). A large proportion of COVID-19 cases are accompanied by different severity of neurological symptoms. Madeline et al. reported that cortical astrocytes, which had lowest ACE2 levels and elevated DPP4 levels were significantly and mainly infected. Inhibition of DPP4 can attenuate viral infection and decrease the expression levels of the cell stress marker, ARCN1. DPP4 mediates SARS-CoV-2 tropism to human astrocytes, resulting in reactive glial hyperplasia injury (61). Vankadari et al. predicted the interactions between DPP4/CD26 and the S1 domain of SARS-CoV-2 spike protein, thereby elucidating on the complex docking model of SARS-CoV-2 spike glycoprotein and DPP4 (62). This implies that the complementary virus-host interaction was in addition to main interactions between ACE2 and the S protein.

Indeed, Tai et al. (63) reported that the RBD of SARS-CoV-2 can bind 293 T cells expressing human ACE2, but cannot bind 293 T cells expressing human DPP4. Flow cytometry analysis further showed that the binding of SARS-CoV-2 RBD to 293 T cells can be significantly blocked by the sACE2 protein, but not by sDPP4. Furthermore, the SARS-CoV-2 RBD cannot prevent MERS-CoV pseudovirus from entering 293T cells expressing hDPP4 (63). Hence, in the absence of experimental verification, bioinformatics data should be interpreted with caution. Although it is necessary to clarify the direct relationship between DPP4 and SARS-CoV-2 infection, evidence suggests that the DPP4 inhibitor (DPP-4i) can regulate inflammation and exert antifibrotic activities. These properties may have potential applications in preventing progression to an over-inflammatory state associated with severe COVID-19.

DIABETES INCREASES COVID-19 SEVERITY

Diabetes does not increase the risk for SARS-CoV-2 infection, but significantly increases COVID-19 severity and associated

mortality rates. These outcomes are mediated by hyperglycemia and blood glucose fluctuations, expressions of furin proteins and ACE2 receptor, ACE2 autoantibodies production, imbalances in immune and inflammatory pathways, diabetes-related complications as well as lung injury in diabetes. In addition, demographic features (e.g., age, gender) affect COVID-19 prognosis **(Figure 4)**.

Hyperglycemia and Glucose Fluctuations

Baseline blood glucose and continuous glycemic control in hospitalized patients, rather than T2DM itself seem to be associated with COVID-19 progression (64). Fasting blood glucose levels at admission, rather than previously diagnosed diabetes was found to be an independent predictor for severe illness (65), death (66), or adverse outcomes (67) in hospitalized COVID-19 patients.

Elevated plasma glucose levels and glycolysis in human monocytes enhance and sustain SARS-CoV-2 replication (68) while elevated plasma glucose levels promote viral replication (69). These findings imply that hyperglycemia may inhibit antiviral immune responses (70), which may explain the prolonged rehabilitation time among diabetic patients with COVID-19.

In addition, SARS-CoV2 causes acute glucose fluctuations in diabetic patients, and glycemic variability may exert negative effects on patient rehabilitation (71, 72). Fluctuations in blood glucose levels are also true for diabetic patients. Therefore, in addition to long-term glycemic control (73), acute hyperglycemia may play a key role in worsening COVID-19 prognosis (74).

Immune Dysfunction and Cytokine Storm

Congenital and adaptive immunity, inflammation and coagulation abnormalities are significantly more in diabetic COVID-19 patients than in non-diabetic COVID-19 patients (75), which promotes COVID-19 progression, independent of other complications (76), and are associated with blood glucose levels (77). Nevertheless, delayed immune responses, the disproportionate number of immune cells and high levels of inflammation in diabetic patients may exacerbate them to 'cytokine storm', which seems to be directly correlated with multiple organ dysfunction, death and COVID-19 severity (78–80).

Expression of ACE2 and Furin in Diabetes

SARS-CoV-2 binds ACE2, which is subsequently cleaved by proteases, such as TMPRSS2 and furin, leading to internalization of virosome complexes (81). One of the mechanisms through which diabetes increases the risk of serious infection may be attributed to increased expressions of ACE2 receptor and furin, which may promote the entry and replication of SARS-CoV-2 (81, 82). Due to the increase in efficient virus entry, viral load increases, resulting in a poor prognosis of COVID-19. An elevated serum furin level is a marker of diabetes progression, and is correlated with metabolic abnormalities as well as an increased risk of premature diabetes-associated death (83). In diabetic rodent models, ACE2 expressions were also reported to be elevated in the lungs, kidneys, heart and pancreas (84, 85). The relationship between diabetes and ACE2 expression in human lungs has not been fully established. Rao et al. (86) conducted a full-phenotypic Mendel randomization study and found that diabetes has a causal relationship with elevated ACE2 expression levels in the lungs, which may promote susceptibility to severe complications of SARS-CoV-2 infections. While some evidence shows that ACE2 levels in renal tissues of diabetic patients are down-regulated (87, 88), suggesting that ACE2 expressions in diabetic patients vary among tissues. ACE2 has been found to be expressed in multiple organs - a possible reason for multiple organ failure in some COVID-19 patients.

ACE2 Autoantibodies

ACE2 autoantibodies were found in SARS-CoV-2-infected patients (89–91). Once the body generates antibodies against SARS-CoV-2 RBD, antibodies that recognize and inhibit self ACE2 may also be produced. Through this mechanism, the virus triggers autoimmune diseases (92). Therefore, ACE2 autoantibodies may be considered anti-idiotypic antibodies. Autoantibodies against ACE2 (AA-ACE2) can be used to reflect the severity of COVID-19. The AA-ACE2 level in patients with COVID-19 was significantly higher than that in the control group without infection. Of note, AA-ACE2 levels in moderate and severe COVID-19 patients were significantly higher than in mild patients (91). Similarly, Wang et al. found that AA-ACE2 levels were higher in COVID-19 patients, especially in severe patients, than in healthy controls. The AA-ACE2 can alter the course of COVID-19 by disrupting immune response to SARS-CoV-2 and tissue homeostasis (93).

The AA-ACE2 level in patients with diabetes (Median 16.630 [IQR 10.480–27.356] U/mL) was significantly higher than that in non-diabetic patients (Median 7.957 [IQR 4.339–19.715] U/mL) (91). Notably, there were differences in AA-ACE2 levels among patients with the same severity level, this may be caused by individual differences in immune response as well as consequences of previous complications (such as diabetes). The relationship between AA-ACE2 and disease severity is also applicable for COVID-19 patients with diabetes (91).

Plasma from patients with ACE2 autoantibodies inhibited exogenous ACE2 activity whereas that obtained from patients without ACE2 autoantibodies did not (91). This inhibition may reduce the activity of membrane-bound ACE2 and soluble ACE2 (89). In COVID-19, circulating ACE2 levels were increased as a function of virus-induced ACE2 shedding. The elevated ACE2-SARS-CoV-2 complex level in circulation may enhance the production of AA-ACE2. High levels of AA-ACE2 may further decrease the activity of membrane-bound ACE2 in lung and other tissues, which is likely to increase Ang II levels and immune system activation, thereby transforming the RAS balance to the pro-inflammatory axis. Hence, AA-ACE2 (by reducing the activity of transmembrane ACE2 and transformation of Ang II to Ang 1-7) increased the activity of AT1 receptor, enhanced pro-inflammatory response, and ACE2 shedding (94, 95), thereby aggravating the severity of COVID-19.

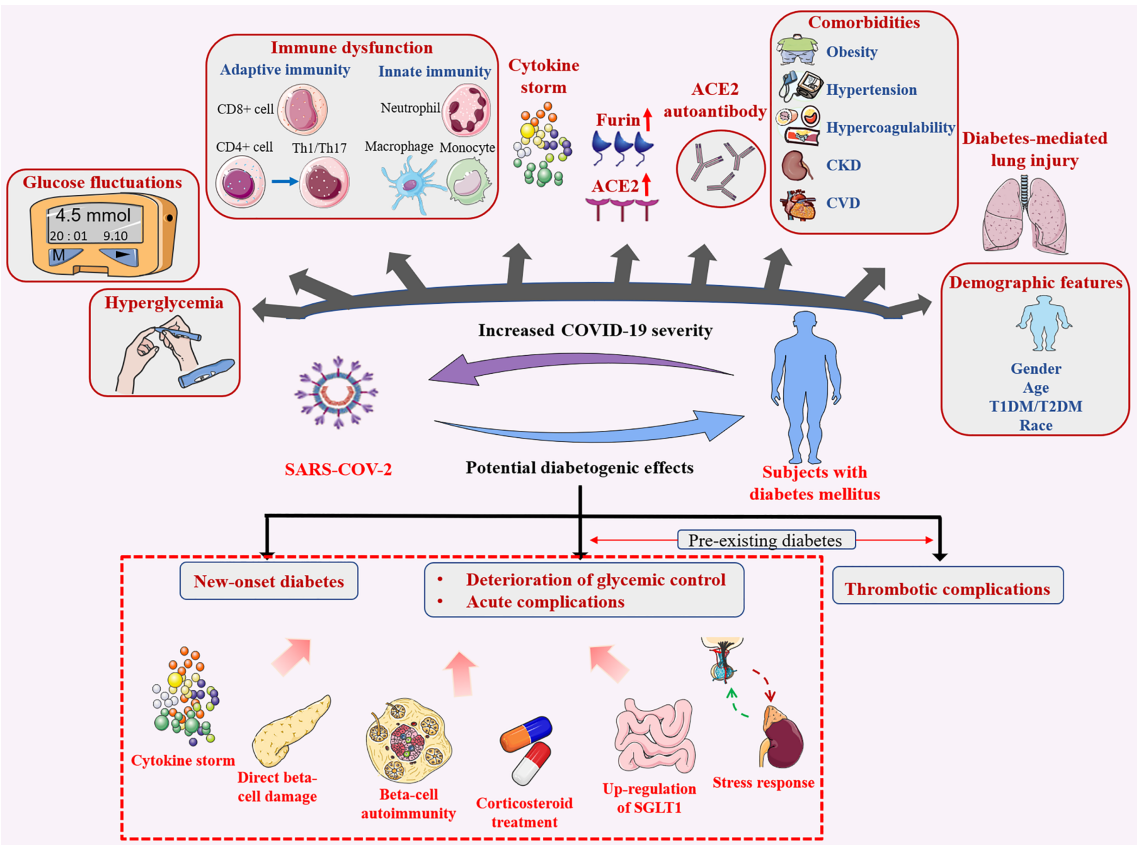

FIGURE 4 | Bidirectional effects between diabetes mellitus and SARS-CoV-2 infection. Diabetes mellitus contributes to poor infection outcomes, SARS-CoV-2 infection has potential diabetogenic effects.

Diabetes-Related Complications

In addition to the negative impact of hyperglycemia on COVID-19, diabetes-related chronic complications such as obesity, hypertension, coronary arterial disease and chronic kidney disease may further deteriorate COVID-19 progression (96–103). Diabetes and related complications are also associated with increased fibrinolytic enzyme expression levels. The fibrinolytic enzyme is a protease that can cleave the S protein of SARS-CoV2, which is beneficial for the virus to combine with ACE2 and enter cells to increase the virulence and infectivity of SARS-CoV-2 (104). Furthermore, fibrinolytic enzyme-associated degradation of blood fiber proteins leads to elevated D-dimer levels and other degradation products of blood fiber proteins, which is a feature of severe disease (104). Complications of diabetic patients are independent of diabetes itself, and they are associated with poor COVID-19 prognosis (73, 105).

Lung Injury in Diabetes

Diabetes is associated with physiological and structural abnormalities in lung tissues and impaired lung functions (106). Forced vital capacity is reportedly low in T1DM patients, which is thought to be associated with poor glycemic control (107). Philips et al. (108) found that in animal models, diabetes was correlated with changes in lung structure, increased pulmonary vascular permeability and contributed to alveolar epithelial collapse. Therefore, given the impaired respiratory function in diabetic patients and the tendency of SARS-CoV-2 to attack lung tissue cells, it may aggravate COVID-19 pulmonary complications.

Demographic Features

Gender

Global Health 50% data tracker (109) provides data on COVID-19 confirmed cases and deaths from countries around the world according to gender. As of April 2021, the number of male and female COVID-19 confirmed cases was generally comparable, however, the mortality rate among male patients was found to be high. Two reports from the United States (83, 110) and one report from Italy (111) showed that the number of COVID-19-associated deaths in males is higher than in females, however, the incidence of SARS-CoV-2 infection in females is higher. These studies did not classify infections based on age. Risk assessment data of COVID-19 confirmed cases from the European Union/European Economic Zone (EU/EEA) countries and the United Kingdom (UK) showed that the proportion of males requiring hospitalization, intensive care, respiratory support and death was

higher than that of females. And the overall ratio of male to female confirmed cases was 0.9, the proportion of males in infected elderly cases was higher, while the proportion of females in infected young cases was higher (112). Indeed, reports from Switzerland and Germany confirmed increased incidences of COVID-19 among men aged over 60 years (113, 114). Nevertheless, the equal absolute number of cases between men and women may indicate a higher incidence of SARS-CoV-2 infection in young women.

These gender differences may be attributed to variations in expression levels of ACE2 and TMPRSS2, and the influence of hormones on immune responses (115). Based on existing evidence, we only analyzed the differences in expressions of the two forms (mACE2 and sACE2) of the main receptor ACE2, and proposed two mutually exclusive mechanisms for explaining gender susceptibility and infection outcomes for COVID-19.

Poor outcomes among males with SARS-CoV-2 infections were speculated to be mainly related to insufficient mACE2 and elevated sACE2 levels. i. Older age, male gender and higher BMI may enhance mACE2 shedding, leading to relative mACE2 deficiency. Jehpsson et al. (116) reported that renin levels increase with age and from puberty, these levels are elevated among men than in women with a positive correlation with sACE2 levels. In circulation, renin levels and activities are correlated with AngII levels (117). Renin may promote ADAM-17-induced mACE2 shedding by affecting AngII levels. Hence, in COVID-19, increased RAS signals with age may lead to elevated AngII/α integrin and ADAM-17-induced mACE2 shedding. Therefore, potential age and gender differences in RAS signals between children and adults as well as between males and females (118) may theoretically lead to mACE2 deficiency among young men (compared to children and women), which may increase the risk for severe COVID-19. These findings are in tandem with those found in rat models (17). ii. Male gender, smoking, older age, high blood pressure and BMI are associated with higher sACE2 levels in circulation. And sACE2 at near-physiological concentration has been proposed as a potential biomarker for COVID-19 severity and can enhance SARS-CoV-2 infection. Moreover, the sACE2-virus complex can be endocytosed by host cells, leading to a sharp decline in sACE2 levels. The subsequent rapid increase in Ang II levels aggravates disease severity through the Ang II-AT1R axis. iii. Low androgen levels among women may inhibit TMPRSS2 expressions, implying that it may be a further protective factor against COVID-19 (119).

Reasons as to why young women have a higher SARS-CoV-2 infection incidence have not been established. However, we postulate that it could partially be due to elevated ACE2 gene expression levels. Through analysis of the GTEx and other public data in 30 organizations of thousands of individuals, expression levels of the ACE2 gene in young people, especially women, were found to be high, while in men, they were low, and the ACE2 gene expressions further decreased with age and T2DM occurrence (120). The data involving humans and mice (120) further revealed that T2DM and inflammatory cytokines are involved in inhibition of ACE2 expressions. Estrogen significantly increases the expressions of the ACE2 gene, while androgen moderately elevates its expression. Age-associated decrease in sex hormone levels can lead to suppressed ACE2 expressions while elevated ACE2 mRNA levels may increase ACE2 activity. Therefore, as a receptor for SARS-CoV-2, elevated ACE2 gene expressions among women may increase the viral load by increasing viral invasion, thereby increasing the risk of SARS-CoV-2 infection.

However, from the perspective of sACE2, males exhibit higher plasma sACE2 levels (26, 27). sACE2 at near-physiological concentration has the ability to bind SARS-CoV-2 and mediate viral infections. However, the half-life of natural sACE2 in circulation is short and it occurs in very low levels (there is little difference between males and females). Therefore, mACE2 plays a leading role in mediating SARS-CoV-2 infections. Nevertheless, it should be noted that there is evidence for mismatches between mRNA levels of ACE2 and ACE2 activity or protein expression in many tissues (121). Thus, the susceptibility of SARS-CoV-2 cannot be fully explained based on ACE2 mRNA expression pattern alone.

Age

The severity of infection significantly increases with age. Inferences from age-specific COVID-19-related mortality data from 45 countries revealed that mortality rates among children aged 5-9 years were the lowest, and there was a strong logarithmic linear relationship between age and mortality risk in individuals aged 30-65 years (122). Another study showed that the death risk for patients aged 80 and above was 12 times that of patients aged 50-59 (123). Age-related immune dysfunction, namely immunosenescence and inflammation, plays an important role in increasing vulnerability to severe COVID-19 outcomes among the elderly (124).

T1DM/T2DM

Evidence for COVID-19 in T1DM and T2DM is inconclusive. A large National Health Agency (NHS) study in England evaluated COVID-19 mortalities in T1DM (n = 364) and T2DM (n = 7,434) patients. Multiple ORs of 2.86 (95% CI: 2.58-3.18) and 1.80 (95% CI 1.75-1.86), respectively, were reported (125). These findings are supported by a cohort study based on COVID-19 infection, which reported a higher severity and risk of death in T1DM patients than in T2DM patients (73). In another prospective cohort study involving all Scottish populations, OR values of COVID-19 risk for T1DM and T2DM patients with fatal or intensive care unit (ICU) treatment were 2.40 (95% CI 1.82, 3.16) and 1.37 (95% CI 1.28, 1.47), respectively (126). In contrast, multicenter French Coronavirus SARS-CoV-2 and Diabetes Outcomes (CORONADO) study did not reveal differences in COVID-19 primary outcomes between T1DM and T2DM patients. However, this study only involved 39 T1DM patients (105). Moreover, Wargny et al. reported that the risk of death in hospitalized T1DM patients with COVID-19 on day 7 was only half of that for T2DM patients (10.6% *vs* 5.4%) (127), this study based on the CORONADO Study. Most of the current findings indicate a higher risk of death in T1DM patients, although other studies are yet to confirm this finding.

Race

In addition to the abovementioned factors, the race is also a factor associated with increased COVID-19 severity. Among the three major global ethnic groups (African, Asian, White), expression levels of ACE2 differ. Among East Asians, the most important quantitative expression locus (eQTL) contributing to elevated ACE2 expressions is close to 100%, which is more than 30% higher than that of other ethnic groups (120).

COVID-19 POTENTIAL DIABETOGENIC EFFECT

The SARS-CoV-2 infection has a huge impact on diabetes progression. It alters immune system responses and promotes inflammation, which may, in turn, lead to glucose imbalance among patients with previous diabetes and even new-onset diabetes. This is an amplification cycle and forms a vicious cycle (**Figure 4**).

COVID-19 Induced New-Onset Diabetes

Some viruses could cause autoimmune type 1 diabetes in patients with genetic susceptibility after infection, and even fulminant diabetes due to massive damage to islet β cells (128). SARS-CoV-1 can cause secondary hyperglycemia (129). Yang et al. compared SARS patients without T2DM history and steroid treatment with matched healthy compatriots. They found that, during hospitalization, more than 50% of patients suffered from diabetes due to SARS-CoV infection. After three years of recovery from viral infections, only 5% of patients still suffered from diabetes (130). Immunostaining of the ACE2 protein also revealed that it was strongly expressed in the islets, but weakly expressed in exocrine tissues, indicating that SARS-CoV might bind ACE2 receptors in the pancreas, leading to acute diabetes by damaging the islets (130). Similarly, the initial diabetes diagnosis is very common in SARS-COV-2 infections, and is not associated with a previous history of diabetes or with the use of glucocorticoids (131), this newly-occurring hyperglycemia is an independent predictor of death (132). According to a meta-analysis involving eight studies, approximately 14% of COVID-19 patients develop new-onset diabetes after hospitalizations (133). By measuring plasma amylase and lipase levels, Wang et al. found pancreatic injury in 9/52 patients with SARS-CoV-2 associated pneumonia, with 6/9 of them exhibiting a moderate increase in blood glucose levels (134). Another study reported that 17% of severe COVID-19 patients had elevated lipase and amylase levels while 7.5% had focal pancreatic enlargement or pancreatic duct dilatation (135). ACE2 is highly expressed in the liver, endocrine pancreas, adipose tissues, kidneys and in the small intestines (136, 137). Therefore, we postulate that SARS-CoV-2 may bind ACE2 in important organs such as the liver and pancreas, and play a potential role in β-cell damage and insulin resistance, thereby inducing new-onset diabetes or worsening diabetes prognosis.

After recovery from COVID-19, it has not been determined whether blood glucose levels return to normal. Long-term assessment of pancreatic β-cell function is recommended to identify future diabetes mellitus development. The COVID-19-related diabetes global registration system (COVIDIAB project) has been established, which may clarify the course and prognosis of new-onset diabetes in COVID-19.

Deterioration of Blood Glucose, Severe Metabolic Complications and Thromboembolism Induced by COVID-19

Deterioration of Glycemic Control and Acute Complications

In diabetic patients infected with SARS-CoV-2, insulin doses increase while blood glucose levels become difficult to control (76, 131, 138, 139). Zhou et al. (138) retrospectively analyzed capillary blood glucose (BG) levels in 881 diabetic patients with COVID-19. They reported that 69.0% of the patients had unsatisfactory BG levels, while 10.3% had at least one hypoglycemia attack. Hypoglycemia is associated with higher cardiovascular-related mortality in diabetic patients (140). In addition, COVID-19 is associated with severe diabetic metabolic complications, including DKA and HHS (76, 141). Hyperglycemia and/or DKA often occur in T1DM patients after COVID-19 infections (142).

Inflammatory storms, β-cell damage, triggering of beta-cell autoimmunity, SGLT1 dysfunction, elevated stress levels and corticosteroid treatment after SARS-CoV-2 infection may act com-binatorially, which not just induces new-onset diabetes, also aggravates hyperglycemia and abnormal blood glucose fluctuations, even leads to DKA, HHS and hypoglycemia.

Inflammatory Storms

The relationship between COVID-19 and hyperglycemia in T2DM patients might reflect metabolic inflammation and excess release of cytokines. In diabetic patients, basal cytokine levels, such as IL1-β and IL-6, are elevated, and they are in a mild chronic inflammatory state, which is further amplified by SARS-CoV-2 infection, thereby promoting the vicious circle of cytokine release and rapid increase in blood glucose levels. Elevated cytokine levels (especially IL-6) cause invasive inflammatory reactions referred to as "cytokine storms", which result in more extensive multiple organ damage. At the same time, it also alters the AMPK/mTOR signaling pathway in diabetic patients, which may aggravate insulin resistance and development of diabetes-related complications. In addition, subclinical inflammatory responses, especially elevations in both IL-1β and IL-6, have been shown to occur prior to the onset of T2DM (143), which further suggests that COVID-19 may increase the risk of new-onset diabetes.

Direct β-Cell Damage

Islet β cell injury is associated with various factors. i. As earlier mentioned, ACE2 is also expressed in islet β cells, SARS-CoV-2 enters islet cells by binding ACE2 receptors, thereby resulting in inflammatory cytokine release, β-cell apoptosis and reduced insulin secretion (30). Similarly, studies by Müller et al. have shown that SARS-CoV-2 infects and replicates in cultured human islet cells, leading to morphological and functional

abnormalities (144). ii. Interestingly, ACE2 knockout mice were shown to be more susceptible to β-cell dysfunction (145), which explains why SARS-CoV-2 infection can lead to secondary hyperglycemia in patients without previous diabetes. RAS plays a key role in liver and adipose tissue inflammation, insulin resistance and poor glucose tolerance (146, 147). The unique interactions between SARS-CoV-2 and RAAS may provide another mechanism. Down-regulation of ACE2 after SARS-CoV-2 infection may lead to excess Ang II accumulation. It is worth noting that AT1R exists in pancreatic β and α cells of both mice (148) and human beings (149). Therefore, through ACE-Ang II-AT1R axis, it may reduce blood flow and insulin secretion while increasing oxidative stress in pancreatic cells, thereby promoting low inflammation, leading to insulin resistance and islet β cell impairment (150). iii. Regarding deteriorations in blood glucose levels among COVID-19 patients with previous T2DM, a recent study (151) reported that mRNA and protein expression levels of ACE2 in human islet β cells were significantly elevated under the actions of inflammatory cytokines, which may make β cells to be more vulnerable to severe infections.

Triggering Beta-Cell Autoimmunity

In addition to direct β-cell damage, SARS-CoV-2 invasion in pancreatic β-cells triggers β-cell autoimmunity in susceptible individuals, leading to development of type 1 diabetes (T1DM) (79).

SGLT1 Dysfunction

Sodium-glucose cotransporter 1 (SGLT1 or SLC5A1) is physiologically involved in the active absorption of glucose across the intestinal epithelium. ACE2-mediated SGLT1 imbalance in the intestinal epithelium may explain why COVID-19 aggravates diabetes complications and increases the risk of death (137, 152, 153). The existing data support the elevated expression levels of ACE2 in human gastrointestinal tissues (136), the high expressed ACE2 levels in intestinal mucosal cells and gallbladder makes these organs potential sites for viral entry and replication. Continuous viral replication at these ACE2 enrichment sites may be the basis for recurrence in some reported patients (believed to have been cured). ACE2-mediated downregulation of SGLT1 in the intestinal epithelium has been shown to prevent hyperglycemia in diabetic rats (154, 155). Although there is no direct evidence on effects of the combination of SARS-CoV-2 and ACE2 on its signaling cascade reactions, evidence from SARS-CoV-1 infections suggests that it can down-regulate ACE2 expressions (12), which may lead to up-regulation of SGLT1, thereby, aggravating hyperglycemia (154, 155). Since Ang II up-regulates while Ang-(1-7) suppresses the up-regulation of SGLT1 (154, 155), it is postulated that down-regulation of ACE2 expressions by binding SARS-CoV-2 leads to increased Ang II levels and to a corresponding decrease in Ang-(1-7) levels, which up-regulates SGLT1, increases intestinal glucose absorption, and promotes the development of hyperglycemia in COVID-19 patients.

Elevated Stress Levels

SARS-CoV-2 infections in diabetic patients may elevate stress levels and promote hyperglycemic hormone secretion, such as glucocorticoids and catecholamines, resulting in elevated glycemia and abnormal glucose variability (156).

Corticosteroid Treatment

Corticosteroids, including dexamethasone, are effective in preventing clinical deterioration and in reducing COVID-19-associated mortality rates. However, they are considered to be highly diabetogenic drugs, leading to uncontrolled hyperglycemia and even DKA or HHS.

Thrombotic Complications

Another feature of COVID-19 potential diabetogenic effect is diabetes-related endothelial dysfunction, which is characterized by hypercoagulable states, high incidences of thrombosis as well as microvascular complications.

SARS-CoV-2 infections activate immune responses, mediate the release of pro-inflammatory cytokines, over-activation of coagulation cascades as well as platelet aggregation, and induce micro- and macrovascular thrombosis, which are the main pathological characteristics of COVID-19. This greatly increases the likelihood of thromboembolic events, which are leading causes of death. Incidences of venous thromboembolism in COVID-19 patients are high (157). First, SARS-CoV-2 infections are associated with excess inflammation, and IL-6-related cytokine storms (158) can lead to high blood viscosity, thereby increasing the risk of stroke. Second, SARS-CoV-2 can also infect endothelial (54) and myocardial cells (136) through ACE2 receptors expressed in the vascular endothelium, myocardium and arterial smooth muscles, causing endothelial cell apoptosis, inflammatory cell infiltration, myocardial inflammation and injury, which increases the risk of thrombosis and stroke.

Hypercoagulable and fibrinolytic markers were found to be significantly increased in diabetic patients, and platelet activities as well as adhesion to endothelial walls were increased. These patients are at an increased risk of thromboembolic events or stroke (159, 160), especially in acute hyperglycemia (161) or high glucose variability states (162). Therefore, SARS-CoV-2 infections may promote their susceptibility to these conditions. Moreover, elevated levels of D-dimer, ferritin, IL-6 and other inflammatory markers in COVID-19 (163) may increase the risk of micro- and macrovascular complications in diabetic patients, which are as a result of low-grade vascular inflammation (164).

DRUG THERAPY FOR DIABETES AND RELATED-COMPLICATIONS IN PATIENTS WITH COVID-19

Diabetes and related metabolic complications are highly associated with severe COVID-19 development. Therefore, in addition to continuous strict blood glucose management to prevent acute complications **(Table 1)**, taking into account complications and specific thrombosis tendencies, diabetic patients with COVID-19 should be provided with appropriate antihypertensive, lipid-lowering and anticoagulant therapies to

TABLE 1 | A summary of classes of antidiabetic medications and their effects on ACE2 expression, inflammation, cardiovascular and kidney outcomes, effects in SARS-CoV-2 infection as well as issues prompting cautious use in patients with COVID-19.

Drugs	ACE2 expression	Anti-inflammatory	Cardiovascular and kidney outcome	Other beneficial effects	Effects in SARS-CoV-2 infection	Issue requiring caution in patients with COVID-19	Use in SARS-CoV-2 infection
Metformin	Increased expression and stability of ACE2	+	Benefit	i)Reduced uncomplicated DKA ii)Reduced microvascular complications and thrombotic events	i)Reduced disease severity and mortality ii)Improved prognosis iii)Potentially Inhibits virus-host protein interaction	i)Lactic acidosis ii)Deterioration of renal function iii)Gastrointestinal adverse events	Continue in hemodynamic stability and avoid in severe COVID-19 patients with hypoxia, hypoperfusion, septicemia, severe hepatic and renal impairment or hemodynamic instability
DPP-4 inhibitors	NR	+	Neutral	Reducing the risk of hypoglycemia	Not enough data (controversial, not defined)	i)Vildagliptin is associated with interstitial lung injury ii)Sitagliptin is associated with increased risk of venous thromboembolism	Continue in mild to moderate COVID-19 and avoid in critically ill
Thiazolidinediones	ADAM-17 down-regulation subsequently up-regulates mACE2	+	Benefit	i)Attenuates pulmonary fibrosis and ALI ii)Reducing the risk of thromboembolism events	Targeted 3CLpro and potentially inhibited SARS-CoV2 RNA synthesis and replication	i)Fluid retention ii)Peripheral edema iii)Heart failure	Discontinue in hospitalized COVID-19 patients with the risk of acute heart failure by current illness
GLP-1RAs	up-regulation mACE2	+	Benefit	i)Reduced hypoglycemia and glucose variability ii)Reducing the risk of AS and thromboembolism events	Not enough data	i)Gastrointestinal adverse events ii)Slow efficacy	May consider continuing in COVID-19 patients without associated gastrointestinal symptoms
SGLT2 inhibitors	up-regulation mACE2	+	Benefit	i)Reducing the risk of hypoglycemia ii)Reducing the risk of MACE and heart failure iii)Reduced all-cause mortality	i)Not enough data for disease prognosis ii)Inhibits cell Na+/H+ exchangers, reduces lactate serum levels, thus reducing cell acidosis and SARS-CoV-2 activation at acidic pH	i)DKA ii)Dehydration iii)kidney injury iv)genitourinary infection	Can be used in well-controlled, uncomplicated patients and avoid in moderate to severe patients requiring strict body fluid balance control
Insulin	down-regulation mACE2 correspondingly up-regulation sACE2	+	Neutral	Low risk of uncontrolled hyperglycemia and DKA	Not enough data (Possible increases the risk of poor prognosis and mortality)	i)Hypoglycaemia ii)weight gain	Can be used in patients requiring strict glycemic control
Sulfonylureas	NR	+	Inconclusive		Neutral	i)Hypoglycemia ii)Potential adverse cardiovascular effect	It is best to avoid using SUs, especially in critically ill patients
Glinides	NR	+	Neutral		Potentially inhibits the replication and transcription of SARS-CoV-2	The same as SUs	The same as SUs
Alpha-glucosidase inhibitors	NR	+	NR		Potentially inhibits virus-host protein interaction, inhibits the replication and transcription of SARS-CoV-2	Gastrointestinal adverse events	Avoid in patients with obvious gastrointestinal symptoms

NR, not report; ACE2, angiotensin-converting enzyme 2; DKA, diabetic ketoacidosis; COVID-19, coronavirus disease 2019; 3CLpro, 3-chymotrypsin-like protease; DPP4, dipeptidyl peptidase-4; SGLT2, sodium-glucose cotransporter 2; GLP-1RAs, glucagon-like peptide 1 receptor agonists; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; MACE, major adverse cardiovascular events; AS, atherosclerosis.

ensure better clinical outcomes. However, clinical administrations of glucocorticoids must weigh the beneficial effects of anti-inflammation against the potentially harmful effects of impaired immunity.

Antidiabetic Drugs

Metformin

The mechanisms of metformin involve AMPK-independent and AMPK-dependent pathways (165). Indirectly, metformin suppresses chronic inflammation by improving insulin resistance and hyperglycemia. One of the mechanisms through which it exerts its anti-inflammatory effects involves inhibition of AGEs formation, which will promote inflammation and glucose oxidation (166). Moreover, to exert its anti-proliferative and immunomodulatory effects, it inhibits nuclear factor-κB (NF-κB) through the AMP-activated protein kinase (AMPK) activation pathway (167–170). Besides, it regulates glucose and lipid metabolism (171). The activation of AMPK can also phosphorylate ACE2, which may alter the conformational and functional of the receptor, thereby reducing the binding of RBD to the ACE2 receptor (172), thus alleviating SARS-CoV-2 infections. Metformin up-regulates ACE2 expression and increases its stability in COVID-19.

A large-scale retrospective cohort study involving 6256 patients showed that compared to male patients, metformin treatment is significantly correlated with decreased mortality rates in female T2DM patients with COVID-19 (173). Other studies have supported the clinical benefits of metformin in diabetic patients with COVID-19 (174, 175).

Continuing metformin administration in hemodynamically stable COVID-19 patients may be safe, however, it should be avoided by critically ill patients with sepsis, severe liver and kidney damage, hypoxemia, inadequate perfusion or by those exhibiting hemodynamic instabilities due to the risk of lactic acidosis (176).

DPP4 Inhibitors

Apart from ACE2, DPP4 may also be involved in SARS-CoV-2 infections (62). It represents a potential target for mitigating the severity of COVID-19 by blocking viral transmissions and attenuating inflammatory responses. However, receptor inhibitors that are designed to lower glycemia may not apply to the binding interface between the virus and the receptor. Anti-DPP4 antibodies have been shown to inhibit hCoV-EMC infections of Huh-7 cells and human bronchial epithelial cells, while DPP-4is, such as vildagliptin, sitagliptin and saxagliptin were not shown to prevent this infection (60).

Rhee et al. reported that DPP-4i is associated with better clinical outcomes in COVID-19 patients (177). In a multicenter retrospective case-control study involving 338 consecutive T2DM patients with COVID-19, sitagliptin therapy reduced mortality and improved clinical outcomes (178). Mirani et al. (179) reported that DPP-4is are significantly and independently correlated with a low mortality risk. Diabetic patients administered with DPP-4i exhibited a lower severity of COVID-19, a lower risk of mortality as well as a lower need for mechanical ventilation. Multiple retrospective case-control studies have shown that DPP-4i does not affect the risks of hospitalization, ICU admission, mortality and clinical outcomes in T2DM patients with COVID-19 (175, 180). Currently, evidence for the use of DPP-4i in diabetic patients with COVID-19 is inconclusive. Two ongoing clinical trials are assessing whether addition of DPP-4 inhibitors on the basis of insulin therapy can help improve the severity of COVID-19 (NCT04341935; NCT04542213), which may also be extended to patients without diabetes mellitus.

DPP-4i mainly affects postprandial blood glucose levels and lowers hypoglycemia risk. In stable patients with good food intake, DPP-4i can be continued (139), however, it should be suspended in patients with severe discomforts (181). DPP-4i can be administered in impaired renal function patients, however, for COVID-19 patients who clinically manifest fluid depletion or systemic sepsis, DPP-4i doses should be adjusted for optimal outcomes. Sitagliptin is associated with an increased risk of venous thromboembolism, which may require discontinuation in hospitalized COVID-19 patients (182).

Thiazolidinedione

Pioglitazone downregulates ADAM-17, a mACE2 cleaving enzyme in the human skeletal muscles, leading to elevated mACE2 levels. Moreover, pioglitazone has been shown to elevate ACE2 expression in animal models, especially in the liver and adipose tissues (183, 184), raising concerns about increased susceptibility to SARS-CoV-2 infection. Computer-based virtual bioinformatics analysis revealed that pioglitazone might target 3-chymotrypsin-like protease (3CLpro) to inhibit SARS-CoV-2 RNA synthesis and replication (185).

Thiazolidinediones (such as pioglitazone and rosiglitazone) have the potential risk of fluid retention and peripheral edema, which may increase the risk of heart failure. Therefore, they should be avoided in COVID-19 patients with a risk of acute heart failure from existing diseases (186). The antihyperglycemic effects of thiazolidinedione last for several weeks after drug withdrawal (just like the effect of maintaining fluid), therefore, temporary interruption of treatment has little effect on glycemic control (187). More clinical trials should be performed to optimize the risk-benefit ratio of pioglitazone in COVID-19 patients.

GLP-1 Receptor Agonist

GLP-1RAs have been reported to increase ACE2 expressions in the lungs and hearts of type 1 diabetic rats (188), therefore, it can offset the down-regulation effects of diabetes on the expressions of ACE2 in the lungs. However, it has not been established whether it can affect clinical progressions of COVID-19. Indeed, clinical evidence for GLP-1RAs administration in SARS-CoV-2 infections is inconclusive. Given the beneficial effects of GLP-1RA (189) in prevention of cardiovascular and kidney diseases have been defined (190), these drugs may be a priority for treatment of diabetic patients with this risk. GLP-1RAs treatment can lower hypoglycemia risk, reduce glucose variability as well as catabolism by suppressing glucagon levels (191), which may exert a protective effect on critically ill patients in the ICU. However, it is not recommended to start or maintain such therapies in acute or critical situations (e.g. severe COVID-

19), as they may work slowly and are associated with increased gastrointestinal adverse events. Studies in animal models have reported that GLP1 analogues can inhibit atherosclerosis formation, stabilize the plaque of carotid artery and aortic arch (189, 192). The REWIND study also showed that dulaglutide can decrease stroke incidences in T2DM patients (193). Taken together, it will be beneficial for diabetic patients with COVID-19 to be administered with antidiabetic drugs that can reduce the risk of thromboembolism.

SGLT2 Inhibitors

Evidence suggests that SGLT-2i promotes the expression of ACE2 in the heart and kidneys (194), and then increases Ang1-7 levels. Ang1-7 can effectively expand blood vessels, exert antioxidant and anti-fibrotic effects, and is involved in attenuation of cytokine storms as well as in prevention of ARDS, which is also considered to be a possible heart and kidney protection mechanism for these drugs. Although SGLT-2i alleviates inflammatory injury and endothelial dysfunction, its potential beneficial effects in diabetic patients with COVID-19 have not been fully established. Evidence from DARE-19 (195), a randomized, double-blind, placebo-controlled study of dapagliflozin for delaying disease progression and prevention of major clinical events in hospitalized COVID-19 patients with cardiometabolic risk factors, clarified that dapagliflozin treatment did not significantly improve clinical recovery or reduce the risk of organ dysfunction as well as death, but was well tolerated.

SGLT-2i has important cardiovascular and renal protective functions and may protect important organs during COVID-19. Similarly, the results from the DARE-19 study do not support the conventional discontinuation of dapagliflozin in COVID-19. Concerns about the administration of SGLT2is in COVID-19 are associated with reduced blood volume, increased risk of renal insufficiency, genitourinary tract infections and ketoacidosis. DKA with normal blood glucose has been reported in SGLT-2i administered T1DM and T2DM patients (196, 197). Drug withdrawal must be considered if glomerular filtration rate decreases and renal function deteriorates. SGLT-2i is associated with dehydration and anorexia, therefore, it is not recommended for diabetic patients with moderate to severe COVID-19 who need strict body fluid balance control in conditions of inadequate food intake, dehydration and insufficient blood volume.

Insulin

Insulin is widely used in COVID-19 patients with hyperglycemia, especially in severe cases. However, recent conflicting evidence suggests that insulin therapy may contribute to the death of diabetic patients with COVID-19. A retrospective study involving 689 T2DM patients with COVID-19 reported that insulin therapy is significantly associated with increased mortality, accompanied by aggravated systemic inflammation and exacerbated damage to vital organs (198). Nonetheless, residual confounders cannot be ruled out. Patients receiving insulin therapy are usually in severe conditions, and have high blood glucose levels, therefore insulin is preferred.

Immunofluorescence analysis confirmed elevated ACE2 and ADAM17 levels in the kidneys of diabetic Akita mice and decreased ACE2 levels in the kidneys of insulin-treated Akita mice (199). Helena et al. (84) reported that insulin down-regulated the expression of mACE2 in non-obese diabetic mice. In a longer follow-up period, insulin administration restored serum sACE2 levels. At near-physiological concentrations, sACE2 enhances SARS-CoV-2 infection, and it may be a potential biomarker for COVID-19 severity. Therefore, increased severity of COVID-19 after insulin administration may be partly attributed to the ACE2 receptor. However, evidence for these outcomes is limited. Large, prospective, randomized, placebo-controlled clinical trials should be performed to elucidate on the harmful effects of insulin therapy in COVID-19 patients. Tight glycemic control is important because poor-outcomes are correlated with high blood glucose levels in COVID-19. Taken in sum, fuzzy evidence on insulin should not prevent its use in hospitalized patients that are in need of tight glycemic control.

Sulfonylureas (SUs)

Feng et al. (200) predicted that glibenclamide (SUs) and tolazamide (the first generation of sulfonylurea drugs) might be used to treat COVID-19 coexisted with T2DM, but their findings, which are based on computer simulations, need *in vitro* experimental validation. Multiple studies have reported that SUs are harmless or unhelpful to COVID-19 patients (105, 201–203), while some studies report that SUs administration may lead to poor prognostic outcomes in patients with myocardial infarction (MI) (204). On the contrary, some studies have refuted the association between SUs and MI-induced death (205, 206). Besides, SUs increase the risk of hypoglycemia, which is worsened in patients with impaired renal functions or insufficient calorie intakes. Given the risk of severe hypoglycemia and cardiovascular safety, SUs should be avoided by COVID-19 patients, especially critically ill patients.

Glinides and Alpha-Glucosidase Inhibitors

Through virtual screening, repaglinide (207) and acarbose (208, 209) were found to inhibit SARS-CoV-2 replication and transcription. However, molecular structure information differs from clinical effectiveness, therefore, careful interpretation is needed. The action mode of glinides is similar to that of SUs, therefore, they should be cautiously administered, especially in COVID-19 patients with related cardiac injuries. Given the gastrointestinal side effects, α-glucosidase inhibitors are not suitable for COVID-19 patients with obvious gastrointestinal symptoms.

Therapeutic Options for Related-Complications

ACE Inhibitor (ACEi) and Angiotensin Receptor Blockers (ARBs)

Previous report had shown that lisinopril and losartan are associated with a significant increase in ACE2 levels (210). Concerns raised about the up-regulation of ACE2 expression might increase the risk of COVID-19 infection. Contrarily, it has

been proposed that elevated ACE2 levels after RAAS inhibitor treatments might actually be beneficial for COVID-19 induced lung injury (211). Elevated mACE2 levels are associated with upregulated Ang1-7, which has vasodilation, anti-fibrosis as well as protective effects on lung injury (212). However, SARS-CoV-2 infection down-regulates mACE2, leading to over-accumulation of AngII toxicity, which may aggravate inflammation and thrombosis (13), and lead to more severe lung injury (213).

Through feedback regulation, the accumulation of Ang II in COVID-19 patients may lead to low mRNA expression levels of ACE2. Gallagher et al. reported that Ang II over-expression reduces ACE2 mRNA expressions in rat cardiomyocytes and fibroblasts, which was blocked by losartan, an ARB (214).

With aging and disease, the ACE-Ang-II-AT1R axis controls ACE2 - Ang (1-7) -MasR axis. After SARS-CoV-2 infection, virus-mediated endocytosis further reduces mACE2 expressions, resulting in Ang II surge, thereby triggering another round of mACE2 reduction through up-regulation of ADAM-17-mediated shedding by AT1R. Therefore, in COVID-19 patients with comorbidities, viral entry and Ang II accumulation downregulates ACE2, which transfers RAS balance to the harmful end. ARBs and ACEI turn the balance to the beneficial axis, attenuate Ang II accumulation, and prevent AT1R-mediated mACE2 loss as well as harmful cascades. Due to the administration of ARB and ACEI, this recovered ACE2 was mistakenly considered to be over-expressed.

Losartan was shown to prevent severe lung injury and pulmonary edema in ACE2 knockout mice (215). A retrospective study of hospitalized patients with COVID-19 and hypertension showed that ACEI and ARBs ameliorated clinical outcomes and alleviated cytokine storms (216, 217). A multicenter retrospective study involving 1128 adult hypertensive patients diagnosed with COVID-19 showed that hospitalized patients with ACEI/ARB had a lower risk of all-cause death compared to those without ACEI/ARB (218). A recent meta-analysis suggested that ACEI therapy reduced the risk of SARS-CoV-2 infection and that blocking RAS might reduce all-cause mortality in COVID-19 patients (219). Multicenter, blinded RCTs on the impacts of losartan on clinical prognosis of COVID-19 patients who need/do not need hospitalization (NCT04312009, NCT04311177), and the ongoing trials on whether the lead-in of ACEi in *de novo* may help ameliorate COVID-19 outcomes (NCT04366050) will elucidate on these aspects.

Experts strongly recommend treatment continuation with ACEi and ARBs. Substantial evidence indicates that RAAS inhibitors elevate the expressions of ACE2, but they are not associated with increased SARS-CoV-2 infection risks or poor prognosis of COVID-19 (220–223), on the contrary, it is even helpful to some extent. A sudden withdrawal of ACEI/ARB may cause greater harm, especially in patients with a high risk of renal and cardiovascular diseases. Accordingly, to maintain their antihypertensive and cardiorenal protective effects in COVID-19 patients, it is not recommended to discontinue these drugs.

Aspirin

Due to reports on acute respiratory tract infections previously, concerns were raised about the possibility of increased risks of adverse events (such as multiple organ failure, ARDS and death) induced by NSAID treatments in COVID-19 patients (224, 225). Nonetheless, given the lack of evidence that they may lead to serious adverse consequences in COVID-19, major scientific societies worldwide have issued advisories to discourage either avoidance or suspension of NSAIDs in COVID-19 (226–228).

On the contrary, aspirin, a non-steroidal anti-inflammatory drug, has various pharmacological properties that can exert potential beneficial effects for COVID-19 patients. First, its analgesic and antipyretic effects may help alleviate the specific symptoms of COVID-19. Second, it may exert anti-inflammatory, antithrombotic and antiviral effects (229–233), which may be useful in preventing pathophysiological processes of severe clinical manifestations involved in COVID-19. Much solid evidence from *in vitro* and experimental models support that aspirin reduces the synthesis and replication of several RNA-encapsulated viruses (including human CoV-229E and MERS-coronavirus) in infected cells, thereby reducing viral titers and virulence (229). Moreover, excess Ang II signaling in COVID-19 may activate the STING pathway (234), which promotes hypercoagulation through the secretion of interferon-β and tissue factors by monocyte-macrophages. Aspirin has been found to directly inhibit the STING pathway (235), thereby reducing tissue factor procoagulant activities.

Aspirin improves survival outcomes for patients with different types of infections, which is characterized by hyperactivation of inflammatory cascades and increased platelet reactivities (236–238). Zhou et al. reported that COVID-19 severity can be alleviated by aspirin. After investigating a cohort of adult COVID-19 patients admitted to several hospitals in the United States, aspirin administration was found to reduce mechanical ventilation rate, ICU occupancy rate and hospital mortality rates (239). A cross-sectional study using data from the Leumit Health Services database observed that the possibility of COVID-19 infection, disease duration and mortality are negatively correlated with aspirin administration for primary prevention (240).

Prophylactic anticoagulation is a potential conventional treatment option for diabetic patients with COVID-19. For hospitalized patients with moderate to severe COVID-19, initiation of anticoagulant therapy may be prudent, but may not be necessary for mild patients. Anticoagulant therapy (e.g., aspirin, low molecular weight heparin) for severe COVID-19 patients with a high risk of thromboembolism (e.g., D-dimer increased patients) (241) has been associated with better prognostic outcomes. A randomized clinical trial, RECOVERY II, is underway to test the effectiveness of low-dose aspirin as an antithrombotic and anti-inflammatory option for COVID-19 patients (242). Given the increased risk of thromboembolism among patients with COVID-19 and diabetes, we recommend that physicians consider the administration of antiplatelet or anticoagulant therapies more actively.

Heparin

Heparin sulfate (HS) promotes the recruitment of SARS-CoV-2 to the cell surface, thereby increasing its local concentrations to effectively bind ACE2 and increase viral entry (243, 244). The

SARS-CoV-2 spike protein can simultaneously bind heparin and ACE2. Therefore, heparin has the ability to compete with HS for SARS-CoV-2 S protein binding, which is important in inhibiting SARS-CoV-2 infection.

COVID-19 patients usually present with thrombosis complications, including microthrombosis, venous thromboembolism and stroke, and often receive therapeutic unfractionated heparin (UFH) or low molecular weight heparin. Both drugs can block viral infection (244, 245). Plasma heparin levels of 0.3 – 0.7 unit/mL (equivalent to 1.6-4 μg/mL) can achieve effective anticoagulation, and this concentration is sufficient to prevent the S protein from binding cells. However, it does not prevent SARS-CoV-2 infection, but rather, attenuates infection according to viral load (243). Plasma heparin levels of ≥ 0.5 μg/mL can effectively reduce SARS-CoV-2 infection by more than 4 times. UFH and degradation of cell surface HS was shown to reduce infection by more than 5 times without affecting cell viability (243). These findings further emphasize on the potential of using UFH or other non-anticoagulant heparins to prevent SARS-CoV-2 adhesion.

Statins

Statins have been shown to restore the lowered ACE2 levels that are suppressed by high lipids such as low-density lipoprotein or lipoprotein(a) (246). Upregulated ACE2 levels may help alleviate multiple organ injuries after SARS-CoV-2 infections.

Besides, lipid-lowering effects of statins can improve hyperlipidemia associated with antiretroviral and immunosuppressive drugs that are based on protease inhibitors in COVID-19.

The lipid-lowering effect of statins is beneficial for cardiovascular diseases. Besides, statins modulate immune responses and alleviate inflammation as well as oxidative stress, which may help in attenuating cytokine storms. MYD88, a protein connector of the downstream inflammatory signaling pathways of Toll-like receptor and IL-1 receptor family members, plays a key role in activation and amplification of innate immune responses (247). SARS-CoV-1 induces inflammatory host responses by activating the Toll-like receptor (TLR)-MYD88-NF-κB pathway (248). Studies involving animal models have shown that statins inhibit myeloid differentiation of MYD88 and NF-κB activation. Inhibition of MYD88 was shown to suppress pneumonia-associated damage and improved the survival rate of mice infected with SARS-CoV and MERS-CoV (249).

Elevated C-reactive protein (CRP) levels are a risk factor for increased mortality in diabetic patients with COVID-19 (175). In individuals without COVID-19 or hyperlipidemia but with elevated CRP levels, rosuvastatin was found to decrease the risk of major cardiovascular events by 44% (250), which was attributed to its pleiotropic anti-inflammatory effects (251). A Chinese study reported that statins administration was correlated with low risk of all-cause mortality and good recovery characteristics in hospitalized patients with COVID-19 (252). Nevertheless, clinical benefits of statins should be further verified in RCTs.

The regulation of ACE2 expression affects SARS-CoV-2 infection and disease-associated mortality. Since the long-term benefits of statins may include weakening cytokine storms by suppressing IL-6 and IL-1β levels, it should not be discontinued. Moreover, COVID-19 is associated with higher cardiovascular-related mortality rates, particularly in patients with higher risk factors (including hypertension and T2DM). In these patients, statins can be administered to maintain or optimize lipid management and improve endothelial dysfunctions (253). Given the risk of hyperlipidemia, which is associated with antiretroviral immunosuppressive drugs, and cardiovascular disease, we recommend diabetic COVID-19 patients continuing the current statin therapy. Most statins are metabolized by CYP3A4 in the liver, however, in cases of simultaneous administrations of CYP3A4 inhibitors for COVID-19, such as ritonavir, it is recommended to start with lower doses of statins, while monitoring creatine kinase and transaminase levels.

Corticosteroids

Corticosteroids play an important role in the treatment of ARDS and sepsis, which are also the therapeutic targets for severe COVID-19 infections. COVID-19 is characterized by strong inflammatory responses. Corticosteroids may play an important role in inhibiting inflammation in the lungs and other tissues, thereby regulating inflammation-mediated lung injury and reducing the risk of ARDS as well as death by attenuating cytokine production.

A recent meta-analysis revealed that systemic glucocorticoid therapy reduces short-term all-cause mortality in severe COVID-19 patients (254). The RECOVERY study reported that dexamethasone administration reduced mortality by 36% (95% CI 0.5 - 0.81) in hospitalized COVID-19 patients receiving invasive mechanical ventilation (255). However, there are conflicting findings. Glucocorticoids therapy in COVID-19 patients was shown to induce delayed viral RNA clearance, increased mortality and incidences of complications (256). Moreover, glucocorticoids administration in diabetes patients with COVID-19 has been associated with poor prognostic outcomes, and meanwhile, the results also confirmed the harmful effect of hyperglycemia on disease outcomes (257, 258). The possible explanation is that, systemic corticosteroids therapy may decrease the expression of Ang1-7 and Mas receptors, leading to deterioration of hyperglycemia, which aggravates metabolic control (259). The possibility that hyperglycemia offsets mortality benefits of systemic corticosteroids cannot, therefore, be ruled out. Effectiveness of corticosteroid therapies in diabetic patients with COVID-19 has not been established, however, further long-term studies are needed to confirm this result.

Although corticosteroids reduce lung inflammation, they also inhibit immunity and pathogen clearance (256). Viral shedding after SARS-CoV-2 infection seems to be high in the early stages, and then decreases (260–262). Glucocorticoid treatment might be ineffective or even harmful in the early stages of infection (by increasing viral load), therefore, it is not recommended to use corticosteroids at the outset of the COVID-19 pandemic. For 1-week post-SARS-CoV-2 infection patients, or those receiving respiratory support, dexamethasone has been shown to have greater survival benefits, indicating that inflammation is

predominant and viral replication is secondary at this stage. The WHO provisional guidance on clinical management of severe acute respiratory infections induced by SARS-CoV-2 suggests that corticosteroids should not be used outside clinical trials.

CONCLUSIONS AND PERSPECTIVES

This review preliminarily summarizes current research progress on receptors related to COVID-19 with diabetes, especially ACE2. The mutual physiological effects between COVID-19 and diabetes, and commonly used medications have been updated and summarized. More RCT studies in the future will enable us to have a more accurate understanding of these findings. Optimal and timely blood glucose management can improve the clinical course of diabetic patients with COVID-19. Insulin is the preferred hypoglycemic agent for hospitalized and critically ill patients, however, the effects of insulin on ACE2 expressions and COVID-19 progression remains uncertain. For patients with COVID-19, AT1 receptor blocker treatment should not be interrupted. Considering that ACE2 autoantibodies increase the severity of COVID-19, it raises an important question whether AT1 receptor blockers or ACEI should be initiated for patients at risk of infection or diagnosed with COVID-19.

The biological functions of sACE2 (protective or risk factor biomarkers) in COVID-19 are poorly understood. The reasons as to why mACE2 proteins vary between different genders and ages leaving much to be explored. Whether for effective antiviral therapies, vaccines development or the progression of diabetes as well as related complications, ACE2 will remain the target for research as well as drug development for COVID-19 in the future. ACE2 is a complex site for diabetic nephropathy and can affect its progression, however, it has not been determined whether SARS-CoV-2 infection accelerates diabetic nephropathy progression through ACE2. In particular, long-term proteinuria and GFR monitoring should be performed for COVID-19 patients with diabetes. With genetic variations in the novel coronavirus, in order to contain its spread and reduce its associated severity and mortality, efforts should be aimed at developing effective vaccines. In this regard, it may be necessary to determine whether therapeutic options for diabetic patients have any impact on antibody responses and antibody levels of various vaccines for COVID-19. Besides, the impact of these vaccines on diabetic patients remains to be further explored.

AUTHOR CONTRIBUTIONS

LX drafted and wrote the manuscript. ZZ revised the manuscript. QW, YC, and DL reviewed the manuscript and provided critical comments. WW conceived and organized the work, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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High Fasting Blood Glucose Level With Unknown Prior History of Diabetes Is Associated With High Risk of Severe Adverse COVID-19 Outcome

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Background: We aimed to understand how glycaemic levels among COVID-19 patients impact their disease progression and clinical complications.

Methods: We enrolled 2,366 COVID-19 patients from Huoshenshan hospital in Wuhan. We stratified the COVID-19 patients into four subgroups by current fasting blood glucose (FBG) levels and their awareness of prior diabetic status, including patients with FBG<6.1mmol/L with no history of diabetes (group 1), patients with FBG<6.1mmol/L with a history of diabetes diagnosed (group 2), patients with FBG≥6.1mmol/L with no history of diabetes (group 3) and patients with FBG≥6.1mmol/L with a history of diabetes diagnosed (group 4). A multivariate cause-specific Cox proportional hazard model was used to assess the associations between FBG levels or prior diabetic status and clinical adversities in COVID-19 patients.

Results: COVID-19 patients with higher FBG and unknown diabetes in the past (group 3) are more likely to progress to the severe or critical stage than patients in other groups (severe: 38.46% vs 23.46%-30.70%; critical 7.69% vs 0.61%-3.96%). These patients also have the highest abnormal level of inflammatory parameters, complications, and clinical adversities among all four groups (all p<0.05). On day 21 of hospitalisation, group 3 had a significantly higher risk of ICU admission [14.1% (9.6%-18.6%)] than group 4 [7.0% (3.7%-10.3%)], group 2 [4.0% (0.2%-7.8%)] and group 1 [2.1% (1.4%-2.8%)], (P<0.001).

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Compared with group 1 who had low FBG, group 3 demonstrated 5 times higher risk of ICU admission events during hospitalisation (HR=5.38, 3.46-8.35, P<0.001), while group 4, where the patients had high FBG and prior diabetes diagnosed, also showed a significantly higher risk (HR=1.99, 1.12-3.52, P=0.019), but to a much lesser extent than in group 3.

Conclusion: Our study shows that COVID-19 patients with current high FBG levels but unaware of pre-existing diabetes, or possibly new onset diabetes as a result of COVID-19 infection, have a higher risk of more severe adverse outcomes than those aware of prior diagnosis of diabetes and those with low current FBG levels.

Keywords: COVID-19, FBG levels, diabetes, glycaemia control and treatment, complications

INTRODUCTION

As of August 10, 2021, more than 204 million cases of coronavirus disease 2019 (COVID-19) have been reported worldwide, including more than 4.3 million COVID-19 related deaths (1). Patients with SARS-CoV-2 infection may develop severe complications, such as acute respiratory distress syndrome (ARDS) and multiple organ failures during hospitalisation, often resulting in deaths (2). Previous studies have established that COVID-19 patients with diabetes are more susceptible to inflammatory storms, leading to worse severe adverse clinical outcomes (2–4). These patients reportedly have a consistently higher risk of complications during hospitalisation, with more frequent mechanical ventilation, intensive care unit (ICU) admission, and deaths than otherwise (3–5). In particular, the odds of ICU admission are 36-fold higher when blood glucose levels increase from 5 to 10 mmol/L among COVID-19 patients (6, 17).

Numerous studies have reported that SARS-CoV-2 can infect pancreatic endocrine cells that express Angiotensin-converting enzyme 2 (ACE2), the well characterised receptor of this virus, thus directly affecting insulin production and glucose metabolism (7–11). SARS-CoV-2 infection is also known to stimulate the vast release of cytokines, thereby leading to hyperinflammatory syndrome and triggering an inflammatory cytokine storm (12). Furthermore, the cytokine storm is often associated with the dysregulation in glucose metabolism, manifested as diabetes mellitus or elevation in blood glucose level, leading to metabolic disorders (13). Indeed, common pro-inflammatory molecules, such as IL-6 and CRP, are involved in pathways leading to inflammatory cytokine storm as a result of SARS-COV-2 infection and in diabetes contributing to an aberrant glucose metabolism (14). Taken together, the interaction among overlapping pathways affected by COVID-19 and diabetes may synergise to trigger cytokine storms and adversely affect the prognosis of individuals with COVID-19 and diabetes.

Indeed, diabetes mellitus is a common comorbidity associated with deleterious clinical outcomes in COVID-19 patients (15). Some of these patients presented themselves with a newly diagnosed diabetes, showing an elevated FBG level without a prior history of diabetes. Firstly, these individuals might have had a pre-existing diabetes which was not previously diagnosed. Indeed, it was found in a survey in 2010 in China that ~70% of the Chinese participants living with diabetes had no diagnosis of diabetes, thus they did not know that they had diabetes (16, 17). Secondly, SARS-COV-2 infection is likely to cause new onset diabetes in those with no prior diabetes as well as increase the severity of pre-existing diabetes and pre-diabetes (9, 18–25), leading to elevation of blood glucose levels.

A number of recent studies have shown that COVID-19 patients with an elevated FBG level are at a higher risk of more severe COVID-19 than that seen in subjects with normal glucose levels (26–29). These studies have clearly shown that elevated glycemic levels and previous diagnosis of diabetes are associated with adverse and more severe outcome of COVID-19. More interestingly, new onset diabetes defined by an elevated glycemic level and a normal HbA1c level on admission has been shown to be associated with more severe outcome than those with pre-existing diabetes. However, it is unclear whether the COVID-19 patients who present with an elevated glycemic level on hospital admission, but were unaware of prior status of diabetes, have a higher risk of severe COVID-19 outcome than those who had a prior diagnosis of diabetes. In this study, we analysed the clinical outcomes of 2,366 COVID-19 patients in a major site of the initial COVID-19 epidemic, the Huoshenshan hospital, Wuhan, China, and specifically investigated whether the current FBG level and prior awareness of diabetic status can predict the risk of clinical complications, length of hospital stay, ICU admission, and death in COVID-19 patients.

METHODS

Participants, Inclusion and Exclusion Criteria

We established a retrospective observational study cohort based on 3,059 COVID-19 patients admitted to the Huoshenshan hospital in Wuhan between February 04 and April 15, 2020. Subjects who had either a positive result of severe acute respiratory syndrome coronavirus two (SARS-Cov-2) detections in respiratory specimens by the reverse transcriptase-polymerase chain reaction assay or the presence of specific IgM and IgG antibodies to

SARS-Cov-2 in serum were included in the study. We applied the following exclusion criteria on participants: 1. Subjects who had neither positive SARS-Cov-2 detection result nor anti-SARS-Cov-2 antibodies detected as specified above in the inclusion criteria. 2. Patients who were referred to other medical institutions during hospitalisation; 3. Patients who were admitted to the hospital multiple times; 4. Patients younger than 18 years old; 5. patients without historical records of diabetes diagnosis at admission and laboratory data of FBG within 72 hours after admission.

Clinical and Outcome Indicators

Demographic, clinical, pre-existing comorbidities, laboratory test data, complications and outcome data were obtained from the hospital's electronic clinical medical records. At admission, demographic, clinical, laboratory data and pre-existing comorbidities (coronary heart disease, cancer, chronic bronchitis, cerebrovascular disease, chronic kidney disease, chronic obstructive pulmonary disease, diabetes, hepatitis and hypertension) were collected within the first day after admission. Pre-existing comorbidities were defined according to the ICD10-CM code based on the patients' clinical records and medical history (30).

We defined ICU admission as a key clinical outcome in this study. Clinical complications (acute respiratory distress syndrome, acute myocardial injury/heart failure, acute hepatitis/liver failure, respiratory failure, shock and acute kidney injury) and clinical outcomes (the length of stay, intensive care unit and death) were also collected during the hospital course from admission to the study endpoints. Detailed definitions for clinical symptoms and complications are provided in the supplemental materials section.

Clinical Discharge Criteria

Patients had to meet all the following criteria before being discharged: (1) body temperature returned to normal (<37.5°C) for three consecutive days; (2) respiratory symptoms improved substantially; (3) pulmonary imaging showed an obvious resolution of inflammation; and (4) two consecutive laboratory tests showing negative detection of SARS-Cov-2 by RT-PCR, each at least 24 hours apart.

Stratification of the Participants for Analysis

The subjects were stratified into 4 groups based on their FBG levels and awareness of prior status of diabetes. Group 1: patients with FBG<6.1mmol/L and unknown diabetes; Group 2: patients with FBG <6.1mmol/L and known diagnosis of prior diabetes; Group 3 patients with FBG≥6.1mmol/L and unknown diabetes; Group 4: patients with FBG≥6.1mmol/L and known diagnosis of prior diabetes. FBG 6.1 mmol/L was chosen as a cut point in stratification of subjects considering that FBG 6.1-6.9 mmol/L is defined to reflect impaired fasting glucose for Chinese population, from which the participants were recruited, according to the Guideline for the prevention and treatment of type 2 diabetes mellitus in China (2020 edition) (31), which is also consistent with the criteria of the World Health Organization.

Statistical Analysis

We presented continuous variables as the median and interquartile range (IQR) and examined the differences among the groups using the Kruskal-Wallis one-way ANOVA. We presented categorical variables with the corresponding percentage and examined the differences using the $\chi 2$ or Fisher's exact test. A multivariable cause-specific Cox proportional hazard model, adjusted for age, sex, chronic obstructive pulmonary disease, chronic kidney disease, cerebrovascular disease, coronary heart disease, cancer, chronic bronchitis, hepatitis and hypertension, was used to assess the associations between the FBG levels and prior diabetes status and ICU admission. Cumulative incidence curves were plotted to compare the incidence of ICU admission in 4 groups. A P-value of <0.05 was considered statistically significant. Statistical analyses were conducted using the R software (version 3.6.1).

RESULTS

Demographic and Clinical Characteristics of Patients

Of the 3,059 patients, we excluded 214 patients who were diagnosed as having COVID-19 based on clinical symptoms without laboratory tests, 46 patients who were transferred to other medical institutions, 16 patients who were admitted to the hospital multiple times, 6 patients who were <18 years of age. We further excluded 395 patients who had no record of FBG levels determined within 72 hours after admission or had no information to show whether they had a prior history of diabetes. A total of 2,366 COVID-19 patients were included for analysis (**Figure 1**).

The demographic characteristic, symptoms, pre-existing comorbidities, laboratory test results, complications and clinical outcomes are shown in **Table 1** for all the 4 stratified groups. The subjects who had <6.1 mmol/L FBG and had no record of prior diagnosis of diabetes at admission were stratified to Group 1. The subjects in this group had a significantly younger age than those in the other groups (median age: 58 years versus 63-65 years, Kruskal-Wallis one-way ANOVA, p<0.001) and had the lowest percentage of male participants (47.9% vs 53.4%-56.4%, p=0.051).

The subjects who had ≥6.1 mmol/L FBG and had no record of prior diagnosis of diabetes at admission were stratified to Group 3. This group had the highest proportion of patients with severe and critical clinical severity than that in other groups (38.5% vs 23.5-30.7% with severe clinical severity, and 7.7% vs 0.6-4.0% with critical clinical severity, p<0.001). Subjects in Group 3 had the highest levels of key inflammatory parameters among the four groups (p<0.001), such as C-reactive protein, D-dimer, neutrophils count, lactate dehydrogenase, direct bilirubin, fibrinogen, alkaline phosphatase, creatinine and cystatin C (**Table 1**). There were highest proportion of patients in Group 3 who had clinical complications, such as acute respiratory distress syndrome (4.3% vs 0.2-1.7%, p<0.001), acute myocardial injury/failure (3.8% vs 1.2-2.0%, p=0.012), acute hepatitis/liver failure (3.8% vs 1.4-2.6%, p=0.034), respiratory failure (6.0% vs 0.9-3.9%, p<0.001) and shock (2.1% vs 0.3-1.0%,

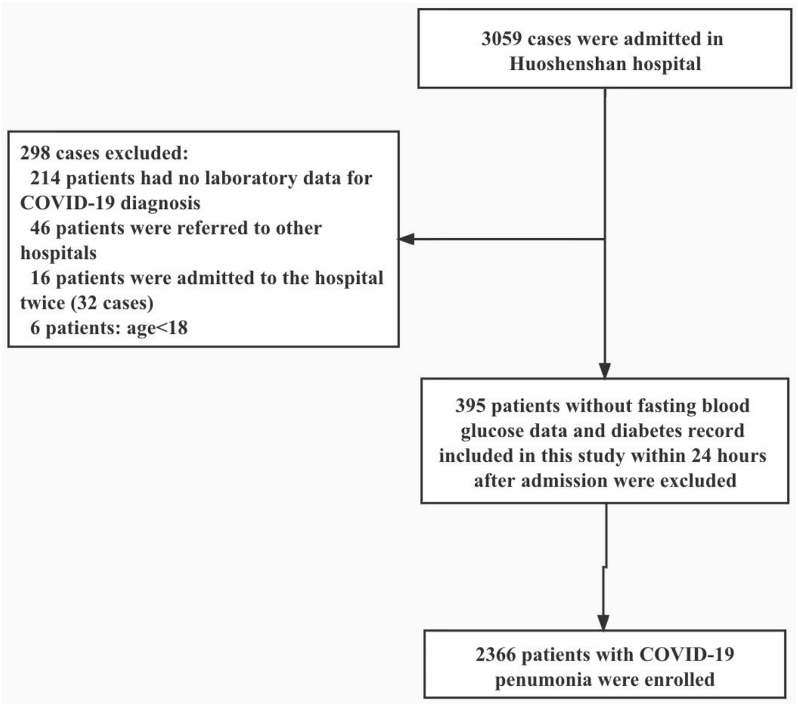

FIGURE 1 | Flowcharts for the selection of study participants.

p=0.009). People in Group 3 had the longest hospital stay (17 vs 12-14 days, p<0.001), the highest rate of ICU admission (14.1% vs 2.2-7.0%, p<0.001) and death (9.4% vs 0.8%, 4.0%, 4.0% in groups 1, 2 and 4, respectively; p<0.001).

Risk of ICU Admission

Hazard ratio was analysed to show the relative risk of ICU admission, an indicator of critical severity of COVID-19 patients in various groups. People in Group 3 were found to have an >5 times higher risk of ICU admission during hospitalisation than group 1 (HR=5.38, 95% CI 3.46-8.35, P<0.001, **Table 2**). People in Group 4, like people in Group 3, had FBG≥6.1mmol/L on admission, but, unlike group 3, they knew that they had pre-existing diabetes. Interestingly, group 4, albeit showing higher odds than that seen in group 1, had lower odds than Group 3 (1.99, 1.12-3.52, P=0.019). Furthermore, the risk of ICU admission during hospitalisation was not significantly different (1.06, 0.38-2.98, P=0.915) between the 2 groups with <6.1mmol/L FPG, the Groups 1 and 2 regardless of their awareness of prior diagnosis of diabetes. After excluding the patients who required ICU admission within 24 hours after hospital admission, group 3 still demonstrated a significantly higher risk of ICU admission than group 1 (4.79, 1.98-11.59, P<0.001, **Table 2**). In contrast, group 4 did not show any statistically significant difference in risk of ICU admission, when compared to group 1 (1.46, 0.44-4.88, P=0.540).

As a part of the sensitivity analysis, we additionally adjusted for demographic characteristics, symptoms, pre-exisiting comorbidities and laboratory indexes for the calculation of the Hazard Ratio. However, the findings of the adjusted model did not alter any of our conclusions (**Figures S1**, **S2** and **Table S1** in supplemental materials). Further detailed analyses and results were also provided in the supplemental materials.

Cumulative Incidence Curve on the FBG Levels and Prior Diabetic Status to ICU Admission

Figure 2 demonstrated the cumulative incidence of ICU admission events in all participants stratified by prior diabetic status and current FBG levels at hospital admission.

Group 3 had the highest risk of ICU admission at day 21 of hospitalisation. Its incidence was [14.1%, (9.6%-18.6%)], significantly higher than that of group 4 [7.0%, (3.7%-10.3%)], group 2 [4.0%, (0.2%-7.8%)] and group 1 [2.1%, (1.4%-2.8%)], (P<0.001, **Figure 2A**). After excluding the 71 patients who were admitted to ICU within 24 hours after hospital admission, group 3 still showed the highest risk of ICU admission at day 21 of hospitalisation. Its incidence was 2.4%, [0.1%-3.8%]). It is significantly higher than that seen in group 4 [1.4%, (0.0%-3.0%)], group 1 [0.7%, (0.3%-1.1%)] or group 2 [0.0%, (0.0%-0.0%)], (P=0.063) (**Figure 2B**).

DISCUSSION

Our study has evaluated whether FBG level in COVID-19 patients with or without awareness of the previous diagnosis of diabetes can

TABLE 1 | Basic demographic characteristics, signs and symptoms, comorbidities, laboratory findings, treatment and clinical outcomes of patient groups in COVID-19. (Unit of FBG is mmol/L).

Variable	FBG<6.1 No prior diabetes Group 1 (N = 1803)	FBG<6.1 Diagnosed diabetes Group 2 (N = 101)	FBG≥6.1 No prior diabetes Group 3 (N = 234)	FBG≥6.1 Diagnosed diabetes Group 4 (N = 228)	P-value
Demographic characteristics at admission					
Age (yr)	58.00 (48.00,67.00)	63.00 (57.00,71.00)	64.00 (55.00,70.00)	65.00 (57.00,72.00)	<0.001**
					<0.001**
<45 —no. (%)	368 (20.41)	2 (1.98)	14 (5.98)	7 (3.07)	
45-59 —no. (%)	570 (31.61)	34 (33.66)	72 (30.77)	63 (27.63)	
60-74 —no. (%)	687 (38.10)	54 (53.47)	111 (47.44)	113 (49.56)	
>74 —no. (%)	178 (9.87)	11 (10.89)	37 (15.81)	45 (19.74)	
Male gender —no. (%)	864 (47.92)	57 (56.44)	125 (53.42)	125 (54.82)	0.051
Respiratory rate >20 min —no. (%)	490 (27.28)	25 (24.75)	91 (39.06)	86 (37.72)	<0.001**
Pulse rate >100 per min —no. (%)	276 (15.36)	17 (16.83)	44 (18.88)	44 (19.30)	0.282
Systolic blood pressure ≥140 mmHg —no. (%)	405 (24.49)	29 (31.52)	65 (29.41)	93 (45.15)	<0.001**
Diastolic blood pressure ≥90 mmHg —no. (%)	395 (23.90)	26 (28.26)	42 (19.00)	50 (24.27)	0.281
Severity of COVID-19 patients —no. (%)					<0.001**
Mild severity	24 (1.33)	0 (0.00)	0 (0.00)	0 (0.00)	
Moderate severity	1345 (74.60)	67 (66.34)	126 (53.85)	153 (67.11)	
Severe severity	423 (23.46)	30 (29.70)	90 (38.46)	70 (30.70)	
Critical severity	11 (0.61)	4 (3.96)	18 (7.69)	5 (2.19)	
Signs and Symptoms —no. (%)					
Fever	22 (1.23)	2 (1.98)	11 (4.72)	7 (3.08)	<0.0001**
Cough	1000 (55.5)	60 (59.4)	123 (52.6)	119 (52.2)	0.549
Fatigue	682 (37.83)	31 (30.69)	97 (41.45)	100 (43.86)	0.087
Diarrhea	50 (2.77)	1 (0.99)	10 (4.27)	3 (1.32)	0.198
Chest tightness	216 (11.98)	6 (5.94)	36 (15.38)	31 (13.60)	0.094
Shortness of breath	419 (23.24)	33 (32.67)	59 (25.21)	64 (28.07)	0.078
Pre-existing comorbidities —no. (%)					
Coronary heart disease	87 (4.83)	11 (10.89)	17 (7.26)	35 (15.35)	<0.0001**
Cancer	19 (1.05)	4 (3.96)	3 (1.28)	12 (5.26)	<0.0001**
Chronic bronchitis	42 (2.33)	2 (1.98)	6 (2.56)	1 (0.44)	0.246
Cerebrovascular disease	46 (2.55)	9 (8.91)	18 (7.69)	21 (9.21)	<0.0001**
Chronic kidney disease	33 (1.83)	6 (5.94)	5 (2.14)	11 (4.82)	0.004*
Chronic obstructive pulmonary disease	18 (1.00)	0 (0.00)	4 (1.71)	1 (0.44)	0.511
Hepatitis	24 (1.33)	1 (0.99)	3 (1.28)	3 (1.32)	0.993
Hypertension	435 (24.13)	57 (56.44)	86 (36.75)	131 (57.46)	<0.0001**
Laboratory findings					
Fasting blood glucose (mmol/L)	4.69 (4.38,5.09)	5.14 (4.78,5.68)	7.16 (6.57,8.44)	8.67 (7.19, 11.48)	<0.001**
C-reactive protein (mg/L)	1.73 (0.66,5.23)	2.19 (1.00,9.70)	8.52 (2.09,55.77)	3.67 (1.23,16.71)	<0.001**
D-dimer (mg/L)	0.34 (0.18,0.66)	0.46 (0.24,0.74)	0.68 (0.39,1.64)	0.49 (0.28,1.03)	<0.001**
White blood cell count (10^9/L)	5.60 (4.60,6.80)	5.50 (4.50,6.60)	6.30 (4.90,8.90)	6.30 (5.10,7.53)	<0.001**
Lymphocyte count (109/L)	1.53 (1.17,1.90)	1.53 (1.08,1.81)	1.15 (0.74,1.60)	1.44 (0.99,1.86)	<0.001**
Neutrophils count (10^9/L)	3.33 (2.62,4.34)	3.31 (2.71,4.28)	4.36 (3.16,6.35)	3.73 (3.09,5.34)	<0.001**
Monocyte count (10^9/L)	0.43 (0.34,0.54)	0.42 (0.35,0.53)	0.40 (0.29,0.56)	0.46 (0.37,0.57)	<0.001**
Lactate dehydrogenase (IU/L)	171.95 (148.80,205.10)	171.75 (150.97,203.03)	212.10 (175.30,296.40)	187.00 (159.72,243.15)	<0.001**
Thrombinogen time (s)	12.80 (12.23,13.46)	12.94 (12.40,13.65)	12.84 (12.23,13.87)	12.67 (12.06,13.52)	0.315
Total bilirubin (μmol/L)	9.50 (7.30,12.30)	9.80 (7.27,12.20)	9.80 (7.30,12.38)	9.40 (7.20,12.60)	0.967
Direct bilirubin (μmol/L)	3.30 (2.50,4.30)	3.45 (2.77,4.43)	3.60 (2.80,5.18)	3.50 (2.58,4.70)	0.001*
Albumin (g/L)	38.40 (35.60,40.70)	38.60 (36.15,40.90)	36.40 (32.73,39.00)	37.55 (34.55,40.40)	<0.001**
Alkaline phosphatase (IU/L)	68.30 (57.30,82.10)	70.50 (60.00,88.75)	78.40 (62.70,97.45)	75.10 (63.38,92.98)	<0.001**
Fibrinogen (g/L)	2.89 (2.57,3.28)	3.10 (2.88,3.46)	3.30 (2.80,3.82)	3.14 (2.80,3.50)	<0.001**
Creatinine (μmol/L)	64.10 (55.10,75.30)	64.60 (56.15,74.80)	65.00 (54.35,76.80)	62.90 (53.60,73.65)	0.433
Creatine kinase (U/L)	51.70 (37.50,72.45)	46.30 (34.25,75.30)	47.00 (33.70,75.50)	45.55 (33.62,67.92)	0.102
Creatine kinase-MB (IU/L)	8.40 (6.80,10.50)	9.00 (7.00,11.50)	9.50 (7.60,12.30)	9.50 (7.82,12.20)	<0.001**
Cystatin C (mg/L)	0.92 (0.82,1.05)	0.97 (0.87,1.08)	0.99 (0.84,1.15)	0.95 (0.84,1.10)	<0.001**
Platelets count (10^9/L)	220.00 (182.00,268.25)	217.00 (184.00,255.50)	226.00 (178.00,290.00)	216.00 (177.00,260.25)	0.248

(Continued)

TABLE 1 | Continued

Variable	FBG<6.1 No prior diabetes Group 1 (N = 1803)	FBG<6.1 Diagnosed diabetes Group 2 (N = 101)	FBG≥6.1 No prior diabetes Group 3 (N = 234)	FBG≥6.1 Diagnosed diabetes Group 4 (N = 228)	P-value
Complications —no. (%)					
Acute Respiratory distress syndrome	4 (0.22)	2 (1.98)	10 (4.27)	4 (1.75)	<0.001**
Acute myocardial injury/failure	21 (1.16)	3 (2.97)	9 (3.85)	4 (1.75)	0.012*
acute hepatitis/liver failure	25 (1.39)	2 (1.98)	9 (3.85)	6 (2.63)	0.034*
Respiratory failure	16 (0.89)	1 (0.99)	14 (5.98)	9 (3.95)	<0.001**
shock	6 (0.33)	1 (0.99)	5 (2.14)	2 (0.88)	0.009*
Acute kidney injury	3 (0.17)	1 (0.99)	2 (0.85)	1 (0.44)	0.045*
Clinical outcomes					
The length of stady (day)	12.00 (8.00,18.00)	13.00 (8.00,20.00)	17.00 (10.25,22.00)	14.50 (9.00,21.25)	<0.001**
ICU —no. (%)	39 (2.16)	4 (3.96)	33 (14.10)	16 (7.02)	<0.001**
Death —no. (%)	15 (0.83)	4 (3.96)	22 (9.40)	9 (3.95)	<0.001**

**P-value is between 0.05 and 0.001; **P-value < 0.001.*

TABLE 2 | Adjust Hazard Ratio of FBG levels and diabetes to ICU admission in COVID-19 patients. (Unit of FBG is mmol/L).

Groups	HR# (95%CI)	P-value	HR## (95%CI)	P-value
FBG<6.1 & No prior diabetes (Group 1)	reference	reference	reference	reference
FBG<6.1 & Diagnosed diabetes (Group 2)	1.06 (0.38-2.98)	0.915	–	–
FBG≥6.1 & No prior diabetes (Group 3)	5.38 (3.46-8.35)	<0.001**	4.79 (1.98-11.59)	<0.001**
FBG≥6.1 & Diagnosed diabetes (Group 4)	1.99 (1.12-3.52)	0.019*	1.46 (0.44-4.88)	0.540

*Adjust parameters: age, sex, pre-existing comorbidities (chronic obstructive pulmonary disease, chronic kidney disease, cerebrovascular disease, coronary heart disease, cancer, chronic bronchitis, hepatitis and hypertension); *Compared with the above reference the P-value is between 0.05 and 0.001; **Compared with the above reference, the P-value < 0.001; #All COVID-19 patients; ##COVID-19 Patients were excluded by admitted to ICU at admission; Unit of FBG is mmol/L; ¯HR is not analysed due to the number of this group patients is lower.*

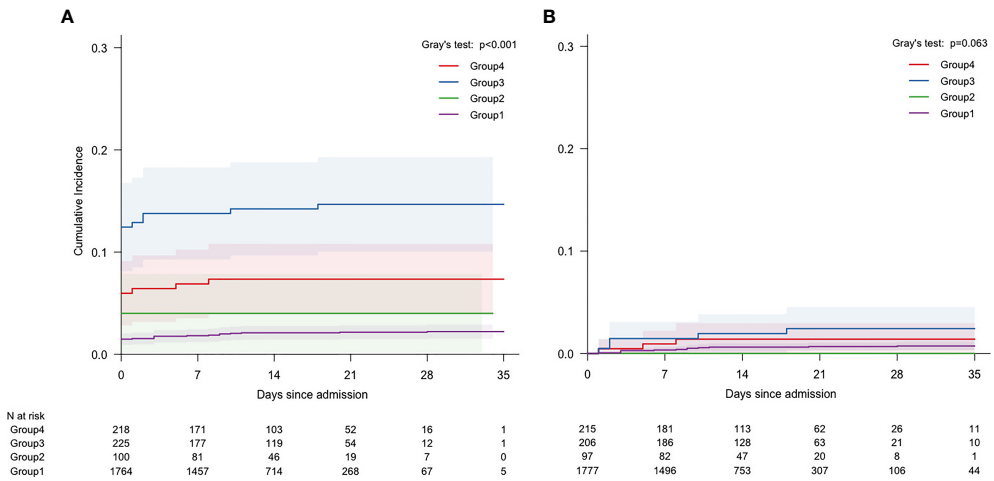

FIGURE 2 | Cumulative incidence of ICU admission stratified by patients with FBG levels and diabetes: **(A)** All COVID-19 patients; **(B)** Remaining COVID-19 Patients after excluding those admitted to ICU on admission. (Unit of FBG is mmol/L).

predict an adverse outcome during hospitalisation. Our results demonstrate that patients who had a high FBG level (≥6.1mmol/L) at admission without a prior diagnosis of diabetes (group 3) have the highest risk of a severe or critical severity of COVID-19, such as a severe inflammatory response and multiple clinical complications. Patients in group 4 with pre-existing diabetes who presented with a high FBG level (≥6.1mmol/L) on admission have higher risk of higher severity of COVID-19 than those with a low FBG. In contrast, patients having a low FBG (<6.1 mmol/L) on admission had the lowest risk of clinical complications regardless of their awareness of the prior diagnosis of diabetes (groups 1 and 2). This finding of association between the elevated current FBG level and risk of adverse COVID-19 outcome is consistent with the previously reported findings (5, 6, 32, 33).

Interestingly, the patients in group 2, albeit having a prior diagnosis of diabetes, presented on admission with normalised

FBG levels, indicating an adequate fasting glycaemic control. The group showed no significant differences in the proportion of patients developing clinically adverse outcomes compared to those from group 1, a group having no prior diagnosis of diabetes and presenting with a normal FBG level on admission. This result emphasizes a potentially important role of low current glycemic level, which could be a result of adequate glucose management in the past, in preventing a severe adverse outcome of COVID-19.

In contrast, the patients with uncontrolled FBG levels (≥6.1 mmol/L) and no prior diagnosis of diabetes (group 3) have the highest risk of an adverse prognosis, including a severe inflammatory response, having various clinical complications, long duration of hospitalisation, higher probablity of ICU admission and an increase in COVID-19 related death. This group of patients had a worse outcome than those in group 4 with pre-existing diabetes who had a similar high FBG on admission. This result is consistent with those from the previously reported studies where COVID-19 patients with a new onset diabetes, presented with hyperglycemia [(FBG >7mmol/L) and a normal HbA1c level (<5.6%)], are reported to have a worse COVID-19 outcome than those with pre-existing diabetes (27–29). Unlike this study, these previous studies did not examine the association between the unawareness of prior diabetes and the adverse outcome of COVID-19 in patients with high current FBG levels. In this study, the hyperglycaemic patients in group 3 had no awareness of their prior glycaemic status and there could be a significant proportion of patients in this group having pre-existing diabetes, considering a high prevalence and low diagnosis rate of diabetes as well as >50% adults having pre-diabetes in the general population in China (17). Inevitably, this group would also contain patients with new onset diabetes, as shown by another study on the same population in the same period of time where 21/82 (26%) COVID-19 patients had new onset diabetes with FBG ≥7.0 mmol/L and HbA1c <5.6% (28).

It has been observed that diabetes and/or current hyperglycaemia can be associated with deleterious outcome of COVID-19 (15, 33). The interaction between COVID-19 and diabetes is considered to be bi-directional (23). COVID-19 is also associated with increased severity of pre-existing diabetes leading to severe complications such as diabetic ketoacidosis (19–21, 34) as well as multi-organ injury (35, 36). Furthermore, a number of studies have shown that SARS-Cov-2 is able to infect multiple organs including those responsible for glucose metablolism, such as the pancreas leading to damage of pancreatic islets and impairment of β cell function (9, 25). Therefore, the elevation of glycemic level in the COVID-19 patients is not only possible to play a biological role leading to increased severity of Convid-19, it may also reflect the multi-organ injury as a result of SARS-Cov-2 infection, thus predicting a high risk of adverse outcome.

The specific identification of persons with diabetes at the time of COVID-19 diagnosis or those for the first time having an elevated FBG has not been part of the routine assessment of COVID-19 subjects. For example, the current Chinese guidelines for Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (37) do not specifically address patients with diabetes or elevated FBG levels. The 2017 Chinese guidelines for the Prevention and Control of Type 2 Diabetes (38), which was published 2 years before the COVID-19 pandemic started, have not been amended to recommend specific treatment for COVID-19 patients. Bornstein et al. (22) have suggested that patients without diabetes, but have a higher risk for the metabolic disease should be monitored for new-onset diabetes triggered by COVID-19. Mary et al. (39) have developed a pragmatic approach for inpatient diabetes management and recommended insulin therapy for COVID-19 patients when their FBG level is above 10.00 mmol/L. This means, the patients without known diabetes but with FBG levels of ≥6.10 - <10.00 mmol/L, who are shown to be on risk (median 7.16 [IQR: 6.57, 8.44]) in this study, would not be specifically considered for their glycaemic management. Therefore, our finding provides evidence to suggest consideration of refining the clinical protocol to include this catogery of patients as on risk and eligible to receive glucose lowering management. Furthermore, the current World Health Organization guideline on drugs for COVID-19 strongly recommends the use of systemic corticosteroids in patients with severe and critical COVID-19 (40). Since previous studies demonstrate that corticosteroids can induce an elevation in blood glucose by promoting insulin resistance, further increasing the blood glucose level and the risk of type 2 diabetes (41, 42). Our findings suggest that these patients receiving corticosteroid treatment should be closedly monitored and carefully managed for their blood glucose in order to improve their prognosis. We consider the failure to provide timely glucose lowering management for patients with elevated FBG levels but not previously diagnosed diabetes as a missed opportunity to intervene to potentially improve the prognosis of these patients. This needs to be further confirmed in an intervention study.

Our findings further suggest that people with elevated FBG levels, particularly those without a previous diagnosis of diabetes, should be identified in the general population by FBG screening and considered as a prioritised group for COVID-19 vaccination in order to reduce the risk of COVID-19 infection, due to their higher risk of the severe adverse outcomes if being infected. However, it should be noted that this is solely based on the data from the hospitalized patients with overall severe disease. Whether this is applicable to the ambulatory patients in the general community should be further investigated. Nevertheless, at least 13 different COVID-19 vaccines have been registered by the WHO, and an estimated 11% of the world population has received at least one dose of the COVID-19 vaccine (43, 44). COVID-19 vaccines have been shown to be both effective and cost-effective in prevention new infections and severe COVID-19 diseases (45). The International Diabetes Federation strongly urges governments to prioritise vaccine access for people with diabetes (46). Based on our findings, we further propose to identify people with diabetes who have inadequately controlled FBG levels as well as to increase screening of the general population to identify those with elevated FBG levels, albeit they have not been previously diagnosed with diabetes. They should be prioritised as at risk subgroups for COVID-19 vaccination. The US CDC reported that among 86 million adults in the US who have prediabetes, 90% are not aware of

their prediabetic status (47). In this population, 15-30% will progress to type 2 diabetes within five years (47). Even among adults with type 2 diabetes, only about a half have been diagnosed, according to the WHO (48) and the diabetes diagnosis rate can be much lower in some parts of the world, as shown by the Chinese survey several years ago (17). Hence, routine glucose screening at a population level to identify prediabetes or diabetes would be an important means to increase the awareness of individual glymaemic status, allowing them to receive adequate glucose lowering treatment to normalize their FBG level in order to reduce their risk of deleterious outcome if being infected by SARS-Cov-2. Equally important, these people can be identified for prioritising vaccination to be protected from infection, leading to a reduction of COVID-19-related mortality.

We appreciate there are several limitations to this study. Firstly, very few COVID-19 patients had HbA1c measured at Huoshenshan hospital; therefore, the average glucose control of the patients during the prior 3 months was unknown. The COVID-19 patients with uncontrolled FBG levels at admission cannot be diagnosed specifically as prediabetes, diabetes or COVID-19 associated new-onset diabetes. Secondly, our study only analyses FBG levels at three time points during the course of hospitalisation and may not fully reflect the variation of FBG levels. Thirdly, this cohort of COVID-19 patients was admitted to Huoshenshan hospital in the early phase of the pandemic, and the treatment options for COVID-19 and glucose management for elevated FBG may have been limited during the acute initial phase of the COVID-19 pandemic in Wuhan. Fourthly, this is a retrospective cohort study, which can only provide analytic results to show association among the current FBG levels, awareness of prior diabetes and outcome of COVID-19, and specifically designed intervention studies are required to establish a causal role for the current FGB level and/or awareness of individual's prior glymcaemic status in an adverse outcome of COVID-19. Finally, we appreciate that obesity is recognized to be a potential risk factor for severe adverse outcomes in COVID-19. However, we don't have obesity data for this cohort of patients at the early stages of the pandemic. We consider lack of obesity data for analysis to be a limitation in this study. Therefore, it would be worth considering additional cohort studies on patients with COVID-19 to specifically address the potential confounding issue in association with obesity.

CONCLUSION

In conclusion, our study shows that COVID-19 patients with high FBG levels on admission with no awareness of their prior diagnosis of diabetes have the highest risk to develop severe inflammatory responses and complications of COVID-19 among patients with controlled FBG, including those with a known diagnosis of diabetes. Indeed, those with diagnosed diabetes but with FBG levels under control showed only a minimal risk of clinical adverse outcomes. COVID-19 patients presenting with an elevated FBG on admission with known diagnosis of prior diabetes would have a lower risk than those who were unaware of their prior glycaemic status. Thus, we recommended that there is a review of the current treatment guidelines for COVID-19 patients, emphasising glucose monitoring/awareness, optimising glycaemic management and prioritising COVID-19 vaccination for those who cannot obtain adequate glycemic control.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

All methods of this study were carried out in accordance with relevant guidelines and regulations. This study was approved by Ethical Review of Scientific Research Projects of the Medical Ethics Committee of the Chinese PLA General Hospital, and obtained a waiver of one or more elements of informed consent form. The need for informed consent was waived by Medical Ethics Committee of the Chinese PLA General Hospital. The name of the ethics committee, The Medical Ethics Committee of the Chinese PLA General Hospital. The approval committee's reference number, No. S2020-162-01.

AUTHOR CONTRIBUTIONS

Participants of the study should "contribute" as follows. WW, study design, data collection, data analysis, and writing. ZC, MC, PZZ, writing. HG, JD, FY, XL, XC, KZ, QZ, ZL, PFZ, ZW, data collection. XG, data collection and writing. LZ and KH, study design, data collection, data analysis, and writing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.791476/full#supplementary-material

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A Randomized Clinical Trial of Linagliptin vs. Standard of Care in Patients Hospitalized With Diabetes and COVID-19

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Objective: To assess the effect of linagliptin vs. standard therapy in improving clinical outcomes in patients hospitalized with diabetes and coronavirus disease 2019 (COVID-19).

Materials and Methods: We did an open-label, prospective, multicenter, randomized clinical trial in 3 Israeli hospitals between October 1, 2020, and April 4, 2021. Eligible patients were adults with type 2 diabetes mellitus and a diagnosis of COVID-19. A total of 64 patients, 32 in each group, were randomized to receive linagliptin 5 mg PO daily throughout the hospitalization or standard of care therapy. The primary outcome was time to clinical improvement within 28 days after randomization, defined as a 2-point reduction on an ordinal scale ranging from 0 (discharged without disease) to 8 (death).

Results: The mean age was 67 ± 14 years, and most patients were male (59.4%). Median time to clinical improvement was 7 days (interquartile range (IQR) 3.5-15) in the linagliptin group compared with 8 days (IQR 3.5–28) in the standard of care group (hazard ratio, 1.22; 95% CI, 0.70–2.15; p = 0.49). In-hospital mortality was 5 (15.6%) and 8 (25.0%) in the linagliptin and standard of care groups, respectively (odds ratio, 0.56; 95% CI, 0.16–1.93). The trial was prematurely terminated due to the control of the COVID-19 outbreak in Israel.

Conclusions: In this randomized clinical trial of hospitalized adult patients with diabetes and COVID-19 who received linagliptin, there was no difference in the time to clinical improvement compared with the standard of care.

Clinical Trial Registration: ClinicalTrials.gov, identifier NCT04371978.

Keywords: COVID-19, diabetes, linagliptin, DPP-4 inhibitors, hospital management

INTRODUCTION

The coronavirus disease 2019 (COVID-19) is an emerging pandemic in 2020–2021 caused by a novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1, 2). Diabetes confers a significant additional risk for COVID-19 patients (3–5). Currently, there are only a few specific effective therapeutic agents for the treatment of COVID-19 despite a large number of clinical trials that evaluated a wide range of therapies (6–10).

Dipeptidyl peptidase 4 (DPP-4) is a transmembrane glycoprotein expressed ubiquitously in many tissues and originally known as T-cell surface marker cluster of differentiation 26 (CD26) (11). In addition to its effect on glucose levels, DPP-4 has various effects on the immune system and several diseases, including lung diseases (11, 12). DPP-4 serves as the functional receptor for the Middle East respiratory syndrome coronavirus (MERS-CoV) (13), and potential interactions of SARS-CoV-2 spike glycoprotein and DPP-4 may play a significant role in the viral process of hijacking the human host cells (14).

In addition to their role in treating diabetes, several studies have evaluated the potential of DPP-4 inhibitors as immune-modulating agents and as a treatment for chronic allograft dysfunction following lung transplantation (11, 15). The anti-inflammatory effects of DPP-4 inhibitors and the possible involvement in the process of entering human cells are the basis for the assumption that these agents may be beneficial for the treatment of COVID-19 (16, 17). Two retrospective studies from Italy found that the use of DPP-4 inhibitors as a group or sitagliptin specifically reduced mortality and improved outcomes of patients hospitalized with COVID-19 (18, 19). Nevertheless, a meta-analysis based on retrospective studies concluded that the current data are insufficient and that the combined estimate of the risk ratio for mortality reduction by DPP-4 inhibitors is neutral (20).

Linagliptin is a DPP-4 inhibitor approved by the Food and Drug Administration in 2011 as a treatment for type 2 diabetes (21) and is as effective as other DPP-4 inhibitors (22). In contrast to other DPP-4 inhibitors, no dosage adjustment is necessary for renal or hepatic impairment, and linagliptin does not affect the risk of heart failure (23). In this multicenter randomized, controlled, open-label trial, we investigated the safety and efficacy of linagliptin in hospitalized patients with COVID-19 and diabetes.

MATERIALS AND METHODS

Design

This was an investigator-initiated, multicenter, open-label randomized clinical trial aimed to assess the safety and efficacy of linagliptin vs. standard therapy in hospitalized patients with diabetes and COVID-19. A total of 3 hospitals in Israel enrolled patients between October 1, 2020, and April 4, 2021, from designated COVID-19 departments. Patients were randomized by the study coordinators using a web-based system with blocks of variable size to a 1:1 allocation ratio. Patients were randomized to receive linagliptin 5 mg daily PO plus standard of care or standard of care alone. The standard of care for patients with diabetes included holding oral drugs and initiating insulin therapy according to a basal bolus protocol (24). The goal of therapy was to keep blood glucose levels in the range of 140–180 mg/dl (25). COVID-19-specific treatments in both groups were according to the updated evidence (26). Treatment with linagliptin was continued from randomization to hospital discharge. The randomization was stratified by the hospital and in each hospital by age (≤70 or >70 years) and oxygen use at randomization (if supplemental oxygen was required or not). Patients were followed up until 28 days following randomization when they were contacted by the study staff by telephone. This trial followed the Consolidated Standards of Reporting Trials (CONSORT) reporting guidelines. Due to the control of the COVID-19 outbreak in Israel (27, 28), no patients were enrolled after April 4, 2021, and the trial was terminated prematurely on May 4, 2021.

Patients

Hospitalized patients 18 years and older, with a diagnosis of COVID-19 confirmed by a positive reverse-transcriptase PCR (RT-PCR) assay for SARS-CoV-2 in a respiratory tract specimen, were all evaluated for eligibility for the study. Other inclusion criteria were a previous diagnosis of type 2 diabetes mellitus or a diagnosis initially made during hospitalization, according to the American Diabetes Association (ADA) guidelines (29), 10 days from COVID-19 symptom onset or within 48 h after positive RT-PCR test. Exclusion criteria included a need for mechanical ventilation or vasopressor medications prior to randomization; expected immediate need of intensive care unit (ICU) admission or immediate surgical intervention; current treatment with any DPP-4 inhibitor; pregnancy; or known hypersensitivity to DPP-4 inhibitors.

Outcomes

The primary clinical endpoint was time to clinical improvement within 28 days after randomization. Clinical improvement was defined as a 2-point reduction in patients' admission status on a 9-point ordinal scale. This scale has been used in different COVID-19 therapeutic trials (30–32) and was recommended by the R&D Blueprint of the WHO (33). The 9-point scale was as follows: 0, discharged, no clinical or virological evidence of infection; 1, discharged, no limitation of activities; 2, discharged, limitation of activities or ambulatory oxygen use; 3, hospitalized, no oxygen therapy; 4, hospitalized, oxygen by mask or nasal prongs; 5, hospitalized, non-invasive ventilation or high-flow oxygen; 6, hospitalized, intubation and mechanical ventilation; 7, hospitalized, ventilation + additional organ support—vasopressors, renal replacement therapy, extracorporeal membrane oxygenation; and 8, death.

Secondary outcomes were the proportion of patients with 2-point reduction in patients' admission status on a 9-point ordinal scale; all-cause mortality at 28 days; in-hospital death; length of hospitalization; ICU admissions; mechanical ventilation; supplemental oxygen-free days at 28 days; ventilator-free days

at 28 days; and the proportion of patients with 50% decrease in C-reactive protein (CRP) levels. Safety outcomes included treatment-related adverse events, serious adverse events, and premature discontinuations of the study drug.

Statistical Analysis

During the initial design of the study and based on the trial of Cao et al. (30), assuming a decrease in the primary endpoint measure from a median of 16 days to a median of 8 days between the two trial groups with overall power of 80% and significance of 0.05, the sample size required for the trial was 88 patients. After publications of further trials, it was assumed that a larger sample size is required for such power, but the wide vaccine campaign and outbreak control in Israel (27, 28) led to premature termination of the trial.

Primary endpoint analysis was based on intention-to-treat analysis and included all the patients who had undergone randomization. The time to clinical improvement was assessed after all patients had reached day 28, with failure to reach clinical improvement or death before day 28 and before clinical improvement was considered as right-censored at day 28. The time to clinical improvement was portrayed by the Kaplan–Meier plot and compared with a log-rank test. Hazard ratios with 95% CIs were calculated by Cox proportional hazards model. Secondary outcomes such as mortality at 28 days, the proportion of ICU admission, the proportion of mechanical ventilation, and the proportion of CRP reduction were compared using the χ^2 test. Only the p-value for the primary efficacy analysis (2-tailed; significance defined as $p \leq 0.05$) was reported, and bilateral 95% CI was not adjusted for multiplicity for all the other comparisons. Statistical analyses were performed with Stata, version 13.0 (StataCorp, Texas, USA) and SPSS, version 25 (IBM Corp). This trial is registered with ClinicalTrials.gov, NCT04371978.

RESULTS

Patients

A total of 64 patients with COVID-19 from three hospitals in Israel underwent randomization and were included in this analysis. All patients were included in the intention-to-treat analysis (**Figure 1**). No patient withdrew from the trial. Demographic and baseline clinical characteristics are summarized in **Table 1**. Most of the patients were male (59.4%) with a mean age of 66.95 ± 13.93 years. Generally, the baseline clinical and laboratory parameters were balanced between the two groups, except for HbA1c, which was lower in the linagliptin group. COVID-19 disease classification was defined according to the National Institute of Health guidelines (10), and more than 70% of patients were classified as severe on admission. Of the patients, 84.4% and 81.3% received dexamethasone and 43.8% and 56.3% received remdesivir in the linagliptin and standard of care groups, respectively.

Primary Outcome

A total of 26 patients (81.3%) in the linagliptin group and 23 (71.9%) in the standard of care group showed clinical improvement, i.e., 2-point reduction in the WHO scale, within 28 days of randomization (odds ratio, 0.59; 95% CI, 0.18–1.91) (**Table 2**). The time to improvement was also similar between the groups—median time of 7 days (interquartile range (IQR) 3.5–

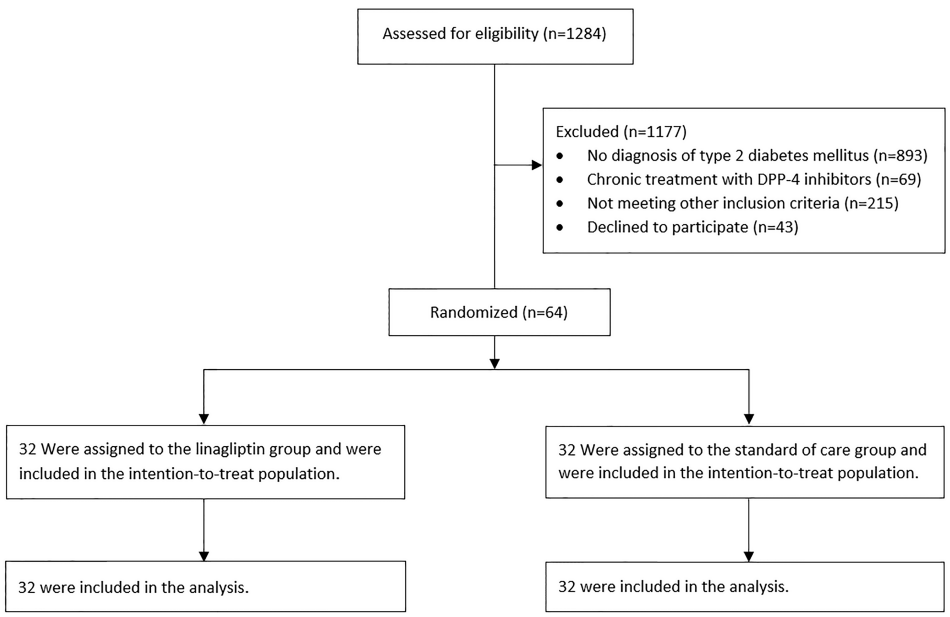

FIGURE 1 | Flowchart of the study.

TABLE 1 | Characteristics of the patients at baseline.

Characteristic	All patients (N = 64)	Linagliptin (n = 32)	Standard of care (n = 32)	p-Value
Age (years, mean ± SD)	66.95 ± 13.93	65.53 ± 16.02	68.38 ± 11.54	0.42
Male sex, n (%)	38 (59.38%)	21 (65.63%)	17 (53.13%)	0.31
Body mass index, median (IQR)	29.1 (26.6–33.3)	28.5 (26.3–33.3)	29.9 (26.7–33.3)	0.96
Oxygen support on admission, n (%)	41 (64.06%)	21 (65.63%)	20 (62.5%)	0.79
Duration of diabetes, years, median (IQR)	13.5 (8–19)	12.5 (5.5–20)	13.5 (8–18)	0.89
Diabetes complications, n (%)	28 (43.75%)	12 (37.5%)	16 (50%)	0.31
HbA1c median (IQR)				**0.02**
%	7.5 (6.7–8.8)	7.4 (6.6–7.9)	8.2 (6.8–9.3)	
mmol/mol	58 (50–73)	57 (49–63)	66 (51–78)	
Smokers, n (%)				0.11
Never	36 (56.25%)	16 (50%)	20 (62.5%)	
Former	23 (35.94%)	15 (46.88%)	8 (25%)	
Current	5 (7.81%)	1 (3.13%)	4 (12.5%)	
Charlson Comorbidity Index median (IQR)	7 (5–8.8)	6 (4–9)	7 (5–8)	0.69
Duration of symptoms prior to admission, days, median (IQR)	6 (2–8)	7 (2–8)	6 (3–8)	0.53
Disease severity on admission, n (%)				0.17
Mild	10 (15.63%)	3 (9.38%)	7 (21.88%)	
Moderate	8 (12.5%)	6 (18.75%)	2 (6.3%)	
Severe	46 (71.88%)	23 (71.88%)	23 (71.88%)	
Chronic medications				
Statins, n (%)	49 (76.56%)	24 (75%)	25 (78.13%)	0.77
Diuretics, n (%)	20 (31.25%)	8 (25%)	12 (37.5%)	0.28
Beta-blockers, n (%)	24 (37.5%)	11 (34.38%)	13 (40.63%)	0.61
Anti-platelets, n (%)	41 (64.06%)	18 (56.25%)	23 (71.88%)	0.19
Calcium channel blockers, n (%)	21 (32.81%)	11 (34.38%)	10 (31.25%)	0.79
ACEI/ARB, n (%)	39 (60.94%)	21 (65.63%)	18 (56.25%)	0.44
Steroids, n (%)	13 (20.31%)	7 (22.58%)	6 (18.75%)	0.71
COVID-19 treatments				
Remdesivir, n (%)	32 (50%)	14 (43.75%)	18 (56.25%)	0.32
Dexamethasone, n (%)	53 (82.81%)	27 (84.38%)	26 (81.25%)	0.74
Convalescent plasma, n (%)	7 (10.94%)	4 (12.5%)	3 (9.38%)	0.69
Laboratory values on admission				
Lymphocytes, absolute, median (IQR)	0.8 (0.6–1.2)	0.9 (0.6–1.3)	0.8 (0.5–1.2)	0.77
C-reactive protein, mg/dl, median (IQR)	9.6 (5.5–17.9)	9.9 (5.6–20.5)	8.5 (5–15.9)	0.32
LDH U/L, median (IQR)	682.5 (487.5–816.3)	687.5 (502.8–834)	633 (450–816.3)	0.74
Ferritin mg/ml, median (IQR)	439.4 (232.9–1,000.7)	574.2 (306.9–1,028.9)	363.2 (177–808.5)	0.64
D-dimer ng/ml, median (IQR)	1,024 (638.5–1,699)	926.5 (696.3–1,668.5)	1,114 (576–1,798)	0.27
Troponin ng/l, median (IQR)	20 (12–44)	12 (12–37.5)	23 (12–60)	0.33

ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; HbA1c, hemoglobin A1C; LDH, lactate dehydrogenase; IQR, interquartile range.
Bold p-values mean statistically significant p-value <0.05.

15) in the linagliptin group compared with 8 days (IQR 3.5–28) in the standard of care group (hazard ratio, 1.22; 95% CI, 0.70–2.15; p = 0.49, **Figure 2**). Different sensitivity analyses showed similar results.

Adverse events related to the study drug were rare. Only two patients had a related adverse event, hypoglycemia, with one in the linagliptin group and the other in the standard of care group.

Secondary Outcomes

There were no major differences in the rate of ICU admission between the groups, with 7 (21.9%) in the linagliptin group and 4 (12.5%) in the standard of care group (odds ratio, 1.96; 95% CI, 0.51–7.50) (**Table 2**). All-cause mortality at 28 days was 7 (21.9%) and 9 (28.1%) in the linagliptin and standard of care groups, respectively (odds ratio, 0.72; 95% CI, 0.23–2.23). The length of hospitalization was also similar in both groups with a median of 7 days (IQR 3–12) for the entire cohort (mean difference, 0.44; 95% CI, −2.83 to 3.71). One patient in the linagliptin group was still hospitalized in the ICU after 28 days from randomization.

DISCUSSION

Observational studies have suggested that DPP-4 inhibitors are effective in reducing mortality in patients with COVID-19 (18, 19). However, this randomized trial found that in hospitalized adults with COVID-19 and diabetes, linagliptin treatment added to the standard of care failed to significantly improve the time to resolution of symptoms or day 28 mortality. Furthermore, no difference between the study groups was observed for any of the secondary outcomes, including the proportion of patients admitted to an ICU, mechanical ventilation rates, length of hospitalization, and supplemental oxygen use. To our

TABLE 2 | Outcomes in the intention-to-treat population.

Outcome	Linagliptin (n = 32)	Standard of care (n = 32)	Odds ratio (95% CI)	Mean difference (95% CI)	p-Value
Death at 28 days, n (%)	7 (21.9%)	9 (28.1%)	0.72 (0.23–2.23)		
In-hospital death, n (%)	5 (15.6%)	8 (25.0%)	0.56 (0.16–1.93)		
ICU admissions, n (%)	7 (21.9%)	4 (12.5%)	1.96 (0.51–7.50)		
Number of patients with 2-point reduction in WHO scale, n (%)	26 (81.25%)	23 (71.88%)	0.59 (0.18–1.91)		
Oxygen supplementation during the admission, n (%)	29 (90.6%)	25 (78.1%)	2.71 (0.63–11.59)		
Mechanical ventilation, n (%)	6 (18.8%)	4 (12.5%)	1.62 (0.41–6.38)		
CRP reduction of over 50% from admission to discharge, n (%)	14 (43.75%)	11 (34.38%)	0.67 (0.25–1.85)		
Length of hospitalization					
Mean ± SD	8.81 ± 6.98	8.38 ± 6.08		0.44 (–2.83 to 3.71)	0.88
Median (IQR)	7 (3.5–11.5)	6.5 (3–12.75)			
Supplemental oxygen-free days at 28 days					
Mean ± SD	0.84 ± 1.71	1.44 ± 2.58		–0.59 (–1.69 to 0.5)	0.23
Median (IQR)	0 (0–1)	0 (0–2)			
Ventilator-free days at 28 days					
Mean ± SD	7.56 ± 5.90	7.25 ± 5.52		0.31 (–2.54 to 3.17)	0.77
Median (IQR)	6 (3–9)	6 (3–11.25)			
ICU-free days at 28 days					
Mean ± SD	7.75 ± 8.31	7.47 ± 5.29		0.28 (–3.2 to 3.76)	0.77
Median (IQR)	6 (3–9)	6 (3–11.25)			

ICU, intensive care unit; CRP, C-reactive protein; IQR, interquartile range.

knowledge, this is the first randomized clinical trial evaluating the efficacy and safety of a DPP-4 inhibitor in patients with COVID-19.

As in many COVID-19 trials (31, 34–36), our study also had a male predominance in both groups, but the mean age was higher. The death and complications rates of our patients were higher than in most other clinical trials since our population included only patients with diabetes mellitus, a known risk factor for severe COVID-19 (3–5). The number of comorbidities was also high as reflected in the Charlson Comorbidity Index with a median score of 7, which is a strong predictor for mortality (37). As in other places in the world (38), hospital resources became scarce during the pandemic, and ambulatory treatment models were widely adopted. That narrowed hospitalizations only to those who needed them the most and explains why more than 70% of the patients were classified with severe disease on admission. Patients with a mild or moderate disease on admission were at high risk of progression to severe disease and/or with several additional acute illnesses.

The timing of linagliptin administration during the course of the disease might also be of importance. There are two suggested mechanisms for the effects of linagliptin on COVID-19. The first

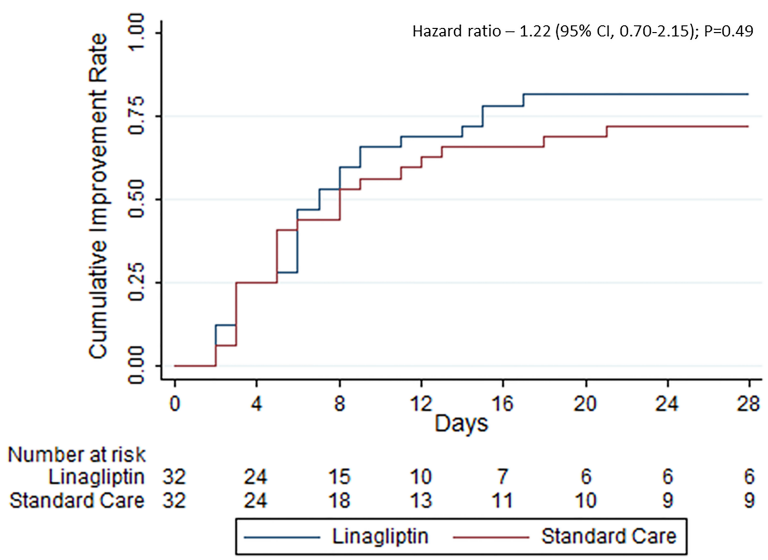

FIGURE 2 | Kaplan–Meier estimates of cumulative clinical improvement.

is the interaction of the virus with the human host cell (14), and the second is the anti-inflammatory effect (39). The first encourages use during the initial infection, while most patients are asymptomatic/presymptomatic, while the second is aimed for a later stage in the natural history of the disease, the inflammatory stage (40). Since we enrolled only hospitalized patients, treatment during the presymptomatic stage was not practical, but we enrolled patients only until the 10th day of symptoms. The results of previous observational studies support the hypothesis of the anti-inflammatory effect, as patients treated with DPP-4 inhibitors prior to COVID-19 infection had similar outcomes compared with those not being treated, as opposed to the studies in which DPP-4 inhibitors were used during the hospitalization, in which mortality reduction was shown (20). In our study, analysis of patients with symptoms shorter or longer than 5 days prior to admission showed no significant difference in the primary outcome. In addition, since most patients in our study were hospitalized with a disease classified as severe, it is possible that treatment of patients with diabetes and mild COVID-19 in the symptomatic stage may lead to different clinical outcomes. The use of DPP-4 inhibitors as anti-inflammatory agents in patients without diabetes may also lead to different clinical outcomes than those presented in this manuscript. Glucagon-like peptide 1 receptor (GLP-1R) agonists may have a similar anti-inflammatory effect in patients with diabetes and COVID-19, as they are known to reduce the levels of several anti-inflammatory markers (41). This should be assessed in further studies.

The Italian observational studies that showed mortality reduction with DPP-4 inhibitors were conducted during the early phase of the pandemic, February–April 2020 (18, 19). The rapid changes of clinical guidelines as new data emerged weekly led to a substantial difference between the treatment of COVID-19 patients in April 2020 and April 2021 (26, 42). Thus, it is possible that DPP-4 inhibitors have a beneficial effect on COVID-19 compared with supportive care alone, but this effect is eliminated when other therapies such as remdesivir and dexamethasone are added, as was for most of the patients in our study. The relation between the immunomodulatory effect of DPP-4 inhibitors and corticosteroids is unknown, and it is possible that dexamethasone diminished the immunomodulatory effect of linagliptin (43, 44). It should be noted that while sitagliptin specifically was evaluated in a previous study (18), we evaluated linagliptin, but if DPP-4 inhibitors are beneficial, then this is probably a class effect.

The very low rate of adverse events can be explained by the fairly good safety profile of linagliptin, especially with regard to severe adverse events (45). Another explanation is the short treatment period, with most patients treated for less than 10 days, while for diabetes, it is a chronic treatment, and most trials that evaluated the safety of the drug followed up patients for 6–24 months.

Our study has several limitations. The first limitation is the sample size and the premature discontinuation of the trial due to the end of the COVID-19 outbreak in Israel (27, 28). This makes our study underpowered to detect possible differences in the primary outcome and in mortality. The second limitation is the fact that the trial was open-label. We considered conducting a double-blind placebo-controlled trial, but that was not feasible due to the time constraints.

CONCLUSIONS

The administration of linagliptin in patients with COVID-19 and diabetes did not improve the time to clinical improvement or 28-day mortality. Further large-scale, blinded, placebo-controlled randomized clinical trials are needed to confirm the results and to explore possible applications of DPP-4 inhibitors in different stages of the disease in patients with and without diabetes.

DATA AVAILABILITY STATEMENT

The data used in the analysis of this study are not publicly available due to the requirements of the IRB Committee but are available from the corresponding author upon request.

ETHICS STATEMENT

The trial was approved by the institutional review board of Rabin Medical Center (confirmation number 0303-20-RMC) and Shamir Medical Center (confirmation number 0010-21-ASF). Written informed consent was obtained from all patients. The trial was conducted in accordance with the principles of the Declaration of Helsinki. The authors were responsible for designing the trial and for compiling and analyzing the data. The authors vouch for the completeness and accuracy of the data and for the adherence of the trial to the protocol. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization: RA, AG, and IA-D. Methodology: RA and IA-D. Formal analysis: RA, NZ, MK, and DD. Investigation: RA and AG. Resources: AG. Data curation: RA. Writing—original draft preparation: all authors. Writing—review and editing: RA, AG, and RK. Supervision: AG and RK. Funding acquisition: AG. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.794382/full#supplementary-material.

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Clinical Characteristics of COVID-19 Patients in a Regional Population With Diabetes Mellitus: The ACCREDIT Study

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Objective: To identify clinical and biochemical characteristics associated with 7- & 30-day mortality and intensive care admission amongst diabetes patients admitted with COVID-19.

Research Design and Methods: We conducted a cohort study collecting data from medical notes of hospitalised people with diabetes and COVID-19 in 7 hospitals within the Mersey-Cheshire region from 1 January to 30 June 2020. We also explored the impact on inpatient diabetes team resources. Univariate and multivariate logistic regression analyses were performed and optimised by splitting the dataset into a training, test, and validation sets, developing a robust predictive model for the primary outcome.

Results: We analyzed data from 1004 diabetes patients (mean age 74.1 (± 12.6) years, predominantly men 60.7%). 45% belonged to the most deprived population quintile in the UK. Median BMI was 27.6 (IQR 23.9-32.4) kg/m^2. The primary outcome (7-day mortality) occurred in 24%, increasing to 33% by day 30. Approximately one in ten patients required insulin infusion (9.8%). In univariate analyses, patients with type 2 diabetes had a higher risk of 7-day mortality [$p < 0.05$, OR 2.52 (1.06, 5.98)]. Patients requiring insulin infusion had a lower risk of death [$p = 0.02$, OR 0.5 (0.28, 0.9)]. CKD in younger patients

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(<70 years) had a greater risk of death [OR 2.74 (1.31-5.76)]. BMI, microvascular and macrovascular complications, HbA1c, and random non-fasting blood glucose on admission were not associated with mortality. On multivariate analysis, CRP and age remained associated with the primary outcome [OR 3.44 (2.17, 5.44)] allowing for a validated predictive model for death by day 7.

Conclusions: Higher CRP and advanced age were associated with and predictive of death by day 7. However, BMI, presence of diabetes complications, and glycaemic control were not. A high proportion of these patients required insulin infusion warranting increased input from the inpatient diabetes teams.

Keywords: diabetes, COVID-19, mortality, CRP, risk factors, observational study

INTRODUCTION

As of 8 June 2021, an estimated 172 million people have been afflicted by coronavirus disease 2019 (COVID-19) with a death toll of 3.7 million worldwide (1). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), with ACE-2 serving as its cellular receptor, presents with a wide spectrum of clinical manifestations from asymptomatic cases to severe life-threatening pneumonia and acute respiratory distress syndrome (ARDS). Moreover, there is ongoing surveillance for variants of concern and episodic surges of cases globally (2). Therefore, the relevance of continued research about COVID-19 and its prognosis remain.

At present over 150,000 people in the United Kingdom (UK) have died with COVID-19, one of the highest case fatalities in Europe (3). According to multiple reports, risk factors for poor outcomes appear to be male sex, advanced age, diabetes, hypertension, obesity, and cardiovascular disease among others (4–6). In addition, there is evidence suggesting an increased risk of mortality among Black, Asian, and Minority Ethnic (BAME) populations (7).

The burden of diabetes on people with COVID-19 has been highlighted in several reports. In 2019, an estimated 463 million people aged between 20 to 79 years are living with diabetes worldwide and its prevalence is predicted to increase in the coming years (8). In the UK, more than 4.7 million people have diabetes (9). It is well established that certain characteristics are associated with higher mortality in patients with diabetes - in particular, cardiovascular disease (CVD), microalbuminuria, socioeconomic deprivation and foot ulceration (10, 11).

According to the ongoing International Severe Acute Respiratory and Emerging Infections Consortium (ISARIC) World Health Organization (WHO) Clinical Characterisation Protocol UK (CCP-UK) study covering 208 UK acute hospitals with over 20,000 patients, diabetes is the second most prevalent comorbidity, present in 20% of COVID-19 patients needing hospital admission. Furthermore, 26% mortality throughout the hospital inpatient population has been reported in this study (5). Therefore, identifying which clinical factors are associated with greater morbidity and mortality is of paramount importance in tackling the ongoing COVID-19 pandemic.

Two recent nationwide observational studies from France and the United Kingdom found obesity was independently associated with increased mortality and endotracheal intubation in diabetes patients with COVID-19 (12, 13). In the NHS England study, Holman and colleagues also found hyperglycaemia was associated with increased mortality. Another NHS England study found a 3-fold and 2-fold increased risk of COVID-19-related deaths amongst type 1 diabetes and type 2 diabetes respectively when compared to the general population (14). These studies provide insight into important modifiable characteristics, which can potentially improve outcomes from COVID-19 in people with diabetes. Medications commonly used in diabetes and cardiovascular disease have generated much interest especially given the integral role of ACE2 in COVID-19 pathogenesis. ACE inhibitors (ACEi) and angiotensin receptor blockers (ARB) were initially hypothesised to affect outcomes in COVID-19. However, evidence from observational studies is still largely equivocal (15–17). Whilst these studies demonstrate significant results, it remains imperative to further study regional data given the demographic variety of populations across the UK.

This study aimed to identify clinical and biochemical characteristics associated with mortality and the need for intensive care in people with diabetes and COVID-19 admitted to secondary and tertiary UK National Health (NHS) hospitals within the Cheshire and Merseyside regions of North West England.

RESEARCH DESIGN AND METHODS

Study Design and Data Collection

This was a multicentre, retrospective, observational cohort study involving secondary and tertiary hospitals in Cheshire and Merseyside regions in the North West of England. All patients admitted to 7 hospitals in the region with COVID-19 between 01 January 2020 to 30 June 2020 with a known diagnosis either of type 1 diabetes, type 2 diabetes, other forms of diabetes, or newly diagnosed diabetes on admission were included irrespective of the reason for admission. Attribution of COVID-19 was defined as a positive SARS-CoV-2 Reverse Transcriptase PCR test. The endpoints of the study were 7 and 30 days from the date of

admission and, if discharged before 7 days, general practitioners (GPs) were contacted for the occurrence of any endpoint. The study was approved by the Regional Research Ethics Committee. As ACCREDIT study has a purely non-interventional observational study design and in the context of the ongoing COVID-19 pandemic, written informed consent was not obtained from patients. The study data were collected by physicians and associates involved in the direct care of COVID-19 patients in the respective study centres from medical case notes, hospital electronic health records, local ISARIC data and the local GP practice records. Information bias due to missing data may occur. To address this, we specifically highlighted variables whose significance should be interpreted with caution.

Clinical Outcomes

The primary outcome of the study was death by day 7 of admission. Secondary outcomes include intensive care for invasive/non-invasive ventilation, inotropic support or renal replacement within 7 days of admission and death within 30 days of admission.

We also explored diabetes-related events including insulin infusion requirement, the incidence of diabetic ketoacidosis (DKA) or ketosis, incidence of severe hypoglycaemia and the need to escalate anti-hyperglycaemic therapy (as defined by the unit increase in preadmission total daily insulin dose and/or switch from oral medication to insulin therapy).

Statistical Analyses

Continuous variables are expressed as means (SD) and medians (IQR) and categorical variables as numbers and percentages. In determining the sample size, we used the Clopper-Pearson estimate and determined that a sample size of 700 participants with due consideration for an attrition rate of 20% for missing data and a percentage of 16% of our main outcome, would give us a 95% CI equal to 13.1% to 19.4%.

Progression of the outcome over time was characterised with Kaplan-Meier curves. To establish the robustness of the multivariate analyses, the cohort was split into separate datasets for model development and validation, with a further split into training and testing data to allow for model optimisation on out-of-sample data. Age and CRP were log-transformed before the analysis to account for skew. Multivariate logistic regression was applied using SPSS 26.0 with model selection by stepwise feedforward modelling with appropriate regularisation (Akaike and Bayesian information criteria) (18).

RESULTS

The ACCREDIT study included 1004 participants with diabetes and microbiologically confirmed COVID-19 admitted to 7 North West hospitals in the UK from 01 January 2020 to 30 June 2020. A total of 241 patients met the primary outcome of death within 7 days of hospital admission. The overall event rates are 24% for death by day 7 and 33% by day 30. One hospital was unable to obtain data on CRP, BMI, weight, and presence of COPD (n = 123). Upon statistical review of these data, they were found to be missing completely at random and were not included in further statistical analysis.

The clinic characteristics of this cohort are shown in **Table 1**. Mean age was 74.1 ± 12.6 years and these patients were predominantly men (60.7%). The great majority had type 2 diabetes (93.8%), followed by type 1 diabetes (4.8%), and the rest had other forms of diabetes (1.3%). The median BMI was 27.6 (25th-75th percentile 23.9 - 32.4) kg/m^2. The mean HbA1c value was 61.2 ± 19.7 mmol/mol (7.7 ± 4%).

The comorbidities for this group included hypertension (60.8%), ischaemic heart disease (43.3%), peripheral vascular disease (13.9%), cerebrovascular disease (18.6%), chronic kidney disease (30.4%) and COPD (17.2%). Microvascular and macrovascular complications of diabetes were evident amongst 49.6% and 56.2% of the cohort respectively. For smoking status, 6.7% were current smokers and 32.6% were ex-smokers.

For pre-admission pharmacotherapy, 27.4% were prescribed insulin therapy whilst 18.1% received dipeptidyl peptidase 4 (DPP-4) inhibitors. ACE inhibitors were prescribed in 27.9% of patients whilst 12.9% were on ARBs.

The distribution of socioeconomic status is heavily skewed with 45% amongst 959 participants belonging, respectively, to the two most deprived deciles in the UK by Index of Multiple Deprivation (19). The majority of the participants were White (96.8%).

Focusing on parameters during admission, as expected, a median CRP of 76.5 (34-136) mg/ml was observed as evidence of an ongoing inflammatory process in the majority of cases reviewed. The median absolute lymphocyte count was 0.88 (0.6-1.2) x 10^9/l. Median random non-fasting blood glucose at the time of admission was 8.2 (6.1-11.4) mmol/l. Only 16.5% and 23.7% of the study population had available D-dimer and troponin level respectively. The median D-dimer was 1071 (524-2120) mg/ml and the median troponin was 27.2 (13-58.3) mg/l at the time of COVID-19 diagnosis. 12.5% of the cohort received corticosteroid therapy during their hospital stay.

Focusing on diabetes-related events, 9 patients were diagnosed with new diabetes during their admission with COVID-19. Escalation of anti-hyperglycaemic therapy was necessary for 117 patients (11.9%), and 97 patients required intravenous insulin infusion (9.8%). A composite of diabetic ketoacidosis and ketosis was evident in 27 patients (2.7%) and 52 patients developed severe hypoglycaemia (5.2%).

Factors Associated With the Primary Outcome

Univariate analysis revealed that amongst the pre-admission variables, age was significantly associated with the primary outcome of death by day 7 (mean age 74.1, p = <0.01). **Figure 1A** shows a clear separation for the groups stratified by age. BMI was not found to be significantly associated with mortality even after stratification based on age and WHO classification. Univariate logistic regression with intensive care admission as the outcome variable showed statistical significance

TABLE 1 | Clinical characteristics of ACCREDIT participants according to the primary outcome of death within 7 days of admission.

Clinical features	Number of patients	Mean [± SD] or proportion (%)	Median [± IQR]	Primary outcome (*n* = 241)	Odds ratio
Pre-admission variables					
Age (years)	1004	74.1 [61.5, 86.7]	77 [66-84]	<0.001	
Sex	1004			>0.05	
Male		609/1004 (60.7)			
Female		395/1004 (39.3)			
Type of diabetes	1003				
Type 1		48/1003 (4.8)		0.05	0.43 [0.18, 1.01]
Type 2		941/1003 (93.8)		0.04	2.52 [1.06, 5.98]
Other		6/1003 (0.5)		–	–
Diagnosed on admission		9/1003 (0.8)		–	–
Weight (kg)	736	81.2 [79.6, 82.8]	78.5 [66.1, 92.3]	0.47	
BMI	687	28.9 [28.4, 29.4]	27.6 [23.9, 32.4]	0.52	
<18.5 kg/m^2		16/687 (2.3)			
18.5-24.9 kg/m^2		207/687 (30.1)			
25-29.9 kg/m^2		205/687 (29.8)			
30 kg/m^2 and above		259/687 (37.7)			
Ethnicity	1004			>0.05	
White		952/1004 (94.8)			
Black African, Caribbean, or Black British		11/1004 (1.1)			
Asian		9/1004 (0.9)			
Mixed or multiple ethic groups		3/1004 (0.2)			
Other ethnic group		8/1004 (0.7)			
Unknown		21/1004 (2.1)			
Index of Multiple Deprivation[a]	959			>0.05	
1		296/959 (30.9)			
2		138/959 (14.4)			
3		84/959 (8.8)			
4		91/959 (9.5)			
5		54/959 (5.6)			
6		58/959 (6.0)			
7		60/959 (6.3)			
8		58/959 (6.0)			
9		63/959 (6.6)			
10		57/959 (5.9)			
Smoking status	1004			>0.05	
Never	364	364/1004 (36.3)			
Current	67	67/1004 (6.7)			
Ex-smoker	327	327/1004 (32.6)			
Unknown	246	246/1004 (24.5)			
Duration of diabetes (in years)	696	12.4 [11.8, 13.0]	12 [6, 17]	0.61	
Latest HbA1c (mmol/mol)	943	61.2 [60.0, 62.5]	56 [47, 71]	0.05	
UACR (mg/mmol)	595	23.4 [16.8, 30.0]	2.5 [0.77, 9.8]	0.86	
Insulin		273/997 (27.4)		0.38	0.86 [0.62, 1.20]
DPP4 inhibitor		181/1001 (18.1)		0.81	0.95 [0.65, 1.40]
ACE inhibitor		278/998 (27.9)		0.21	0.81 [0.58, 1.13]
ARB		129/999 (12.9)		0.49	1.16 [0.76, 1.77]
Hypertension		599/986 (60.8)		0.15	0.80 [0.60, 1.08]
COPD		150/870 (17.2)		0.12	1.37 [0.93, 2.04]
Documented foot ulcers		95/928 (10.2)		0.08	0.6 [0.34, 1.06]
IHD		429/991 (43.3)		0.56	1.09 [0.81, 1.46]
CVD		186/998 (18.6)		0.17	1.29 [0.90, 1.84]
PVD		133/955 (13.9)		0.28	1.25 [0.83, 1.90]
CKD		302/994 (30.4)		0.02	1.45 [1.06, 1.97]
Peripheral neuropathy		144/783 (18.4)		0.98	1.01 [0.66, 1.54]
Retinopathy		221/734 (30.1)		0.34	0.83 [0.57, 1.21]
Microvascular complications[b]		498/1004 (49.6)		0.17	1.23 [0.92, 1.64]
Macrovascular complications[c]		564/1004 (56.2)		0.21	1.21 [0.90, 1.62]
Admission variables					
Random non-fasting blood glucose (mmol/l)	949	9.5 [9.2, 9.8]	8.2 [6.1, 11.4]	0.87	
CRP (mg/l)	858	97.2 [91.4, 103.0]	76.5 [34, 136]	<0.001	

(Continued)

TABLE 1 | Continued

Clinical features	Number of patients	Mean [± SD] or proportion (%)	Median [± IQR]	Primary outcome (*n* = 241)	Odds ratio
Absolute lymphocyte count (10^9/l)	992	1.06 [1.0, 1.15]	0.88 [0.6, 1.2]	0.001	
Troponin (ng/l)	238	168.0 [48.9, 287.1]	27.2 [13, 58.3]	0.27	
D-dimer (ng/ml)	166	1952 [1528, 2376]	1071 [524, 2120]	0.04	
Insulin infusion required		97/988 (9.8)		0.02	0.5 [0.28, 0.9]
DKA or ketosis		27/996 (2.7)		0.49	0.7 [0.26, 1.88]
Escalation of insulin or anti-hyperglycaemic therapy		117/980 (11.9)		0.22	0.74 [0.46, 1.20]
Severe hypoglycaemia		52/998 (5.2)		0.40	0.74 [0.37, 1.50]
Steroid use during admission		124/991 (12.5)		0.64	0.9[0.57, 1.40]
Oxygen use during admission		654/880 (74.3)		<0.001	7.76 [4.24, 14.21]

[a]*Index of Multiple Deprivation presented in deciles, 1 being the most deprived and 10 being the least deprived.*
[b]*composite of CKD, retinopathy, and neuropathy.*
[c]*composite of IHD, CVD, and PVD.*
UACR, urine albumin:creatinine ratio; DPP4, dipeptidyl peptidase-4; ARB, angiotensin receptor blocker; COPD, chronic obstructive pulmonary disease; IHD, ischaemic heart disease; CVD, cerebrovascular disease; PVD, peripheral vascular disease; CKD, chronic kidney disease; CRP, C-reactive protein; DKA, diabetic ketoacidosis.

for BMI (p-value = 0.007) when used alone in the model. However, when the model was adjusted for age and sex, BMI was no longer significant (p-value = 0.168). Most recently recorded HbA1c, presence of CKD, and a diagnosis of either type 1 diabetes or type 2 diabetes were modestly associated with the primary outcome (p<0.05). Moreover, when stratified by age, younger patients with CKD (age < 70 years) showed an increased risk of the primary outcome [OR 2.74 (1.31-5.76)]. Duration of diabetes, UACR, presence of macro- and microvascular complications, and use of insulin, DPP4 inhibitor, ACE inhibitor, and ARB were not associated with the primary outcome. Within our review, a series of Kaplan-Meier charts were performed for key variables identified in the univariate analysis. In this exploratory analysis, the Kaplan-Meier charts were for death up to 30 days.

Amongst the individual admission variables, only CRP was significantly associated with the primary outcome (p = <0.001). For the Kaplan-Meier curve (**Figure 1B**), CRP values were stratified into quartiles. There is an association between a greater CRP numeric value (top quartile >136 mg/L), and mortality at 30 days. This key association between CRP and mortality at seven days follows the same trajectory for up to 30 days (see **Figure 1B**). The use of supplemental oxygen was also associated with the primary outcome (p < 0.001, OR 7.76 [4.24, 14.21]. D-dimer, absolute lymphocyte count, oxygen requirement, and insulin infusion requirement were found to be significant albeit to a lesser degree (p<0.05). The use of corticosteroid therapy during admission was not associated with mortality.

Multivariate Analysis

We determined sample sizes of 564, 245 and 195 for the training, testing, and validation datasets, respectively, with the incidence listed in **Table A3** (see **Appendix**) and without any rows with missing values gives prevalence [CIs] for the event rates of 25.25% [21.94%, 29.10%], 23.23% [18.46%, 28.80%] and 20% [14.99%, 26.17%]. The validation data set contains exclusively cases from different hospitals from those contributing to the training and test data sets.

Among pre-admission variables, the multivariate study identified age to be significantly associated with death by day 7 (p-value < 0.05). Further multivariate analysis including admission variables found that CRP was the most significant independent effect for death by day 7, followed by age. All models were fitted to the training dataset, identifying the most significant variables using AIC and BIC applied to both the training and test datasets. Performance statistics were calculated for all three data sets.

We can verify the discriminant boundary for the two variables selected by direct visualisation in **Figure 2**.

DISCUSSION

The ACCREDIT study aimed to identify characteristics associated with 7-day mortality and intensive care admission in people with diabetes and COVID-19. Our study found CRP to be the most significant independent variable associated with 7-day mortality more so than age, and we have assessed that both parameters combined provide reasonable predictive power for this outcome. This is in keeping with other whole observational studies and metanalyses, which have identified that advanced age and CRP predisposes COVID-19 patients to a higher risk of ventilator support and death in people with or without diabetes (12, 13, 20, 21). CRP has previously been found to be positively correlated with the diameter of lung lesion and severe presentation of COVID-19 (22).

The 7-day mortality rate amongst our cohort (24%) was beyond double the rate found in the CORONADO study, which was 10.3% on day 7 of admission (12). One notable difference between the cohorts was the mean age of the CORONADO study cohort was 69.8 compared to 74.1 for the ACCREDIT study cohort (12). We also note significant socioeconomic deprivation within our cohort. Both of these

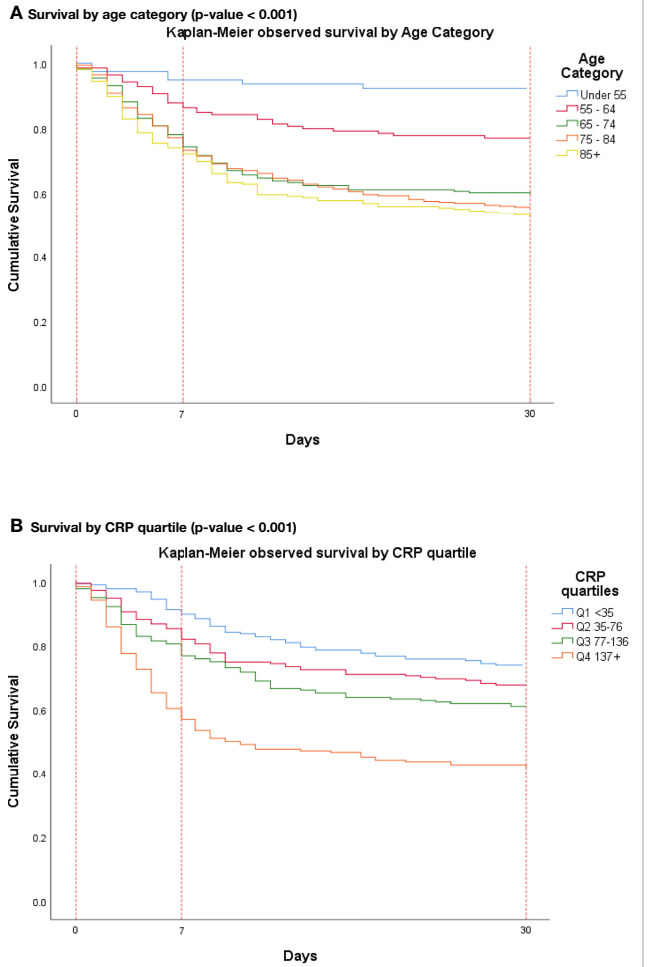

FIGURE 1 | Kaplan-Meier cumulative survival probability in hospitalised patients with diabetes and COVID-19 by day 30. **(A)** Mortality was significantly higher amongst patients with advancing age. **(B)** Mortality was significantly higher amongst patients with higher CRP levels. Confidence intervals are found in the **Appendix** section (**Tables A1, A2**).

factors may partially explain the significantly increased mortality revealed in the ACCREDIT population. Furthermore, data specific for persons with diabetes extrapolated from the ISARIC study revealed a 14-day in-hospital mortality rate over 29% in comparison to an estimated 26% in persons without diabetes (5). Although this finding is not directly comparable to our outcomes, it remains consistent with the mortality trend over time of this cohort.

A particular area of interest was the effects of glycaemic control on COVID-19 outcomes. Findings from previous studies have shown an association between poorer long-term and inpatient glycaemic control and greater mortality (4, 13, 23). However, in this study, both HbA1c and random blood glucose on admission were not associated with the primary outcome. Interestingly, almost a quarter in our cohort had HbA1c recorded below 48 mmol/ml, which may have contributed to the lack of association. It is noteworthy that this study did not have a strict preceding time limit on HbA1c values to be included as compared to the CORONADO group who allowed data for HbA1c to be recruited in the six months before admission only. Even then, the CORONADO study did not demonstrate an independent association between long-term glucose control and mortality at 7 days (12). This study also found an increased risk of death amongst younger patients with diabetes complicated by CKD. A population-based study shows higher probabilities of death and intubation from COVID-19 amongst patients with diabetic kidney disease (DKD) compared to other causes of CKD (24). These findings were attributed to a chronic pro-inflammatory state and immune dysregulation. Moreover, renal tubular ACE2 receptors were found to be overexpressed in DKD as compared to the general population, possibly enhancing viral entry (25, 26). There are concerns regarding the use of corticosteroid therapy in the setting of COVID-19 and diabetes given its potential to worsen glycaemic control. The RECOVERY trial showed the use of dexamethasone in moderate to severe COVID-19 improved clinical outcomes (27). Our study did not find any association between steroid use and 7-day mortality [p=0.64, OR 0.9 (0.57,1.40)]. However, only a small proportion of the cohort (12.5%) received steroids. This is despite the majority of the patients (74.3%) needed oxygen therapy. It is important to note that our data were gathered during the time when steroids use in COVID-19 was still largely under investigation. It was only later during the data collection period when dexamethasone was recommended as a national guideline. Larger and more focused studies are recommended to further elucidate its effect on diabetes and COVID-19 outcomes. Newly diagnosed diabetes amongst COVID-19 patients conferred a higher risk of adverse outcomes, according to some studies (28, 29). However, the very small number of this subgroup in this cohort makes it difficult to draw any significant conclusion from it.

Due to the limited numbers admitted to intensive care (7.5%) we did not proceed with further statistical analysis for this outcome because any significant result will likely be underpowered. Advanced care planning continues to play an important role in tackling COVID-19 and decisions surrounding escalation should be appropriately made within the wider clinical context including assessment of co-morbidities, performance status and resource allocation.

The ACCREDIT study population had higher deprivation and obesity levels in comparison to the rest of the UK population, which makes the results prone to selection bias. With regards to socioeconomic deprivation, the NHSE study found that there were substantially more deaths in the most deprived quintile - especially for those with type 1 and type 2 diabetes, and to a lesser extent for those without diabetes (13). However, our study did not show an increased risk of death with higher deprivation. It is worth noting that almost half of the ACCREDIT cohort belonged to the two most deprived deciles, which is higher compared to the regional population (45% versus 34.75%) (19). This may explain the absence of such association. Obesity levels were noted to be higher in comparison to the general UK population, especially amongst the under-55 age group (30). Throughout this pandemic, obesity has consistently been highlighted as a risk factor for worse outcomes with COVID-19 (2, 6). From the CORONADO study, the

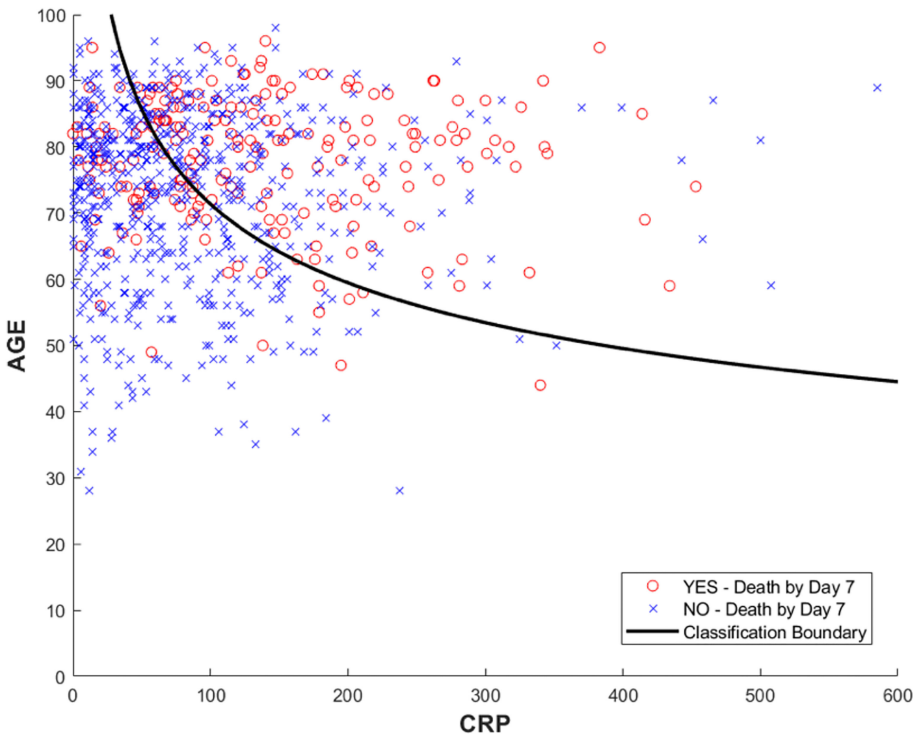

FIGURE 2 | The figure shows the individual values in the training data for Age and CRP colour coded for outcome, with observed death by day 7 from admission highlighted in red. The curved line shows where the logistic regression model prediction is equal to the prevalence of events on the training data. The curved boundary results from the use of logarithmically transformed variables in the original logistic regression model. Stratifying the observations to the right of the curve and those to the left into high- and low-risk cohorts results in an odds ratio of 3.44 [2.17, 5.44] for the increase in probability of death by day 7.

population median BMI was 28.4 kg/m^2 and they found a positive and independent association with tracheal intubation and/or death within 7 days. However, this outcome was largely driven by tracheal intubation (12). Similarly, obesity (BMI ≥ 30 kg/m^2) had a higher HR for death in the NHS England study (13). The median BMI for our patient cohort was lower at 27.6 kg/m^2 and did not replicate the findings from both the above studies. Moreover, Holman and colleagues identified a higher HR for death in patients with low BMI (< 20 kg/m^2) (13). We did identify a trend, albeit not significant, towards low BMI and 30-day mortality.

One strength of our study is the data collection strategy, which involved direct perusal of patient notes by clinicians thereby assuring the accuracy of recording clinically relevant data. However, there were limitations; it should be stressed that the ACCREDIT data will reflect the demographics of the local population attending hospitals throughout the region during these six months. As mentioned before, this cohort had higher levels of deprivation. Moreover, there were a higher proportion of people of White ethnicity. This makes it underpowered for further analysis focusing on the BAME demographic group. It is also worth noting that increased D-dimer levels appeared to show a trend towards increased 30-day mortality. However, there was a significant number of missing values D-dimer within the dataset leading to insufficient statistical power. This may be because we were not consistently looking for venous thromboembolic events during admission in the first wave of COVID-19. Regarding glycaemic control, there was no time limit defined in determining the latest HbA1c level to be included in this study, which may have contributed to the relatively low mean HbA1c level in this cohort. Also, whilst we were able to accrue data from admission to include glucose and CRP measurements, we did not collect serial measurements throughout the admission and we are unable to assess the trend of these results and their relationship to relevant outcomes. Lastly, this study did not include other commonly used medications amongst people with diabetes and other cardiovascular conditions including metformin, sodium-glucose co-transporter-2 inhibitors, and statins.

Our study highlights substantial resources are required in tackling COVID-19 for this patient cohort with diabetes. Nearly 10% patients needed intravenous insulin infusion. This is higher compared to the recent 2017 National Diabetes Inpatient Audit in 2017, wherein there was a steady decline of the use of insulin infusions from 11% in 2011 to 8.2% in 2017 (31). Of note, the rate of severe hypoglycaemia in this study (5%) was lower compared to 7% seen in the above audit. The significance of which remains to be a question. These measures represent increased demand on the inpatient diabetes services across the region including increased numbers of giving sets for intravenous infusions, greater inpatient diabetes nursing provision and education within the hospital setting.

Amongst patients with diabetes presenting with COVID-19 in the hospital setting, CRP and age are useful markers associated with an increased risk of death within the first week of admission, specifically amongst the most deprived regions in the community. Since these variables are easily identified early on during hospital admission, they can aid in determining treatment escalation plans and risk stratification models thereby potentially improving patient outcomes. Further work will allow clinician-researchers to establish a more robust risk stratification model on admission and perhaps a lower threshold for intensification of treatment for this patient cohort. This information may also aid in further prognostic studies looking into new variants and subsequent surges of COVID-19. Precedence has been previously set with the introduction of the ISARIC 4C Mortality Score (32). The ACCREDIT study results may aid in further development of a similar yet specific prognostic tool for diabetes patients with COVID-19 infection. Additional research is needed to elucidate any relationship between glycaemia and the prognosis of diabetes patients admitted to hospital with COVID-19. We suggest that future mechanistic studies could be considered to further explore the increased mortality for the diabetes cohort as compared to the non-diabetes population.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by North East - York Research Ethics Committee NHSBT Newcastle Blood Donor Centre Holland Drive Newcastle upon Tyne NE2 4NQ Telephone: 0207 1048091. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

DL and RW contributed to the study equally and should be regarded as co-first authors. DL, RW, HS, and SN conceived the study and conducted literature search. DL and RW developed the database for data collection and collation. All authors contributed to writing the study protocol. PL and IJ conducted the statistical analysis and contributed the figures. SN, HS, DL, and RW led the collection of data. At their respective study sites, the following authors conducted data collection from the patients' medical notes: StW, JN, DS, DK, SR, ScW, RN, NF, SaW, RY, and ZQ. Lastly, all authors collaborated in interpreting the results. DL, RW, HS, and SN drafted the manuscript and all authors contributed to critical revisions in the manuscript. All authors contributed to the article and approved the submitted version.

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We recognise the invaluable work of healthcare staff across the region in the fight against COVID-19.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.777130/full#supplementary-material

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ORIGINAL RESEARCH
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Omicron Variant of SARS-CoV-2 Virus: *In Silico* Evaluation of the Possible Impact on People Affected by Diabetes Mellitus

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The Omicron variant of SARS-CoV-2 (Spike mutant B.1.1.529) carrying more than 30-point mutations in its structure, of which 15 are localized in the receptor-binding domain (RBD), allows to hypothesize a relevant change in interactivity with ACE2. In previous reports we hypothesized that the worse outcome of the COVID-19 disease in diabetes mellitus condition could be related to the non-enzymatic glycation of ACE2 receptor and an *in silico* evaluation led to the demonstration that the number of interactions is decreased in comparison to the unmodified model, possibly shifting the virus attack through different, multiple alternative entry routes. Given the evidenced features of this variant, we aimed to investigate with a computational approach the characteristics of Omicron SARS-CoV-2 with respect to its binding to human ACE-2 receptor, in a particular population, namely people affected by diabetes mellitus, at risk for unfavorable outcomes of the COVID-19. The computational analysis, considering the case in which all the lysine residues in the system are subjected to non-enzymatic glycation, confirmed that lysine glycation causes a general loss of interactivity between wild-type (WT)-Spike-RBD and ACE2. In the Omicron variant, Lys417 mutates into an asparagine, preventing the possible non-enzymatic glycation of this residue. Therefore, if non-enzymatic glycation seemed to cause a shift in the way in which the virus enters the cell from the ACE2-mediated mechanism to other pathways, in the case of the Omicron variant the ACE2-mediated approach of the virus seems to remain an important event to take into account. Indeed, interaction profile analysis, together with molecular mechanics–generalized Born surface area (MM-GBSA) calculations, suggests that the Omicron-Spike-RBD maintains a higher affinity for ACE2 subsequently to non-enzymatic glycation with respect to WT-Spike-RBD. The finding of the present computational study may suggest a different clinical relevance of the Omicron variant for the diabetes mellitus field, also in the possible direction of a lower severity of the disease.

Keywords: COVID-19, diabetes mellitus, glycation, human ACE2 receptor, Maillard reaction, Spike protein

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INTRODUCTION

The unprecedented occurrence of a set of several genetic mutations in the Omicron variant of the SARS-CoV-2 virus has drawn particular attention within scientists and media. Omicron-Spike mutant B.1.1.529 carries more than 30-point mutations in its structure, of which 15 are localized in the receptor-binding domain (RBD) (1). Significant changes have occurred, also with respect to the Delta-Spike variant (**Figure 1**). Moreover, 11 of these mutations are located at the contact surface with ACE2, allowing us to hypothesize that Omicron-Spike could have a relevant change in interactivity with ACE2. Some of these mutations, such as Q498R and N501Y, have already proven to lead to an increased affinity with ACE2 in respect to WT-Spike (2).

The Technical Advisory Group on SARS-CoV-2 Virus Evolution (TAG-VE) has advised WHO that the Omicron variant should be designated as a variant of concern (VOC), due to the epidemiological parameters initially reported in South Africa, and now spreading around the world (3). This is the fifth VOC to be reported since the beginning of the pandemic (4); following the experience with the previously reported variants associated with new worsening of the pandemic, a great concern has arisen whether a relevant change in transmissibility and binding affinity is to be expected with the new Omicron variant. Moreover, most recent data suggest that mutations occurring at RBD influence negatively the activities of neutralizing antibodies induced by vaccines, or administered as monoclonal antibody therapy (5). However, preliminary observations from South Africa suggest that the SARS-CoV-2 Omicron variant is linked to a reduced risk of severe disease when compared to the Delta variant (6, 7).

Since the first times of the pandemic, increased morbidity has been evidenced in people affected by diabetes mellitus (8–11), and the reason for this phenomenon is at present still debated. In a previous report, we hypothesized that the worse outcome of the COVID-19 disease in diabetes mellitus condition could be related to the non-enzymatic glycation of ACE2 receptor, due to hyperglycemic environment, triggering a higher interaction with virus Spike protein (12). However, a specific *in silico* evaluation of the interaction between ACE2 receptor and SARS-CoV-2 Spike protein under different conditions of non-enzymatic glycation has led to the demonstration that the number of interactions is decreased in comparison to the unmodified model (13), possibly shifting the virus attack through different, multiple alternative entry routes. In particular, the interaction of SARS-CoV-2 with human cells has been suggested also be mediated by Transmembrane Protease Serine-2 (TMPRSS2) activity (14, 15), as well as by other receptor pathways, such as Neuropilin-1 (NRP1) (14, 16), dipeptidyl peptidase 4 (DPP4) also known as cluster of differentiation 26 (CD26) (14, 17), the transmembrane glycoprotein CD147 (basigin 2) (14), and glucose-regulated protein 78 (GRP78) (18). However, the role of these alternative routes of virus entry in diabetes is still a matter of debate and investigation (13).

In view of the evidenced features of this variant, we aimed to investigate with a computational approach the characteristics of Omicron SARS-CoV-2 with respect to its binding to human ACE-2 receptor, in a particular population, namely people affected by diabetes mellitus, at risk for unfavorable outcomes of the COVID-19. The *in silico* analysis, after the recognition of the main interactions occurring between virus and ACE2 receptor, was directed to the specific evaluation of the impact

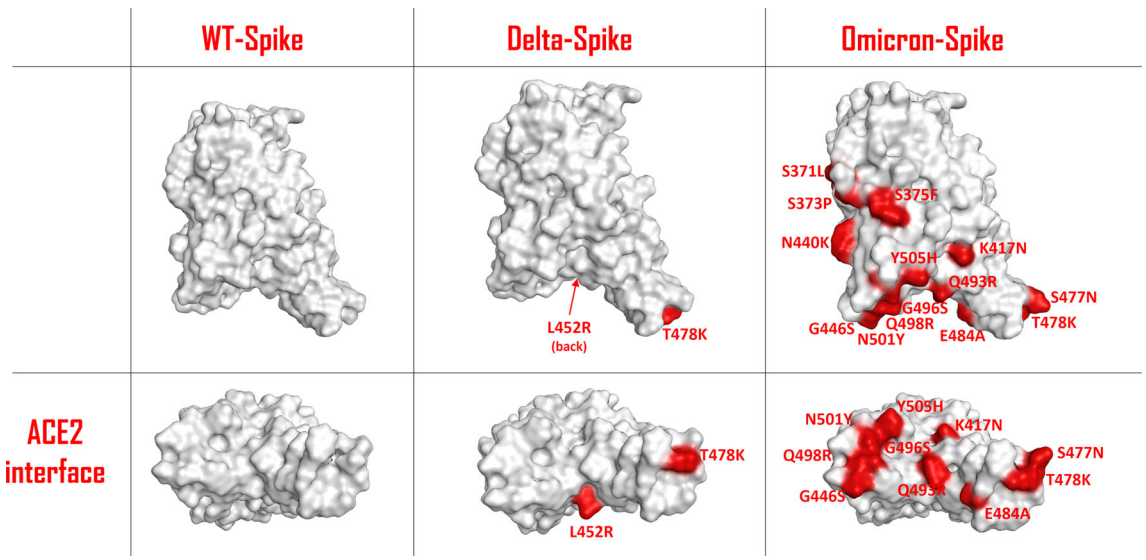

FIGURE 1 | Schematic representation of the SARS-COV-2 Spike protein receptor-binding domain (RBD) surface for the wild type (WT), the Delta variant and the Omicron variant. The residues involved in mutations are colored in red and are also labelled. The top panels represent a lateral view of the Spike-RBDs considered, while the bottom panels highlight the protein surface facing the ACE2 interaction interface for each situation examined.

to the affinity of the system, induced by a hyperglycemic environment, conditioning non-enzymatic glycation at lysine residues of both Omicron-Spike-RBD and ACE2 receptor.

MATERIALS AND METHODS

The computational analysis presented in this study was conducted starting from two different experimental structures, which were both downloaded from the Protein Data Bank (PDB) (19). The first of these systems represents the wild-type (WT) form of SARS-CoV-2 Spike RBD complexed with ACE2 receptor (PDB code: 6M0J; method: X-ray diffraction; resolution: 2.45 Å) (20), while the second involves the SARS-CoV-2 Spike-RDB Omicron variant forming a complex with ACE2 (PDB code: 7T9L; method: cryo-electron microscopy (Cryo-EM); resolution: 2.66 Å) (21). The proteins were prepared for molecular modeling with the "Structure Preparation" tool implemented in the Molecular Operating Environment (MOE) suite (22). The missing hydrogen atoms were added exploiting the MOE "Protonate 3D" tool, which assigns to each residue the most probable protonation state at the chosen pH, which in our case was set at the value of 7.4. These hydrogen atoms were then minimized using the AMBER10:EHT (23) force field implemented in MOE. To create the glycated forms of both WT-Spike-RBD/ACE2 and Omicron-Spike-RBD/ACE2 systems, the lysine residues of the prepared complexes 6M0J and 7T9L were manually changed. This operation involved the addition of a cyclic amino sugar moiety, which results from the Maillard reaction between the lysine amino acid and D-glucose, followed by the Amadori rearrangement (24) (as depicted in **Supplementary Figure 1**). This step allowed us to obtain the four systems of interest for our study, considering various conditions of non-enzymatic glycation (here labeled as "glyco"). These complexes are WT-Spike-RBD with ACE2, WT-Spike-RBD-glyco with ACE2-glyco, Omicron-Spike-RBD with ACE2, and Omicron-Spike-RBD-glyco with ACE2-glyco. Once the different systems were obtained, the glycated lysine residues were energetically minimized under the AMBER10:EHT (23) force field implemented in MOE. This passage was essential in order to allow the newly introduced sugar groups to be properly orientated in the environment.

After these preliminary steps, the interactions between Spike-RBD and ACE2 were analyzed both visually and with the "GetContacts" tool (25). This last program can extrapolate and classify all the interactions between the biological entities of a system. In our case, the specific contacts between Spike-RBD and ACE2 were evaluated for all the cases.

To give a better view of the macroscopic changes brought by the mutation and the glycation of the systems, the electrostatic surface of both ACE2 and Spike-RBD were also calculated for all the situations considered (as depicted in **Figure 2**).

To further inspect the effect of glycation on the interaction between Spike-RBD and ACE2 receptor for both WT and Omicron variants, molecular mechanics–generalized Born surface area (MM-GBSA) calculations were executed in the complexes using the Schrödinger Prime application (26). This method consists of the estimation of the binding free energy between two entities in an environment, exploiting an implicit solvation model. In our case, the Surface Generalized Born Model and Variable Dielectric (VSGB) solvation model (27) was used, and the force field in which the complexes were evaluated was the OPLS4 force field (28) implemented in Prime.

RESULTS

A visual representation of the complexes created considering various conditions of non-enzymatic glycation on the SARS-CoV-2 Spike RBD/ACE2 receptor system is reported in **Supplementary Figures 2–5**. A brief report of the number of the interactions (expressed in terms of the number of pairs of residues that are in contact between the two proteins) in each system is reported in **Table 1**.

The interaction pattern occurring in the native WT-Spike-RBD/ACE2 system (**Supplementary Figure 2**) is characterized by polar bonds (comprising both salt bridges and hydrogen bonds) as well as non-polar van der Waals interactions. The WT-Spike-RBD-glycated/ACE2-glycated system (**Supplementary Figure 3**), mimicking the maximum level of glycation attainable in a hyperglycemic condition in the case of native SARS-CoV-2, is characterized by a reduction in the number of interactions, mainly of a non-polar entity.

The effect of glycation on the new Omicron SARS-CoV-2 variant (compare **Supplementary Figure 4** and **Supplementary Figure 5**), produces a diminished number of bonds as well, occurring in virus/receptor interaction. As depicted from the results obtained, while for the WT-Spike-RBD/ACE2 situation the reduction in the number of non-polar interactions due to glycation is not linked to an overall change in the number of polar contacts, for the Omicron variant, the small decrease in the number of hydrogen bonds subsequent to the non-enzymatic glycation of lysine amino acids seems to be compensated by an increase in non-polar interactions.

Consequently, we can hypothesize that, if the decrement in non-polar contacts between WT-Spike-RBD/ACE2 and WT-Spike-RBD-glyco/ACE2-glyco systems is not balanced by a significant strengthening of the polar component of the interaction, for the Omicron variant, where the number of polar bonds is higher with respect to the WT in both native and glycated forms, the interaction between the two glycated proteins could be more efficiently preserved. This is also supported by the MM-GBSA calculation results, reported in **Table 2**, which highlight the loss in interaction energy that both the WT-Spike-RBD/ACE2 and the Omicron-Spike-RBD/ACE2 complexes experienced after glycation. The data coming from this analysis confirm that Omicron-Spike-RDB is able to form with ACE2 a more stable complex in respect to the WT-Spike-RBD (with a difference in the binding free energy of about 11 kcal/mol). Moreover, the binding free energies obtained demonstrate that glycation causes a general loss in interactivity towards ACE2 for both for WT-Spike-RBD and Omicron-

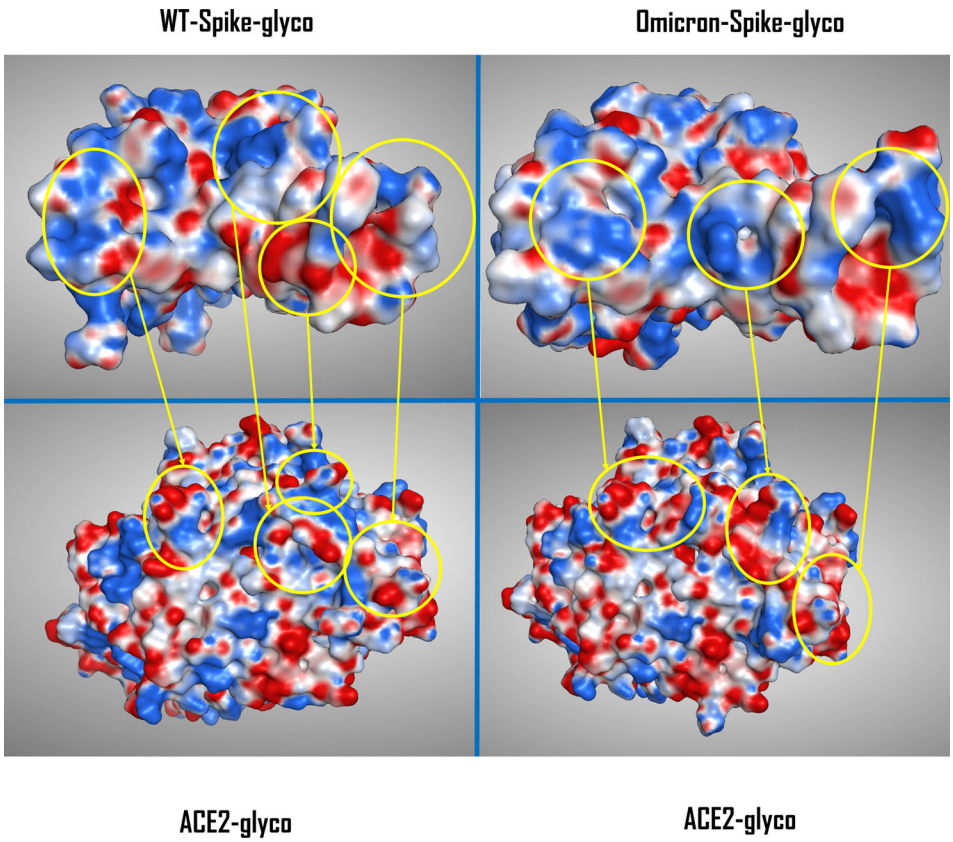

FIGURE 2 | Comparison between the electrostatic contact surface between the WT-Spike-RBD-glyco/ACE2-glyco system (top and bottom left) and the Omicron-Spike-RBD-glyco/ACE2-glyco complex (top and bottom right). The areas of highest variation in electrostatic distribution between glycated WT and Omicron Spike-RBDs are circled in yellow and are then connected with the corresponding ACE2-glyco interacting surface portion. The Spike-RBD/ACE2 backbone conformation for the WT variant comes from the crystal with PDB code 6M0J, while for the Omicron variant the data come from the Cryo-EM structure with PDB code 7T9L. WT, wild type; RBD, receptor-binding domain; PDB, Protein Data Bank; Cryo-EM, cryo-electron microscopy.

TABLE 1 | Number of interactions (expressed in terms of the number of residues pairs that are in contact between the two proteins) extrapolated from the four systems considered in the study presented (WT-Spike-RBD on ACE2, WT-Spike-RBD-glyco on ACE2-glyco, Omicron-Spike-RBD on ACE2, and Omicron-Spike-RBD-glyco on ACE2-glyco).

Interaction type	WT-Spike-RBD on ACE2	WT-Spike-RBD-glyco on ACE2-glyco	Omicron-Spike-RBD on ACE2	Omicron-Spike-RBD-glyco on ACE2-glyco
Salt bridge interactions	1	0	2	1
Hydrogen bonds	9	10	11	10
van der Waals interactions	30	21	32	37

WT, wild type; RBD, receptor-binding domain.
The"GetContacts" tool was exploited in order to calculate all the contacts between Spike and ACE2 in each of the scenarios considered.

TABLE 2 | Free energy of binding (expressed in kcal/mol) obtained with the MM-GBSA calculations executed on the different complexes considered in this study (WT-Spike-RBD/ACE2, WT-Spike-RBD-glyco/ACE2-glyco, Omicron-Spike-RBD/ACE2, and Omicron-Spike-RBD-glyco/ACE2-glyco).

Interaction type	WT-Spike-RBD on ACE2	WT-Spike-RBD-glyco on ACE2-glyco	Omicron-Spike-RBD on ACE2	Omicron-Spike-RBD-glyco on ACE2-glyco
MM-GBSA total free energy of binding (kcal/mol)	-114.37	-89.29	-125.59	-111.83

MM-GBSA, molecular mechanics–generalized Born surface area; WT, wild type; RBD, receptor-binding domain.

Spike-RBD, and that this drop is much more significant for the WT variant.

To allow a more comprehensive view of the overall changes caused by the Omicron mutation and the glycation of the systems, the electrostatic surface of both ACE2 and Spike-RBD were calculated for all the situations considered, and the differences between the electrostatic distribution were highlighted (**Supplementary Figure 6** and **Figure 2**). As illustrated by these pictures, it is interesting to notice the linkage between the change in the surface features of the proteins and the locations in which glycation takes place. To represent this parallelism, the glycation sites were highlighted on the Spike-RBD surface, and the results are reported in **Figure 3**. As depicted, the only Spike-RBD lysine residue directly in contact with ACE2 is Lys417 in the native form. Although in the Omicron variant this lysine mutates into an arginine, Thr478 mutates into lysine (T478K), forming another glycation site on the ACE2 interface.

DISCUSSION

The computational analysis allowed us to evaluate the changes in the interaction pattern between Spike-RBD and ACE2 receptor considering both mutation (focusing on the Omicron variant) and the presence of a hyperglycemic environment. The modified affinity between these two biological entities, which seems higher for Omicron-Spike-RBD to WT-Spike-RBD, has been reported (21, 29, 30). In a hyperglycemic environment, typical of diabetes, we considered the case in which all the lysine residues in the system are subjected to non-enzymatic glycation. The behavior of the proteins will so change with respect to the position of the lysine amino acids. A recent work of our group highlighted the possibility that diabetic patients in which this glycation process takes place are less prone to be infected by SARS-CoV-2 in an ACE2-dependent way (13). Indeed, our analysis here confirms the result and suggests that lysine glycation causes a general loss of interactivity between WT-Spike-RBD and ACE2.

One of the main WT-Spike-RBD residues involved in the interaction with ACE2 is Lys417, which is the lysine amino acid that is most exposed to the ACE2 interface when Spike-RBD approaches the host cells. Indeed, glycation at this residue could lead to a weakening of the contacts between the two entities, mainly due to the loss of the strong salt bridge between the positive-charged nitrogen of Spike-RBD Lys417 and the negative-charged oxygen of ACE2 Asp30. Moreover, the increased hindrance of the glycated moiety at the contact interface can contribute to a general decrease of the interaction strength between Spike-RBD and ACE2 residues at the interface.

In the Omicron variant, Lys417 mutates into an asparagine, preventing the possible non-enzymatic glycation of this residue. This variation, together with several mutations on Spike-RBD that were linked to an increased affinity for ACE2 (such as S477N, Q498R, and N501Y) (31), is predicted to contribute to an

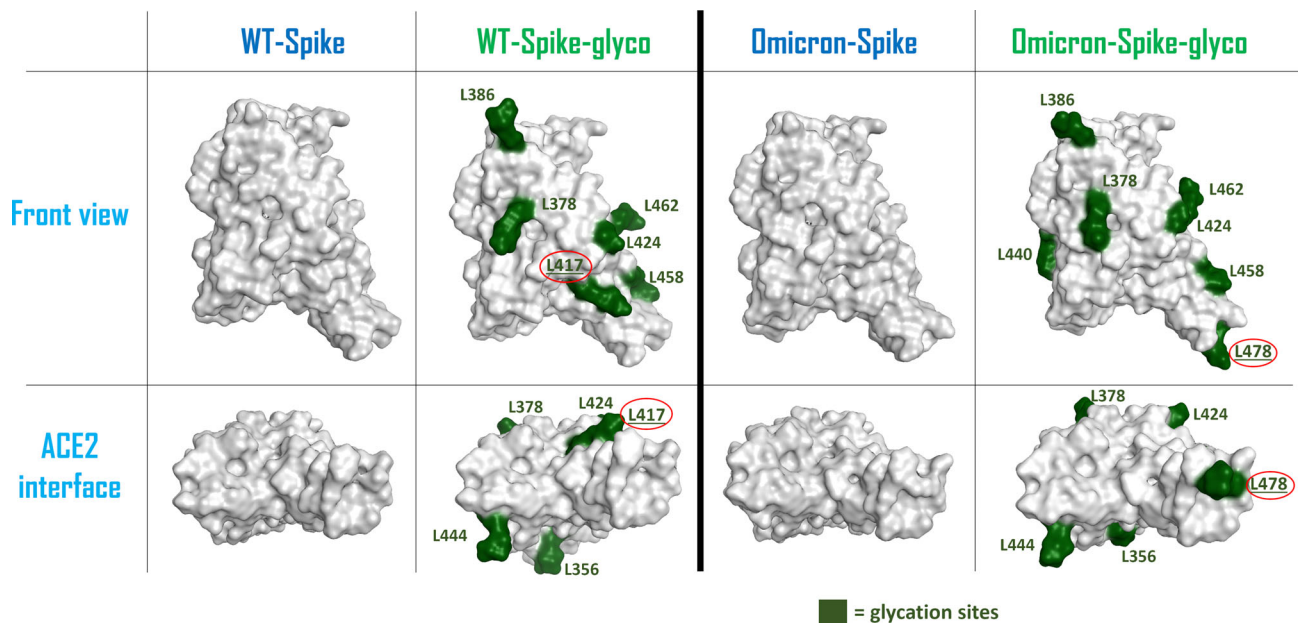

FIGURE 3 | Schematic representation of the surfaces of the possible forms of Spike-RBD considered in this computational study. From the left, the systems considered are: wild-type (WT) Spike-RBD, the lysine-glycated form of wild-type (WT) Spike-RBD, the Omicron variant Spike-RBD and the glycated form of Omicron variant Spike-RBD. For the glycated systems, the surface of lysine amino acids has been colored in dark green and has also been labeled. The lysine which are located on the contact interface with ACE2 (Lys417 for the WT-Spike-RBD-glyco and Lys478 for the Omicron-Spike-RBD-glyco) have their label underlined and circled in red. The top figures represent a lateral view of the Spike-RBDs considered, while the bottom figures highlight the protein surface facing the ACE2 interaction interface for each situation examined. RBD, receptor-binding domain.

increase in the interactivity between Omicron-Spike-RBD and ACE2 in a hyperglycemic environment. This behavior is shown by **Supplementary Figures 2**–**5**, in which the number of contacts between Spike-RBD and ACE2 (derived from the calculation executed with the "GetContacts" tool) is reported for the different systems considered. **Table 1** summarizes the overall number of residues pairs in contact between each kind of Spike-RBD/ACE2 molecular complex. First, as mentioned, both interaction analysis and MM-GBSA calculations highlight the higher affinity that Omicron exerts for ACE2 with respect to the WT variant. The data coming from the MM-GBSA calculation show that the reduction in the number of contacts between WT-Spike-RBD and ACE2 subsequent to glycation causes an increase in the binding free energy which is more significant than the one experienced by Omicron-Spike-RBD. The glycation, as expected, causes in both WT and Omicron variants a reduction in interaction strength with ACE2 receptor, and this can be attributed to different molecular reasons. For WT-Spike-RBD, the glycation causes mainly a loss in non-polar interaction, which could be also associated with a loss in the efficiency of hydrogen-bonding, leading to an overall noticeable increase in binding free energy. In the case of Omicron-Spike-RBD, where the loss in the number of polar contacts is compensated by an increase in non-polar interaction, and the number of non-polar contacts not only does not decrease, but also increases, the loss in binding free energy seems to be fully attributable to a drop in the efficiency of hydrogen bonding. Indeed, the increased hindrance created by the glycated residues present on the Omicron-Spike-RBD-glyco/ACE2-glyco interface (e.g., ACE2 Lys353) could destabilize the web of polar contacts that, even if the number is retained, decrease in strength. In any case, the data coming both from the analysis of the number of interactions and from MM-GBSA calculations highlight higher stability of the Omicron-Spike-RBD-glyco/ACE2-glyco complex over the WT-Spike-RBD-glyco/ACE2-glyco system.

The results of our analysis allow us to hypothesize that the affinity between the viral protein Spike and the human receptor ACE2 is higher for the Omicron variant in respect to the WT both in native conditions and also in the case of non-enzymatic glycation, typical of the hyperglycemic environment.

The results of the computational analysis here conducted allow us to hypothesize that, if non-enzymatic glycation seemed to cause a shift in the way the virus enters the cell from the ACE2-mediated mechanism to other pathways, in the case of the Omicron variant the ACE2-mediated approach of the virus seems to remain an important event to take into account. Indeed, both our number and our MM-GBSA analysis suggest that, even if a loss in interactivity is noticeable between the Omicron-Spike-RBD/ACE2 and the Omicron-Spike-RBD-glyco/ACE2-glyco, these systems are able to maintain a higher affinity with each other in respect to, respectively, the WT-Spike-RBD/ACE2 and WT-Spike-RBD-glyco/ACE2-glyco complexes.

The finding of the present computational study may suggest several consequences of potential clinical relevance for the diabetes mellitus field.

Non-enzymatic glycation is a well-documented phenomenon occurring in diabetes, and the fast kinetics of the event has been proven. Atanasova et al. (32) demonstrated that non-enzymatic glycation of protein amino groups (Maillard reaction) can occur at high glucose concentrations very quickly, already after few minutes. Therefore, a non-enzymatic glycation of ACE2 receptor in target tissues exposed to virus entry is very likely a fast spreading-out process, and the virus Spike protein can be glycated as well very quickly when high glucose concentrations occur.

If on one side the glycation might prevent the ACE2-Spike interaction in diabetes mellitus, as previously suggested (13), on the other side the mutations of the Omicron-variant Spike protein, leading to the loss of a lysine glycation site, may be responsible for a relatively augmented binding affinity to ACE2 (compare with **Figure 3**). Therefore, the risk of a more pronounced virus binding to ACE-2 receptor may occur in patients with diabetes, possibly conditioning a higher susceptibility to SARS-CoV-2 infection, as indicated in our first hypothesis (12). Moreover, in patients with decompensated diabetes, the greater affinity of Omicron Spike for ACE2 could on the one hand enhance the contact with the virus and therefore infection rate, but on the other hand, may reduce the possibility of entry through alternative routes and thus modify the overall course and severity of the disease. The new suggested roles of a novel furin cleavage site, exploited by SARS-CoV-2 to become fully active (33), could represent a possible escape mechanism used by the virus to produce an infection that, in the case of diabetes mellitus, may assume a pernicious evolution. The possible occurrence of alternative ways of virus entry in people affected by diabetes, as recently suggested (13), makes the new Omicron virus mutation of peculiar interest, not only in the general population, but also particularly in diabetes and pre-diabetes areas. However, the present results seem to point towards a lower severity of the disease with the Omicron variant also for patients with diabetes mellitus, supporting very recently published data (7), which show that diabetes did not appear to be a co-morbidity factor influencing disease severity, but only age. The severity of COVID-19 in diabetes remains a clinical query, also because the overall course of the infectious disease is influenced by a pre-existing reactivity, characterized by an increased pro-inflammatory profile (34), also linked to an excess of adipose tissue, which is associated with augmented lymphocyte activation and cytokine production (35). Whether this new variant may influence the real-world clinical evolution of COVID-19 in people affected by diabetes mellitus is still a matter of speculation. As suggested by the comment of Karim and Karin (4), we await knowledge of how this new variant will develop.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

DB, ER, GS, and SM contributed to the conception and design of the research. DB and SM performed the molecular modeling experiments. DB, ER, SM, and GS drafted the paper. AL and SM supervised and edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.847993/full#supplementary-material

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Metformin Use in Relation to Clinical Outcomes and Hyperinflammatory Syndrome Among COVID-19 Patients With Type 2 Diabetes: A Propensity Score Analysis of a Territory-Wide Cohort

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Aim: This study was conducted in order to evaluate the association between metformin use and clinical outcomes in type 2 diabetes mellitus (T2DM) patients hospitalized with coronavirus disease 2019 (COVID-19).

Methods: Patients with T2DM with confirmed diagnosis of COVID-19 and admitted between January 21, 2020, and January 31, 2021 in Hong Kong were identified in our cohort. Exposure was defined as metformin use within 90 days prior to admission until hospital discharge for COVID-19. Primary outcome was defined as clinical improvement of ≥1 point on the WHO Clinical Progression Scale (CPS). Other outcomes were hospital discharge, recovery, in-hospital death, acidosis, hyperinflammatory syndrome, length of hospitalization, and changes in WHO CPS score.

Results: Metformin use was associated with greater odds of clinical improvement (OR = 2.74, $p = 0.009$), hospital discharge (OR = 2.26, $p = 0.009$), and recovery (OR = 2.54, $p = 0.005$), in addition to lower odds of hyperinflammatory syndrome (OR = 0.71, $p = 0.021$) and death (OR = 0.41, $p = 0.010$) than control. Patients on metformin treatment had a shorter hospital stay (–2.76 days, $p = 0.017$) than their control counterparts. The average WHO CPS scores were significantly lower in metformin users than non-users since day 15 ($p < 0.001$). However, metformin use was associated with higher odds of acidosis.

Conclusions: Metformin use was associated with lower mortality and lower odds for hyperinflammatory syndrome. This provides additional insights into the potential mechanisms of the benefits of metformin use in T2DM patients with COVID-19.

Keywords: type 2 diabetes, metformin, COVID-19, SARS-CoV-2, in-hospital mortality, hyperinflammatory syndrome

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has infected over 208 million people globally as of August 19, 2021 (1). Type 2 diabetes mellitus (T2DM) is associated with worse clinical outcomes in COVID-19 patients, such as requirement of intensive care unit (ICU) admission and all-cause mortality (2–4). Patients with diabetes are more vulnerable, owing to the associated hyperglycemia causing dysregulated innate immunity and the associated low-grade chronic inflammation which increases the likelihood of cytokine storms in COVID-19, hence leading to adverse clinical outcomes (3, 5, 6).

As metformin is the first-line antidiabetic medication, it is important to understand the benefits and risks of its use among COVID-19 patients. While retrospective cohort studies have mostly revealed better outcomes associated with metformin use among COVID-19 patients with diabetes, such as intubation, ICU admission, and mortality (7–9), clinical data providing mechanistic links are limited. The potential beneficial effects of metformin probably extend beyond its glucose-lowering effect. In fact, metformin has also been proposed to possess antiviral effects and influence the level of inflammatory cytokine production (6). Nevertheless, one has to consider the potential adverse effects due to metformin use. Lactic acidosis is among one of the most commonly raised concerns (4, 10–12), which may be relevant in patients with renal impairment despite being extremely rare in clinical settings (10).

Recommendations differ regarding metformin use in COVID-19 patients: while the Joint British Diabetes Societies for inpatient care has proposed to continue metformin use, possibly due to being associated with better clinical outcomes (13), one published review article suggested stopping metformin in case of acute illness and respiratory distress given the concerns about risk of acidosis (11). Hence, a more in-depth evaluation of the potential effects of metformin use in a cohort of COVID-19 patients with diabetes will shed light on this issue.

Hence, we initiated this analysis of a territory-wide cohort of COVID-19 patients with T2DM to evaluate the impact of metformin use in relation to clinical outcomes, hyperinflammatory syndrome, and viral loads.

MATERIALS AND METHODS

Data Source and Study Population

Our data were extracted from a territory-wide cohort of patients with anonymized electronic health records provided by the Hong Kong Hospital Authority (HA). The public health ordinance in Hong Kong required all patients diagnosed with COVID-19 to be isolated in public hospitals, including those detected on contact tracing and the universal community testing program, regardless of symptoms. All COVID-19 cases would be captured as the HA is the only public-funded healthcare provider managing COVID-19 patients in Hong Kong.

In this study, all patients who had been diagnosed with T2DM and were tested positive for COVID-19 were included, if they were admitted to public hospitals between January 21, 2020, and January 31, 2021 in Hong Kong SAR, China. COVID-19 was confirmed by positive SARS-CoV-2 viral nucleic acid detected using real-time reverse transcription-polymerase chain reaction (RT-PCR) assay, performed by the Public Health Laboratory under the Department of Health. Patients with T2DM were captured using the International Classification of Primary Care, Version 2 (ICPC2) code T90 or the International Statistical Classification of Diseases and Related Health Problems, 9th Revision, Clinical Modification (ICD-9-CM) codes 250.x0 or 250.x2. This cohort has been used for studying pharmacoepidemiology of drug treatment for COVID-19 (14).

Each eligible patient was observed from the date of hospital admission (baseline, day 0) to the date of in-hospital death, hospital discharge, or data cutoff date (April 30, 2021), whichever came first.

Definition of Metformin Exposure

Patients were classified as metformin users and non-users according to their exposure to metformin. Metformin users were patients who had received metformin from 90 days prior to admission to the day of discharge due to SARS-CoV-2 infection. T2DM patients who had not received or used metformin during the stated period were categorized as non-users.

Definition of Covariates

Pre-existing comorbidity profile was represented by the Charlson comorbidity index; hypertension; chronic lung, heart, and kidney diseases; liver disease; malignancy; and obesity and was captured based on ICD-9-CM diagnosis codes. Long-term medications taken by the patients in the past 3 years included angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB), anticoagulants, antiplatelets, lipid-lowering agents, and non-steroidal anti-inflammatory drugs (NSAID). The use of concomitant antidiabetic agents from 90 days prior to admission to hospital discharge was also recorded [namely, glucagon-like peptide 1 receptor agonists (GLP1RA), insulin, sulfonylureas (SU), thiazolidinediones (TZD), acarbose, dipeptidyl peptidase 4 inhibitors (DPP4i), and sodium-glucose cotransporter-2 inhibitors (SGLT2i)].

A score of baseline COVID-19 severity was assigned based on the WHO Clinical Progression Scale (15). Disease severity was categorized into 1) not requiring any oxygen therapy, score 4; 2) requiring supplemental oxygen without ventilation, score 5–6; and 3) requiring mechanical ventilation, score 7–9 (15). COVID-19 drug treatments were documented (remdesivir, interferon-β-1b, dexamethasone, and tocilizumab). The clinical condition of each patient at baseline was recorded, including the need for ICU admission, admission *via* the emergency department, initiation of extracorporeal membrane oxygenation (ECMO), and dialysis, as well as the occurrence of acute respiratory distress syndrome (ARDS) and hyperinflammatory syndrome [as defined by Webb et al., which consists of macrophage activation, hematological dysfunction, coagulopathy, and hepatic inflammation (16)].

A comprehensive panel of hematological and biochemical laboratory parameters was obtained, coupled with regular assessments during COVID-19. These included white blood cell, neutrophil, lymphocyte, platelet, lactate dehydrogenase, creatine kinase, total bilirubin, C-reactive protein, estimated glomerular filtration rate (eGFR), alkaline phosphatase (ALP), alanine transaminase (ALT), hemoglobin, prothrombin time, random glucose, and glycated hemoglobin (HbA1c). The presence of SARS-CoV-2 was confirmed in all patients by reverse transcription-polymerase chain reaction (RT-PCR) from the nasopharyngeal swab or deep throat saliva. A standardized real-time RT-PCR assay was used to detect the E gene of SARS-CoV-2. The cycle threshold (Ct) value represents the number of cycles required for a gene target or a PCR product to be detected. While viral loads were not directly measured with a dedicated quantitative RT-PCR assay in this analysis, studies have shown a good correlation between Ct values and SARS-CoV-2 viral loads, such that the lower the Ct values, the higher the viral loads (17).

Definition of Outcomes

The primary outcome of this study was clinical improvement, defined as a reduction of at least one point on the WHO Clinical Progression Scale.

The secondary outcomes were as follows: i) hospital discharge; ii) recovery without the need for oxygen therapy; iii) in-hospital death; iv) incidence of acidosis; v) incidence of hyperinflammatory syndrome [defined by Webb et al., including macrophage activation, hematological dysfunction, coagulopathy, and hepatic inflammation (16)]; vi) length of hospitalization; vii) clinical status as measured by the WHO Clinical Progression Scale scores on days 0, 7, 15, 30, 60, and 90; viii) WHO Clinical Progression Scale scores on days 0, 7, 15, 30, 60, and 90; ix) Ct values on days 0, 7, and 15; x) proportion of patients with IgG antibody on days 3, 7, and 15; and xi) cumulative direct medical costs incurred on days 0, 7, 15, 30, 60, and 90. The cumulative healthcare cost was calculated based on the unit cost of medication and healthcare services sourced from the Hong Kong SAR Government Gazette and the Hospital Authority (**Supplementary Table 1**).

Data Analysis

Descriptive statistics of baseline characteristics between the treatment and control groups were presented as mean and standard deviation for continuous variables and count and proportion for categorical variables.

Data completion rates of patient characteristics at baseline are shown in **Supplementary Table 2**. For the missing baseline covariates upon admission, multiple imputation by chained equations (MICE) was employed in the treatment and control groups. Each missing value of laboratory data was imputed 20 times using other variables that might have an impact on the study outcomes.

The propensity scores of each patient in the cohort with the aforementioned covariates were calculated with a logistic regression model. To minimize the outcome bias caused by variations in baseline characteristics, inverse probability of treatment weighting (IPTW) was then implemented to balance the covariates between groups using the calculated propensity scores. After propensity-score weighting, the balance of baseline covariates between treatment groups would be further evaluated using the standardized mean difference (SMD). SMDs ≤0.2 indicated a sufficient balance between groups.

A multivariable logistic regression model weighted by IPTW was adopted in order to estimate the effects of exposure on binary outcomes in odds ratios (OR) and their 95% confidence intervals (CI). The effects of exposure on Ct values, hospital length of stay among survivors, and healthcare costs were all estimated using the multivariable linear regression model weighted by IPTW.

Lastly, sensitivity and subgroup analyses were conducted. Sensitivity analyses included removing hospital discharge as a censoring criterion and limiting the follow-up period to a maximum of 90 days. Subgroup analyses were done on several patient subgroups, namely, age (≤65 and >65 years), sex, initiation of invasive mechanical ventilation or ECMO, ICU admission, any concomitant use of other medications (insulin, SU, remdesivir, interferon-β-1b, and dexamethasone), in-hospital use of metformin without prior metformin use, and without concomitant use of insulin.

All statistical analyses were performed using STATA Version 16 (StataCorp LP, College Station, TX, USA). p-value <0.05 was considered statistically significant.

RESULTS

In total, 1,214 T2DM patients were admitted for confirmed COVID-19 between January 21, 2020, and January31, 2021 in Hong Kong, while 786 patients (64.7%) were metformin users. After multiple imputation and propensity score weighting, all patient characteristics were balanced between groups at baseline with SMDs ≤0.2 (**Table 1**). **Supplementary Figure 1** demonstrates that propensity score density was highly overlapped after propensity score weighting. Overall, the median follow-up period of this patient cohort was 16 days with 30,035 person-days. The incidence rates of outcome events by exposure and control groups are presented in **Supplementary Table 3**. There were 97.1% of patients achieving clinical improvement in the metformin group and 80.4% in the control group.

Table 2 summarizes the rate of outcomes among metformin users and non-users. Metformin use was associated with significantly higher odds of clinical improvement compared with control (OR: 2.74, 95% CI 1.31 to 5.71, p = 0.009), in addition to hospital discharge (OR: 2.26, 95% CI 1.24 to 4.12, p = 0.009) and recovery (OR: 2.54, 95% CI 1.34 to 4.80, p = 0.005). Besides, metformin users had a significantly shorter length of hospital stay compared with their control counterparts (−2.76 days, 95% CI −5.02 to −0.51, p = 0.017). While the odds of acidosis were significantly higher among metformin users (OR: 6.82, 95% CI 2.56 to 18.18, p < 0.001), they had significantly lower odds of in-hospital death (OR: 0.41, 95% CI 0.22 to 0.80, p = 0.010) and hyperinflammatory syndrome (OR: 0.71, 95% CI

TABLE 1 | Baseline characteristics of T2DM patients hospitalized with COVID-19 by exposure to metformin after multiple imputation and propensity score weighting.

Baseline characteristics	Before weighting					After weighting				
	Metformin (*n* = 786)		Control (*n* = 428)		SMD	Metformin (*n* = 786)		Control (*n* = 428)		SMD
	N / mean	% / SD	*N* / mean	% / SD		*N* / mean	% / SD	*N* / mean	% / SD	
Age, years[a]	64.0	12.2	67.5	13.7	0.27	65.8	12.5	67.2	12.7	0.12
≤65	418	(53.2%)	183	(42.8%)	0.21	(47.6%)		(45.6%)		0.04
>65	368	(46.8%)	245	(57.2%)		(52.4%)		(54.4%)		
Sex					0.03					0.01
Male	423	(53.8%)	237	(55.4%)		(53.7%)		(53.3%)		
Female	363	(46.2%)	191	(44.6%)		(46.3%)		(46.7%)		
Pre-existing comorbidities										
Charlson index[a,b]	5.0	1.6	5.9	2.2	0.52	5.4	1.8	5.6	1.9	0.07
1–4	264	(33.6%)	92	(21.6%)		(26.9%)		(23.2%)		
5-6	415	(52.8%)	198	(46.4%)		(50.8%)		(52.3%)		
7-14	107	(13.6%)	137	(32.1%)		(22.3%)		(24.5%)		
Hypertension	587	(74.7%)	341	(79.7%)	0.12	(78.0%)		(77.7%)		0.01
Chronic lung disease	55	(7.0%)	72	(16.8%)	0.31	(12.3%)		(10.4%)		0.06
Chronic heart disease	107	(13.6%)	78	(18.2%)	0.13	(14.6%)		(15.4%)		0.02
Chronic kidney disease	63	(8.0%)	95	(22.2%)	0.40	(13.4%)		(15.0%)		0.04
Liver disease	90	(11.5%)	63	(14.7%)	0.10	(16.8%)		(14.4%)		0.06
Malignancy	20	(2.5%)	21	(4.9%)	0.12	(3.8%)		(4.6%)		0.04
Obesity	93	(11.8%)	48	(11.2%)	0.02	(12.2%)		(12.3%)		0.01
Preadmission or in-hospital use										
Metformin	786	(100.0%)	0	(0.0%)	NA	(100.0%)		(0.0%)		NA
GLP1RA	8	(1.0%)	2	(0.5%)	0.06	(0.7%)		(0.3%)		0.07
Insulin	417	(53.1%)	277	(64.7%)	0.24	(54.4%)		(60.1%)		0.11
Oral antidiabetic drugs										
SU	373	(47.5%)	57	(13.3%)	0.80	(36.5%)		(38.4%)		0.04
TZD	70	(8.9%)	12	(2.8%)	0.26	(6.5%)		(5.3%)		0.05
Acarbose	2	(0.3%)	2	(0.5%)	0.04	(0.2%)		(0.5%)		0.04
DPP4i	74	(9.4%)	33	(7.7%)	0.06	(7.3%)		(7.6%)		0.01
SGLT2i	26	(3.3%)	3	(0.7%)	0.19	(2.3%)		(2.0%)		0.02
Long-term medications										
ACEI/ARB	395	(50.3%)	152	(35.5%)	0.30	(45.3%)		(48.9%)		0.07
Anticoagulant	219	(27.9%)	215	(50.2%)	0.47	(36.0%)		(35.7%)		0.01
Antiplatelet	167	(21.2%)	106	(24.8%)	0.08	(20.9%)		(26.4%)		0.13
Lipid-lowering agent	488	(62.1%)	185	(43.2%)	0.38	(53.1%)		(60.3%)		0.15
NSAID	185	(23.5%)	114	(26.6%)	0.07	(22.7%)		(28.6%)		0.14
In-hospital COVID-19 drug use										
Remdesivir	207	(26.3%)	121	(28.3%)	0.04	(27.8%)		(24.7%)		0.07
Interferon-β-1b	503	(64.0%)	306	(71.5%)	0.16	(64.8%)		(68.2%)		0.07
Dexamethasone	341	(43.4%)	255	(59.6%)	0.33	(49.8%)		(47.7%)		0.04
Tocilizumab	41	(5.2%)	66	(15.4%)	0.34	(9.9%)		(10.3%)		0.01
ECMO	4	(0.5%)	8	(1.9%)	0.13	(0.5%)		(0.7%)		0.03
Dialysis	17	(2.2%)	45	(10.5%)	0.35	(3.2%)		(6.4%)		0.15
ICU admission on admission	104	(13.2%)	150	(35.0%)	0.53	(20.2%)		(19.2%)		0.03
Admission *via* the emergency department	368	(46.8%)	236	(55.1%)	0.17	(51.2%)		(50.8%)		0.01
Clinical severity on admission by WHO Clinical Progression Scale										
Score (range 0–10)[a]	4.4	0.8	4.8	1.1	0.53	4.6	1.0	4.5	0.9	0.07
No oxygen therapy (score 4)	647	(82.3%)	255	(59.6%)		(75.0%)		(76.2%)		
Supplemental oxygen without ventilation (score 5–6)	133	(16.9%)	164	(38.3%)		(21.9%)		(23.0%)		

(Continued)

TABLE 1 | Continued

Baseline characteristics	Before weighting					After weighting				
	Metformin (*n* = 786)		Control (*n* = 428)		SMD	Metformin (*n* = 786)		Control (*n* = 428)		SMD
	N / mean	% / SD	*N* / mean	% / SD		*N* / mean	% / SD	*N* / mean	% / SD	
Mechanical ventilation (score 7–9)	6	(0.8%)	9	(2.1%)			(3.1%)		(0.8%)	
ARDS	13	(1.7%)	16	(3.7%)	0.13		(2.3%)		(3.4%)	0.06
Macrophage activation	0	(0.0%)	0	(0.0%)	NA		(0.0%)		(0.0%)	NA
Haematological dysfunction	41	(5.2%)	40	(9.3%)	0.16		(8.8%)		(6.1%)	0.10
Coagulopathy	3	(0.4%)	3	(0.7%)	0.04		(0.7%)		(0.3%)	0.05
Hepatic inflammation	48	(6.1%)	45	(10.5%)	0.16		(7.8%)		(6.3%)	0.06
Hyperinflammatory syndrome	81	(10.3%)	75	(17.5%)	0.21		(14.6%)		(10.2%)	0.13
Laboratory parameters (normal range)[a]										
White blood cell, $\times10^9$/L (3.7–9.2 × 10^9/L)	5.9	2.1	6.4	3.0	0.20	6.1	2.4	6.3	2.8	0.05
Neutrophil, $\times10^9$/L (1.7–5.8 × 10^9/L)	4.0	1.9	4.6	2.8	0.29	4.3	2.4	4.2	2.3	0.05
Lymphocyte, $\times10^9$/L (1.0–3.1 × 10^9/L)	1.2	0.7	1.1	0.8	0.18	1.2	0.7	1.4	1.7	0.16
Platelet, $\times10^9$/L (145–370 × 10^9/L)	208.6	75.8	199.4	77.9	0.12	200.5	76.5	207.5	82.2	0.09
Lactate dehydrogenase, U/L (110–210 U/L)	251.1	109.7	301.7	164.2	0.39	272.1	133.2	268.5	129.0	0.03
Creatine kinase, U/L (26–192 U/L)	186.8	304.1	249.9	503.3	0.16	208.0	342.2	247.9	384.6	0.11
Total bilirubin, μmol/L (5–27 μmol/L)	8.8	6.6	9.9	7.3	0.15	10.2	14.2	9.5	6.0	0.06
C-reactive protein, mg/L (<5 mg/L)	37.2	51.6	50.4	64.3	0.23	41.4	53.8	40.3	57.9	0.02
Cycle threshold value, cycle	22.8	6.4	22.0	6.3	0.12	22.5	6.3	22.2	6.1	0.04
eGFR, ml/min/1.73 m^2 (>90 ml/min/1.73 m^2)	106.4	88.2	99.2	103.4	0.08	102.2	85.5	96.7	73.6	0.07
ALP, U/L (30–120 U/L)	72.6	25.7	80.5	41.3	0.25	72.9	26.8	75.3	30.2	0.08
ALT, U/L (<46.5 U/L)	37.2	41.7	36.8	28.3	0.01	39.4	45.4	37.6	28.5	0.04
Hemoglobin, g/dl (13.4–17.1 g/dl)	13.2	1.6	12.8	1.9	0.24	13.0	1.7	13.1	1.9	0.04
Prothrombin time, s (10–14 s)	11.8	3.0	12.9	4.8	0.28	12.2	3.0	12.1	3.8	0.04
Random glucose, mmol/L (3–11 mmol/L)	10.2	4.9	8.3	4.6	0.38	9.1	5.4	9.8	5.7	0.12
HbA1c, % (4.8%–6.0%)	8.0	2.4	6.5	2.3	0.63	7.3	2.7	7.7	2.5	0.14

SMD of <0.2 indicates covariate balance between metformin and control groups.

ACEI, angiotensin-converting enzyme inhibitor; ALP, alkaline phosphatase; ALT, alanine transaminase; ARB, angiotensin receptor blocker; ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; DPP4i, dipeptidyl peptidase 4 inhibitor; ECMO, extracorporeal membrane oxygenation; eGFR, estimated glomerular filtration rate; GLP1RA, glucagon-like peptide 1 receptor agonist; HbA1c, glycated haemoglobin; ICU, intensive care unit; NA, not applicable; NSAID, non-steroidal anti-inflammatory drug; SD, standard deviation; SGLT2i, sodium-glucose cotransporter-2 inhibitor; SMD, standardized mean difference; SU, sulfonylurea; TZD, thiazolidinedione; T2DM, type 2 diabetes mellitus.

[a]*Age, Charlson index, clinical severity, and laboratory parameters on admission are presented as mean ± SD.*

[b]*The calculation of Charlson index does not include acquired immune deficiency syndrome (AIDS).*

0.53 to 0.95, $p = 0.021$) compared with control. Regarding the criteria for hyperinflammatory syndrome with reference to Webb et al., metformin use was associated with lower odds of macrophage activation (OR: 0.74, 95% CI 0.59 to 0.93, $p = 0.009$) and hepatic inflammation (OR: 0.50, 95% CI 0.38 to 0.65, $p < 0.001$) compared with control. Trends toward a reduction in the odds of hematological dysfunction and coagulopathy were also observed among metformin users. The results of sensitivity were generally comparable to those of the main analysis (**Supplementary Table 4**).

Changes in the clinical status of patients over the follow-up period are illustrated in **Figure 1** by treatment group. As depicted in **Figure 2**, the mean WHO Clinical Progression Scale scores were significantly lower among metformin users compared with non-users since day 15 (2.91 vs. 3.45, $p < 0.001$; day 30: 1.49 vs. 2.53, $p < 0.001$; day 60: 0.99 vs. 1.88, $p = 0.001$; day 90: 0.83 vs. 1.69, $p = 0.002$). Both mean Ct values and the proportion of IgG antibody from baseline to day 15 yielded insignificant results between the exposure and non-exposure groups, despite a trend toward a higher proportion of patients with positive IgG antibodies in metformin users as demonstrated on day 15 (72% vs. 67%, $p = 0.090$). In terms of average cumulative direct medical costs incurred by treatment groups, metformin users had an insignificant increase in cost from baseline to day 7, followed by trends toward incurring insignificantly lower costs from day 7 to day 90 (US$34,199 vs. US$39,950, $p = 0.141$) compared with non-users.

Moreover, significantly increased likelihood of clinical improvement on the WHO Clinical Progression Scale by ≥1 score (HR: 1.27, 95% CI 1.03–1.58), increased risk of acidosis (HR: 8.48, 95% CI 1.48–48.57), and reduced risk of hepatic inflammation (HR: 0.59, 95% CI 0.39–0.89) can be seen in the additional analysis by time-to-event Cox models, which are consistent with the main analysis.

Supplementary Table 5 summarizes the results of subgroup analyses. Significant results for the comparison between with and

TABLE 2 | Comparison of the odds of clinical improvement, in-hospital death, acidosis, and hyperinflammatory syndrome between metformin and control groups.

	Before weighting		After weighting		
	Metformin	Control	Metformin vs. control		
Outcomes	**% (*N*)**	**% (*N*)**	**OR[a]**	**95% CI**	***p*-value**
Clinical improvement on WHO clinical progression scale by ≥1 score	97.1% (786)	80.4% (428)	2.74	(1.31, 5.71)	0.009
Hospital discharge (score ≤ 3)	95.7% (786)	76.6% (428)	2.26	(1.24, 4.12)	0.009
Recovery (score ≤ 4)	85.6% (139)	60.1% (173)	2.54	(1.34, 4.80)	0.005
Outcomes	% (*N*)	% (*N*)	OR[b]	95% CI	*p*-value
In-hospital death	3.6% (786)	20.3% (428)	0.41	(0.22, 0.80)	0.010
Acidosis	1.1% (786)	0.9% (428)	6.82	(2.56, 18.18)	<0.001
Hyperinflammatory syndrome	48.8% (705)	64.3% (353)	0.71	(0.53, 0.95)	0.021
Macrophage activation	30.4% (786)	45.1% (428)	0.74	(0.59, 0.93)	0.009
Hematological dysfunction	27.5% (745)	53.4% (388)	0.77	(0.55, 1.10)	0.142
Coagulopathy	6.1% (783)	15.8% (425)	0.81	(0.58, 1.14)	0.234
Hepatic inflammation	23.0% (738)	47.3% (383)	0.50	(0.38, 0.65)	<0.001

CI, confidence interval; OR, odds ratio.

[a]*OR >1 (or <1) indicates that the metformin group was associated with higher (lower) odds of clinical improvement, hospital discharge, or recovery, compared with the control group.*

[b]*OR >1 (or <1) indicates that the metformin group was associated with higher (lower) risk of in-hospital death, acidosis, and hyperinflammatory syndrome compared with the control group.*

without preadmission use of metformin include higher odds to recovery, lower odds to macrophage activation, hepatic inflammation, and hyperinflammatory syndrome in the preadmission group. Without prior metformin use is associated with significantly lowered odds of mortality and increased odds to clinical improvement, hospital discharge, and acidosis.

DISCUSSION

It is important to review the risk–benefit balance of antidiabetic medication for T2DM patients amid the COVID-19 pandemic. Apart from metformin, drugs such as, but not limited to, DPP4i and SGLT2i have also been discussed. In-hospital DPP4i use was found to reduce the risk of mortality due to its potential anti-inflammatory effect proposed to be beneficial to T2DM COVID-19 patients while being generally well-tolerated with minimal side effects (18). On the other hand, SGLT2i was proposed to be used with greater precaution due to potentially higher risk of euglycemic diabetic ketoacidosis despite showing signs of reduction in production and expression of proinflammatory cytokines in both RCT and observational studies (19–21). Hence, it is of importance to help depict a clearer picture in the clinical treatment of COVID-19 in T2DM patients by understanding of the risks and benefits of metformin use.

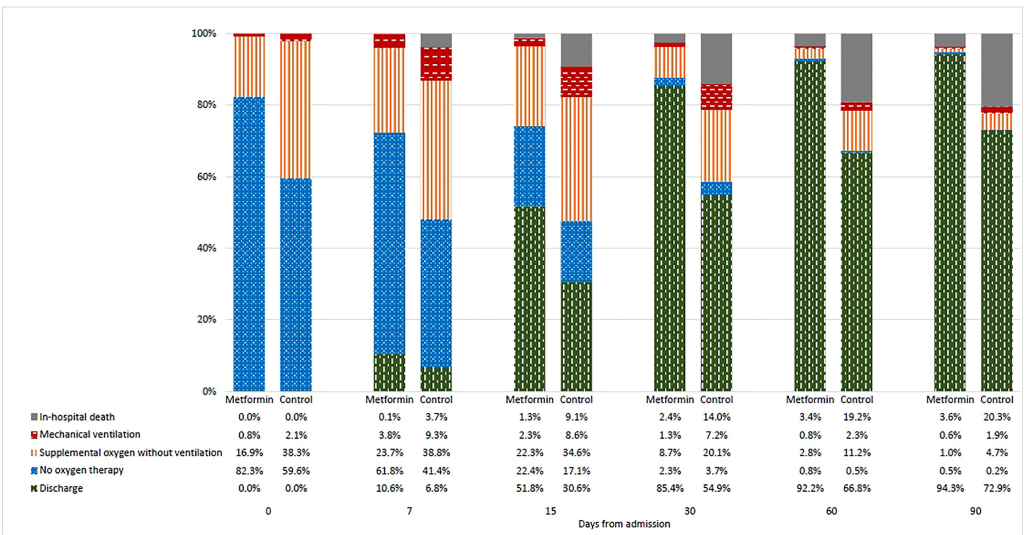

FIGURE 1 | Changes in clinical status of patients as indicated by the WHO Clinical Progression Scale score from baseline to day-90 by treatment groups.

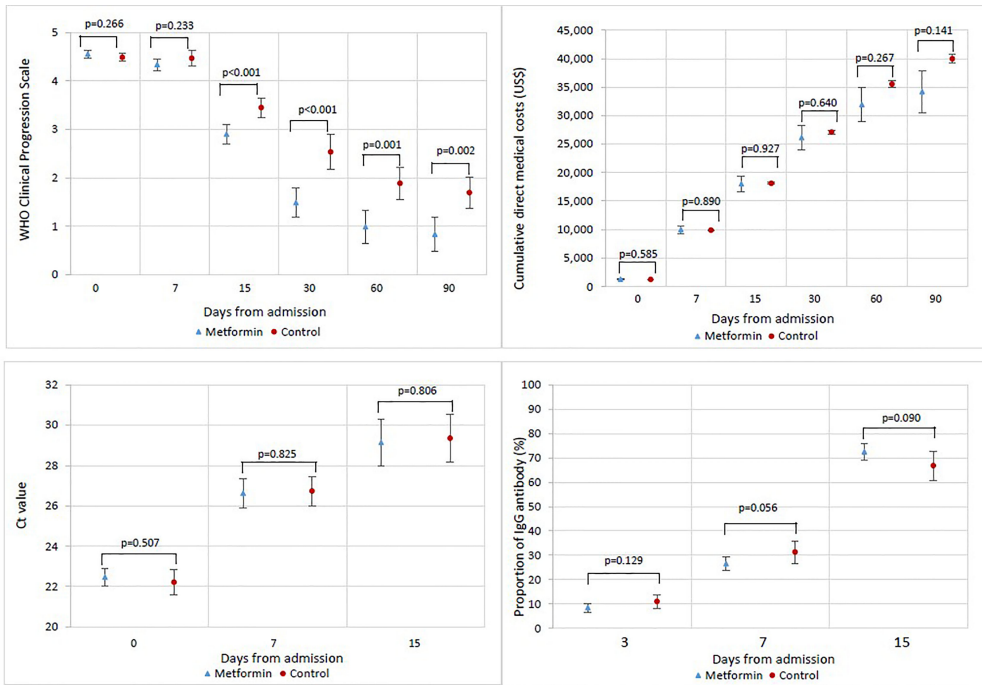

FIGURE 2 | Changes in WHO Clinical Progression Scale score, cumulative direct medical costs, cycle threshold (Ct) value, and proportion of patients with IgG antibody from baseline to day-90 among patients by treatment groups.

In this retrospective, territory-wide cohort of T2DM patients hospitalized with COVID-19, metformin users were found to have significantly greater odds of clinical improvement, hospital discharge, and recovery, as well as a shorter length of hospital stay compared with their control counterparts. Despite significantly increased odds of acidosis, metformin use was associated with lower odds of mortality and hyperinflammatory syndrome compared with weighted control.

To the best of our knowledge, four meta-analyses have been conducted so far on the mortality outcome in metformin users with T2DM and COVID-19, which unanimously agreed that metformin use was associated with a significant reduction in mortality compared with control, and our results were generally in line with such observation (6, 22–24). Furthermore, the meta-analysis done by Yang et al. has demonstrated a significant reduction in disease severity among metformin users (24). Despite accumulating evidence pointing toward better clinical outcomes with reference to the anti-inflammatory effect of metformin and, hence, advocating for its use, several clinical guidelines and individual studies have suggested that it should be prescribed with caution due to its potential side effects of different severities, especially during the course of COVID-19; for instance, it should be withdrawn in cases of respiratory distress, renal impairment, and heart failure, owing to associated risks of lactic acidosis, ketoacidosis, and dehydration (4, 11, 25). Of note, Gao et al. echoed that metformin users might have significantly higher risks of disease progression and life-threatening complications compared with non-users (12).

It has been established that both T2DM and COVID-19 will contribute to a heightened inflammatory state in patients. While SARS-CoV-2 infection triggers acute immune responses, hyperglycemic state and poor blood glucose control in T2DM patients may lead to chronic inflammation characterized by innate immune system dysregulation (26, 27), ultimately increasing the risks of mortality and poor clinical outcomes (28–30). Therefore, the anti-inflammatory effect of metformin has been widely discussed as a viable option for T2DM patients with COVID-19, as users were found to have lower levels of proinflammatory cytokines and higher levels of anti-inflammatory cytokines compared with non-users (31), which may contribute to reducing the incidence of hyperinflammatory syndrome and, hence, widespread organ damage in these patients. To date, limited research has explored the relationship between metformin use and hyperinflammatory syndrome, while our results suggested a significant reduction in the odds of hyperinflammatory syndrome among metformin users when compared with control.

The currently proposed mechanistic explanations with regard to the anti-inflammatory effects of metformin remain disputed as metformin's mechanism of action is closely tied to the expression and activity of ACE2, which has also been identified as a viral receptor for SARS-CoV-2 (25, 32). It is commonly acknowledged that metformin exerts glucose-lowering effects by activating the adenosine monophosphate-activated protein kinase (AMPK) (10). AMPK activates and phosphorylates ACE2 and, hence, evokes anti-inflammatory effects *via* catalyzing the production of

angiotensin-(1-7) peptide of the RAS (33, 34). On the other hand, SARS-CoV-2 downregulates ACE2 expression upon cellular entry (8), which in turn promotes inflammation and potentially exacerbates hyperinflammatory syndrome, also known as cytokine storm, a key component of COVID-19 pathophysiology that is heavily implied in related mortality (35). The clash occurs with whether metformin, by potentially raising ACE2 expression with AMPK and Sirtuin 1 (25, 36), as well as increasing ACE2 stability by reducing ubiquitination and degradation (8, 10), would facilitate viral entry (12) and induce poor clinical outcomes, or would metformin be able to exert immunomodulatory effects to mitigate inflammation and organ damage. It has also been suggested that phosphorylated ACE2 after post-translational modification would be less recognizable by SARS-CoV-2 owing to steric hinderance (8, 10), thus shifting the action of ACE2 toward the cardiopulmonary protective alternative. Apart from acting on the RAS, AMPK activation is also associated with the reduction of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) activity, lowering the release of proinflammatory cytokines and reducing mortality in women according to a recent retrospective cohort analysis (31). Our results are inconclusive regarding the notion of reduced viral entry *via* steric hindrance owing to insignificant differences in mean Ct values between groups; however, the lower odds of macrophage activation and hepatic inflammation, possibly induced by reduced cytokine release and signaling, immune cell activation, recruitment, and activity, seem to override the insignificant differences in viral clearance, hence leading to better clinical outcomes among metformin users.

Despite the positive result of lowering the odds of hyperinflammatory syndrome, our results also indicated significantly increased odds of acidosis in the treatment group compared with control, which has been consistently found in other studies (10, 12). Accordingly, the risk of acidosis might be a concern for specific patient subgroups (such as those with reduced kidney function and those with severe COVID-19). A mechanistic explanation proposed would be metformin's inhibition of mitochondrial cellular respiration to enhance anaerobic respiration (37, 38), where the risk of acidosis may further be exacerbated owing to infection-induced hypoxia upon SARS-CoV-2 infection (39). Metformin use was associated with an increased risk of acidosis, but not mortality, as concluded by Cheng et al. (10), which was in line with the significantly reduced odds of mortality in our study. These results may imply that the protective effects of hyperinflammatory syndrome reduction could potentially override potential harms brought by acidosis. Nevertheless, the potential risk of lactic acidosis should still be meticulously acknowledged with careful monitoring of the patient's condition and safe administration of metformin.

In this retrospective cohort study, all T2DM patients with COVID-19 were captured in the public healthcare system; hence, all eligible cases were included in this analysis regardless of disease severity. Besides, various patient characteristics at baseline were taken into account and balanced with multiple imputation and propensity score weighting, including pre-existing comorbidities and medical treatments of T2DM and COVID-19, as well as laboratory parameters on admission. While our study has provided some preliminary evidence on the association between metformin use and alleviation of hyperinflammatory syndrome in T2DM patients with COVID-19, our study was not without its limitations. Firstly, due to its observational nature, residual confounding might not have been fully addressed after propensity score weighting. Secondly, our patient cohort consisted of mainly Chinese and cases of moderate COVID-19, which will likely undermine the generalizability of our results to other populations or healthcare settings. Lastly, due to limited sample size of cases of "acidosis," the breakdown of the "acidosis" outcome into "non-lactic acidosis" and "lactic acidosis" is not possible. It should be emphasized that although lactic acidosis is a potential adverse event of metformin therapy, it is extremely rare in the clinical setting.

In conclusion, metformin use was associated with significant increases in the odds of clinical improvement, hospital discharge, and recovery when compared with control, in addition to a shorter length of hospital stay. Despite an increased risk of acidosis, lower odds of in-hospital death and hyperinflammatory syndrome were observed among metformin users compared with their control counterparts. Our results demonstrated positive results regarding the management of inflammatory status and eventually clinical improvement of T2DM patients during COVID-19. Notably, as metformin treatment was associated with a significant risk of acidosis, patients with renal and/or pulmonary impairment should be carefully monitored. Prospective studies on the safe use of metformin are required for better clinical management of T2DM patients with COVID-19.

DATA AVAILABILITY STATEMENT

The data that supported the findings of this study were provided by the Hong Kong Hospital Authority but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Hong Kong Hospital Authority.

ETHICS STATEMENT

The study protocol was approved by the Institutional Review Board of the University of Hong Kong/ Hospital Authority Hong Kong West Cluster (Reference No. UW 20-493). Given the extraordinary nature of the COVID-19 pandemic, individual patient informed consent was not required for this retrospective cohort study using anonymized data.

AUTHOR CONTRIBUTIONS

CW reviewed the literature, designed the statistical analysis, conducted the analyses, and wrote the manuscript. DL, AL, AK, ML, and KL reviewed the literature, contributed to the

interpretation and analysis of data, and wrote the manuscript. IA and MC conducted the analyses. XX, EL, and BC contributed to the interpretation and analysis of data. All authors contributed to the interpretation and analysis of data, critically reviewed and revised the manuscript, and approved the final manuscript as submitted. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.810914/full#supplementary-material

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frontiers in Endocrinology

CASE REPORT
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A Case Series of Ketoacidosis After Coronavirus Disease 2019 Vaccination in Patients With Type 1 Diabetes

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Introduction: We report a case series of severe ketoacidosis after COVID-19 vaccination in a type 1 diabetes patients treated with insulin and an SGLT-2 inhibitor.

Case Report: We present two cases of type 1 diabetes mellitus. One patient was treated with insulin therapy and an SGLT-2 inhibitor, and the other patient was treated with insulin therapy alone. Both patients became ill after coronavirus disease-2019 vaccination, making it difficult to continue their diet or insulin injections. On admission, they developed severe diabetic ketoacidosis. This is the first report of ketoacidosis after coronavirus disease-2019 vaccination.

Conclusion: The vaccine should be carefully administered to type 1 diabetes patients receiving intensive insulin therapy and a sodium-glucose transporter due to the high risk ketoacidosis. It is important to instruct patients to drink sufficient fluids and to continue insulin injections when they become sick.

Keywords: coronavirus disease 2019 vaccination, ketoacidosis, type 1 diabetes, sodium-glucose cotransporter 2 inhibitor, clinical diabetes, latent autoimmune diabetes in adults

HIGHLIGHTS

→In patients with type 1 diabetes, COVID-19 vaccination can trigger illness and may be a clinical challenge that requires prompt treatment.

→Prior to COVID-19 vaccination, patients with type 1 diabetes may need to be assured of what to do if they become sick.

→Please instruct the patient to drink sufficient fluids and continue insulin injections. This is posted as a message for patients on the ADA homepage (https://www.diabetes.org/coronavirus-covid-19/know-what-to-do).

INTRODUCTION

COVID-19 infection can have serious consequences for people suffering from diabetes, obesity, malnutrition, Cushing's syndrome (CS), and adrenal insufficiency (1). The coronavirus disease 2019 (COVID-19) vaccine is expected to reduce the incidence and risk of severe disease among patients with diabetes and elderly individuals, who are particularly predisposed to critical illness from COVID-19 infection (2).

On the other hand, the COVID-19 vaccine is reportedly associated with nausea and vomiting as adverse effects (3, 4). Loss of appetite and prolonged general malaise are likely to cause insulin injection failure. The interruption of treatment with insulin injections has reportedly induced diabetic ketoacidosis in type 1 diabetes mellitus patients. In addition, treatment with sodium-glucose transporter 2 (SGLT-2) inhibitors may increase the risk of developing diabetic ketoacidosis in patients with type 1 diabetes (5).

We encountered two cases of severe ketoacidosis associated with COVID-19 vaccination in type 1 diabetes patients. We present those cases because there have been no prior reports on the occurrence of this complication.

CASE REPORT

First Case

A 71-year-old woman was diagnosed with diabetes mellitus at 56 years of age. She had a history of hyperthyroidism due to Basedow disease when she was 52 years of age. After three years of anti-thyroid therapy, her hyperthyroidism went into remission. She received oral drugs after being diagnosed with diabetes mellitus at 56 years of age.

Seven months later, blood examination revealed an elevated level of anti-glutamic acid decarboxylase antibody (869 U/ml). She then received insulin injection therapy for type 1 diabetes mellitus. Her glycated hemoglobin (HbA1c) level was approximately 8%, and she had no history of ketosis or ketoacidosis.

She was treated with insulin glulisine (13 units/day, pre-breakfast 4 units, pre-lunch 4 units, pre-dinner 5 units), insulin degludec (pre-dinner 3 units), and a SGLT-2 inhibitor, ipragliflozin (25 mg/day). Her fasting blood glucose levels in the morning before COVID-19 vaccination ranged from 93 to 169 mg/dl. Her HbA1c levels at approximately three months and one month before admission were 8.3% and 8.1%, respectively. She received the Pfizer-BioNTech COVID-19 (BNT162b2) vaccine (Pfizer, Inc; Philadelphia, Pennsylvania) three days prior to admission. Immediately after COVID-19 vaccination, she developed nausea and fatigue, and her water and dietary intake decreased. From the next day, the patient showed impaired consciousness, which made it impossible for her to inject insulin or take SGLT-2 inhibitor. Her symptoms worsened, and she was taken to the emergency room of Tokyo Medical University Hachioji Medical Center, Hachioji, Tokyo, Japan.

On arrival, she had tachycardia and tachypnea, and her Glasgow Coma Scale was 12 (E3V4M5). A laboratory evaluation showed severe acidosis, ketonuria, ketonemia (**Table 1**). Chest X-ray, electrocardiography, abdominal computed tomography, and urinary sediments showed no abnormalities. Thus, an infectious disease was unlikely. The patient was diagnosed with ketoacidosis. Immediately after admission, continuous intravenous insulin, Ringer's solution, and glucose infusion were initiated in the intensive care unit.

With the above combined treatment, her ketoacidosis gradually improved (**Figure 1**). On admission, the plasma glucose, HbA1c, and glycated albumin levels were 944 mg/dL, 8.0%, and 27.7%, respectively. She tested positive for anti-glutamic acid decarboxylase antibody, but was negative for insulinoma associated antigen-2 antibody and insulin autoantibody (**Table 1**). Serum C-peptide and urinary C-peptide were undetectable. After her ketoacidosis improved, intensive insulin therapy without SGLT-2 inhibitor was resumed. Serum C-peptide was also below the limit of detection in a glucagon load test (**Supplemental Figure 1**). When the patient was discharged, she was treated with insulin glulisine (17 units/day, pre-breakfast 7 units, pre-lunch 6 units, pre-dinner 4 units) and insulin degludec (pre-dinner 4 units).

Second Case

The patient was a 52-year-old woman who received insulin infusion therapy with multiple daily injections after being diagnosed with type 1 diabetes mellitus at 47 years of age. At the time of the diagnosis, she was admitted to another facility due to diabetic ketosis. She noticed thirst and >10 kg body weight loss in comparison to 6 months before her admission. A blood examination showed an elevated HbA1c level (15.5%), an elevated anti-glutamic acid decarboxylase antibody level (1230 U/ml), and an elevated islet antigen 2 antibody level (or*insulinoma-associated protein-2 antibody) (>41 U/mL). The urinary c-peptide level (45 μg/day) indicated that her insulin secretory capacity remained. She was diagnosed with latent autoimmune diabetes in adults and insulin treatment was continued.

She was treated by a family doctor with insulin aspart (25 units/day, pre-breakfast 11 units, pre-lunch 8 units, pre-dinner 6 units) and insulin degludec (before sleeping 12 units). However, the transition of her HbA1c level was poor (10-11%). Her fasting blood glucose levels in the morning before COVID-19 vaccination ranged from 106 to 262 mg/dl. She received her second vaccination the day before admission. For both vaccinations, she received the Pfizer-BioNTech COVID-19 (BNT162b2) vaccine (Pfizer, Inc; Philadelphia, Pennsylvania). She had a drinking habit and consumed approximately 20 g of alcohol on the night before vaccination.

Immediately after the second COVID-19 vaccination, she had symptoms of nausea, palpitation and respiratory distress. Her symptoms worsened, she could not inject insulin or eat from noon on that day. The following day she was taken to the emergency room of Tokyo Medical University Hachioji Medical Center, Hachioji, Tokyo, Japan.

TABLE 1 | The patient's physical examinations and laboratory data on admission.

Physical Examination					
BH 151.8 cm, BW 41.4 kg, BMI 18.0 kg/m^2, RR 24 times/min, BP 118/76 mmHg, Glasgow Coma Scale E3V4M5					
Complete Blood Count		**Urinalysis**		**Arterial Blood Gas Analysis**	
White blood cell count	15300 /μL	Protein	2+	pH	7.049
Neutrophil	72.8%	Glucose	4+	pCO_2	12.4 mmHg
Lymphocyte	15.7%	Ketone body	1+	pO_2	128 mmHg
Red blood cell count	405×10^4 /μL	Urinary osmotic pressure	360 mOsm/L	HCO_3^-	3.3 mmol/L
Hemoglobin	13.3 g/dL	C-peptide (urine)	<0.8 μg/day	Base excess	-26.9 mmol/L
Hematocrit	44%			Lactate	26 mg/dL
Platelet count	17.4×10^4 /μL			Anion gap	28.9 mmol/L
Blood chemistry					
Aspartate aminotransferase	67 IU/L	Sodium	118 mEq/L	Glycoalbumin	27.7%
Alanine aminotransferase	34 IU/L	Potassium	6.7 mEq/L	C-peptide (serum)	<0.03 ng/mL
Lactate dehydrogenase	282 IU/L	Chlorine	79 mEq/L	anti-GAD antibody	>2000 U/mL
Total protein	6.6 g/dL	Calcium	8.4 mg/dL	Islet antigen 2 antibody	N.D
Albumin	4 g/dL	Phosphate	9 mg/dL	Anti-insulin autoantibody	N.D
Total bilirubin	0.6 mg/dL	Magnesium	2.4 mg/dL	Total ketone body	16427 μmol/L
Amylase	9696 U/L	Uric acid	11.7 mg/dL	Acetoacetate	3340 μmol/L
p-Amylase	304 U/L	Blood urea nitrogen	90.9 mg/dL	3-Hydroxybutyrate	13087 μmol/L
Lipase	13 U/L	Serum Creatinine	1.93 mg/dL	TSH	1.01 μU/mL
Trypsin	219 ng/mL	Fasting blood glucose	938 mg/dL	freeT3	1.94 pg/mL
Osmotic pressure	344 mOsm/L	HbA1c	8%	freeT4	1.34 ng/dL

Past Disease History except diabetes

She was diagnosed with Basedow disease at the age of 52, but is now in remission. There was no history such as hypertension or cardiovascular disease.

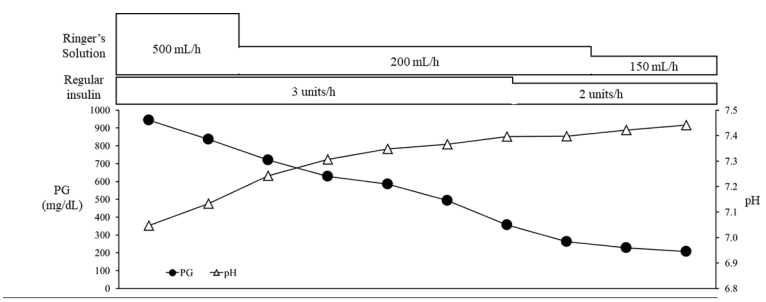
N.D, not detected.

On arrival, she had tachycardia and tachypnea, but her consciousness was clear (E4V5M6). A laboratory evaluation showed severe acidosis, ketonuria, and ketonemia (**Table 2**). The patient was diagnosed with diabetic ketoacidosis. Immediately after admission, continuous intravenous insulin, Ringer's solution, and glucose infusion were initiated in the intensive care unit. With these combined treatments, her ketoacidosis gradually improved (**Figure 2**). On admission, her plasma glucose and HbA1c values were 494 mg/dL and 11.6%, respectively (**Table 2**). Serum C-peptide and the urinary C-peptide were slightly detected. Her thyroid hormone levels were normal. After her ketoacidosis improved, intensive insulin therapy was resumed.

When the patient was discharged, she was treated with insulin glulisine (27 units/day, pre-breakfast 11 units, pre-lunch 8 units, pre-dinner 8 units) and insulin degludec (before sleeping 13 units).

DISCUSSION

We reported two cases of ketoacidosis that occurred after COVID-19 vaccination in patients with latent autoimmune diabetes in adults. The first patient was diagnosed with diabetic ketoacidosis based on a blood glucose level of 944 mg/dL, arterial pH of 7.049, serum bicarbonate of 3.3 mEq/L, and urinal ketone



Time	17:00	19:00	21:00	23:00	0:00	2:00	4:00	6:00	8:00	10:00
PG (mg/dL)	944	836	721	628	585	494	357	263	229	207
pH	7.049	7.133	7.243	7.307	7.348	7.366	7.396	7.398	7.422	7.441
pO2	128	123	112	104	97.4	88.4	87.9	98.8	91.4	93.8
pCO2	12.4	18.7	22.8	28.7	28.8	33.8	33.7	36.3	34.3	33.1

FIGURE 1 | The course in day1-2 in a patient with ketoacidosis who was treated with insulin and SGLT2 inhibitor. PG, plasma glucose.

TABLE 2 | The patient's physical examinations and laboratory data on admission.

Physical examination					
BH 162 cm, BW 60.3 kg, BMI 23.0 kg/m^2, RR 36 times/min, BP 168/82 mmHg, Glasgow Coma Scale E4V5M6					
Complete Blood Count		**Urinalysis**		**Arterial Blood Gas Analysis**	
White blood cell count	24600 /μL	Protein	1+	pH	6.958
Neutrophil	88.3%	Glucose	4+	pCO_2	8.8 mHg
Lymphocyte	3.0%	Ketone body	4+	pO_2	133 mmHg
Red blood cell count	599x10^4 / μL	Urinary osmotic pressure	1.3 μg/day	HCO_3^-	1.9 mmol/L
Hemoglobin	13.9 g/dL			Base excess	-30.4 mmol/L
Hematocrit	45%			Lactate	16 mg/dL
Platelet count	45.8x10^4 / μL			Anion gap	25.9 mmol/L
Blood chemistry					
Aspartate aminotransferase	15 IU/L	Sodium	129 mEq/L	Fasting blood glucose	494 mg/dL
Alanine aminotransferase	12 IU/L	Potassium	6.7 mEq/L	HbA1c	11.6%
Lactate dehydrogenase	361 IU/L	Chlorine	94 mEq/L	Glycoalburnin	N.D
Total protein	9.2 g/dL	Calcium	9.4 mg/dL	C-peptide (serum)	N.D
Albumin	4.6 g/dL	Phosphate	5.3 mg/dL	anti-GAD antibody	123 U/mL
Total bilirubin	0.3 rng/dL	Magnesium	N.D	Islet antigen 2 antibody	N.D
Amylase	112 U/L	Uric acid	N.D	Anti-insulin autoantibody	N.D
		Blood urea nitrogen	17.5 mg/dL	Total ketone body	13856 μmol/L
		Serum Creatinine	0.64 mg/dL	Acetoacetate	3275 μmol/L
				3-Hydroxybutyrate	10581 μmol/L
Past Diseases History except diabetes					
She has no history such as blood pressure or cardiovascular disease					

N.D, not detected.

body positivity on admission. Because the patient did not consume alcohol or excessive soft drinks after vaccination, it was considered unlikely that her ketoacidosis was caused by alcohol ketosis or soft drink ketosis. Her lactate level was 26 mg/dL, suggesting that lactic acidosis was unlikely.

Since her diabetes diagnosis, the patient had never developed ketosis or ketoacidosis. She had also been taking oral drugs for seven months after her diagnosis. The patient likely had residual insulin secretion at the time of the diagnosis of diabetes mellitus, and her insulin secretion gradually decreased. The urinary C-peptide level was undetectable on admission, suggesting that endogenous insulin secretion gradually decreased and became depleted. The authors therefore believe that the patient had latent autoimmune diabetes in adults.

On admission, she had an elevated glycated albumin level of 27.7% and an HbA1c level of 8.0%, suggesting that the blood glucose level rapidly increased with the administration of the COVID-19 vaccine approximately three days before the onset of ketoacidosis. The clinical presentation of this patient was consistent with ketoacidosis.

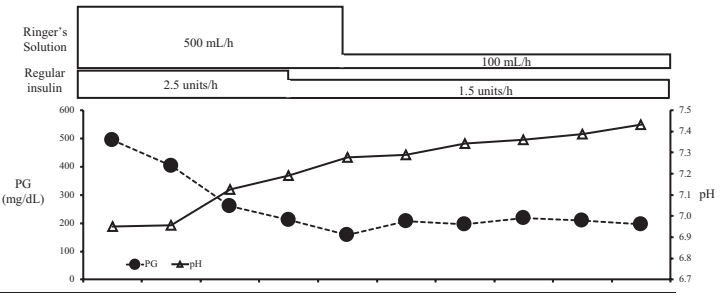
This patient likely had a gradual decrease in insulin secretion. She did not develop ketosis or ketoacidosis, even after receiving SGLT-2 inhibitors for one year and four months before admission. In Japan, SGLT-2 inhibitors have been approved for prescription for type 1 diabetic patients since December 2018.

Since SGLT-2 inhibitors can cause metabolic imbalance, the administration of SGLT-2 inhibitors significantly increases the incidence of diabetic ketoacidosis in patients with type 1 diabetes (6). SGLT-2 inhibitors also reportedly increased endogenous glucose production, serum glucagon levels (7), and serum ketones (8). Although, the patient's carbohydrate and fluid intake in their diet prevented the development of ketosis. In fact, the urinary ketone body was negative at the time of examination two days before the COVID-19 vaccination, and her dietary intake was good.

The administration of the COVID-19 vaccine caused nausea and vomiting, but not ketosis (9). However, nausea and vomiting led to the patient becoming unable to consume carbohydrates. Thus, SGLT-2 inhibitor treatment was discontinued, but her reduced carbohydrate intake and lack of insulin action resulted in ketosis. Ketosis exacerbates nausea, while dehydration exacerbates ketosis, further inducing nausea and vomiting. This vicious cycle resulted in ketoacidosis. The patient also had ketoacidosis, which was complicated by an anaerobic metabolism in the tissues due to rapid metabolic ataxia, probably due to dehydration.

The second case followed a similar course after vaccination without taking SGLT2 inhibitors. Therefore, in addition to the effect of insulin discontinuation as the cause of diabetic ketoacidosis, the effect of the vaccine itself must be examined. We considered the possibility of a temporary decrease in insulin secretion after vaccination common to both patients. And we suspect that is the reason why diabetic ketoacidosis occurred in latent autoimmune diabetes in adults, not acute onset type 1 diabetes.

COVID-19 is known to be a virus that infiltrates and infects cells *via* angiotensin-converting enzyme 2 (ACE2). ACE2 receptors are expressed in various metabolic tissues, including pancreatic beta cells (10) There are also reports of observations of islet cell degeneration in postmortem COVID-19 patients (11).



Time	8:30	9:30	10:30	12:00	14:00	16:00	18:00	20:00	22:00	0:00
PG(mg/dL)	494	403	260	212	158	208	195	219	209	195
pH	6.95	6.958	7.125	7.191	7.276	7.289	7.341	7.36	7.386	7.431
pO2	129	133	135	127	115	106	98.3	90.2	87.8	92
pCO2	14.4	8.8	8.4	9.9	14.4	18.7	23.9	26.5	28.7	28.4

FIGURE 2 | The course in day1-2 in a patient with ketoacidosis who was treated with insulin. PG, plasma glucose.

With viral endocytosis, the activation of the renin-angiotensin system through the downregulation of the ACE2 receptor can impair insulin receptor signaling (12). It has also been reported that a similar reaction in islet cells may occur temporarily upon SARS-CoV-2 antigen presentation after vaccination against COVID-19 (13).

To address the COVID-19 pandemic, vaccines have been administered worldwide. In Japan, by December 26, 2021, approximately 77.8% of total population has received the vaccine twice. The percentage of people who have finished vaccination with a history of diabetes is unknown.

A recent meta-analysis confirmed a strong relationship between COVID-19 severity and blood glucose levels (14). Patients with diabetes have a higher risk of severe COVID-19 infection (15) and benefit from the COVID-19 vaccine. It has already been reported that SARS-CoV-2 replication occurs in human beta cells (16).

On the other hand, it has been pointed out that hyperglycemia occurs 1-6 days after the administration of the COVID-19 vaccine in patients with type 2 diabetes. The frequency of hyperglycemia is unknown, but it has been shown to occur soon after vaccination (17). In the United States, case of non-diabetic patient who developed a hyperosmolar hyperglycemic state 6 days after the second vaccination with an mRNA-based vaccine (BNT162b2) have been reported. This patient had an HbA1c value of 5.6% before vaccination, indicating no overt diabetes (18).

Recently, serious adverse events such as thrombosis and cardiomyopathy after COVID-19 vaccination have been reported (19, 20). Similarly, we need to consider the direct link between COVID-19 vaccination and acute hyperglycemic crisis. Because inflammatory cytokine responses from SARS-CoV-2 proteins due to SARS-CoV-2 infection or vaccination can contribute to direct damage to pancreatic islet cells and impaired insulin receptor signaling.

Decreased insulin secretion can induce diabetic ketoacidosis. Diabetic ketoacidosis is generally known to be a major cause of increased hospitalization and mortality in diabetic patients (21). But educational programs can reduce the frequency of ketoacidosis in patients with type 1 diabetes (22). In addition, compliance with the sick day rule is important for preventing the aggravation of ketoacidosis and reducing the frequency and length of hospital stay. Many people with type 1 diabetes say they are confident in managing their physical condition during sick days. However, it is known that the percentage of those who actually take actions, such as increasing their water intake, adjusting their insulin dose, and measuring urinary ketone bodies is small (23). All physicians treating patients with type 1 diabetes must seek ways to encourage their patients to comply with sick day rules. Diabetic patients treated as type 2 diabetics may include type 1 diabetics who have never had diabetic ketoacidosis before. It is important to check the sick day rule in diabetic patients, even when administering the COVID-19 vaccination. We hope that enlightenment activities will be expanded more than ever to reduce deaths and hospitalizations due to diabetic ketoacidosis.

CONCLUSION

We propose the following hypothesis to explain the development of diabetic ketoacidosis in the present cases. First, the patients were unable to ingest water and sugar in response to the loss of appetite that occurred after vaccination. Second, despite a decrease in insulin secretion or an increase in insulin requirement, the patients were not to possible perform insulin injection appropriately.

Education from healthcare professionals is important for self-management of diabetes. However, caution is required when social problems are present, especially with when associated with reduced adherence. For such patients, it may be necessary to implement a system where the attending physician can be involved in management during sick days, in addition to self-management.

The two cases presented in the present report involved type 1 diabetes; however, temporary pancreatic β-cell hypofunction may have progressed due to the COVID-19 vaccine itself or as

a result of the protein-induced inflammation that occurs after vaccination.

The COVID-19 infection situation is still not stable worldwide, and the occurrence of breakthrough infections after vaccination has become a problem. In Japan, there is an active movement to start the third vaccination in 2021, mainly for elderly people and medical personnel. It is expected that similar cases will increase with increased vaccination coverage. The vaccine should be carefully administered to type 1 diabetes patients receiving intensive insulin therapy and sodium-glucose transporter treatment, as they are at high risk for the development of ketoacidosis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

FY and TM designed the project. FY, TK, and MS obtained written informed consent from the patients. FY and SO clinically characterized the patient and collected clinical information. FY and TM wrote the manuscript. MS, AH, HA, YH, TK, NAw, NAs, TA, SO, and AO were major contributors to the editing of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.840580/full#supplementary-material

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SYSTEMATIC REVIEW
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The Association Between Antidiabetic Agents and Clinical Outcomes of COVID-19 Patients With Diabetes: A Bayesian Network Meta-Analysis

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Aims: This study aimed to assess the impact of different antidiabetic agents on individuals with diabetes and COVID-19.

Methods: We searched PubMed, Web of Science, Embase, and Cochrane Library databases from inception to October 31, 2021 and included seven antidiabetic agents. The data were pooled *via* traditional pairwise meta-analysis and Bayesian network meta-analysis.

Results: The pairwise meta-analysis included 35 studies. Metformin (odds ratio (OR), 0.74; P=0.001), dipeptidyl peptidase-4 inhibitors (DPP4i) (OR, 0.88; P=0.04), sodium-glucose cotransporter-2 inhibitors (SGLT2i) (OR, 0.82; P=0.001), and glucagon-like peptide-1 receptor agonists (GLP1RA) (OR, 0.91; P=0.02) treatment were associated with lower COVID-19 mortality in individuals with diabetes compared to respective non-users. However, insulin treatment resulted in higher mortality (OR, 1.8; P=0.001). Mortality did not significantly differ in sulfonylurea (OR, 0.97; P=0.56) and thiazolidinediones (TZDs) (OR, 1.00; P=0.96) users. Furthermore, due to limited data, we analyzed five antidiabetic agents (metformin, DPP4i, sulfonylurea, insulin, and SGLT2i) and found no association between them and severe disease risk (all P>0.05). The Bayesian network meta-analysis included 18 studies. GLP1RA and SGLT2i had the highest first and second rank probability (67.3% and 62.5%, respectively). Insulin showed the maximum probability of ranking seventh (97.0%). Metformin had the third and fourth highest rank probability of 44.8% and 38.9%, respectively. Meanwhile, DPP4i had the fifth-highest rank probability of 42.4%, followed by sulfonylurea at 45.1%.

Conclusion: Metformin, DPP4i, SGLT2i, and GLP1RA treatments were highly possible to reduced COVID-19 mortality risk in individuals with diabetes, while insulin might be related to increased mortality risk. Sulfonylurea and TZDs treatments were not associated with

mortality. None of the antidiabetic agents studied were associated with the risk of severe disease. Additionally, GLP1RA probably had the most significant protective effect against death, followed by SGLT2i and metformin.

Systematic Review Registration: PROSPERO (CRD42021288200)

Keywords: diabetes, antidiabetic agents, COVID-19, mortality, Bayesian network meta-analysis

INTRODUCTION

The ongoing pandemic of Coronavirus disease-19 (COVID-19) is an emerging, rapidly evolving situation that poses challenges for diabetic patients. Previous studies have demonstrated that diabetes is an independent risk factor for worse outcomes and in-hospital mortality in COVID-19 patients [1]. The percentage of COVID-19 patients with diabetes in hospitalization was high, with adverse effects on their prognosis, and most COVID-19 fatalities occurred among diabetic patients. It is necessary to decide a relatively beneficial treatment plan for diabetic patients with COVID-19 among the massive diabetes treatment plans.

Antidiabetic agents have been demonstrated to exhibit an antidiabetic role and anti-inflammatory and immunomodulatory effects (1, 2). Numerous studies have been conducted to determine the impact of antidiabetic agents on clinical outcomes of diabetic patients with COVID-19. Additionally, some pairwise meta-analyses researched the role of a specific antidiabetic agent on the mortality and severe poor outcomes of COVID-19, including metformin, dipeptidyl peptidase 4 inhibitors (DPP4i), sulfonylurea, insulin, and glucagon-like peptide-1 receptor agonists (GLP1RA) (1, 3, 4). However, these investigations merely compared the outcomes of users with specific antidiabetic agents to those of non-users. The mortality risks between different antidiabetic agents in these patients remain unclear.

To date, no comprehensive network meta-analysis has been performed to explore the associations between different antidiabetic drugs and COVID-19 patients' outcomes. The current analysis aims to evaluate the association between different antidiabetic agents with the risk of COVID-19 mortality, as well as the risk of severe disease outcomes among individuals with diabetes.

MATERIAL AND METHODS

Literature Search

The literatures of any design were searched in PubMed, Embase, and Cochrane Library databases from inception to October 30, 2021, following the Meta-analyses Of Observational Studies in Epidemiology (MOOSE) guidelines as described (5). The PROSPERO registration number is CRD42021288200. The search terms included antidiabetic agents, metformin, DPP4i, sulfonylurea, thiazolidinediones (TZDs), insulin, sodium-glucose cotransporter-2 inhibitors (SGLT2i), GLP1RA, glinides, α-glycosidase inhibitors, and COVID-19. Each search strategy included a combination of one antidiabetic agent and COVID-19. The exact search terms are shown in **Supplementary Table 1** (**S Table 1**). We limited our search by the English language. The retrieved articles were imported into EndnoteX9 and extracted by screening their titles and abstracts. Duplicate studies and multiple reports using the same data were removed. After preliminary screening, nonconforming literature was excluded by reading the full text, and the final remaining articles were included in this study.

Inclusion and Exclusion Criteria

This study comprised studies that met the following criteria: (1) original studies evaluating the clinical outcomes of diabetic COVID-19 patients receiving specific antidiabetic agents at home or in the hospital; (2) study designs including randomized controlled trials (RCT), and observational studies; (3) studies published in English. The exclusion criteria included the following: (1) reviews, systematic reviews, correspondences, or *in vitro* animal studies; (2) studies with incomplete data and indeterminate outcome endpoints; (3) abstracts, unpublished studies, and retracted manuscripts.

Data Extraction and Quality Assessment

Two independent reviewers used a predetermined data collection table to extract relevant data. Disagreements were solved by discussion and following a third author's opinion. The main extracted data included the following: study characteristics (first author, publication date, country, and study design), patient characteristics (sample size, mean age, and male proportion), as well as study's outcomes data. Newcastle-Ottawa Quality Assessment Scale criteria were utilized to assess the risk of bias for the included studies (6, 7). According to this scale, a study can be awarded a maximum of nine stars; four stars for study selection, two stars for comparability, and three stars for outcome sections.

Outcomes of Interest

Our primary outcome was the mortality of individuals with diabetes and COVID-19. The secondary outcome was the incidence of severe disease. Here, we defined the cases with poor outcomes as those requiring intensive care unit admission, mechanical ventilation of acute respiratory distress syndrome, disseminated intravascular coagulation, or others described in the individual study as severe disease.

Statistical Analysis

Meta-analysis and Bayesian network were conducted only in studies >3 per outcome. Meta-analysis was performed using Stata 16.0 (Stata Corp) software. Both chi-squared and I^2 tests estimated heterogeneity between studies. The pooled effect sizes were analyzed using a random-effects model (I^2 >0.5, P <0.1) and fixed-effects model (I^2 <0.5, P >0.1). As reported by individual cohort studies, pooled OR was estimated by combining relative risk (RR) or odds ratio (OR) (hazards ratio was presumably equivalent to RR). If studies included unadjusted and covariate-adjusted data, we selected the latter. To stabilize the variances, we transformed each study's effect estimates and their confidence intervals (CI) to natural logarithms (8, 9). If the number of studies was >5, publication bias was evaluated using Begg's test, Egger's test, and the trim-and-fill method. Subgroup analyses based on data types (adjusted or unadjusted data), effect size types (OR or RR), diabetes types (only T2DM or not only T2DM), and medication use timing (use before admission or in-hospital) would be established when the number of studies in a subgroup was greater than three. Meta-regression analysis was performed to assess the potential impact of the study's sample size, patients' mean age, and gender on the pooled results.

The Bayesian network meta-analysis was performed using R 4.1 software with gemtc and rjags packages as previously described (10). The network meta-analysis employed OR and 95% credibility intervals (95% CrIs). Our study's consistency was determined by comparing the fit of consistency and inconsistency models using deviance information criteria (differences < 5 are considered consistency fitting) (11). We calculated the rank probabilities of the effects of all drugs available in the network. Additionally, the probability of the first to seventh of each drug was visualized using radar plots of ranking probability for the network. $P \leq 0.05$ was considered statistically significant.

RESULTS

Study Selection and Characteristics

The search strategy identified 681 potentially relevant studies, of which 249 were excluded due to duplication. Among the remaining 432 studies, 384 were excluded due to their titles or abstracts. Therefore, 48 studies were eligible for full-text review. After re-excluding six articles, this study comprised 42 full-text articles (**Figure 1**). The vast majority of them were observational studies (6 cross-sectional studies, 1 case-control study, and 34 cohort studies) and only one article was RCT. **Table 1** summarizes the baseline characteristics of patients in the included studies.

Quality Assessment

Newcastle-Ottawa Quality Assessment measured the quality of studies. The quality scores of studies ranged from seven to nine stars with a median of eight stars (**S Table 2**).

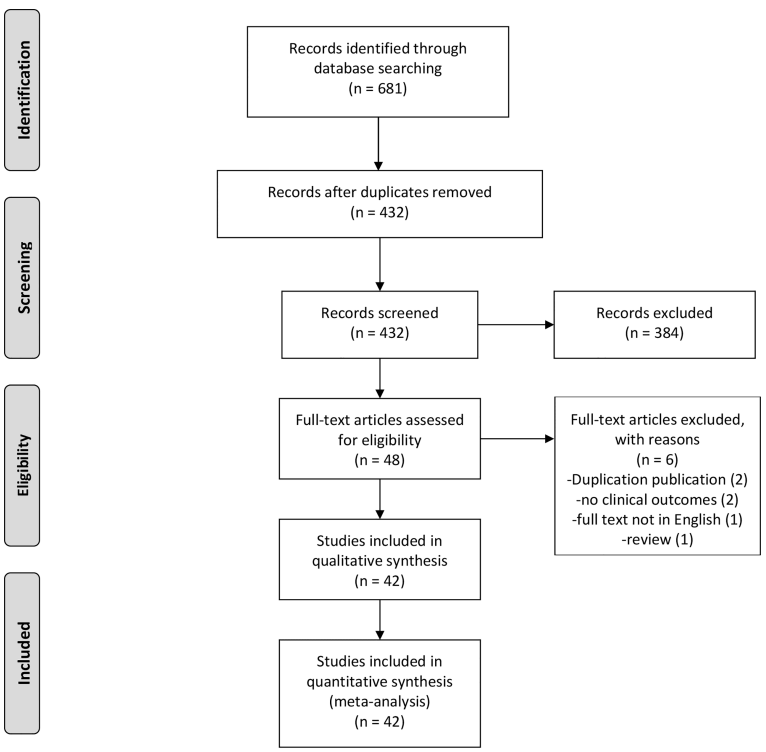

FIGURE 1 | The flow diagram of study selection.

TABLE 1 | Clinical characteristics of the studies included in the review.

Author (year)	Country	Included in	Type of study	Sample	Sample characteristics	Results
Silverii, G. A (2021) (12)	Italy	Bayesian network meta-analysis and pairwise meta-analysis	cross-sectional study	Patients with COVID-19 and DM; Sample size: 159	Mean age: 73.31; Male: 54.1%	No medication for diabetes was associated with differences in risk of COVID-19 fatality, with the only exception of metformin (RR 0.6; 95%CI 0.39-0.93).
Kahkoska, A. R (2021) (13)	USA	Bayesian network meta-analysis	retrospective cohort study	Patients with COVID-19 and DM; Sample size: 12446	Mean age: 58.6; Male: 46.6%; White: 62.5%	Both GLP1-RA and SGLT2i use were associated with lower 60-day mortality compared with DPP4i use (OR 0.54 [95% CI 0.37–0.80] and 0.66 [0.50–0.86], respectively).
Ramos-Rincón, J. M (2021) (14)	Spain	Bayesian network meta-analysis and pairwise meta-analysis	cross-sectional study	Patients with COVID-19 and T2DM and age≥80; Sample size: 790	Mean age: 85; Male: 52.9%;	The preadmission cardiometabolic medications found to be independent protective factors against in-hospital mortality were the use of DPP-4i (adjusted OR 0.502, 95% CI: 0.309–0.815, p = 0.005).
Orioli, L (2021) (15)	Belgium	Bayesian network meta-analysis	retrospective cohort study	Patients with COVID-19 and DM; Sample size: 73	Mean age: 69; Male: 48%; White: 79.5%	Death cases were less often treated with metformin (P<0.036)
Israelsen, S. B (2021) (16)	Denmark	Bayesian network meta-analysis	retrospective cohort study	Patients with COVID-19 and DM; Sample size: 1970	/	Current users of GLP-1 RAs had an adjusted RR of 0.89 (95%CI 0.34-2.33), while users of DPP-4i had an adjusted RR of 2.42 (95%CI 0.99-5.89) for 30-day mortality compared with SGLT-2i use.
Sourij, H (2021) (17)	Austria	Bayesian network meta-analysis and pairwise meta-analysis	combined prospective and retrospective cohort study	Patients with COVID-19 and DM; Sample size: 238	Mean age: 71.1; Male: 63.6%;	With regard to medication use, no difference was observed for any other glucose-lowering medication between people who survived or died.
Nyland, J. E (2021) (18)	USA	Bayesian network meta-analysis and pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and T2DM; Sample size: 29516	Mean age: 60.9; Male: 48.2%; White: 47.9%	Use of glucose-regulating medications such as GLP-1R agonists, DPP-4 inhibitors, or pioglitazone may improve outcomes for COVID-19 patients with T2DM.
Cheng, X (2021) (19)	China	Bayesian network meta-analysis	retrospective cohort study	Patients with COVID-19 and T2DM; Sample size: 50	Mean age: 56.0; Male: 54%; White: 0%	In-hospital insulin usage attempted to increase the invasive ventilation (34.8% vs. 3.7%, adjust P = 0.043), independent of age and blood glucose.
Elibol, A (2021) (20)	Turkey	Bayesian network meta-analysis	cross-sectional study	Patients with COVID-19 and T2DM;	Mean age: 63.3; Male: 45.6%	No oral anti-diabetics was found to be associated with COVID-19 related death.

(Continued)

TABLE 1 | Continued

Author (year)	Country	Included in	Type of study	Sample	Sample characteristics	Results
				Sample size: 432		
Luk, A. O. Y (2021) (21)	China	Bayesian network meta-analysis and pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and T2DM; Sample size: 1220	Mean age: 65.3; Male: 54.3%	Users of metformin and DPP-4 inhibitors had fewer adverse outcomes from COVID-19 compared with non-users, whereas insulin and sulphonylurea might predict a worse prognosis.
Li, J (2021) (22)	China	Bayesian network meta-analysis	retrospective cohort study	Patients with COVID-19 and T2DM; Sample size: 131	Mean age: 66.8; Male: 56.5%; White: 0%	Using metformin (p = .02) and acarbose (p = 0.04), alone or both together (p = 0.03), after admission were significantly more likely to survive than those who did not use either metformin or acarbose.
Lally, M. A (2021) (23)	USA	Bayesian network meta-analysis	retrospective cohort study	Patients with COVID-19 and DM; Sample size: 775	Mean age: 75.6; Male: 97.3%; White: 66.3%	Comparing to those not receiving diabetes medications, residents taking metformin were at significantly reduced hazard of death (adjusted HR 0.48, 95%CI 0.28, 0.84) over the subsequent 30 days from COVID-19 diagnosis. There was no association with insulin (adjusted HR 0.99, 95% CI 0.60, 1.64) or other diabetes medications (adjusted HR 0.71, 95% CI 0.38, 1.32).
Crouse, A. B (2020) (24)	USA	Bayesian network meta-analysis and pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and DM; Sample size: 239	Mean age:/; Male: 50.6%; White: 27.6%	Metformin may provide a protective approach in high-risk population.
Pérez-Belmonte, L. M (2020) (25)	Spain	Bayesian network meta-analysis and pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and T2DM; Sample size: 2666	Mean age: 74.9; Male: 61.9%	In patients with type 2 diabetes mellitus admitted for COVID-19, at-home glucose-lowering drugs showed no significant association with mortality and adverse outcomes.
Wargny, M (2021) (26)	France	Bayesian network meta-analysis and pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and DM; Sample size: 2796	Mean age:/; Male: 63.7%; White: 58.1%	Insulin was associated with a greater risk of death while routine metformin therapy was negatively associated with death.
Khunti, K (2021) (27)	UK	Bayesian network meta-analysis and pairwise meta-analysis	retrospective cohort study	Patients with T2DM; Sample size: 2851465	Mean age:/; Male: 55.9%; White: 66.1%	The adjusted HR associated with recorded versus no recorded prescription was 0.77 (95% CI 0.73–0.81) for metformin and 1.42 (1.35–1.49) for insulin.
Solerte, S. B (2020) (28)	Italy	Bayesian network meta-analysis and pairwise meta-analysis	retrospective case-control study	Patients with T2DM; Sample size: 338	Mean age: 69; Male: 70.4%;	Treatment with sitagliptin at the time of hospitalization was associated with reduced mortality (18% vs. 37% of deceased patients; hazard ratio 0.44 [95% CI 0.29–0.66]; P = 0.0001).
Meijer, R. I (2021) (29)	Netherlands	Bayesian network meta-analysis and pairwise	prospective cohort study	Patients with COVID-19 and T2DM;	Mean age: 69.4; Male: 64.1%;	Outpatient use of a DPP-4 inhibitor does not affect the clinical outcomes of patients with type 2 diabetes who are hospitalized because of COVID-19 infection.

(Continued)

TABLE 1 | Continued

Author (year)	Country	Included in	Type of study	Sample	Sample characteristics	Results
		meta-analysis		Sample size: 565		
Cheng X (2020) (30)	China	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and T2DM; Sample size: 1213	Mean age: 63; Male: 52.1%;	Metformin use was significantly associated with a higher incidence of acidosis, particularly in cases with severe COVID-19, but not with 28-day COVID-19-related mortality.
Luo P (2020) (31)	China	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and DM; Sample size: 283	Mean age: 64; Male: 55.1%;	Antidiabetic treatment with metformin was associated with decreased mortality compared with diabetics not receiving metformin.
Bramante CT (2021) (32)	USA	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and DM; Sample size: 9555	Mean age:/; Male: 47.3%; White: 69.2%	Metformin was associated with a decrease in mortality from COVID-19, in the propensity-matched cohorts, OR 0.38 (0.16, 0.91; p = 0.03).
Ghany R (2021) (33)	USA	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19; Sample size: 1139	Mean age: 71.1; Male: 40.3%	The relative hazard (RH) of death for metformin users was 0.34; 95% CI 0.19–0.59. The RH of ARDS for metformin users was 0.32; 95% CI 0.22–0.45.
Jiang N (2021) (34)	China	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and T2DM; Sample size: 148	Mean age: 65; Male: 47.3%	In the mixed-effected model, metformin use was associated with the lower incidence of ARDS, but no significant association with 30-day all-cause mortality.
Lalau J D (2021) (35)	France	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and T2DM; Sample size: 2449	Mean age: 70.9; Male: 64.0%	The odds ratios for primary outcome and death (OR [95%CI], metformin users vs non-users) were 0.783 [0.615–0.996] and 0.710 [0.537–0.938] on day 28, respectively.
Oh T K (2021) (36)	South Korea	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and T2DM; Sample size: 11892	Male: 44.9%	Metformin use was not associated with hospital mortality (OR: 1.26, 95% CI: 0.81–1.95; P = 0.301).
Bramante CT2 (2021) (37)	USA	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and T2DM; Sample size: 6256	Mean age: 73; Male: 47.2%	In unadjusted analyses, metformin was associated with decreased mortality. But the association was not statistically significant in the adjusted analysis.
Chen Y (2020) (38)	China	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and DM; Sample size: 120	Mean age: 64	None of the glucose-lowering medications (metformin, insulin, α-glycosidase, secretagogues, or DPP-4 inhibitors) were associated with in-hospital death.

(Continued)

TABLE 1 | Continued

Author (year)	Country	Included in	Type of study	Sample	Sample characteristics	Results
Do JY (2020) (39)	South Korea	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and T2DM; Sample size: 1865	Mean age: 61; Male: 58.7%	No definite association could be found between metformin use and clinical outcomes, including survival
Gao Y (2020) (40)	China	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and T2DM; Sample size: 110	Mean age: 65; Male: 41.8%	Antidiabetic therapy with metformin was associated with a higher risk of severe illness (adjusted odds ratio 3.964, 95% confidence interval 1.034–15.194).
Kim MK (2020) (41)	South Korea	Pairwise meta-analysis	cross-sectional study	Patients with COVID-19 and DM; Sample size: 470	Mean age: 69; Male: 42.8%	The use of metformin or insulin tended to be associated with less severe disease and lower mortality, although these findings did not achieve statistical significance
Saygili ES (2021) (42)	Turkey	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and DM; Sample size: 240	Mean age: 69; Male: 52.5%	Preadmission metformin usage is associated with reducing all-cause mortality.
Wander PL (2021) (43)	USA	Pairwise meta-analysis	cross-sectional study	Patients with COVID-19 and DM; Sample size: 64892	White: 66%	Among veterans with diabetes and COVID-19, insulin use were directly associated with adverse outcomes, while use of a GLP1-RA, metformin, and SGLT2i was inversely associated.
Tamura RE (2021) (44)	Brazil	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and DM; Sample size: 188	Male: 62.8%	Patients that used metformin during hospitalization have a better prognosis and reduced risk of death.
Dave JA (2021) (45)	South Africa	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and DM; Sample size: 5708	Mean age: 57; Male: 37.1%	Use of insulin (OR1.49, 95% CI: 1.27; 1.74) was associated with an increased mortality whereas use of metformin (OR 0.77, 95% CI: 0.64; 0.92) was associated with a reduction in mortality.
Cariou B (2020) (46)	France	Pairwise meta-analysis	cross-sectional study	Patients with COVID-19 and DM; Sample size: 1317	Mean age: 69.8; Male: 64.9%	Metformin use was lower in people who died.
Fadini GP (2020) (47)	Italy	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and T2DM; Sample size: 85	Mean age: 70.3; Male: 64.7%	The current study does not support the hypothesis that DPP-4is might be protective against COVID-19.
Rhee SY (2021) (48)	South Korea	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and DM;	Male: 53.5% and 40.3%	DPP-4i is significantly associated with a better clinical outcome of patients with COVID-19.

(Continued)

TABLE 1 | Continued

Author (year)	Country	Included in	Type of study	Sample	Sample characteristics	Results
Dalan R (2021) (49)	Singapore	Pairwise meta-analysis	retrospective cohort study	Sample size: 832 and 704 Patients with COVID-19 and DM; Sample size: 76	/	Patients on DPP-4 inhibitors were more likely to require ICU admission.
Noh Y (2021) (50)	South Korea	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and T2DM; Sample size: 586	/	Compared with use of other second- or third-line antidiabetic drugs, use of DPP-4 inhibitors was not associated with adverse COVID-19-related outcomes among patients with T2DM.
Roussel R (2021) (51)	France	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and T2DM; Sample size: 2449	Mean age: 70.9; Male: 64%	No significant association between the use of DPP-4 inhibitors and the risk of tracheal intubation and death.
Zhou JH (2020) (52)	China	Pairwise meta-analysis	retrospective cohort study	Patients with COVID-19 and T2DM; Sample size: 444	Mean age: 64; Male: 48.6%	The author did not observe any significant difference between patients taking DPP4i drugs and those taking other oral hypoglycemic drugs regarding the incidence or risk of all-cause mortality.
Kosiborod MN (2021) (53)	USA	Pairwise meta-analysis	RCT	Patients with COVID-19 and T2MD; Sample size: 1250	Mean age: 61.4; Male: 42.6%	Dapagliflozin did not significantly reduce the rates of organ dysfunction or death or improve recovery

DM, diabetes mellitus; T2DM, type 2 diabetes mellitus.

Meta-analysis of the Impact of Using Antidiabetic Agents on Patients' Clinical Outcomes

The pairwise meta-analysis included 35 studies (12, 14, 17, 18, 21, 24–53), of which 25 (12, 14, 17, 21, 24–27, 30–46) were related to metformin, 21 (12, 14, 17, 18, 21, 25–29, 36, 38, 41, 43, 46–52) were linked to DPP4i, 10 (12, 17, 21, 26, 27, 36, 41, 43, 46, 49) were associated with sulfonylurea, 5 (12, 18, 27, 36, 43) were related to TZDs, 14 (12, 14, 17, 21, 24–27, 36, 38, 41, 43, 45, 46) were linked to insulin, 8 (12, 14, 17, 27, 41, 43, 49, 53) were correlated with SGLT2i, 7 (12, 14, 18, 26, 27, 43, 46) were linked to GLP1RA, 2 (27, 38) were associated with acarbose (α-glycosidase inhibitors), and one (27) was correlated with glinides. Some of these studies included multiple different medications.

Qualitative meta-analysis revealed that metformin users had a significantly reduced mortality rate than non-users. (**Figure 2A**; pooled OR, 0.74; 95% CI: 0.67-0.81; P=0.00). Subgroup analyses based on data types, effect size types, and diabetes types respectively revealed lower mortality in metformin users in each subgroup (**S Figures 1A**, **2A** and **3A**, all subgroups $P < 0.05$). In the Subgroup analyses based on medication use timing, metformin use before admission was associated with a significantly reduced mortality rate (pooled OR, 0.76; 95% CI: 0.69-0.84), but no such an association was observed in in-hospital use subgroup (pooled OR, 0.41; 95% CI: 0.11-1.60) (**S Figures 4A**). Using DPP4i in individuals with diabetes was also associated with reduced mortality from COVID-19 (**Figure 2B**; pooled OR, 0.88; 95% CI: 0.78-1.00; P=0.04). Further subgroup analysis indicated that DPP4i use remained linked to a statistically decreased mortality after adjusting for age, gender, and other characteristics (**S Figure 1B**; pooled OR, 0.86; 95% CI: 0.75-0.98; P=0.02) whereas was not statistically significant in unadjusted data ($P > 0.05$). However, in subgroup analyses based on effect size types, diabetes types, and DPP4i use timing, there was no statistically significant difference in each subgroup (**S Figures 2B**, **3B**, and **4B**, all subgroups $P > 0.05$). Thus, caution is needed when interpreting the effect of DPP4i on individuals with

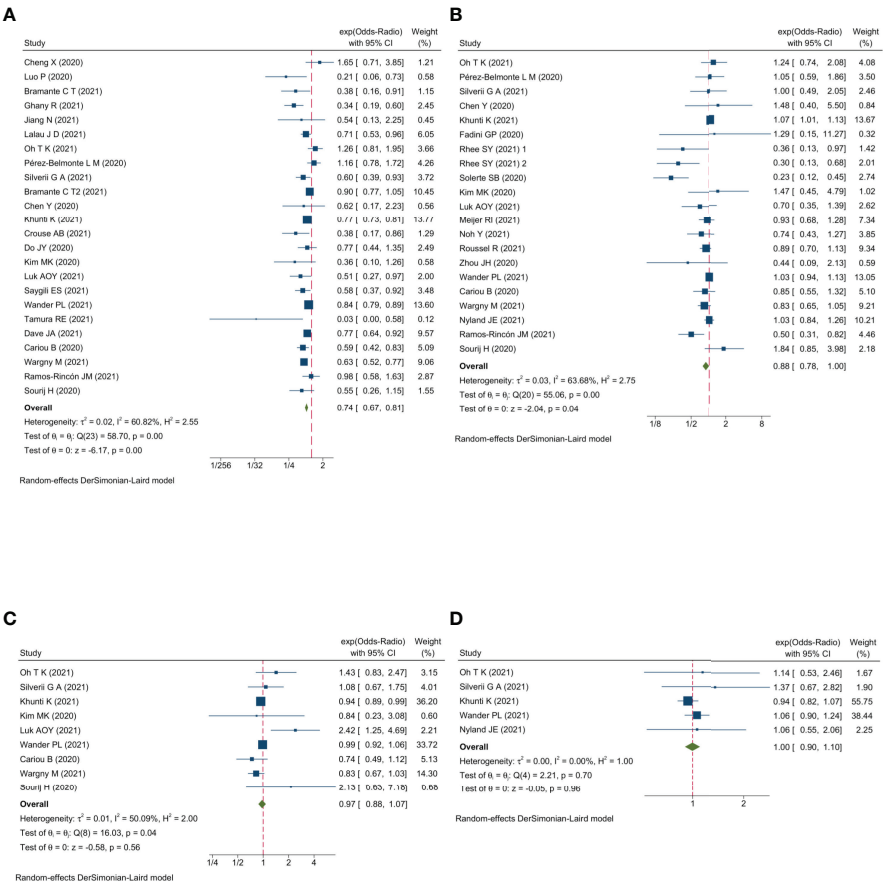

FIGURE 2 | Forest plot showing the association between antidiabetic agents use and mortality. **(A)** Metformin; **(B)** DPP4i; **(C)** Sulfonylurea; **(D)** TZDs.

diabetes and COVID-19. For sulfonylurea or TZDs medications, no significant difference in mortality was observed between medication users and non-users (sulfonylurea: **Figure 2C**; pooled OR, 0.97; 95% CI: 0.88-1.07; P=0.56, TZDs: **Figure 2D**; pooled OR, 1.00; 95% CI: 0.90-1.10; P=0.96). Subgroup analysis based on data types, effect size types, diabetes types, and sulfonylurea use timing demonstrated similar results respectively (**S Figures 1C**, **2C**, **3C**, and **4C**; all subgroups P > 0.05). Insulin treatment was significantly associated with increased mortality compared to non-users (**Figure 3A**; pooled OR, 1.38; 95% CI: 1.24-1.54; P=0.00). In subgroup analysis based on data types, effect size types and diabetes types, there was also higher mortality in each insulin subgroup (**S Figures 1D**, **2D** and **3D**, all subgroups P < 0.05); while subgroup analysis based on insulin using timing revealed that higher mortality was only observed in before-admission use subgroup (**S Figures 4D**, pooled OR, 1.43; 95% CI: 1.25-1.64). Finally, therapy of SGLT2i or GLP1RA was significantly associated with a reduction in mortality compared to non-users (SGLT2i: **Figure 3B**; pooled OR, 0.82; 95% CI: 0.76-0.88; P=0.00, GLP1RA: **Figure 3C**; pooled OR, 0.91; 95% CI: 0.84-0.98; P=0.02). Subgroup analysis was not performed due to insufficient SGLT-2i or GLP-1RA treatment data. Furthermore, meta-regression analysis disclosed that the study's sample size, patients' mean age, and gender did not significantly impact the association of antidiabetic agents on patients' mortality (all P > 0.05) (**Table 2**).

Most studies did not report the outcome of severe disease. Due to a scarcity of data, a qualitative meta-analysis of only five antidiabetic agents was performed (metformin, DPP4i, sulfonylurea, insulin, and SGLT2i). The pooled results demonstrated no significant associations between antidiabetic agent use and risk of severe disease (all P > 0.05) (**S Figure 5**).

Begg's and Egger's tests revealed no publication bias for sulfonylurea, TZDs, insulin, and SGLT2i mortality (all P > 0.05), while Egger's test manifested publication bias for metformin, DPP4i, and GLP1RA (Egger's test P = 0.00, 0.04, and 0.04, respectively). The trim-and-fill analysis suggested no evidence of a significant difference between adjusted and original values of all antidiabetic agents (**Table 2** and **S Figure 6**), implying that the results in this meta-analysis were relatively robust.

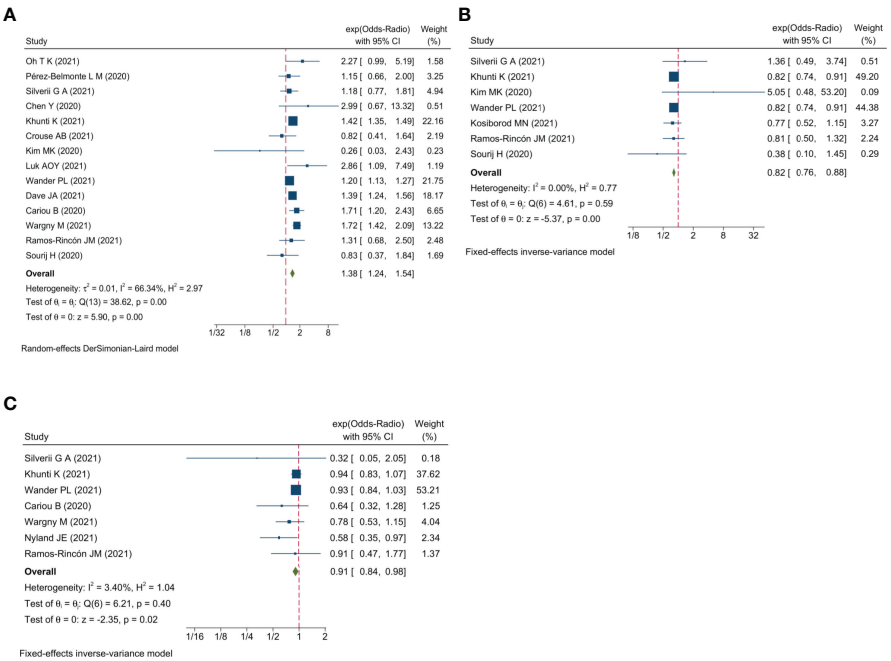

FIGURE 3 | Forest plot showing the association between antidiabetic agents use and mortality. **(A)** Insulin; **(B)** SGLT2i; **(C)** GLP1RA.

TABLE 2 | The results of publication bias and regression analysis for mortality.

Mortality	Studies trimmed/ total studies	OR (95% CI)	P value	Begger's test	Egger's test	Trim-and-fill analysisOR (95% CI)	Regression for Sample size	Regression for mean age	Regression for male proportion
Metformin	7/24	0.74 (0.67-0.81)	0.00	0.14	0.00	0.78 (0.71-0.87)	0.68	0.54	0.78
DPP4i	0/21	0.88 (0.78-0.99)	0.04	0.74	0.04	0.88 (0.78-0.99)	0.28	0.30	0.77
Sulfonylurea	2/9	0.97 (0.88-1.07)	0.56	0.25	0.11	0.94 (0.84-1.07)	0.65	0.37	0.10
TZDs	3/5	1.0 (0.90-1.10)	0.96	0.81	0.35	0.99 (0.90-1.09)	0.19	0.61	0.50
Insulin	0/14	1.38 (1.24-1.54)	0.00	0.44	0.92	1.38 (1.24-1.54)	0.87	0.57	0.47
SGLT2i	0/7	0.82 (0.76-0.88)	0.00	1.00	0.58	0.82 (0.76-0.88)	1.00	0.97	0.76
GLP1RA	3/7	0.91 (0.84-0.99)	0.02	0.13	0.04	0.93 (0.86-1.00)	0.52	0.31	0.99

Bayesian Network Meta-Analysis of the Association Between Antidiabetic Agents and Mortality

The Bayesian network meta-analysis included 18 studies (12–29) and included seven different antidiabetic agents: metformin, DPP4i, sulfonylurea, TZDs, insulin, SGLT2i, and GLP1RA. Due to lack of relevant data, we assessed only the association between different antidiabetic agents and mortality in this Bayesian network meta-analysis and did not examine the risk of severe disease. The network plot is displayed in **Figure 4**.

Using ranking probabilities, Bayesian analysis depicted an overall comparison of the effect of each antidiabetic drug on mortality risk in the network (**Figure 5**). GLP1RA and SGLT2i had the first and second highest rank probabilities of reducing

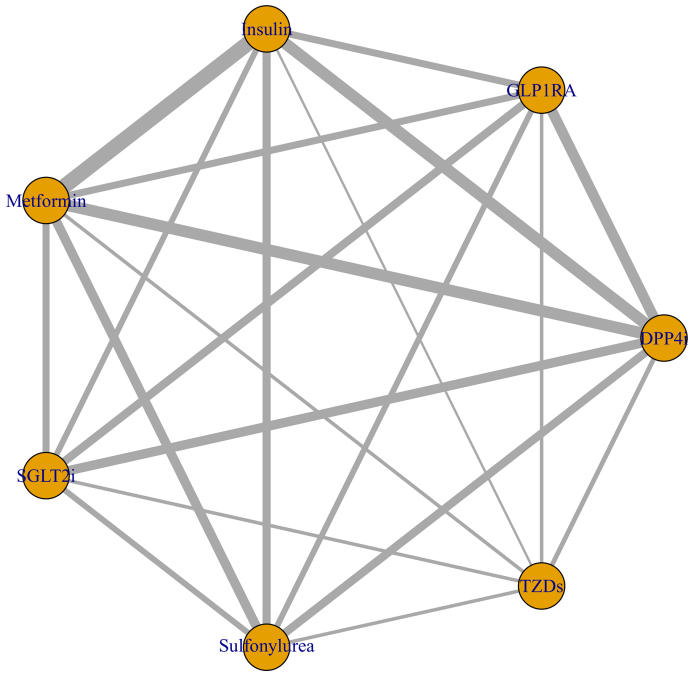

FIGURE 4 | Network plot of the association between different antidiabetic agents and the risk of COVID-19 mortality in diabetic patients. The thickness of lines corresponds to the number of trials.

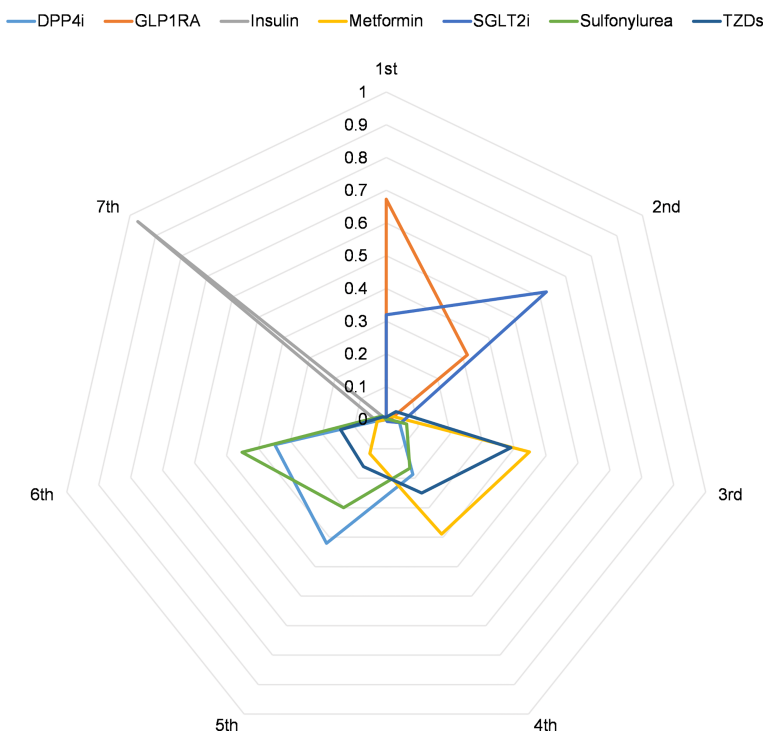

FIGURE 5 | Radar plot of the ranking probability for COVID-19 mortality.

mortality (67.3% and 62.5%, respectively). Insulin had the highest seventh rank probability (97.0%). Metformin had the third and fourth rank probability of 44.8% and 38.9%, respectively. Meanwhile, DPP4i had the fifth-highest rank probability of 42.4%, followed by sulfonylurea at 45.1%.

When taking insulin as a reference, all other antidiabetic agents were associated with a decreased mortality (OR for metformin, 0.56; 95% CrI, 0.42-0.73, OR for DPP4i, 0.66; 95% CrI, 0.49-0.88, OR for sulfonylurea, 0.68; 95% CrI, 0.47-0.97, OR for TZDs, 0.57; 95% CrI, 0.36-0.93, OR for SGLT2i, 0.36; 95% CrI, 0.24-0.55, OR for GLP1RA, 0.33; 95% CrI, 0.23-0.48). Compared with metformin, DPP4i, or sulfonylurea, only GLP1RA and SGLT2i could significantly reduce the mortality, whereas insulin increased the mortality (**Table 3**). All but SGLT2i increased mortality when compared to GLP1RA. Compared with SGLT2i, significantly increased mortality was observed in DPP4i, insulin, metformin, and sulfonylurea treatment groups (**Table 3**).

Consistency results of direct and indirect estimates were detected with relevant P-value larger than 0.05 in any closed loops using the node-splitting method (all $P > 0.05$, **S Figure 7**). The Brooks-Gelman-Rubin plots indicated that the model had a sufficient convergence in the entire network (**S Figure 8**). Global I^2 was employed to evaluate heterogeneity which was 0.4% for mortality. The model fit was estimated by comparing the posterior mean residual deviance with the number of data points, and the results were similar (**S Table 3**). Additionally, network meta-regression analysis revealed that study sample size, mean age, and gender did not significantly impact mortality (**S Figures 9–11**).

DISCUSSION

This is the first comprehensive Bayesian network meta-analysis of different antidiabetic agents for individuals with diabetes and COVID-19. When multiple treatment options are available, network meta-analysis with Bayesian statistics allows for robust mixed treatment comparison analysis. Furthermore, it enables combined direct and indirect comparisons of competing treatments while retaining the advantages of randomization (54). In this study, we assessed the association between various antidiabetic agents and mortality as well as the risk of severe disease in individuals with diabetes and COVID-19. This analysis included 42 studies and seven antidiabetic agents: metformin, DPP4i, sulfonylurea, TZDs, insulin, SGLT2i, and GLP1RA. The results revealed that metformin, DPP4i, SGLT2i, and GLP1RA treatment exhibited lower mortality than respective non-users, while insulin was linked to a higher mortality rate. Treatment with sulfonylurea and TZDs had no impact on mortality. Due to limited data, a meta-analysis of five antidiabetic agents (metformin, DPP4i, sulfonylurea, insulin, and SGLT2i) revealed no association with the risk of incidence of severe disease. Additionally, GLP1RA had the most significant protective effect against mortality among individuals with diabetes and COVID-19, followed by SGLT2i and metformin. Insulin was associated with the highest risk of mortality.

In this meta-analysis, metformin use was associated with decreased COVID-19 mortality, consistent with several previous studies (1, 3, 55). Moreover, statistical significance was observed between subgroups in the subgroup analyses, demonstrating that the overall results about metformin were stable and reliable. Inflammation has long been recognized as a critical factor in the development and severity of COVID-19. Severe COVID-19 is correlated with a hyperinflammatory state and cytokine storm, which can cause multi-organ damage (56). Metformin is the first-line drug in the treatment of diabetes. It has anti-inflammatory characteristics and has been shown to decrease circulating inflammatory biomarkers (2), which might explain why it is beneficial in diabetic patients with COVID-19. Furthermore, some scholars pointed out that metformin's ability to reduce neutrophil counts and neutrophil extracellular traps might contribute to these protective mechanisms (57, 58).

In addition, we found that DPP4i use was associated with lower mortality, even when subgroup pooled adjusted data were included. A consistent conclusion has not been drawn in the previously published meta-analyses about DPP4i. The study (59) of Yang et al. concluded that DPP4i increased the mortality of patients with COVID-19 and diabetes, while Rakhmat's study (60) concluded that DPP4i use was linked to decreased mortality. The other two meta-analyses (56, 61) suggested that DPP4i use was unrelated to mortality. The result of this contradictory evidence could be explained by the uncertain anti-inflammatory properties of DPP4i. In some studies, DPP4i treatment attenuated inflammasome activation and decreased human plasma levels of inflammatory biomarkers such as IL-6, IL-18, CRP, and so on (28, 62). However, some studies concluded that DPP4i did not affect human plasma levels of inflammatory biomarkers (56, 63). Additionally, the impact of DPP4i on active intact cytokines and chemokines can be paradoxical.

TABLE 3 | The results of Bayesian network analysis.

	Metformin	DPP4i	Sulfonylurea	TZDs	Insulin	SGLT2i	GLP1RA
Metformin	–	0.84 (0.63, 1.1)	0.82 (0.58, 1.2)	0.98 (0.6, 1.6)	**0.56 (0.42, 0.73)**	**1.6 (1.0, 2.3)**	**1.7 (1.2, 2.5)**
DPP4i	1.2 (0.89, 1.6)	–	0.98 (0.69, 1.4)	1.2 (0.73, 1.8)	**0.66 (0.49, 0.88)**	**1.8 (1.2, 2.6)**	**2.0 (1.4, 2.8)**
Sulfonylurea	1.2 (0.85, 1.7)	1.0 (0.72, 1.5)	–	1.2 (0.71, 1.9)	**0.68 (0.47, 0.97)**	**1.9 (1.2, 2.9)**	**2.1 (1.4, 3.1)**
TZDs	1.0 (0.65, 1.7)	0.86 (0.55, 1.4)	0.84 (0.51, 1.4)	–	**0.57 (0.36, 0.93)**	1.6 (0.94, 2.6)	**1.7 (1.1, 2.9)**
Insulin	**1.8 (1.4, 2.4)**	**1.5 (1.1, 2.0)**	**1.5 (1.0, 2.1)**	**1.8 (1.1, 2.8)**	–	**2.8 (1.8, 4.1)**	**3.1 (2.1, 4.4)**
SGLT2i	**0.64 (0.44, 0.98)**	**0.54 (0.38, 0.81)**	**0.53 (0.35, 0.84)**	0.63 (0.38, 1.1)	**0.36 (0.24, 0.55)**	–	1.1 (0.74, 1.7)
GLP1RA	**0.59 (0.41, 0.85)**	**0.49 (0.36, 0.7)**	**0.48 (0.32, 0.74)**	**0.57 (0.35, 0.93)**	**0.33 (0.23, 0.48)**	0.91 (0.59, 1.4)	–

An odds ratio lower than 1 favors the row-defining treatment. Statistically significant results are in boldface.

By preventing inflammatory factors from being degraded enzymatically by DPP4, DPP4i may potentially enhance the activity of certain inflammatory networks (64), which might be the underlying reason for the high mortality observed by Yang et al. (59).

The pooled results of sulfonylurea from nine studies revealed no connection between sulfonylurea treatment and COVID-19 mortality. This finding contradicts a previous meta-analysis, in which five studies were included (3). Luk AOY et al. (21) reported a database that captured all COVID-19 patients in China Hong Kong, revealing that sulfonylurea use was associated with increased mortality. However, no statistical significance was observed in the remaining included studies. The specific potential mechanism remains unclear. TZDs is another glucose-lowering agent with the properties of modulating inflammatory and oxidative stress. However, some scholars pointed out that TZDs therapy was associated with weight gain, oedema, and heart failure, implying that it was unsuitable for COVID-19 patients (65). There was no meta-analysis about the effect of TZDs on COVID-19 so far. In this study, pooled results from five studies did not show any significant association between TZDs treatment and COVID-19 mortality as well as the risk of severe disease in diabetic patients.

In our meta-analysis, insulin administration was significantly associated with increased COVID-19 mortality, consistent with previous studies (1, 66). The underlying mechanism between the association of insulin use and COVID-19 remains unclear. Insulin exerted variable antioxidant and anti-inflammatory effects, including induction of nitric oxide production and inhibition of reactive oxygen species (67). Additionally, insulin therapy was proved reducing reactive oxygen species and macrophage infiltration into the liver and omentum in a diabetic rat model, resulting in a reduction in systematic inflammatory status (68). But in another sepsis model of diabetic rats, insulin showed a pro-inflammation ability in the lung (69). The lung was the main targeted organ affected by COVID-19. From a clinical perspective, some scholars worried that because insulin therapy was typically administered to patients with late-stage diabetes, it is difficult to exclude the negative effect of late-stage diabetes on the increased mortality. For instance, when insulin users were admitted to the hospital, they showed higher inflammatory markers, more comorbidities and lower lymphocyte counts than non-users (21). Based on the above, careful assessment of the benefits and adverse effects of insulin was required.

It is known that GLP1RA not only lowers glucose but also controls inflammation-induced lung injury through pulmonary protective effects and reduces major cardiovascular complications (70). Due to the prevalence of cardiovascular disease in diabetic patients, GLP1RA might be beneficial for glucose management in diabetic patients with COVID-19 (2). Moreover, GLP1RA works on GLP1 receptors primarily located on epithelial of the lung and immune cells. Rogliani et al. (71) reported that GLP1RA improved lung function in diabetic patients, while non-GLP1RA treatment or insulin treatment did not. Our meta-analysis corroborated the benefits of GLP1RA in managing COVID-19: lower mortality in GLP1RA users was observed compared to non-users.

SGLT2i is a new class of glucose-lowering agents, which has remarkable cardiorenal protective properties, especially in populations with atherosclerosis, heart failure, and reduced renal function (2, 72). Meanwhile, it has been shown to suppress systemic inflammation and fibrosis and alleviate organ damage (72, 73). The only RCT (53) included showed that patients treated with dapagliflozin had a lower but non-significant mortality. Our meta-analysis identified a significantly reduced mortality in SGLT2i users compared with non-users. Before this study, there have been no meta-analyses on the effect of SGLT2i on individuals with diabetes and COVID-19.

By conducting a Bayesian network meta-analysis on these seven antidiabetic agents to compare COVID-19 mortality between different antidiabetic agents, we discovered that GLP1RA had the most significant protective effect on COVID-19 mortality among individuals with diabetes, followed by SGLT2i and metformin. In contrast, insulin was associated with the highest risk of mortality. When metformin was used as a reference, GLP1RA and SGLT2i reduced mortality, whereas insulin increased mortality; no statistical significance was observed in other agents. Our Bayesian network meta-analysis demonstrated a good consistency of direct and indirect comparisons, indicating that the overall results were stable and reliable.

Although our comprehensive meta-analysis established a link between antidiabetic agents and the risk of COVID-19 clinical outcomes, our study has numerous limitations. First, most included studies were observational, including some confounding variables. We performed regression analysis on sample size, mean age, and male proportion. These results showed that these factors did not influence meta-analysis results. However, other confounding factors may remain. Second, in our pairwise meta-analysis, we did not perform an OR-to-RR effective size conversion and pooled OR and RR together. But we performed a subgroup analysis based on OR and RR. The results of subgroup analyses were consistent with those from the overall meta-analysis. Third, most of the included studies were observational, while only one RCT was included in the currently available literatures; thus, these results should be interpreted with caution.

CONCLUSION

In conclusion, our thorough pairwise meta-analysis and Bayesian network meta-analysis revealed that using metformin, DPP4i, GLP1RA, and SGLT2i was high likely associated with decreased mortality in individuals with diabetes and COVID-19, while insulin treatment might contribute to the increased mortality. Using Sulfonylurea or TZDs had no statistically significant association with mortality. Furthermore, the meta-analysis found no association between the risk of severe disease and five antidiabetic agents (metformin, DPP4i, sulfonylurea, insulin, and SGLT2i). Comparative data between these antidiabetic

agents revealed that GLP1RA probably had the most significant protective effect on COVID-19 mortality among individuals with diabetes, followed by SGLT2i and metformin. Insulin was correlated with the highest risk of mortality. More rigorous randomized controlled design investigations are required to further validate these findings.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

YC, XL, and SL: Conceptualization, methodology, data curation, investigation, and writing - original draft preparation. MA and MD: Supervision, writing - reviewing and editing, and funding acquisition. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.895458/full#supplementary-material

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COVID-19 Lockdown and Lifestyle Changes in Saudi Adults With Types 1 and 2 Diabetes

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Objective: We aimed to evaluate and compare the impact of COVID-19 lockdown on lifestyle changes and other common related effects of the lockdown in Saudi adults with diabetes mellitus (DM), both type 1 (T1D) and type 2 diabetes (T2D).

Methods: 265 T1D and 285 T2D individuals were included in this cross-sectional survey during lockdown using an online questionnaire and compared with 297 participants without DM. Variables included demographics, treatment changes, use of supplements, change in sleeping habits and physical activity, dietary changes, social and mental health, and education and awareness during COVID-19 lockdown.

Results: The COVID-19 lockdown was associated with more treatment doses in people with T1D but not in those with T2D ($p = 0.003$). More participants with T1D and T2D than the control group reported that they felt symptoms of depression during lockdown (ORs of 1.83, $p = 0.008$ and 2.2, $p = 0.001$, respectively) and that lockdown affected them psychologically (ORs of 1.64, $p = 0.019$ and 1.85, $p = 0.005$, respectively). More participants with T1D than controls reported that their physical activity decreased during lockdown (OR of 2.70, $p = 0.024$). Furthermore, significantly lesser participants in both DM groups than controls agreed that the health education regarding COVID-19 covered everything (ORs of 0.41, $p < 0.001$ and 0.56, $p < 0.001$, respectively for T1D and T2D groups). Regarding dietary habits, the DM groups reported more changes in either the number of daily meals, meal content, or mealtimes than the control group.

Conclusions: COVID-19 lockdown-associated lifestyle changes were more prevalent in individuals with T1D and T2D compared to control. Findings may assist public health authorities in outlining their responses in pandemics and promote healthy lifestyle adaptations in this high-risk cohort to limit adverse effects in future lockdowns.

Keywords: COVID-19, diabetes, SARS-CoV-2, lifestyle, diet, physical activity

INTRODUCTION

In December 2019, Wuhan became the first city in China to record some unidentified pneumonia cases, which was later declared a global pandemic, referred to as the COVID-19 pandemic, by the World Health Organization (WHO) (1). A novel coronavirus called the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was responsible for this pandemic (2). The pandemic had an unprecedented impact on global health and is responsible for more than 6.1 million human deaths at the time of writing this manuscript (3). Coronaviruses belong to the well-studied Coronaviridae family with a single-stranded positive-sense RNA genome surrounded by an extracellular membrane that contains a case of spike glycoproteins (4, 5). Coronavirus strains known for their lower respiratory tract severe disease characteristics include SARS-CoV (severe acute respiratory syndrome), MERS-CoV (Middle East respiratory syndrome), and the recently identified SARS-CoV-2 (COVID-19) (6–8).

By and large, the global population has had an unprecedented experience of the measures taken by the governments and the local authorities to mitigate the health impact of this pandemic (9). In the same essence, the Saudi authorities implemented a nationwide lockdown in multiple phases in the Kingdom of Saudi Arabia (KSA) on March 15, 2020, following the emergence of the COVID-19 pandemic (10). Though most cases report mild to moderate symptoms; however, those with an established risk of severe COVID-19 symptoms include elderly persons, especially with comorbidities including diabetes mellitus, cardiovascular diseases, and micronutrient deficiencies such as vitamin D deficiency (11–14). Diabetes mellitus (DM) is a chronic disease categorized by abnormally high blood glucose levels triggered by a deficiency in insulin action and/or insulin secretion, and its prevalence worldwide and in the Middle East is estimated to be around 536.6 million (10.5%) and 72.7 million (16.2%), respectively (15). DM usually raises the risk for infectious severity and susceptibility due to its deleterious effects on host immunity (16). Therefore, individuals with diabetes are at a higher risk of viral and bacterial infections resulting in hospitalization and mortality.

Consequently, poorly controlled DM is a risk factor for several infectious diseases (17). Furthermore, the highly proactive measures taken during the pandemic contributed to fear, anxiety and/or depression in the general population (18–20). The pandemic lockdowns during the year 2020 affected the lifestyle of everybody in general and individuals in the high-risk group in particular. There are reports of increased feelings of stress or anxiety due to the lockdown in this group (21). In Saudi Arabia, multiple studies reported the effects of COVID-19 lockdown on the lifestyles of the population in general, with many highlighting changes in sleep hours, physical activity, diet, stress and anxiety levels, etc. (22–25).

The research related to COVID-19 infection and the effects of resulting lockdowns is a rapidly evolving field at a global level. Several studies have reported the impact of COVID-19 on DM patients, implying that COVID-19 may increase the complications of the disease among those with DM (26, 27). COVID-19 complications in people with diabetes are generally caused by an imbalance in the angiotensin-converting enzyme 2 (ACE2) activation pathways, which leads to an inflammatory response. In the pancreas, this imbalance leads to acute-cell dysfunctionality and complications such as vasculopathy (28). As a result, individuals with diabetes were advised to take specific preventive measures during the pandemic lockdowns (29, 30). However, much is unknown about the dynamics of this disease, and more research to better understand the clinical determinants of disease severity can help improve patient care throughout the healthcare system (31).

It is reasonable to assume that COVID-19 lockdown and associated restrictions may have altered dietary habits, physical activity levels, mental wellbeing, treatment plans, ease of getting medicines and medical advice on time, and sleeping patterns in patients with DM (32, 33), however, there is limited data on such information, particularly in this region. As a result, the current study sought to assess the impact of the COVID-19 lockdown in KSA on individuals with type 1 (T1D) and type 2 (T2D) diabetes compared with the general population using various lifestyle and health parameters such as changes in physical activity habits and dietary patterns to identify high risk timely tailored parameters in the two DM cohorts.

MATERIALS AND METHODS

Study Participants and Study Design

In KSA, an online electronic survey during the COVID-19 pandemic lockdown was conducted between March 23 and June 20, 2020. This survey was done in collaboration with the Saudi Charitable Association of Diabetes (SCAD), which provides healthcare services to the diabetes population in KSA. The population chosen for our study was the subscribers of SCAD from different regions (Northern, Center, Western, Eastern, and Southern) in KSA. Participants voluntarily joined in this survey, and individuals with diabetes all vary in the disease's progression and treatments. The inclusion criteria included adult Saudis who were in KSA during the lockdown and had access to the internet. In addition, those with previously diagnosed diabetes were categorized as T1D or T2D groups, and the rest of the respondents with no history of diabetes were categorized as the control group. There were no additional exclusion criteria other than failure to complete the questionnaire.

Ethical approval for this study was obtained from the Ethics Committee for Scientific Research and Post Graduate Studies at the College of Science, King Saud University (KSU), Riyadh, KSA. The online electronic survey also included the informed consent form, which had to be agreed upon by the respondents before participating in the survey.

Questionnaire

A standardized questionnaire was adapted from earlier studies (23, 34–36), and the online link of the Arabic version of the questionnaire prepared was made available by SCAD to its subscribers for distribution through different social media platforms throughout KSA. The questionnaire used had three sections:

TABLE 1 | Anthropometric and demographic differences in the study groups.

Parameter	Study groups			*P*-value		
	Control	T1D	T2D	T1D vs. control	T2D vs. control	T2D vs. T1D
N	**297 (92/205)**	**265 (117/148)**	**285 (177/108)**			
Age (years)	38.7 ±10.1	43.2 ± 15.0	52.3 ± 9.7	<0.001	<0.001	<0.001
BMI (kg/m^2)	28.9 ± 8.7	26.9 ± 6.3	29.9 ± 5.6	0.004	0.50	<0.001
Region						
North	16 (5.4)	11 (4.2)	7 (2.5)			
Central	132 (44.4)	134 (50.6)	148 (51.9)			
West	41 (13.8)	55 (20.8)	65 (22.8)	<0.001	<0.001	0.76
East	58 (19.5)	51 (19.2)	49 (22.8)			
South	50 (16.8)	14 (5.3)	16 (5.6)			
Occupation						
In-person?	148 (49.8)	67 (25.3)	100 (35.1)	<0.001	<0.001	0.016
Working remotely?	148 (49.8)	108 (40.8)	97 (34)	0.034	<0.001	0.12
Retired?	18 (6.1)	65 (24.5)	114 (40.0)	<0.001	<0.001	<0.001

Data presented as mean ± SD for continuous variables and frequency (%) for categorical variables. T1D and T2D represent type 1 and 2 diabetes groups, respectively. P < 0.05 is considered significant.

Section 1

This section included questions for getting demographic and anthropometric data, where information on participants' gender, age, weight, height, and the region was asked (information presented in **Table 1**).

Section 2

This section included information on COVID-19 pandemic-related parameters like medication and treatment doses, micronutrient supplements and lifestyle habits, etc., during the lockdown were asked through yes/no questions (information presented as **Table 2**).

Section 3

This section had 16 closed format questions in the Likert scale divided into four sub-sections. It provided information about the level of agreement in "COVID-19 measures," "dietary changes," "changes in social and mental health," and "awareness" among participants during COVID-19 lockdown derived from "4," "6," "3" and "3" questions respectively (information presented as **Table 3**). The scale used to calculate the level of agreement was "strongly agree," "agree," "neutral," "disagree," and "strongly disagree."

The reliability of the Arabic version of the questionnaire (**Supplementary File 1**) was tested in a pilot study, also organized by the SCAD, on 50 samples. The reliability test in all four sub-sections of the Linkert scale section of the questionnaire achieved a Cronbach's alpha of >0.7 with an overall score of 0.84 for a total of 16 items (**Supplementary Table S1**).

Data Analysis

Sample size calculation was done using Raosoft online. The sample size needed was calculated using an error margin of 2.5%, and the prevalence of T1D and T2D was 0.03 and 18.3%, respectively. To get a confidence level of 95%, a minimum of 500 sample size was set to achieve the study's objectives. All calculations and data analysis was done using SPSS version 16.5. The data in three study groups were compared using chi-square test for categorical variables and the independent student's *t*-test for continuous variables. The agreement levels for sections "COVID-19 measures," "Dietary changes," "Social and Mental Health during COVID-19 lockdown," and "Health education and awareness" were presented as frequency (%), and an average value of scores for all the items in each section was presented as mean ± standard deviation.

Logistic regression analysis (univariate and multivariate) was run separately with T1D/T2D versus control as dependent variable and close-ended questions as independent variables. In the multivariate model, adjustment was made with age, gender, BMI, and demographic variables. A 95% confidence interval (95% CI) for the odds ratio (OR) was checked to evaluate significant independent categorical variables. Statistical significance was set at $p < 0.05$.

RESULTS

Anthropometric and Demographic Differences Between the Study Groups

Table 1 shows the anthropometric and demographic differences among study groups. The subjects in the T2D group were significantly older and had higher BMI compared to both T1D and controls s ($p < 0.001$). Majority of all participants (about 50%) were from the central region. The control group also had

TABLE 2 | Differences in responses to yes/no questions in the study groups.

Parameter	Study groups			*P*-value		
	Control	T1D	T2D	T1D vs. control	T2D vs. control	T2D vs. T1D
N	**297 (92/205)**	**265 (117/148)**	**285 (177/108)**			
DM treatment during pandemic						
Taking medication		249 (94.0)	267 (93.7)	—	—	0.52
Insulin		199 (75.1)	88 (30.9)	—	—	<0.001
Pills		96 (36.2)	248 (87.0)	—	—	<0.001
DM management plan changed during pandemic?		34 (12.8)	38 (13.3)	—	—	0.48
Treatment doses increased during pandemic?		52 (19.6)	27 (9.5)	—	—	0.003
Treatment doses decreased during pandemic?		28 (10.6)	30 (10.5)	—	—	0.91
Whether respondents on diet management?	88 (43.1)	42 (15.8)	55 (19.3)	<0.001	<0.001	0.31
Whether respondents on weight loss management?	127 (42.8)	36 (13.6)	48 (16.8)	<0.001	<0.001	0.020
On diet + weight loss management?	108 (46.6)	55 (20.8)	58 (20.4)	<0.001	<0.001	0.68
Supplement use						
Vitamin D?	150 (50.5)	103 (38.9)	122 (42.8)	0.004	0.038	0.20
Vitamin C?	201 (67.7)	151 (57.0)	171 (60.0)	0.006	0.033	0.26
Zinc?	119 (40.1)	55 (20.8)	66 (23.2)	<0.001	<0.001	0.28
Psychological changes						
Insomnia?	121 (40.7)	99.4 (37.4)	107 (37.5)	0.43	0.44	0.52
Depression?	62 (20.9)	47 (17.7)	49 (17.2)	0.20	0.15	0.48
Sleeping habits						
Sleeping hours increased during Covid-19?	129 (43.4)	122 (46.0)	103 (36.1)	0.55	0.08	0.02
Was your sleep continuous?	133 (44.8)	121 (45.7)	124 (43.5)	0.86	0.80	0.61
Do you take sleeping pills?	23 (7.7)	9 (3.4)	14 (4.3)	0.030	0.18	0.40
Physical activity						
Did exercise increase during lockdown?	118 (39.7)	102 (38.5)	81 (28.4)	0.79	0.005	0.014
Did exercise decrease during lockdown?	128 (43.1)	97 (36.6)	134 (47.0)	0.12	0.36	0.015
Walking?	194 (65.3)	171 (64.5)	184 (64.6)	0.87	0.86	0.99
Cycling?	21 (7.1)	22 (8.3)	20 (7.0)	0.63	0.98	0.63
Home sports?	159 (53.5)	137 (51.7)	96 (33.7)	0.67	<0.001	<0.001
Swimming?	23 (7.7)	29 (10.9)	22 (7.7)	0.24	0.99	0.24
Length of exercise during Covid-19						
<10 min?	82 (27.6)	58 (21.9)	91 (31.9)!	0.12	0.28	0.009
10–30 min?	87 (29.3)	80 (30.2)	69 (24.2)	0.85	0.19	0.13
30–45 min?	29 (9.8)	34 (12.8)	27 (9.5)	0.29	0.90	0.22
46–60 min?	22 (7.4)	26 (9.8)	32 (11.2)	0.36	0.12	0.68
More than 60 min?	6 (2.0)	11 (4.2)	9 (3.2)	0.22	0.44	0.65
Methods preferred in health education						
Remote communication?	140 (47.1)	100 (37.7)	85 (29.8)			
Social media?	129 (43.4)	141 (52.2)	177 (62.1)	0.06	<0.001	0.10
Others?	28 (9.4)	24 (9.1)	23 (8.1)			
Entities followed for COVID-19 information						
SCAD?	90 (30.3)	45 (17.0)	40 (14.0)			
YouTube?	81 (27.3)	60 (22.6)	90 (31.6)	<0.001	<0.001	0.06
Other associations?	26 (8.8)	35 (13.2)	25 (8.8)			
Others?	100 (33.7)	125 (47.2)	130 (45.6)			
Were you diagnosed with COVID-19?	41 (13.8)	41 (15.5)	44 (15.4)	0.63	0.64	0.99
If yes, did you experience						
Fever?	14 (34.1)	16 (39.0)	19 (43.2)	0.82	0.51	0.83
Cough?	11 (26.8)	15 (36.6)	11 (25.0)	0.48	0.85	0.35
Loss of taste and smell?	11 (26.8)	12 (29.3)	16 (36.4)	0.81	0.36	0.50
Shortness of breath?	7 (17.1)	5 (12.2)	12 (27.3)	0.76	0.31	0.11
Throat pain?	7 (17.1)	11 (26.8)	16 (36.4)	0.42	0.05	0.36

Data presented as frequency (%). T1D and T2D represent type 1 and type 2 diabetes groups, respectively. P < 0.05 is considered significant.

TABLE 3 | Differences in the levels of agreement according to COVID-19 lockdown.

Questions	Control (297)	T1D (265)	T2D (285)	*P*-value
COVID-19 measures during lockdown				
1. Adherence to COVID-19 preventive measures?	271 (91.2)	254 (95.8)*	270 (94.7)	0.02
2. Did you maintain social distance?	225 (75.8)	176 (66.4)	187 (65.6)	0.06
3. Did your family maintain social distance?	206 (69.4)	187 (70.6)	168 (58.9)$	0.03
4. Do you think diabetes is a risk factor for corona?	195 (65.7)	171 (64.5)	179 (62.8)	0.43
Total score (Maximum 4)	3.18 ± 1.1	3.27 ± 0.9	3.11 ± 0.9	0.17
Dietary changes during lockdown				
5. Eating habits changed?	153 (51.5)	90 (34.0)*	99 (34.7)*	<0.001
6. Mealtimes changed?	148 (49.8)	82 (30.9)*	73 (25.6)*	<0.001
7. Number of meals changed?	146 (49.2)	81 (30.6)*	70 (24.6)*	<0.001
8. Meal content changed?	148 (49.8)	76 (28.7)*	84 (29.5)*	<0.001
9. More fast food intake?	196 (66.0)	168 (63.4)	171 (60.0)	0.68
10. Decrease in homemade food intake?	16 (5.4)	10 (3.8)	18 (6.3)	0.69
Total score (Maximum 6)	2.81 ± 1.8	2.0 ± 1.6*	1.90 ± 1.6*	<0.001
Changes in social and mental health during lockdown				
11. Relationship with family got affected?	18 (6.1)	10 (3.8)	11 (3.9)	0.37
12. Has lockdown affected you psychologically?	135 (45.5)	132 (49.8)	133 (46.7)	0.25
13. Felt symptoms of depression?	91 (30.6)	95 (35.8)	92 (32.3)	0.51
Total Score (Maximum 3)	1.01 ± 0.9	1.14 ± 0.9	1.10 ± 0.9	0.28
COVID-19 education and awareness during lockdown				
14. Do you think health awareness for Covid-19 was carried out previously?	225 (75.8)	151 (57.0)*	176 (61.8)*	<0.001
15. Did you make maximum use of health education?	226 (76.1)	146 (55.1)*	167 (58.6)*	<0.001
16. Do you think health education for Covid-19 covered all educational needs?	211 (71.0)	148 (55.8)*	173 (60.7)*	0.003
Total score (Maximum 3)	2.23 ± 1.1	1.68 ± 1.3*	1.81 ± 1.3*	<0.001

*Data presented as frequency (%) for categorical variables and mean ± SD for the total score in each section. T1D and T2D represent type 1 and 2 diabetes groups, respectively. * and $represent the differences compared to the "control" and "T1D" groups. P < 0.05 is considered significant.*

the highest prevalence of employed participants (be it working remotely or in-person) than DM groups with a higher prevalence of "retired from work" participants.

Differences in Responses to Yes/No Questions in the Study Groups

Table 2 shows the information obtained *via* yes/no questions. As expected, most DM groups took medications with higher proportions of insulin and oral hypoglycemic pills in the T1D and T2D groups, respectively ($p < 0.001$ for both). Dietary and weight loss management were overwhelmingly from the control group. The use of dietary supplements such as vitamins D, C, and zinc was highest in the control group ($p < 0.05$ for all). There was no difference in the use of supplements between the DM groups. When asked whether sleeping hours increased during the lockdown, less than half in all groups (43.4, 46, and 36.1% in control, T1D and T2D, respectively) said "yes," and a small number indicated that they were on sleeping pills. Participants whose exercise decreased during lockdown were highest in the T2D group (47.0%). Those with <10 min/per day of exercise were higher in T2D than T1D participants ($p = 0.009$). When asked if they were infected with COVID-19, around 15% of study groups answered "yes." There was no difference in the prevalence of opting for different COVID-19 symptoms experienced.

Levels of Agreement for COVID-19-Related Questions

Table 3 shows the level of agreement of participants with respect to the COVID-19-related questions. The complete data for these sections in the questionnaire has been tabulated as **Supplementary Table S2**. The majority in all groups agreed that they followed COVID-19 preventive measures during the lockdown (>90% in each group). There was no difference statistically when the total score for all four items of the section "COVID-19 measures" was checked. More participants from the control group agreed that their eating habits changed, as well as mealtimes, meal content, and the number of meals than both diabetes groups ($p < 0.001$ for all). No difference was observed between groups related to changes in social and mental health issues during the lockdown. Lastly, the majority (>55%) agreed that health awareness campaigns by SCAD for COVID-19 were carried out previously. A lower proportion in both diabetes groups than control agreed that awareness campaigns covered all aspects of COVID-19-related information and that they maximized the information given ($p < 0.01$ in both).

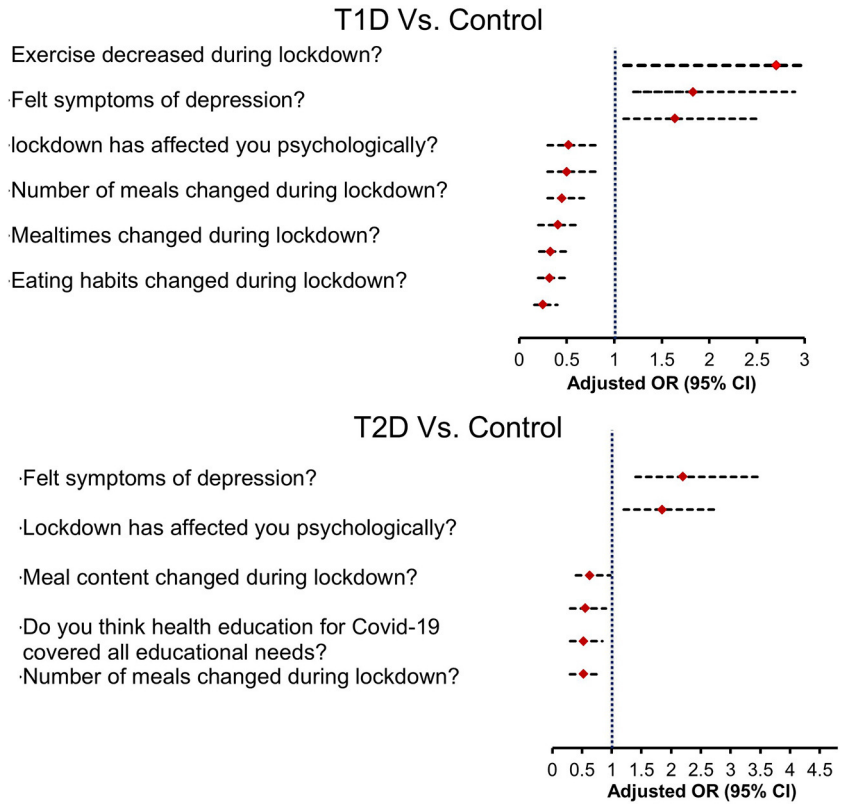

FIGURE 1 | Forest plot depicting adjusted odds of responses related to lifestyle changes in diabetes vs. control groups.

Multinomial Regression for Calculating the Odds Ratio of Lifestyle Changes in Diabetes vs. Control Groups

Multinomial logistic regression was done to examine differences between DM and control groups in the responses. Only significant independent variables were plotted as a forest plot in **Figure 1**. Data of the logistic regression analysis, including OR, 95% confidence interval, and *p*-values, has been provided as **Supplementary Table S3**. The regression model was adjusted with the participants' age, sex, BMI, and demographic status to extract the multivariate odds plotted in the figure. The analysis showed that factors like decreased exercise, depression, and use of supplements showed greater odds in diabetes groups compared to the control groups. However, the dietary changes like changes in meal content, number of meals, and mealtimes during lockdown showed lesser odds in diabetes groups compared to the control group. Also, the DM groups felt a lack of sufficient COVID-19 related information and health awareness during the lockdown than the control group.

DISCUSSION

The consequences of COVID-19 lockdown on the lifestyle of the general population and high-risk groups have been the area of interest because of this pandemic's massive impact on humankind. However, less has been investigated primarily in this population, where there is a huge prevalence of diabetes. Therefore, the present study aimed to evaluate and compare lifestyle changes due to COVID-19 lockdown in a population of adult Saudis with T1D and T2D. The study suggests lifestyle changes in terms of dietary, physical activity, sleeping hours, etc., were more prevalent in diabetes groups compared to the control. Also, the DM groups reported higher prevalence of insufficient health education and awareness related to COVID-19. Besides, the self-assessed changes in the psychological status of the respondents, like insomnia and depression, were also reported by a sizable proportion in all groups.

Diabetes, a chronic disease, is highly prevalent in this part of the world (18.3% of adults according to the International Diabetes Federation) (37), and it imposes a significant burden at the individual, familial, community, and global levels. Compliance with medical treatment is one essential lifestyle component for those with diabetes, and complications caused by uncontrolled glucose levels may occur because of this non-compliance. Our study suggested that the treatment doses increased during lockdown for those with T1D than T2D. This might have resulted from increased uncontrolled glycemia in T1D patients during the lockdown because of less availability of insulin medications. The study by Magliah et al. (38) reported that 66% of T1D patients had difficulty obtaining at least one type of their insulin medication supply during the lockdown.

Reports also suggested that COVID-19 lockdown negatively affected weight and glucose control in individuals with diabetes, mainly in patients on insulin (39). In this lockdown, the emergency services were prioritized, and routine care services, which form the core of managing chronic diseases like diabetes, were jeopardized, increasing the risk of associated complications. Furthermore, the COVID-19 lockdown affected other aspects of the pharmacological and non-pharmacological management of DM, including access to doctor consultation, counseling services, availability of recommended dietary items, social interaction, restricted physical activity, amongst others (40, 41).

A large proportion of participants indicated that they had insomnia (>35%) and irregular sleep (>55%) during the lockdown. Besides, fewer subjects in the T2D group reported that the sleeping hours increased during lockdown compared to the T1D group. These results align with multiple studies that reported an increase in insomnia during COVID-19 lockdown (42–44). The primary cause for this has been the fear of getting infected, the economy-related stress, the social isolation and changes in social rhythms (44, 45). Sleep quantity and quality, though important in the general population, are more critical in managing diseases like diabetes, which require a multidimensional approach including adequate and time-sensitive quality sleep, a lack of which may increase diabetes severity and cardiovascular comorbidities (46, 47). Lack of proper sleep may also aggravate anxiety and depression, and a sizable proportion, even though self-assessed, in all three groups (>45%), agreed that the lockdown affected them psychologically. Therefore, an integrated multidimensional approach, including counseling and training on self-care behaviors, may be needed to mitigate the mental health decline in individuals with diabetes during the COVID-19 lockdown (48, 49).

Physical activity and exercise rates decreased during the lockdown in all groups. Apart from the glycemia-controlling medicines, exercise training programs especially aerobic exercises, have emerged as a useful tool in managing diabetes (50). In line with the results of our study, many recent studies highlighted a significant increase in physical inactivity in both the general population and in individuals with chronic diseases during lockdown (51). Studies indicated that lockdown measures were associated with significantly reducing physical activity levels in chronic patients like those on hemodialysis (52). Others, like one from the United Kingdom, reported a significant decrease in physical activity during this lockdown in the general population (53). Simple physical activity regimens developed and promoted to the public may help mitigate this problem in future pandemics (54).

The dietary guidelines announced by the World Health Organization to boost immunity during the COVID-19 outbreak included taking a balanced diet with the recommended amount of macro and micronutrients; however, the literature suggests that the lockdown resulted in less consumption of fresh produce and more consumption of comfort foods (55). This may have both short and long-term health effects. Earlier reports from Italy also reported that 46.1% of the study participants felt they ate more during the lockdown, particularly high-calorie foods such as chocolate, ice cream, and desserts (56). In this study, more individuals reported changes in eating habits, mealtimes, meal quantity, and quality in the control group compared to the diabetes groups. Sedentary behaviors, including increased screen time, prevalent during confinement, may directly be associated with an increase in unhealthy dietary patterns in lockdown (57). While important in the general population, good dietary practices are a prerequisite for managing the HbA1c levels in diabetes (58). Thus, stakeholders involved in diabetes care must conduct interventions aimed at increasing awareness in diabetes patients about appropriate self-care.

The results of this study could help health authorities and diabetes organizations like SCAD, which provide health care services to people with diabetes in Saudi Arabia, better handle these services in the event of a pandemic. According to the American Diabetes Association, conventional diabetes care for patients involves glucose management and monitoring, timely advice on food and physical exercise, availability, and distribution of medicines, and efforts to prevent infection in high-risk populations. This lockdown taught us a lot about how to better handle a future pandemic by showing us what not to do when it comes to access and availability of glycemic monitoring supplies like glucose strips, glucometers, and needles, among other things. In the event of future pandemics, findings of this study on dietary supplements may assist DM management organizations in emphasizing the need to focus on logistics to ensure that there are enough dietary supplements available and that these supplements are disseminated to patients. Participants in both DM groups believed that COVID-19 information was insufficient, which might assist the authorities in creating better lifestyle interventions for diabetics and other high-risk persons in future pandemics. The Findings on depression and the psychological effects of lockdown in diabetics may also help these organizations develop solutions for mental health, anxiety levels, the significance of enough sleep, and so on in high-risk individuals.

The strengths of the study included a relatively large number of respondents and the subjects recruited from different geographical regions of KSA. To the best of the authors' knowledge, this study is the first report of lifestyle changes during COVID-19 lockdown in both T1D and T2D Saudi subjects. At the same time, the authors would like to acknowledge some limitations which should be considered in interpreting the study findings. Firstly, this study was cross-sectional, which might introduce a potential risk of recall bias. However, our study intended to be a comparison analysis between the study groups. Secondly, participants in this study self-reported lifestyle changes during lockdown with no specific personality assessment like ones for psychological statuses like insomnia and depression, and they might have underestimated these changes. Thirdly, members of SCAD were recruited as participants for the study, which might have had some bias in the socio-economic class of overall Saudi subjects. Nevertheless, the study revealed some important findings about lifestyle changes in DM individuals during pandemic. The findings may help the concerned authorities and organizations prioritize interventions to handle DM management in future lockdowns.

CONCLUSIONS

Our results show that the lockdowns imposed as restrictive measures against COVID-19 negatively affected the lifestyle and daily habits of Saudi individuals with DM. The present findings should help public health authorities and stakeholders involved in diabetes care to plan for future pandemics and promote healthy lifestyle changes in high-risk populations during lockdowns.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Post-Graduate Studies, College of Science, King Saud University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

NA-D, AA, and SS designed the study. DA and GA worked on the methodology. MK did the statistical analysis. AA, HA, AA-H, and DA helped in the data curation. AA and KW wrote the first draft. NA-D, HA, and SS revised the manuscript. NA-D, NA, and HA did the study supervision. All authors contributed and approved the final version of the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.912816/full#supplementary-material

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Unmanaged Diabetes and Elevated Blood Glucose Are Poor Prognostic Factors in the Severity and Recovery Time in Predominantly Hispanic Hospitalized COVID-19 Patients

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Keywords: COVID-19, hyperglycemia, type 2 diabetes (T2D), hispanic, unmanaged diabetes, blood glucose

Diabetes is considered to influence the severity and length of hospital stay (LOS) in COVID-19 patients. It is not known whether lack of diabetes management may magnify the severity and LOS in COVID-19 patients with diabetes. The purpose of the study was to determine the impact of unmanaged diabetes and acute glycemia on severity and LOS among hospitalized COVID-19 patients. This retrospective study used medical records from patients admitted to the University Medical Center, El Paso, TX with COVID-19. Glycemic control was assessed by fasting blood glucose (acute) and HbA1c (chronic); COVID-19 severity was measured by quick sepsis-related organ failure assessment (qSOFA) and the LOS was determined by the number of days spent in the hospital. Diabetes management with medication was self-reported by patients. There was no significant difference in severity and LOS between patients with and without diabetes. However, patients with unmanaged diabetes showed significantly greater severity and LOS compared to patients who managed diabetes. Patients with an elevated fasting blood glucose level also had a greater COVID-19 severity and LOS. In conclusion, unmanaged diabetes and blood glucose showed worsened severity and prolonged hospitalization in COVID-19 patients. Diabetes management should be considered in treatment of COVID-19 patients.

INTRODUCTION

After the recognition of Coronavirus disease (COVID-19), the virus has rapidly spread and became a pandemic. Diabetes is a significant risk factor for elevated mortality rates in a wide array of diseases, including infectious diseases (1). Poor glycemic control and chronic hyperglycemia have been associated with morbidity and mortality (2). Diabetes and unmanaged glycemia were reported as significant predictors of severity and deaths in patients infected with viruses such as the 2009

pandemic influenza A virus (H1N1) and Middle East respiratory syndrome coronavirus (MERS-CoV) (3). While some studies reported no clear association between severity of COVID19 with diabetes (4, 5), others reported a greater risk for COVID-19 severity, length of hospital stay (LOS), and mortality among patients with diabetes (6–8). Patients with diabetes may be susceptible to more severe SARS-CoV-2 infection due to immune system dysfunction (9). Viral infections could also induce a diabetes state, or worsen hyperglycemia in people with diabetes, which may adversely influence prognosis (10).

Whereas an alarming proportion of the US population is diagnosed with diabetes, a significant proportion of the population is undiagnosed or does not manage diabetes. Yet, nearly 1 in 5 Americans with diabetes report that due to the increased financial constraints of the pandemic, they had to choose between buying food or buying medications and medical supplies required to manage their diabetes (11). Moreover, the Hispanic/Latino population is disproportionately impacted by diabetes, a population 50% more likely to have diabetes and 2.4 times more likely to die of COVID-19 than white Americans (11). Management of diabetes with hypoglycemic medication plays an important role in reducing diabetes-related diseases (12). Although it is clear from the present literature that both acute and chronic management of glycemia are important, it is not clear how unmanaged diabetes and acute glycemic control may affect the severity and the LOS in COVID-19 patients. Therefore, the purpose of this study was to determine the impact of unmanaged diabetes and acute glycemia on the severity and LOS among hospitalized, predominantly Hispanic and Latino, patients with COVID-19.

MATERIALS AND METHODS

Study Design and Population

This is a single-center, retrospective, observational study that used data from the medical records of 858 confirmed COVID-19 cases admitted at the University Medical Center, El Paso, Texas between August, and September 2020. This study was approved by the institutional review board at the University of Texas at El Paso. From 858 patients who were initially included in this study, 369 patients met the inclusion criteria of having both hemoglobin A1c (HbA1c) and fasting blood glucose (FBG) data available at the time of admission (age 60.0 ± 16.6 years; BMI 30.3 ± 7.16 Kg/m^2). Diabetes status was determined by following ADA guidelines for diabetes classifications of HbA1c level 6.5% or above at the time of the hospital admission. Patients with diagnosed diabetes (n=246) were further categorized into managed (n=182) and unmanaged (n=64) diabetes groups based on their self-reported diabetes management status with hypoglycemic medication (e.g. insulin sensitizer, insulin) at the time of hospitalization. In this study we have defined hyperglycemia in the context of diabetes following the American Diabetes Association criteria for diagnosis of diabetes using FBG≥126mg/dL or HbA1c≥6.5% (13). To evaluate the role of acute and chronic glycemic control on COVID-19 severity and LOS, all patients (n=364) were categorized into 4 groups based on their diabetes status evaluated by acute (FBG) and chronic (HbA1c) glycemic status at the time of hospitalization (**Figure 2**); Group 1 (G1): HbA1c<6.5%), FBG<126 mg/dl, Group 2 (G2): HbA1c<6.5%, FBG≥126 mg/dl, Group 3 (G3): HbA1c≥6.5%), FBG<126 mg/dl), and Group 4 (G4): HbA1c≥6.5%), FBG≥126 mg/dl).

Data Collection

Clinical records and laboratory information for all patients hospitalized with confirmed COVID-19 were collected for this study. Information on patient demographics and health history data were collected. Acute and chronic glycemic control were assessed using FBG and HbA1c respectively during the time of hospital admission. The severity of the COVID-19 outcome was assessed by quick sepsis-related organ failure assessment (qSOFA) score (14) which identifies patients at high risk for in-hospital mortality based on systolic blood pressure, respiratory rate, and altered mental status from baseline. A qSOFA score of 2-3 indicates a high risk for in-hospital mortality compared to a lower score (15). The LOS was determined by the number of days spent in the hospital.

Statistical Analysis

Data were analyzed using PRISM Graph Pad Software, version 8.0 (Graph Pad Software, La Jolla, California). Unpaired Student's T-test and one-way ANOVA with Tukey *post hoc* analysis were used to compare means among two or more groups respectively, as appropriate. p-values less than 0.05 was considered statistically significant. All tables and figures represent data as mean and standard deviation (SD).

RESULTS

No Difference in COVID-19 Severity and Length of Hospital Stay Based on Diabetes Status

The severity of COVID-19 measured by qSOFA score was not different between patients without and with diabetes groups (0.34 ± 0.49 vs. 0.28 ± 0.5; p=0.30) groups (**Table 1**). LOS was also similar between the two groups (8.15 ± 8.09 vs. 8.9 ± 9.53 days; p=0.47) (**Table 1**).

Lesser COVID-19 Severity and Shorter Duration of Hospital Stay In Patients Who Managed Diabetes With Hypoglycemic Medication

Patients who reported to have managed diabetes with hypoglycemic medication showed significantly lesser severity, assessed by qSOFA index, compared to those who reported to not manage diabetes (0.22 ± 0.44 vs. 0.44 ± 0.62; p<0.01) (**Figure 1A** and **Table 2**). Patients who managed diabetes also had significantly shorter LOS compared to patients with unmanaged diabetes (8.18 ± 8.04 vs. 10.98 ± 12.77 days; p<0.05) (**Figure 1B**). Consistent with these findings, when

TABLE 1 | Study subject characteristics.

	No Diabetes/Prediabetes	Diabetes	p value
Number of subjects	123	246	
Sex (male/female)	70/53	140/106	
Age (years)	59.41 ± 17.80	60.33 ± 14.51	0.60
Ethnicity (Hispanic/Non-Hispanic)	114/9	224/22	
Body Mass Index (BMI) (kg/m^2)	27.23 ± 9.44	30.65 ± 6.22	0.18
Fasting Blood Glucose (mg/dl)	120.6 ± 42.01	214.9 ± 120.1	<0.0001****
Glycated Hemoglobin (HbA1c) (%)	5.9 ± 0.44	9.0 ± 2.40	<0.0001****
Blood Pressure (Systolic) (mmHg)	128.6 ± 25.89	135.3 ± 24.32	0.01*
Blood Pressure (Diastolic) (mmHg)	73.05 ± 14.32	75.8 ± 14.52	0.08
Heart Rate (beats/min)	93.86 ± 19.77	97.13 ± 17.65	0.11
Hemoglobin (Hgb) (g/dL)	13.05 ± 2.56	13.85 ± 6.84	0.21
Alanine aminotransferase (ALT) IU/L	48.55 ± 43.15	44.95 ± 48.71	0.49
Aspartate aminotransferase (AST) IU/L	58.2 ± 46.25	54.47 ± 57.30	0.53
Blood Urea Nitrogen (BUN) (mg/dL)	22.30 ± 18.25	23.17 ± 17.73	0.66
Hospital stays (days)	8.15 ± 8.09	8.88 ± 9.53	0.47
qSOFA index	0.34 ± 0.49	0.28 ± 0.50	0.30

*qSOFA, quick sepsis-related organ failure assessment; Data Presented as mean ± SD. *p<0.05; ****p<0.0001.*

severity was compared among three groups including patients without diabetes (one-way ANOVA), unmanaged diabetes group showed significantly greater severity compared to patients with managed diabetes (0.22 ± 0.44 vs. 0.44 ± 0.61; p<0.05). Characteristics of the patients with managed and unmanaged diabetes is provided in **Table 2**.

Role of Acute and Chronic Glycemic Control on COVID-19 Severity and Length of Hospital Stay

Patients were categorized for the acute and chronic glycemic status (**Table 3** and **Figure 2**). Patients diagnosed with diabetes by FBG but not by HbA1c (G2) experienced a greater COVID-19 severity, measured by qSOFA, compared to the other 3 groups. (G2: 0.61 ± 0.79 vs. G1: 0.24 ± 0.46; p<0.01, G2: 0.61 ± 0.79 vs. G3: 0.16 ± 0.42; p<0.01, and G2: 0.61 ± 0.79 vs. G4: 0.31 ± 0.52; p<0.05) (**Figure 3A**). Patients in G2 also had significantly longer LOS compared to patients in G1 (G2: 12.91 ± 11.61 vs. G1: 6.36 ± 5.21 days; P<0.01) (**Figure 3B**).

DISCUSSION

This study aimed to gain insight into the impact of unmanaged diabetes and acute and chronic glycemic control on COVID-19 infection severity and recovery in COVID-19 patients. Our study reveals unmanaged diabetes and acute glycemia are key factors in COVID-19 severity and complications, among predominantly Hispanic and Latino populations.

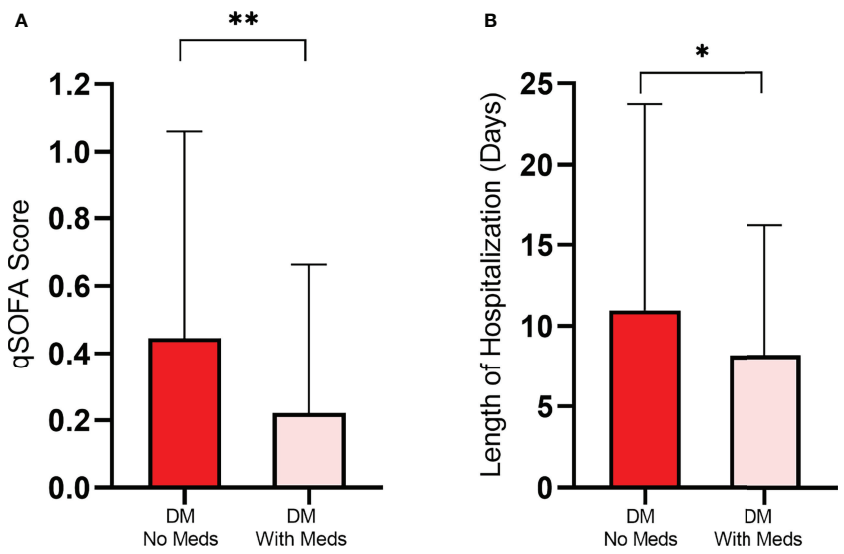

FIGURE 1 | Severity **(A)** and length of hospital stay **(B)** among hospitalized COVID-19 patients with diabetes (DM) were significantly greater in patients with unmanaged diabetes (without medication) compared to patients who managed their diabetes with medication. Data presented as mean ± SD. *p<0.05, **p<0.01.

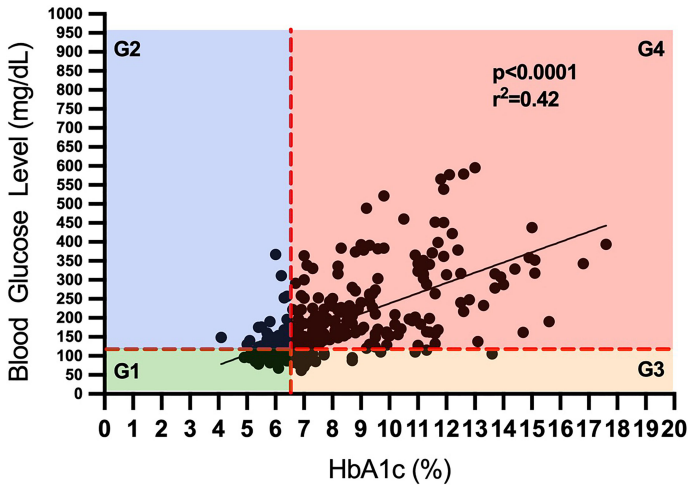

FIGURE 2 | Categorization of patients for the diabetes status based on their fasting blood glucose and glycated hemoglobin level.

We report no difference in COVID-19 severity and LOS among hospitalized patients based on diabetes status determined by HbA1c level in a predominantly Hispanic and Latino study population. Several meta-analyses have indicated greater severity of COVID-19 in patients with diabetes (16, 17) whereas, similar to our report, others have shown no difference in COVID-19 severity based on diabetes status (18) or established cardiovascular disease (19). Here using qSOFA, an objective clinical score identifying patients with suspected infection at greater risk for a poor outcome and increased in-hospital mortality, we report no difference in COVID-19 severity and LOS in a predominantly Hispanic/Latino population.

Although COVID-19 severity and LOS were not impacted by diabetes status in our study population, diabetes management status significantly impacted COVID-19 severity and LOS among COVID-19 patients with diabetes. Patients with unmanaged diabetes showed significantly greater severity of COVID-19 and longer LOS compared to those who managed diabetes with hypoglycemic medications. Our results are in line with other reports that indicated uncontrolled diabetes patients are at a greater risk for severe illness and COVID-19 severity (7, 20). Our data also clearly demonstrate the importance of diabetes management on COVID-19 severity and LOS. Although insulin therapy is an effective method for achieving glycemic targets and improving the clinical outcome of COVID-19 (20), a significant number of patients with diabetes report financial challenges to manage diabetes, which may have a greater impact on poor prognoses of patients with limited financial means (11).

Given that uncontrolled diabetes reflected worsened the severity of COVID-19, we further evaluated how acute and chronic glycemic control may impact COVID-19 severity and LOS irrespective of the diabetes status of the study population. Patients were categorized into 4 groups based on low or high acute (measured by FBG) and chronic (measured by HbA1c)

TABLE 2 | Study subject characteristics.

	Diabetes (managed)	Diabetes (not managed)	p value
Number of subjects	182	64	
Sex (male/female)	102/80	40/24	
Age (years)	61.88 ± 14.14	58.27 ± 15.39	0.09
Ethnicity (Hispanic/Non-Hispanic)	166/16	58/6	
Body Mass Index (BMI) (kg/m^2)	31.04 ± 6.6	29.40 ± 4.75	0.07
Fasting Blood Glucose (mg/dl)	216.4 ± 101.5	160.9 ± 63.11	<0.0001****
Glycated Hemoglobin (HbA1c) (%)	9.4 ± 2.42	7.76 ± 1.85	<0.0001****
Blood Pressure (Systolic) (mmHg)	136.3 ± 24.32	132.4 ± 24.46	0.27
Blood Pressure (Diastolic) (mmHg)	76.08 ± 15.13	74.65 ± 12.56	0.50
Heart Rate (beats/min)	96.46 ± 18.54	98.63 ± 14.62	0.40
Hemoglobin (Hgb) (g/dL)	13.87 ± 7.83	13.74 ± 2.49	0.90
Alanine aminotransferase (ALT) IU/L	43.82 ± 49.70	47.79 ± 46.30	0.58
Aspartate aminotransferase (AST) IU/L	52.55 ± 56.49	59.22 ± 59.90	0.43
Blood Urea Nitrogen (BUN) (mg/dL)	23.23 ± 15.64	23.19 ± 22.93	0.99
Hospital stays (days)	8.18 ± 8.04	10.98 ± 12.77	0.04*
qSOFA index	0.22 ± 0.44	0.44 ± 0.61	0.002**

*qSOFA, quick sepsis-related organ failure assessment; Data Presented as mean ± SD. *p<0.05; **p<0.01; ****p<0.0001.*

TABLE 3 | Characteristics of patients categorized into 4 groups based on their fasting blood glucose and glycated hemoglobin level.

Parameters	G1 (n = 87) FBG<126mg/dl HbA1c<6.5%	G2 (n = 35) FBG≥126mg/dl HbA1c<6.5%	G3 (n = 49) FBG<126mg/dl HbA1c≥6.5%	G4 (n = 193) FBG≥126mg/dl HbA1c≥6.5%
Sex (male/female)	48/39	21/14	28/21	104/89
Age (years)	56.47 ± 18.18[a]	66.29 ± 14.99[b]	60.43 ± 15.09	60.46 ± 14.29
Ethnicity (Hispanic/ Non-Hispanic)	79/8	34/1	43/6	177/16
BMI (kg/m^2)	29.87 ± 9.82	28.85 ± 5.37	30.51 ± 5.64	30.69 ± 6.38
Medications (n (%))	10 (11.5%)	6 (17%)	30 (61%)	137 (71%)
FBG (mg/dl)	103.7 ± 11.16[a]	165.9 ± 53.02[b]	105 ± 17.88[abc]	241.8 ± 110.2[c]
HbA1c (%)	5.8 ± 0.37[a]	5.9 ± 0.59[ab]	7.6 ± 1.91[c]	9.4 ± 2.40[d]
Hospital stays (days)	6.36 ± 5.21[a]	12.91 ± 11.61[b]	9.49 ± 10.86[ab]	8.81 ± 9.26[ab]
qSOFA index	0.24 ± 0.46[a]	0.61 ± 0.79[b]	0.16 ± 0.43[a]	0.31 ± 0.52[a]

BMI, body mass index; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; qSOFA, quick sepsis-related organ failure assessment; Data presented as mean ± SD. [a-d] Values with different superscript letters in each row are significantly different (p<0.05).

glycemic control at the time of hospitalization. Patients with higher acute glycemia but controlled chronic glycemia (G2) had the greatest COVID-19 severity and longer LOS. Our results are consistent with recent studies that suggested uncontrolled glycemia to be a significant risk factor for the severity and morbidity of COVID-19 (21–23). Our results also suggested a lesser severity of COVID-19 with a high acute glycemia (FBG≥126mg/dL) in patients with relatively uncontrolled (HbA1c≥6.5%) chronic glycemia, compared to controlled (HbA1c ≤ 6.5%) chronic glycemia. This may perhaps be explained by better management of diabetes with hypoglycemic medication in the latter group (71% vs. 17%). In-hospital hyperglycemia has been known to be an important marker of poor clinical outcome, mortality, and longer stay in hospital in patients with or without a prior history of diabetes (21, 22, 24). It should be noted that the possible hyperglycemia with COVID-19 has also been suggested by underlying subclinical pulmonary remodeling (diabetic lungs) with COVID-19 infection that leads to hyperglycemia (25). Therefore, we cannot conclude whether a high blood glucose is a consequence of COVID-19. But it is clear from our data that unmanaged glycemic control is associated with worse COVID-19 severity and recovery time. Our study is the first study, to our knowledge, that investigated the effects of acute and chronic glycemic control concurrently on COVID19 severity and LOS. As glucose control helps prevent and control infections and their complications, well-managed blood glucose may lead to an improved outcome for patients with COVID-19.

Racial and ethnic disparities data indicate that COVID-19 has disproportionately infected Hispanic and Black communities. The Hispanic and Black populations are at a greater risk of experiencing COVID-19 severity, intensive care unit (ICU) admission, and risk of invasive mechanical ventilation (IMV) than non-Hispanic white patients (26). Diabetes is a prevalent disease among these racial/ethnic minority communities. Yet, many of them with diabetes often remain undiagnosed or are not able to properly manage their diabetes due to a lack of health insurance or financial resources to support their treatment. This circumstance is likely to expose them to the greater risk for COVID-19 severity and poor clinical outcomes. For example, El Paso has a predominately Hispanic and Latino population (82%) and is characterized as a low-income city where 69% of the population has a household income of less than $20,000 and 23%

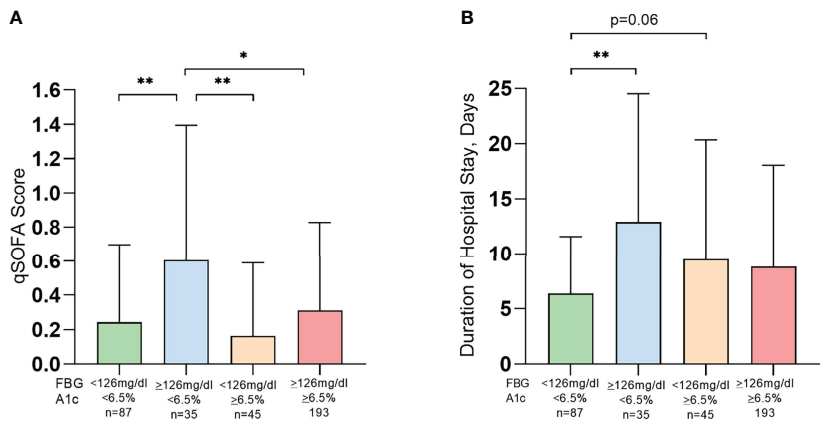

FIGURE 3 | Patients with acute glycemia represented higher severity **(A)** and length of hospital stay **(B)** among hospitalized COVID-19 patients. FBG, fasting blood glucose; HbA1c, glycated hemoglobin; qSOFA, quick sepsis-related organ failure assessment; Data presented as mean ± SD. *p<0.05, **p<0.01.

of the population has not graduated from high school (27). It is important to consider that our data represent a predominantly Hispanic and Latino population (88%), who are at a greater risk to develop diabetes (28). Our study findings highlight the importance of diabetes care in COVID-19 patients, particularly for the communities with a high prevalence of diabetes and limited resources.

One limitation of our study is that categorization of managed and unmanaged diabetes was done based on self-reported medication data. The information on previous diagnosis of diabetes and duration of diabetes were not available. Finally, we cannot completely rule out the impact of diabetes medication as we interpret our results on glycemic control impacting the severity and LOS. However, our data clearly demonstrate the impact of unmanaged hyperglycemia and acute glycemic control on the severity and length of recovery of COVID-19 in a predominantly Hispanic and Latino population.

In conclusion, our study clearly demonstrates that uncontrolled diabetes and acute glycemia worsened severity and the rate of recovery in COVID-19 patients. Our results highlight the importance of assessing, monitoring, and controlling blood glucose in hospitalized COVID-19 patients from the start, specifically for vulnerable populations already at risk of comorbidities. Therefore, during the pandemic of COVID-19, blood glucose management can facilitate the assessment of prognosis and early intervention of hyperglycemia to help improve the overall outcomes in the treatment of COVID-19.

DATA AVAILABILITY STATEMENT

This study used data from the medical records of confirmed COVID-19 cases. Any request for deidentified data should be directed to the corresponding author.

ETHICS STATEMENT

This study was approved by the institutional review board at the University of Texas at El Paso.

AUTHOR CONTRIBUTIONS

SB conceived and designed the study; SB and AM wrote the first draft of the manuscript; all authors contributed to revision of the manuscript; HK, SC, and RM extracted the data, SB, AM, and AW analyzed the data, AW assisted with statistical analyses, all authors contributed to interpretation of the data. SB is responsible for the content of the article. Some of the results from this study were presented at the Texas chapter of American College of Sports Medicine (ACSM) conference, 2021 and American Diabetes Association (ADA) annual conference, 2021 and were published in abstract form. All authors contributed to the article and approved the submitted version.

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REVIEW
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Respiratory Tract Infections in Diabetes – Lessons From Tuberculosis and Influenza to Guide Understanding of COVID-19 Severity

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Patients with type-2 diabetes (T2D) are more likely to develop severe respiratory tract infections. Such susceptibility has gained increasing attention since the global spread of Coronavirus Disease 2019 (COVID-19) in early 2020. The earliest reports marked T2D as an important risk-factor for severe forms of disease and mortality across all adult age groups. Several mechanisms have been proposed for this increased susceptibility, including pre-existing immune dysfunction, a lack of metabolic flexibility due to insulin resistance, inadequate dietary quality or adverse interactions with antidiabetic treatments or common comorbidities. Some mechanisms that predispose patients with T2D to severe COVID-19 may indeed be shared with other previously characterized respiratory tract infections. Accordingly, in this review, we give an overview of response to Influenza A virus (IAV) and to *Mycobacterium tuberculosis* (Mtb) infections. Similar risk factors and mechanisms are discussed between the two conditions and in the case of COVID-19. Lastly, we address emerging approaches to address research needs in infection and metabolic disease, and perspectives with regards to deployment or repositioning of metabolically active therapeutics.

Keywords: diabetes, infection, influenza, COVID – 19, tuberculosis, inflammation

1 INTRODUCTION: TYPE-2 DIABETES AND SEVERITY OF RESPIRATORY TRACT INFECTIONS

Diabetes is a chronic condition characterized by persistently high levels of glucose in blood due to insufficient insulin action or secretion. The international diabetes federation (IDF) estimates that there will be 783 million adults with diabetes in 2045, which is 46% higher compared to the number of cases in 2021 (1). The IDF also reported Type-2 Diabetes (T2D) to be the most prevalent form,

accounting for 90% of all cases worldwide (1). T2D is a condition of insulin resistance, defective insulin secretion and β-cell destruction, these are associated with inflammation and metabolic stress. It results from several risk-factors, such as aging, lifestyle and genetic predisposition (2). Metabolic stress due to loss of glycemic homeostasis results in detrimental microvascular and macrovascular complications and hepatic comorbidities (3). Studies have also shown that T2D increases patient susceptibility to severe infections, with this being attributed to microenvironmental dysmetabolism impairing the immune responses (4, 5). Importantly, this applies to several forms of infection: skin and soft tissue infections, urinary tract infections and respiratory infections, with increased hospitalization and mortality rates (6, 7).

Respiratory infections are considered one of the major severe infections associated with diabetes. Hyperglycemia and increased protein glycosylation were found to be associated with microangiopathic alterations in the lungs of patients with T2D (8). Based on the pneumonia severity index, a higher proportion of diabetic patients suffer severe respiratory infections, compared to non-diabetic patients (52.3% vs. 9.4% of patients) (9). In addition, continuous exposure to high glucose also leads to formation of advanced glycation end-products (AGEs) which are implicated in the development of diabetic vascular complications, induce reactive oxygen species (ROS) and development of pulmonary fibrosis (10). These effects in the lung have been observed in common respiratory diseases that gravely affect patients with diabetes. These include asthma (11), pneumonia (7), tuberculosis (12), influenza (5) and Coronavirus Disease (COVID)-19 (13).

In such conditions, immune responses are often studied for potential impairments and as mechanistic targets contributing to severity (14). In normal physiology, immunity coordinates detection of pathogens, phagocytosis, antigen presentation and production of specific antibodies. In diabetes, the increased susceptibility to severe infection has been associated with defects in these functions at multiple levels (4). Results from a cross-sectional study showed that pulmonary functions were reduced in people with inadequate glucose control and high levels of inflammatory markers like TNFα, IL-6 and C-Reactive Protein (CRP) (15). These inflammatory markers are elevated in diabetes, under non-infectious conditions, and associated with hyperglycemia (16). It was also found that hyperglycemia suppressed IL-2, IL-6 and IL-10 in peripheral blood mononuclear cells (PBMCs) suggesting impaired cellular defense mechanism in patients with diabetes (17). Such markers in circulation reflect localized problems at a given site of infection, such as the lungs. Alveolar macrophages, that populate the lungs, are among the first points of contact with respiratory pathogens, and their dysfunction is a characteristic abnormality in severe cases of COVID-19 (18). These macrophages are specialized tissue resident cells of the innate immune system, generally detecting and disposing of invading foreign debris or pathogens. They also retain the capacity to raise an alarm *via* signaling to cells in the microenvironment (e.g., endothelial or dendritic cells, T-cells etc.).

Every stage of the immune response also relies on micronutrient balance or availability. Micronutrients have synergistic roles with the molecular machinery that facilitates immune effector functions. Vitamin D has been gaining attention as one of the most important micronutrients contributing to efficiency of immune reponses (19). Vitamin D is most widely known as a regulator of calcium and phosphate levels in the body and is necessary for healthy teeth, bones and muscles. However, Vitamin D also ensures integrity of innate immune responses at mucosal barriers, these are the first line of defense against invading pathogens (19). It also plays a role in cell-mediated processes, decrease pro-inflammatory cytokines expression and has inhibitory actions against pathogens (19). The inhibitory effect of the active form of vitamin D [Calcitriol ($1,25\ (OH)_2D_3$)] is modulated by vitamin D receptors (VDRs) that are present in the heart, brain, pancreatic islets, immune cells, muscles and adipose tissue. These are all common target organs in the development of diabetes and its complications (20). Typically, during infection immune cells would trigger VDR signaling, to convert vitamin D to calcitriol, then induce the production of antimicrobial proteins such as cathelicidin (LL-37) *via* Toll-like receptor (TLR) activation (21).

Several studies have reported an inverse relationship between vitamin D status and T2D incidence (22–24) as its deficiency is associated with β-cell dysfunction and insulin resistance (25). Results from an observational study showed that the prevalence of hypovitaminosis D was higher in T2D compared to control (39% vs 25%) (26). Similarly, Plataki et al. also reported that T2D patients tend to have low levels of vitamin D, as well as of LL-37, providing a mechanism for impaired immune responses and antimicrobial peptide production (27). Such alterations of vitamin D could also increase susceptibility to severe respiratory infections especially in patients with asthma and pulmonary diseases (28). Interestingly, the lung's epithelium was found to "self-generate" the active form of vitamin D that increases LL-37 expression. Dysfunction of this process can also contribute to severity of respiratory infections (29).

Such immune-mediated mechanisms, and others, have been a central focus of COVID-19 research. Indeed, T2D is one of the top 3 risk-factors leading to severe infection and undesirable outcome of COVID-19 across all adult age groups (13). Interactions between infection, immunity, and metabolic disease has been studied to decipher which criteria contribute to risk and by which mechanisms does diabetes impair response to infection. However, prior to the emergence of SARS-CoV-2, decades of research into respiratory infection in T2D have given a wealth of information. This review brings together current knowledge in T2D and respiratory infections, mainly interactions between systemic metabolism and the immune system's response to bacterial and viral infectious pathogens. We discuss tuberculosis (TB) and influenza, that predate COVID-19, as longer-studied conditions well-known to be affected by diabetic status. We discuss risk-factors and mechanisms that contribute to severity in both conditions, then relate this to the current knowledge on COVID-19.

2 MYCOBACTERIUM TUBERCULOSIS AND METABOLIC DISEASE

The observation that metabolic factors determined the various outcomes of COVID-19, with severe COVID-19 manifesting primarily in people with metabolic syndrome (30, 31), indicates how host metabolic fitness underlines immune function. A similar spectrum of infection outcomes is observed in TB, caused by infection with the intracellular bacteria Mycobacterium tuberculosis (Mtb). These range from early clearance to latent tuberculosis infections (LTBI) to active TB and severe disease (32). In recent years, T2D emerged as a major risk-factor for developing severe disease, with TB-diabetes recently classified as a syndrome by WHO, particularly prevalent in Southeast Asia (33). Although both pre-diabetes and T2D can enhance risk of contracting TB in the form of LTBI in low burden TB countries (34), in areas where TB is endemic T2D certainly increases risk of active TB and is associated with poorer responses to treatment (12, 35, 36). Interestingly, in these environments, particularly Asia, T2D is associated with worse TB disease (37) irrespective of body mass index (BMI), while obesity in the absence of hyperglycemia is associated with a better prognosis (38). These observations suggest that multiple factors associated with metabolic syndrome including hyperglycemia, insulin resistance and hyperlipidemia can affect TB host immunity differently and ultimately affect disease outcome. Therefore, TB can serve as a case study for how metabolic factors promote severe infection risk. Insights into metabolic factor contribution to TB immunity, from clinical data and limited animal models, are discussed below (**Figures 1A**, **2A**).

2.1 Factors Affecting Tuberculosis in Diabetes

2.1.1 Hyperglycemia

Hyperglycemia affects immune responses to Mtb infection in mouse models and glycemic control has emerged as critical to disease outcome in patient cohorts (**Figure 1A**). Poorer glycemic

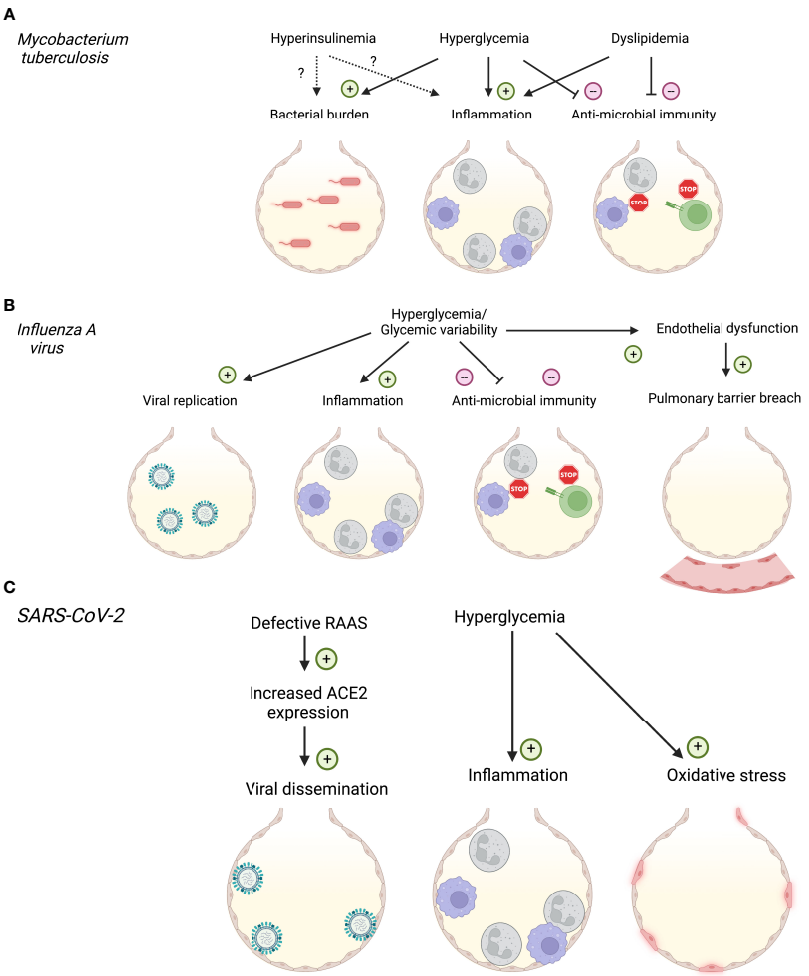

FIGURE 1 | Impact of diabetes and systemic metabolic factors on respiratory infections. **(A)** Impact of hyperinsulinemia, hyperglycemia and dyslipidemia on *Mycobacterium tuberculosis* infection. **(B)** Impact of hyperglycemia on Influenza A virus infection. **(C)** Impact of hyperglycemia and renin-angiotensin-aldosterone system (RAAS) dysfunction on SARS-CoV-2 infection. Created with BioRender.com.

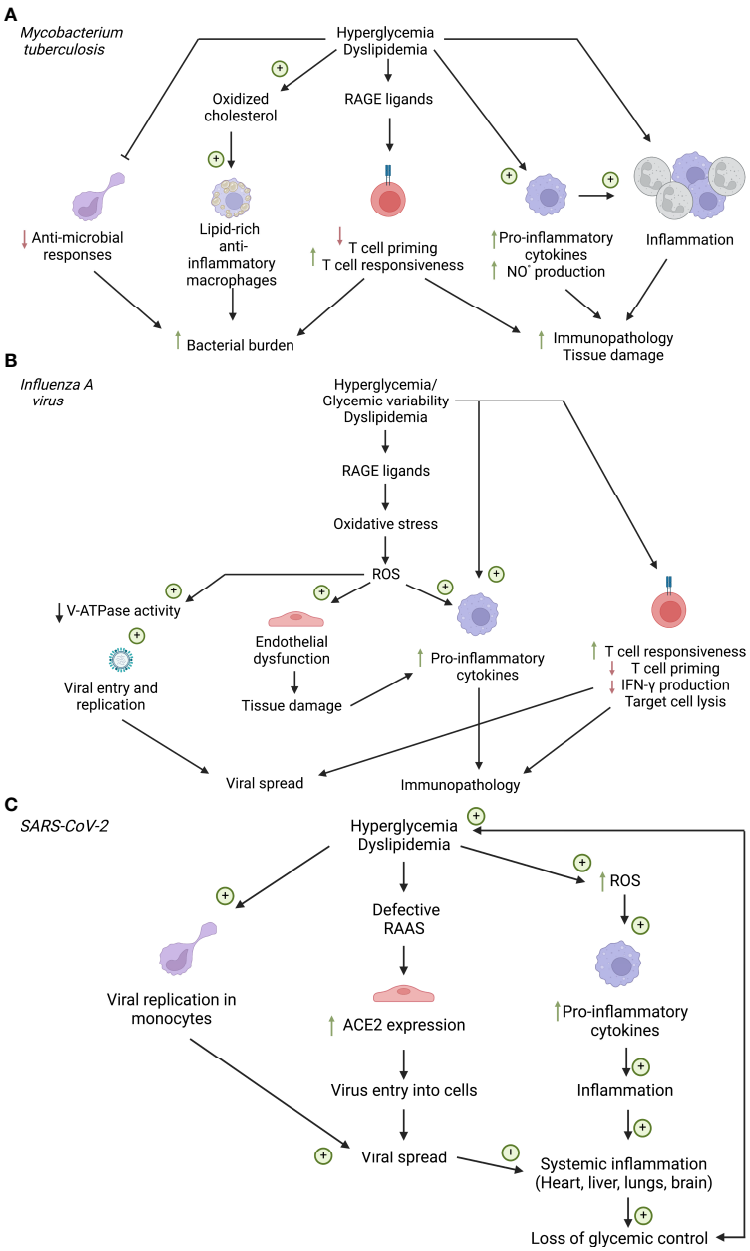

FIGURE 2 | Cellular factors influencing respiratory infections in diabetes. **(A)** Cellular factors influencing *Mycobacterium tuberculosis* infection. **(B)** Cellular factors influencing influenza A virus Infection. **(C)** Cellular factors influencing SARS-CoV-2 infection. ACE2, Angiotensin converting enzyme 2; NO°, nitric oxide; RAAS, renin-angiotensin-aldosterone system; RAGE, Receptor of advanced glycation end products; ROS, reactive oxygen species. Created with BioRender.com.

control is associated with more severe TB disease (39), and strategies to treat this reduce the relative risk. However, glycemia centered strategies do not return relative risk of severe disease to non-T2D levels (40). Mechanistically, peritoneal macrophages from diabetic (db/db) mice display impaired phagocytosis and cytokine responses (41, 42). *In vitro* studies suggest that increasing glucose concentrations enhance Mtb-induced pro-inflammatory cytokine responses in bone-marrow derived macrophages (BMDM), but this is only noted at high glucose concentrations which mimic hyperglycemia (43) (**Figure 2A**).

With the realization that immunometabolic reprogramming underlines macrophage responses in Mtb, these processes should be re-examined. In particular, monocyte-derived macrophages recruited to sites of Mtb infection up-regulate expression of the main macrophage glucose transporter GLUT1 and glucose consumption to promote anti-microbial functions and pro-inflammatory cytokine production (44, 45). Whether this is impacted by the systemic hyperglycemia, characteristic of T2D has not yet been described. However, circulating monocytes derived from pre-diabetic patients display enhanced cytokine

production (46), while monocyte-derived macrophages from patients with T2D have impaired anti-microbial responses to Mtb (47). Similar impairments have been observed in alveolar macrophages derived from diabetic mice (48, 49), these cells seem to rely on oxidative metabolism (45) and are described further in the following sections. *In vivo* Mtb infections in diabetic mouse models display heightened lung inflammation and bacillary burden (50, 51). This correlates with clinical observations of increased lesions, cavities and increased transmissibility of TB in T2D patients (52, 53). Although the antigen-presenting ability of innate cells was not affected by hyperglycemia (54), altered cytokine production can affect the nature of the subsequent T-cell response contributing to poorer bacterial containment (48). Importantly, *in vivo* studies have also found a profound defect in inherent lymphocyte function in streptozotocin-treated hyperglycemic mice. Martinez et al. found that the accumulation of Receptor for Advanced Glycation End-Products (RAGE)-ligands during chronic hyperglycemia activates a p38 dependent pathway that epigenetically reprograms naïve T-cells, making them hyper-responsive to subsequent activation by Mtb antigens (54) (**Figure 2A**). Thus, both myeloid and lymphocyte function is inherently impaired under hyperglycemic conditions and can escape the regulatory mechanisms active under homeostasis.

2.1.2 Hyperinsulinemia

Hyperinsulinemia is the result of insulin resistance, given that pre-diabetes is associated with increased risk of LTBI and that cells from pre-diabetic patients display altered responses to stimulation (40, 46), there is an interest in identifying if hyperinsulinemia in pre-diabetes contributes to altered immune responses (**Figure 1A**). The recent interest in immunometabolism has demonstrated that Mtb-infected macrophages upregulate glycolysis to promote anti-bacterial and pro-inflammatory cytokine functions (44, 45), processes enhanced by T-cell-derived interferon (IFN)-γ and limited by virulent and drug-resistant forms of Mtb (55–57). Although GLUT1 expression is insulin-independent, and insulin-dependent GLUT4 is not expressed in macrophages (58), insulin receptor signaling can have both pro- and anti-inflammatory roles and could impact Mtb immunity. Mtb infection itself can induce transient hyperglycemia through stimulating insulin production which, in guinea pig models, has been linked to more severe disease (59). Identifying these insulin-dependent mechanisms could help explain the complex interdependency between insulin signaling and glucose homeostasis in increased disease severity linked to T2D.

2.1.3 Dyslipidemia

Since glycemic control does not completely reduce risk of severe TB in T2D (40), other metabolic factors have been considered, chiefly, altered lipid homeostasis. Increased BMI contributes to resilience against severe disease (38) and limited human data suggests cholesterol-rich diets may actually protect against TB disease (60). Larger epidemiological studies in Asia have demonstrated that lower total cholesterol levels are linked to more severe TB and poorer treatment outcomes (61). These data suggest a protective role for total cholesterol against TB, which have not been always observed in animal models or *in vitro* studies. Indeed, hyperlipidemic mice display enhanced TB disease with increased lung inflammation characterized by myeloid nitrous oxide production, increased bronchoalveolar fluid (BALF) T helper type 1 (Th1) cytokines, poorer lung histology and higher bacterial burdens and dissemination to organs (62, 63)(**Figure 1A**). The increased myeloid inflammation – both macrophage and neutrophil driven, promotes tissue degradation while simultaneously limiting the development of protective T-cell responses. As a caveat, the mouse models used to illustrate this (Apoe-/-, Ldlr-/-; high-fat fed genetically-deficient mice) display abnormally high cholesterol levels, only seen in humans with familial hypercholesterolemia and may not accurately reflect TB-diabetes.

Mtb itself is a lipid rich bacillus and must obtain cholesterol for biosynthesis and growth from the host (64). This promotes long-term persistence in the host since mutant strains which lack cholesterol transporter systems (e.g. mce4) succumb to the pro-inflammatory effects of IFN-γ (65). The TB granuloma, contains a lipid-rich core surrounded by various foamy macrophage cell types (66). Although initially thought to represent a nutrient source for the mycobacteria, recent lipidomics and single-cell analysis suggests that heterogeneity exists in both the lipid content and inflammatory phenotype of granuloma macrophages (67, 68). While Mtb infection can alter both cholesterol and fatty acid metabolism to favor mycobacterial growth (69, 70), the process of lipid droplet formation appears to be host encoded and augmented by IFN-γ treatment (**Figure 2A**). This drives initiation of triglyceride biosynthesis and lipid uptake to maintain lipid droplets in infected macrophages. This process is linked to the production of eicosanoids which promote host-defence (67). Much of this work has been performed in BMDM cultures and it is now appreciated that the alveolar macrophages, initial host cells rely basally on fatty acid oxidation and do not employ anti-Mtb glycolysis, which is the opposite to infiltrating macrophages. Dodd et al. illustrated that Mtb-infected alveolar macrophages and monocyte-derived macrophages upregulate the lipid transporter CD36 to drive an anti-inflammatory, pro-mycobacterial phenotype (71, 72). Surfactant lipoproteins in the pulmonary space facilitate this and are internalized during infection (71). The lipid-rich environment of the lung itself undoubtedly shapes alveolar macrophage development and may explain the tropism of Mtb for the pulmonary compartment. Whether these processes are altered in the setting of hyperlipidemia is unclear and may depend on the nature of the dyslipidemia. Hypertriglyceridemia in western populations was found to be particularly pathogenic and linked to poorer treatment outcomes than hypercholesterolemia (73). Another confounding factor is that often it is not simply elevated lipid levels themselves, but modified lipid species generated during disease (74). Oxidized forms of low-density lipoprotein (LDL)-cholesterol and sterols have been shown to impact

macrophage Mtb responses and macrophage recruitment to the lung respectively (75, 76).

2.1.4 Vitamin D Deficiency

Alterations in vitamin D levels in individuals with diabetes and Mtb can disrupt the maintenance of immune homeostasis. Several studies have reported that cases of Mtb infection are associated with low levels of vitamin D, in particular active Mtb (77, 78). A randomized controlled trial confirmed that upon vitamin D supplementation, patients had significantly reduced symptoms by 36.6% in the first month (79). Since TLRs are linked to the antimicrobial function of vitamin D, Liu *et al.* investigated this mechanism and found that the induction of antimicrobial peptide LL-37 inhibits Mtb proliferation in human monocytes and macrophages (80). This was confirmed further *in vitro* where the inhibition of LL-37 led to enhanced growth of Mtb, confirming LL-37's suppressive role (81). Very limited number of studies have investigated the status of vitamin D and LL-37 in patients with TB and diabetes. Zhan and Jiang assessed the levels of vitamin D in patients with Mtb and diabetes mellitus and found that the concentration of vitamin D was 35% lower in patients with Mtb and DM compared to the control group (82). In addition, patients with both conditions exhibited high levels of LL-37 that is correlated with a positive acid-fast bacillus smear which indicates the presence of TB (82).

2.2 Chronic Inflammation and Immune Training

As a consequence of both hyperlipidemia and hyperglycemia, systemic inflammation is triggered which underlines many of the metabolic sequelae of T2D. This baseline meta-inflammation could also impact host-defense responses and is already implicated in defective anti-tumor immunity (83). Chronic inflammation inhibits both resolution of inflammation and the development of protective lymphocyte responses and can also alter TB granuloma composition (51, 62). Recently, chronic inflammation associated with hyperlipidemia and western diets was shown to reprogram myeloid progenitor cells for heightened activity through the process of innate immune training (84, 85). Following this, Choudhury and colleagues demonstrated that hyperglycemic but not hyperlipidemic mice also displayed heightened monocyte cytokine production due to epigenetic modification and alterations to bone-marrow myelopoiesis, characteristic of innate immune training (84, 86). This process contributes to the development of T2D, but may also impair host-defense in TB-diabetes. With hematopoietic progenitor cells emerging as a key target of inflammation affecting immune cell composition and fate (87), the chronic inflammation in T2D could alter immune cell fate and affect granuloma composition. Recent profiling studies examining active TB granulomas demonstrate a more regulatory phenotype dominated by immunosuppressive myeloid and Regulatory T cells (Treg) (68) and the impact of meta-inflammation on this warrants examination.

2.3 (Immuno)-Metabolic Treatments for TB-Diabetes

Although control of glycemia in T2D doesn't completely reduce the risk of increased TB (40), metabolic drugs are being assessed for their ability to prevent disease due to their immunometabolic effects. They can also reveal novel targets in pathogenesis which could form the basis for future novel immuno-therapies or vaccine targets. In a normoglycemic mouse model of TB, metformin was shown to exacerbate late-stage disease by ameliorating inflammation (88). In particular, IL-1β expression was reduced, which can be regulated through metformin-induced inhibition of mitochondrial complex 1 activity (89). Similar results were observed in humans receiving metformin, with improved macrophage responses to Mtb infection (90). More pressingly, in the context of T2D, a recent meta-study revealed that diabetes controlled by metformin led to a reduced risk of TB in humans (91). In contrast, a recent mouse study of Mtb infection in diet induced obesity (DIO)-mice suggests that metformin is only beneficial in the hyperglycemic context (92). More recently, cytotoxic $CD8^+$ lymphocytes emerged as targets for metformin in Mtb-infection models in euglycemic mice. Metformin metabolically reprograms these cells toward oxidative metabolism and promotes anti-microbial function. Traditionally TB vaccines target $CD4^+$ Th1 cells, so this study is important as it places these $CD8^+$ T-cells as key in protecting against severe disease, particularly relevant in the metabolically dysregulated context of T2D.

Because of Mtb's tropism for cholesterol, the HMG-CoA reductase targeting drugs, statins, have received attention both as a direct anti-Mtb drug and for use in the context of TB diabetes. By reducing cholesterol biosynthesis at this early rate-limiting step (93), statins lead to compensatory low-denisty lipoprotein receptor (LDLR) expression and reduced serum LDL-cholesterol which should be beneficial in the context of hyperlipidemia and its effect on TB pathogenesis. Clinical studies are examining the feasibility of this (94), however given the controversial role of elevated total cholesterol in TB host defense, the results thus far are unclear. Since targeting HMGR leads to knock-on effects on the production of multiple lipid species (93, 95), statins may affect mycobacterial growth. *In vitro* studies suggest that statins may directly alter axenic Mtb growth in culture (96), Mtb growth in macrophages is also reduced after statin treatment (97). This may be due to cholesterol depletion but also could be mediated through the immunomodulatory functions of statins (95). Finally, novel lymphocyte subsets which present lipid antigens *via* CD1 have recently been associated with early immune responses in TB including γδ T and invariant natural killer T (iNKT) cells (98). The impact of altered systemic lipid homeostasis as well as statin treatment on their function needs to be considered, as does the nature of vaccine strategies used to boost TB immunity, with current targets focusing solely on MHC-restricted peptide antigens that promote $CD4^+$ T-cells.

3 INFLUENZA A VIRUS AND METABOLIC DISEASE

Influenza A virus (IAV) presents a continuous threat to human health, infecting up to 15% of the population annually (99, 100). Influenza infection severity largely depends on the immune and health status of the individual (101). Although considered to be relatively mild, the 2009 H1N1 pandemic served to emphasize that certain host comorbidities increase the risk of severe disease upon influenza virus infection (102, 103). Prior to 2009, reports already emphasized that diabetes increases the risk of lower respiratory tract infections (LRTI) and pneumonia related hospitalization, and indeed led to more severe influenza outcomes (104–106). Both patients with Type 1 Diabetes (T1D) and T2D were at greater risk of developing LRTI (including influenza), while there was no difference in the risk of upper respiratory tract infections over a 12-month time period (105). Interestingly, in this study T2D patients in particular had an increased risk of developing two or more episodes of a LRTI within the 12-month period. This indicates that hyperglycemia *per se* may be an important factor in LRTI severity, but not necessarily in frequency of LRTIs. A 7-year retrospective study reported that individuals with diabetes are at a higher risk of being hospitalized due to pneumonia (104). This increased risk was also associated with increasing blood glucose levels (104). Furthermore, during a one year period, individuals with diabetes were more likely to have influenza or pneumonia listed as cause of death compared to people without diabetes, regardless of race, sex, and socioeconomic status (106). Strikingly, it was estimated that during this time, individuals with diabetes made up more than 10% of the recorded influenza and pneumonia associated deaths in the U.S (106). Together, these studies demonstrate the enhanced risk of severe influenza outcomes in individuals with diabetes during seasonal influenza epidemics.

Much of the current literature regarding the susceptibility of individuals with diabetes to severe IAV infection emerged in response to the 2009 H1N1 pandemic (pH1N1) and has been addressed in a previous review (107). Specifically, diabetes was identified as one of the most common host comorbidities in those hospitalized with pH1N1 (108). During the 2009 pandemic, individuals with diabetes had around a 3- to 4-fold increased risk of hospitalization (109–112), intensive care unit (ICU) admission (109, 113, 114), and death (113–115). Indeed, one study reported up to a 9-fold increased risk of hospital mortality in individuals with diabetes (116). While diabetes was identified as an independent risk-factor for severe pH1N1 outcomes, this risk of ICU admission and death becomes higher in patients with additional underlying medical conditions (117).

3.1 Factors Influencing Influenza in Diabetes

3.1.1 Hyperglycemia

Experimentally, murine models of both T1D and T2D show that diabetic mice have more severe influenza outcomes when compared to healthy controls (107, 118–120), providing experimental evidence that hyperglycemia *per se* is a major contributor to disease severity. Studies using an *in vivo* STZ-induced model of T1D, demonstrated that diabetic mice had increased viral load with a more extensive infection, and lower rates of survival (118, 120, 121). This increase in viral load has also been correlated to increasing blood glucose levels observed in T1D mice (119). Similar results have been observed using a leptin receptor-deficient model for obesity and T2D. Specifically, elevated blood glucose levels correlated with increased viral copy number and an increase in the pulmonary pro-inflammatory cytokine response (122) (**Figure 1B**).

Despite the abundance of data that indicates the role of diabetes in influenza severity, the research into mechanisms remains limited. Chronic hyperglycemia can induce oxidative stress *via* the production of ROS and is thought to be one of the key sources of hyperglycemia-induced diabetes complications (123, 124). There are several mechanisms by which hyperglycemia causes increased oxidative stress, such as the production of AGEs, the activation of Protein Kinase-C (PKC), the accumulation of sorbitol, and the hyperactivity of the hexosamine pathway (125). Cumulatively, these lead to the over production of ROS and a decrease in the endogenous antioxidant defense systems (125). Given the causal role of hyperglycemia-induced ROS has in the development of the non-infectious complications of diabetes, it is likely that hyperglycemia-induced ROS production is a driving factor in increased severity of influenza in individuals with diabetes (**Figure 2B**).

There is evidence that hyperglycemia-driven ROS production alters cellular metabolism that can shape the inflammatory response during viral infections (126), however this has yet to be explored in influenza. Hyperglycemia can upregulate glycolysis (127), which can promote a pro-inflammatory immune cell phenotype (128), and increase viral replication (129) (**Figure 2B**). Despite not coming in direct contact with circulating blood, glucose concentrations in the airway surface liquid increase as a direct result of elevated blood glucose levels (130). High levels of glucose cause a dose-specific increase in IAV infection and replication in Madin-Darby Canine Kidney (MDCK) epithelial cells (129). This was associated with a glycolysis-dependent increase in the assembly of the cellular V-ATPase which is necessary for viral release into the cytoplasm (129). Indeed, increased viral replication has been observed in murine models of both T1D and T2D, and correlated with increased blood glucose levels (119–122). This increase in viral replication as a result of abundance of glucose is likely due to ROS-driven alterations to metabolic function. While this is yet to be directly demonstrated in the context of influenza, high levels of glucose have been shown to increase SARS-CoV-2 viral replication and monocyte cytokine production in a ROS/glycolysis-dependent manner (131).

In addition to altering cellular metabolic function, a history of exposure to hyperglycemia can lead to ROS-driven endothelial dysfunction in diabetes (132). While endothelial cells are not normally infected by influenza virus in humans, they play a crucial role in influenza pathogenesis given their close proximity to the pulmonary alveolar epithelium. Specifically, during IAV infection, it is the pulmonary endothelial cells that are believed to

be a major source of cytokine production in the lungs (133, 134). We have previously demonstrated using both an *in vitro* and *in vivo* model, that exposure to high glucose conditions prior to IAV infection increased virus-induced pulmonary barrier damage (122). This was associated with an increased pro-inflammatory response in endothelial cells and the subsequent damage of the epithelial junctional complex. This is likely to be further exacerbated in conditions of glycemic variability (**Figure 2B**).

3.1.2 Glycemic Variability

Typically, individuals without diabetes have blood glucose levels ranging between 70-100 mg/dl (135). These glucose levels fluctuate during the day, particularly post-meal up to almost 140 mg/dl (135). These glucose fluctuations are referred to as glycemic instability or glycemic variability and can be more extreme and prolonged in individuals with uncontrolled diabetes.

There is mounting evidence that glycemic variability is associated with greater ROS production than steady hyperglycemia (125), and could further impair the immune response (107, 136). However, glycemic variability as a factor in severe influenza infections remains a relatively new topic, with an extremely limited amount of research into its role, and most studies are outside the scope of influenza. Nevertheless, there has been one key study that has investigated the role of glycemic variability in the context of influenza. Using an *in vitro* co-culture model mimicking the respiratory epithelial-endothelial barrier we have shown that compared to hyperglycemia, glycemic variability increased viral replication, cell death, and inflammation of both the epithelial and endothelial cells (136). This was correlated with an increase in a marker of oxidative stress. These results were confirmed using an *in vivo* model, where mice that experience glycemic variability in the weeks leading up to infection suffered more severe influenza (136). Specifically, mice with glycemic variability had increased weight-loss, decreased lung function, and increased apoptotic cell death. This was again associated with increased pulmonary inflammation and oxidative stress. Together, these data suggest a key role of dysregulated glucose levels, both elevated and variable, driving severe influenza in the context of a primary infection (**Figure 1B**).

3.2 Adaptive Immune Responses

In addition to its effect on the innate immune system and viral replication, diabetes may also have direct effects on the cellular adaptive immune response to viral infection. There is evidence that hyperglycemia induces hyperresponsiveness, enhanced activation, and proliferation of T cells (54, 137) (**Figure 2B**). However, there is perhaps a larger body of evidence that hyperglycemia impairs responses, increases the frequency of senescent cells, and impairs the proliferation of T cells (138–140). *In vitro* evidence suggest that high glucose concentrations reduce the production of IFN-γ by $CD8^+$ T cells (141), and reduces their viability (142). Consistent with this, it has been shown that when compared to healthy controls, $CD8^+$ T cells from patients with diabetes had reduced lysis of target cells (143), and using genome-wide expression analysis of PBMCs from donors with diabetes showed a reduction in activity of cytotoxic genes compared to controls (144). Much like what has been observed in innate immunity, there is an emerging role of glycemic variability in negatively shaping the adaptive immune response to influenza. Compared to hyperglycemia, we have shown that glycemic variability increases pulmonary inflammation, oxidative stress and influenza severity following a secondary influenza infection in a murine model (136). It was speculated that this increase in severity could be driven by glycemic variability-induced oxidative stress reducing the $CD8^+$ T cell function. Currently, there is very little known about the effect of both hyperglycemia and glycemic variability on the adaptive immune response to influenza, and further in-depth investigation is warranted.

Taken together these studies suggested that individuals with diabetes suffer more severe influenza as a result of i) metabolic dysfunction, ii) increased viral replication, iii) endothelial dysfunction, and iv) dysregulation of the immune response.

3.3 Response to Vaccination in Diabetes

As individuals with diabetes are at a higher risk of developing serious influenza complication, influenza vaccination is currently recommended by the Centers for Disease Control and Prevention (CDC) for patients with diabetes (145). During recent influenza seasons, almost 30% of adults hospitalized with influenza had diabetes (145), highlighting the need for vaccination in this vulnerable group. During influenza epidemics of the early 1990s, vaccination of individuals with diabetes (both T1D and T2D) reduced hospital admission by almost 80% (146). A more recent meta-analysis determined that when individuals with diabetes (both T1D and T2D) are vaccinated, there is a reduced risk of hospitalization for pneumonia, and a lower mortality rate (147). Furthermore, while individuals with diabetes are at risk of developing cardiovascular complications following influenza virus infection (148, 149), influenza vaccination is associated with a reduced risk of cardiovascular mortality in adults with diabetes (150). While there are rare case study reports of adverse reactions to influenza vaccination in individuals with diabetes, overall, reactogenicity is similar in individuals with diabetes and healthy adults (151, 152). Although influenza vaccines are proven to be safe and reduced the development of severe complications, there have been some questions over vaccine efficacy in people with diabetes. Specifically, there is evidence that whilst patients with T2D were more likely to have received the influenza vaccination in the last 12 months they still experienced a greater number of respiratory infections than their non-diabetic counterparts (153). This is likely due to their reduced T cell and antibody response to influenza vaccines. In a small cohort, patients with T1D had decreased T cell response to influenza A-H1N1 subunit vaccine compared to controls, and this was associated with hyperglycemia (143). A larger study that encompassed both T1D and T2D reported that in the T1D group there was a significant increase in antibody non-responders to two of the three vaccine components (143). While this may be cause for concern, it has been demonstrated *in vivo* that vaccination *via* a higher dose, or a second low dose, increases antigen levels in diabetic mice to the point that they are able to protect against an

otherwise lethal challenge (120, 121). Together, this emphasizes the importance of maintaining up-to-date vaccination in individuals with diabetes. Unfortunately, vaccination rates for influenza are often lagging amongst those with diabetes. The current target vaccination rate for patients with diabetes is 75% (99). However, in developed countries coverage values are around 50-70% (154, 155), with some as low as 10% (156). Recently, in China, the reported vaccination rate of individuals with diabetes was below 8%, despite more than 46% of participants reporting an original intention to receive the vaccine (157). On top of lagging vaccine coverage rates in individuals with diabetes, the overall efficacy of the vaccine is known to change from season to season, as the circulating strains change. For example, the CDC has reported the current influenza vaccination for the 2021-2022 flu season in the U.S. did not reduce the risk of outpatient illness caused by influenza A (H3N2) (158). This suggests that not all influenza associated complications and death in diabetic individuals can be prevented with vaccination alone. Lagging vaccination rates, combined with sub-optimal vaccine efficacy in some influenza seasons means that severe influenza infections remain an issue for those living with diabetes.

4 COVID-19 SEVERITY AND TYPE-2 DIABETES

The above examples of respiratory infections in patients with diabetes clearly indicate a strong interaction between metabolism and successful detection and clearance of invading pathogens from the lung. Whether this be at the level of systemic metabolism or cellular immunometabolic defects that alter immune responses, a wealth of knowledge from cases of influenza and of TB can be used to draw comparisons with COVID-19.

Global events since March 2020 have made COVID-19 a near singular preoccupation of medical and research professionals due to the unprecedented strain on healthcare services. Early in the outbreak, T2D and associated metabolic syndrome were identified as risk factors for severity and death from COVID-19 (159). Diabetes is underpinned by inflammation and systemic dysmetabolism putting patients at-risk of other comorbidities. Although, initial reports focused on the immunology of SARS-CoV-2 infection and clinical trials applied antiviral or anti-inflammatory therapies (160); higher mortality in patients with pre-existing dysmetabolism indicates that metabolic mechanisms are also attractive, to establish risk, or redeploy therapeutics to mitigate severity. Indeed, the increased severity of other respiratory infections (e.g., TB, influenza) in diabetes and the interactions with specific metabolic traits support those common features of metabolic decline that are more important to focus on to mitigate risk.

The earliest report dedicated to COVID-19 patients with T2D revealed that glycemic instability/variability increases risk of severe disease (13). Further meta-analyses found that of the components of metabolic syndrome, diabetes is the biggest contributor to adverse outcome compared to hyperlipidemia, obesity and hypertension (161). Multiple reviews have addressed the interactions between COVID-19 and T2D (162, 163). Here we will cover common points with risk factors for TB and influenza, as well more recent questions regarding metabolically active therapeutics and responses to vaccination in the population with TD2 and COVID-19.

4.1 Factors Influencing COVID-19 in Diabetes

Identified factors that increase risk of COVID-19 include increased inflammation, increased ROS, insulin resistance, hyperglycemia and vascular endothelial damage. All of these factors are pre-existent or accentuated in patients with T2D prior to infection with SARS-CoV-2 (163). These risk factors are generally shared with IAV and Mtb infection (**Figures 1C**, **2C**). The particularity of SARS-CoV-2 is its activation of the renin-angiotensin-aldosterone system (RAAS) *via* the virus's main entry point, the Angiotensin converting enzyme (ACE)-2 (164). The RAAS is defective in T2D and has long been known to contribute to the development of complications in diabetes (nephropathy, macroangiopathy) (165, 166). However, dysfunction of this system cannot solely explain the increased susceptibility of patients with diabetes to a plethora of infectious respiratory conditions. Therefore, other metabolic factors are valuable candidates to look into to better understand risk.

4.1.1 Hyperglycemia

Hyperglycemia increases SARS-CoV-2 replication in circulating monocytes (131). Interestingly, on the immunometabolic front, mitochondrial adaptation also occurs in these cells to produce more ROS, contributing to severity. Our own work demonstrated that patients most severely affected exhibit morphological and functional changes in their monocyte pool (167). These changes are related to hyperinflammation and interferon signaling, associated with severity and are more pronounced in T2D. Thus, potentially *via* an interaction with monocytes, hyperglycemia may be an important mechanism-based risk-factor for severe COVID-19. Of note, glycemic control tends to deteriorate with infection in patients with T2D (**Figure 2C**). Those on insulin will require increasing doses to lower glycemia, and this requirement is associated with increased levels of inflammatory cytokines (168, 169).

Local and systemic inflammation are characteristic of COVID-19. Autopsy studies have reported inflammatory infiltrate, in the form of macrophages and lymphocytes, in a number of tissues, including the lungs, myocardium, liver and the brain (170, 171). Systemically, the cytokine storm syndrome and hyperinflammation are common and potentially life threatening in severe cases (172). The major immune signals that are impaired in COVID-19 at the transcriptional and translational levels are pro-inflammatory IL-6 signaling and the type-1 interferon system (173, 174). Mechanisms shared with other coronavirus infections have been proposed to link immune responses to disturbed metabolic homeostasis and worsening disease course. Notably, the large burden of

inflammatory infiltrate affects key insulin-responsive tissues, including the liver and skeletal muscle (175).

4.1.2 Dyslipidemia

The role of dyslipidemia in COVID-19 severity on a background of T2D is unclear. Studies have found either increased risk of severe COVID-19 with dyslipidemia or no effect (176). The lack of clarity comes from confounding factors (such as multiple treatments, age and comorbid conditions) and from heterogeneity of studied populations.

4.1.3 Vitamin D Deficiency

Hypovitaminosis D is associated with severe COVID-19, increased hospitalization and increased mortality (177). Several studies reported the inverse relationship between vitamin D supplementation and COVID-19 and its role as a preventive measure (178, 179). Generally, the protective effects of vitamin D against COVID-19 are linked to respiratory epithelial cell production of LL-37 that contributes to host-defense mechanisms through the disruption of viral membranes and replication (180). This was confirmed by Roth et al. as they demonstrated the role of LL-37 in preventing viral cell entry by binding to the ACE2 receptor of SARS-CoV-2 (181). It was also noted that vitamin D deficiency leads to a reduction in modulatory potential towards the cytokine storm during viral infection. This phenomenon is implicated in braking the lung epithelium, resulting in alveolar edema (182).

To date, very limited data are available on the link between hypovitaminoses in diabetic individuals and COVID-19. Results from a clinical study found that 76% of patients with vitamin D deficiency and hyperglycemia had severe COVID-19 with increased hospitalization and mortality rates compared to patients with normal vitamin D and glucose levels (183). They also confirmed that hypovitaminosis D in hyperglycemia resulted in worse respiratory parameters and increased levels of IL-1b, IL-6 and IFN-γ (183). Similar results were also found in obese and vitamin D deficient individuals with 72% increase in infection severity compared to the control group (183). These studies suggest that optimal nutrition and supplementation with vitamin D is a promising candidate as a preventive measure or potentially an adjunct treatment for COVID-19 (184).

4.1.4 Obesity

Obesity is considered as a major risk factor for COVID-19 infection due to its significant role in increasing systematic inflammation through the dysregulation of adipose tissue. Compared to non-obese individuals, obese individuals had greater COVID-19 severity, hypoxemic respiratory failure and higher baseline initial serum levels of CRP and IL-6 (associated with low-grade-chronic inflammation) (185). This suggests that obesity leads to worse COVID-19 outcomes that are associated increased inflammation and metabolic dysregulation.

4.2 Response to Vaccination and Metabolically Active Therapeutics

Efficiency of the COVID-19 vaccine response has been evaluated in patients with T2D. Vaccination has been reported to be efficient in patients with good glycemic control compared to patients with uncontrolled T2D. Therefore, the adaptive immune compartment does not seem to be compromised with regards to antibody production in T2D. However, hyperglycemia in uncontrolled diabetes results in weak immunity, indicating that poor glycemic control can indeed impair the antibody producing branch of adaptive immunity. Two independent studies reported similar results (186, 187).

Of the different classes of metabolically active drugs applied in metabolic syndrome and T2D, some have been studied for their contribution to risk or better outcome of infection with SARS-CoV-2. Given the risk factors described above, one would hypothesize that rigorous glycemic control and use of insulin would be beneficial, whilst use of lipid lowering drugs may not affect outcome. Metanalyses have found that user of metformin and sulfonylureas had lower mortality risk (188). Metformin acts by lowering hepatic glucose output, with a number of proposed molecular mechanisms, and Sulfonylureas are insulin secretagogues. Interestingly, some studies report that insulin use was associated with worse outcome (188). This is at odds with the proposed action on stimulating $CD8^+$ T-cell function, however in an already diabetic context, the use of insulin may be a sign of progressed diabetes and thus an overall less healthy metabolic status of the patient. Use of dipeptidyl peptidase-4 inhibitors did not have an effect on disease outcome. So, these studies indicate that use of antidiabetic agents either improve or do not affect outcome in COVID-19 in patients with T2D; unless patients with T2D have progressed to the stage of use of exogenous insulin. With regards to controlling dyslipidemia, the most commonly used class of drugs is Statins and studies have been ambivalent. Some meta-analyses conclude better outcome from retrospective studies (189) whilst others report improvement, worsening or no effect (190).

5 PERSPECTIVES: METABOLOMIC SHIFTS AND METABOLIC DEPRESSION IN COVID-19

Beyond pre-existing T2D, and clinical and immunological factors mentioned above, another way of addressing interactions between metabolism and response to infection is the application of metabolome studies. Such approaches are most valuable to basic research when applied translationally, studies are often data-driven in nature and when combined with clinical and immunometabolic observations, metabolomic approaches may lead to further hypothesis generation to direct future mechanistic work. Two reports by Shen et al (191) and by Wu et al (192) actually characterized the systemic metabolomic, lipidomic and proteomic responses to SARS-CoV-2 infection. Concertedly, these studies show that COVID-19 is characterized by a generalized systemic metabolic depression, although they were carried out in non-diabetic patients, metabolomic and lipidomic changes may indicate which specific pathways are dysregulated in COVID-19, and potentially subject to worsening in patients with metabolic disease.

The study by Shen et al. investigated proteomic and metabolomic profiles of sera from patients with severe and non-severe COVID-19 and non-COVID-19 patients with similar clinical presentation (other respiratory infections). In this study, the COVID-19-dependent proteome and metabolome represented three major pathways: complement system, macrophage function and platelet degranulation (191). Lipoproteins, sphingolipids, glycerolipids, steroid hormones and their intermediates related to macrophage function were downregulated in COVID-19 and correlated to severity. These molecules play important roles in immune function, including signaling, regulating membrane properties, apoptosis, migration and importantly in the resolution of inflammation. This lipid repressive profile is specific to SARS-CoV-2, as studies with other viruses (HCoV-229E, MERS-CoV) found increases of several of these lipid mediators relative to healthy subjects (193). As for liver-derived molecules, increased bilirubin degradation products and bile acid derivatives indicate impaired hepatic detoxification and urea cycle activity is also altered, which is a typical consequence of the interferon response associated with viral infection. Interestingly, the proteomic profile also showed decreased serotonin correlating to disease severity; which would influence sickness behaviors (e.g., lack of appetite, lethargy) also altering systemic metabolism.

The publication by Wu et al (192) carried out kinetic targeted lipidomic and metabolomic profiling of sera from healthy subjects and from COVID-19 patients ranging from mild to severe, and to fatal disease. When compared to healthy subjects, 87 out of 431 metabolites tested were altered in patients with fatal COVID-19 at inclusion, which increased to 162 at last sampling; with fewer metabolites altered in severe and mild cases. Pathway analyses over all severities reflected enrichment of pyrimidine, fructose and mannose and carbon metabolism as well as taste transduction pathways; whereas fatal case features reflected thyroid hormone synthesis and signaling, and purine metabolism pathways. The last time points sampled in fatal cases were marked by an acute reduction of metabolites, with malate and aspartate being the most affected, indicating breakdown of mitochondrial respiration. Targeted lipidomic analyses found most lipids to be upregulated in COVID-19 compared to healthy subjects, with magnitude increasing with severity. Lipids dysregulated over all severities enriched pathways in phosphatidylinositol signaling, inositol phosphate metabolism and long-term depression. Dysregulated lipids in fatal COVID-19 enrich endocannabinoid signaling, bacterial and viral infection and glycerophospholipid metabolism pathways. Interestingly, the serum metabolome did not normalize after recovery. This could be due to residual effects of hyperinflammation, lasting damage to metabolic tissues, a particularity of COVID-19; or a combination of the above. In any case, this indicates that clinical recovery is not dependent on re-establishing metabolic homeostasis.

Taken together the above studies report generalized metabolic depression in COVID-19. Although metabolic diseases were not discussed, it is reasonable to assume that pre-existing dysmetabolism, sub-optimal liver function, impaired glucose homeostasis, dyslipidemia, or any other disequilibrium associated with metabolic disease will adversely affect the metabolic response to COVID-19. The above studies indicate that higher risk of severity in metabolic disease may be due to a lack of metabolic flexibility, as required to efficiently respond to SARS-CoV-2 infection. Indeed, the 80% of COVID-19 cases that are mild or asymptomatic, may be so because of their adaptable metabolism, capable of ramping up or down their substrate production or utilization to cope with pathogen burden.

Several overarching themes can be drawn in the context of these studies. COVID-19 is associated with: i) compromised liver function; ii) dyslipidemia; iii) depression or a depression-like molecular profile; and 4) Altered cellular metabolism. The liver response in releasing alarm molecules is common in severe infection and sepsis, compromised function may arise from high viral loads, congestion due to outstripped filtering capacity, or pre-existing intrahepatic triglycerides that impair liver function. Dyslipidemia may be due to increased lipolysis from a highly inflammatory background, which would be aggravated in T2D where fatty tissues lose sensitivity to insulin's anti-lipolytic effects. Importantly, dyslipidemia and increased lipolysis may also arise due to sickness behaviors that modify energy intake and expenditure, notably a loss of appetite and drop in blood glucose levels causing ketogenesis and reliance on lipid metabolism. Finally, effects were reported on cellular pathways of nucleic acid synthesis, lipid synthesis and oxidative metabolism. The loss of substrates for nucleic acid and lipid synthesis, may be explained by virus-host cell dynamics where viral particles usurp nucleotides for their own replication, and make use of cellular lipids to construct their envelopes. SARS-CoV-2 seems particularly efficient in diverting the use of cellular substrates for its own needs, the authors describe this as a 'hijacking' of host cell metabolic machinery (191). A recent study by Zhang et al. supports such dynamics by demonstrating that intercellular glucose and folate are depleted in SARS-CoV-2 infected cells. This is the result of SARS-CoV-2 infection orienting glucose and folate metabolism towards needs supporting viral replication, destabilizing host mRNA abundance and protein translation processes by its use for viral biosynthesis (194).

To put findings into context, over recent years immuno metabolism research has characterized systemic immunity in metabolic diseases as well as cellular metabolism associated with immune functions. Yet the metabolic demands of viral infection remain relatively understudied. A hallmark publication by Wang et al. in 2017 (130) highlights the systemic metabolism associated with bacterial and viral infection. Whilst both cause similar symptoms or sickness behaviors: loss of appetite, weight loss, fever, lethargy; the energy imbalance and systemic metabolic shift is favourable only in bacterial infection. Of note, fever and marked weight loss are common to SARS-CoV-2, where decreased energy intake is aggravated due to the loss of taste and smell and depression of central reward systems with decreased serotonin reported in the above studies (191, 192, 195). The resulting undernutrition shifts systemic metabolism from glucose-dependence to reliance on ketone bodies and fatty acids, reminiscent of fasting metabolism. This is maladaptive in viral infection, where nutritional supplementation, particularly with glucose, is protective, independently of inflammatory status

or pathogen burden. The systemic metabolic shift in infection is also favorable for viral replication, where free fatty acid substrates are used to form and close virion membranes (196). Whilst all may also apply to COVID-19, the added respiratory depression will also starve the host of oxygen and thus the capacity to maintain efficient oxidative metabolism, largely dependent on lipid-substrates, the mediators of which also sustain lipogenesis.

To date, few specific therapies have proven to be effective against COVID-19 (197, 198). Generally patients receive standard supportive care and antiviral therapies, trials have been ambivalent with regards to alternative therapies including chloroquine, steroids or their related compounds (199). These studies and many others have applied these approaches to classify patient risk, importantly these findings can also be used to redirect trials towards metabolically active therapeutics already applied in metabolic diseases. Analyses from medical databases based around prescription history will also give signs whether lipid lowering, glycogenolytic or anti-hyperglycemic agents may mitigate or aggravate COVID-19, as well as other respiratory infections with a high burden of disease.

AUTHOR CONTRIBUTIONS

AA, FA, RT, FS, KH, KS, JB wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Case report: SARS-CoV-2 infection as a trigger for diabetic ketoacidosis and newly detected pancreatic autoantibodies

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A 39-year-old-woman with a past medical history of type 2 diabetes mellitus (T2DM) on oral hypoglycemic agents presented to the emergency room with nausea, vomiting, shortness of breath, and altered mental status. Seven days prior to presentation, she was diagnosed with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Laboratory workup on presentation confirmed the diagnosis of diabetic ketoacidosis (DKA) (blood glucose 523 mg/dl, beta-hydroxybutyrate 8.91 mmol/l, pH 6.9, bicarbonate 11 mEq/l, anion gap 25 mEq/l, and HbA1c 10.8%). She was managed for DKA with hydration and insulin drip and discharged home. However, to our surprise, at the 2-week follow-up visit, she was found to have positive antibodies for zinc transporter 8 (ZnT8) (samples were collected on day of presentation). The rest of her antibodies associated with T1DM were negative. She was therefore started on a basal-bolus regimen and managed as type 1 diabetes mellitus (T1DM). Our case illustrates that there is an increased risk of T1DM following infection with SARS-CoV-2.

KEYWORDS

COVID – 19, SARS – CoV – 2, type 1 diabetes (T1D), autoantibodies, autoimminity

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Introduction

COVID-19 is caused by SARS-CoV-2 infection and can have severe adverse outcomes, including death (1). The endogenous receptor of the virus, angiotensin-converting enzyme-2 (ACE2), enables the virus to infect other organs including the endocrine system in addition to the lungs. Infection with SARS-CoV-2 is known to act as an infectious trigger for autoimmune conditions such as Guillain–Barré syndrome, systemic lupus erythematosus, thrombotic thrombocytopenic purpura, autoimmune hemolytic anemia, thrombocytopenic purpura, and autoimmune thyroid disease (2, 3). Multicentric studies have confirmed the increased prevalence of new-onset T1DM during the current COVID-19 pandemic (4, 5). Although this increased incidence of T1DM following COVID-19 is well established in epidemiological studies, the current literature lacks data on its pathogenesis. The majority of the published studies are retrospective with significant heterogeneity among them in terms of the study population. Moreover, many of these population studies lack data on immunologic workup, essential to understanding the pathogenesis of T1DM following COVID-19. Hence, there is a gap in our understanding of how infection with SARS-COV-2 may be associated with the development of antibodies against beta-cells, ultimately leading to T1DM. With our case, we want to provide a possible insight into the critical pathophysiologic aspect of the development of T1DM among patients following COVID-19.

Case report

A 39-year-old-woman with a past medical history of T2DM (diagnosed 1.5 years ago prior to presentation and managed with metformin and dapagliflozin) presented to the emergency room with chief complaints of nausea, vomiting, shortness of breath, and altered mental status in March 2022. Seven days prior to presentation, she tested positive for COVID-19 and was in home quarantine. Regarding her history of diabetes, she was diagnosed with type 2 diabetes based on her HbA1c level of 6.6 in 2020, and since then her HbA1c level has ranged between 6.1 and 9.3. On presentation, she had tachycardia with a pulse rate of 115 beats per minute. The rest of her vitals were stable with blood pressure of 109/68 mmHg, saturation of 97% on room air, and temperature of 98.8 F. BMI was 32 kg/m^2. Laboratory workup confirmed the diagnosis of diabetic ketoacidosis (DKA) (blood glucose 523 mg/dl, beta-hydroxybutyrate 8.91 mmol/l, pH 6.9, bicarbonate 11 mEq/l, anion gap 25 mEq/l, potassium 2.9 mEq/l, and HbA1c 10.8%). Rapid nasopharyngeal testing showed a negative result for other common viral etiologies including adenovirus, influenza A/B, parainfluenza, and enterovirus. The complete laboratory workup at presentation is summarized in Table 1. Chest x-ray showed unremarkable results without infiltrates. Considering diagnosis of DKA, she was treated with aggressive intravenous hydration, potassium replacement, and insulin drip per DKA protocol. She was also treated with intravenous bicarbonate. Her hyperglycemia was controlled after 2 days, following which the basal-bolus insulin regimen was initiated. She was discharged in a reasonably comfortable condition on dapagliflozin 10 mg daily, metformin 500 mg twice daily, and premix insulin 70/30 (insulin aspart protamine and insulin aspart) 10 units twice a day before meals. At a 2-week follow-up, her serology results (that were collected on day of presentation) came back positive for zinc transporter 8 (ZnT8) antibodies (67 U/ml; reference range <15 U/ml) and negative for glutamic acid decarboxylase 65 (GAD65) antibodies, insulinoma-associated protein 2 (IA2) antibodies, islet cell antibodies (ICA), and anti-insulin antibodies. Hence, metformin and dapagliflozin were discontinued and diabetes treatment was switched to insulin-only (insulin glargine 20 units daily and insulin lispro 5 units three times a day before meals)

TABLE 1 Laboratory investigation on admission.

Blood investigations (normal range, units)	Values
Blood glucose (random, <200 mg/dl)	523
Sodium (133-144, mEq/L)	137
WBC (3-11.3, K/μL)	13.26
Hemoglobin (10-16, gm/dL)	16.1
Platelet count (182-369, K/μL)	290
Potassium (3.6-5.2 mEq/L)	2.9
Calcium (8.5-10.1, mg/dL)	9.1
Chloride (98-107, mmol/L)	99
Bicarbonate (22-29 mEq/L)	12
Blood urea nitrogen (7-18, mg/dL)	15
Creatinine (0.60-1.30, mg/dL)	1.5
Estimated glomerular filtration rate (60-200, mL/min/m^2)	61.7
Albumin (3.4-5.0, g/dL)	2.5
Alanine transaminase (12-78, U/L)	9
Aspartate transaminase (15-37, U/L)	6
Amylase (25-115, U/L)	86
Lipase (73-393, U/L)	483
Creatinine kinase (21-232, U/L)	49
Troponin I (0-53.7, ng/L)	16.3
Lactic acid (0.4-2, mmol/L)	1.1
Beta hydroxybutyrate (0.02-0.27, mmol/L)	8.91
Hemoglobin A1c (<5.6, %)	10.8
Urinary investigation	
Bacteria (absent, count/HPF)	Trace
Glucose (negative, mg/dL)	4+
Ketone (negative, mg/dL)	4+
Blood (negative, NA)	3+
Protein (negative, mg/dL)	2+
WBC (0-2, mg/dL)	0-2

regimen with a continuous glucose monitoring device for management as T1DM.

Discussion

Acute hyperglycemic states (such as DKA and hyperosmolar hyperglycemia) among patients with diabetes can have catastrophic outcomes. Although DKA is a telltale complication of T1DM, it is not uncommon among patients with T2DM, particularly during insulinopenic states (6, 7). Patients with poorly controlled diabetes (such as our patient with HbA1c of 10.8%) are at a higher risk of DKA in the setting of the acute infection (8). Contrastingly, some studies reported that patients with diabetes who developed DKA following SARS-CoV-2 infection had good glycemic control prior to the hospitalization (9). Our patient had relatively new-onset diabetes (diagnosed within 1.5 years) and presented with DKA following COVID-19 infection, demonstrating a temporal association between onset of T1DM (evidenced by presentation in DKA, and need for insulin) following infection with SARS-CoV-2. Prior to this episode, the patient was treated with oral hypoglycemic agents for T2DM and never had a DKA. Interestingly, the antibodies against ZnT8 were detected upon follow-up visit confirming the diagnosis of T1DM. ZnT8 is a well-known autoantigen leading to the development of T1DM and can be detectable in up to 80% of patients with T1DM. Furthermore, it can be the only detectable autoantibody in approximately 26% of patients with antibody-negative (insulin, GAD, IA-2, and ICA) T1DM (10).

Various studies have demonstrated an increased incidence of newly diagnosed T1DM during the COVID-19 pandemic, compared to the pre-pandemic era (5). In a retrospective study by experts from the Center for Disease Control and Prevention (CDC), an analysis of two databases (IQVIA and HealthVerity) revealed a higher incidence of newly diagnosed diabetes (166% and 33% in IQVIA and HealthVerity databases, respectively) following COVID-19 (4). Besides increased incidence of T1DM, there is increased frequency and severity of DKA at the time of T1DM diagnosis during the current pandemic. Although the increased incidence of T1DM following COVID-19 is well established in epidemiological studies, the current literature still has a paucity of data on its etiopathogenesis with the majority of the published literature being retrospective, with significant heterogeneity, and with a lack of data on immunologic workup.

Multiple studies have hypothesized COVID-19 as a downstream precipitator of autoantibody-positive T1DM, with a possible inciting autoimmune trigger a while ago (11). Some studies have reported virus-mediated apoptosis of beta cells (pancreatic islet) in COVID-19 which has signaling pathways similar to those seen in T1DM patients (12). A stem cell study suggests that the SARS-CoV-2 virus can enter pancreatic islet cells *via* the ACE2 receptor leading to β-cell damage and a new-onset diabetes (13). While our report does not prove a link, we postulate that SARS-CoV-2 exposure could have potentially contributed to the observed effect in our case leading to precipitation of T1DM. Recently, a similar report of autoantibody (GAD and ZnT8)-positive T1DM, following an asymptomatic SARS-CoV-2 (confirmed with molecular testing), was described in a pediatric patient, in which authors postulated SARS-CoV-2 infection to be the precipitating event for T1DM (11).

One limitation of our article is that it lacks information on the variant type of SARS-CoV-2, which led to precipitation of DKA and positive antibodies, which could have helped understand pathogenesis. Nonetheless, our case supports the association between infection with SARS-CoV-2 and the development of pancreatic autoantibodies, adding evidence for the adult population. Although our patient did not have baseline autoantibodies assays, the long-term follow-up of the patient would be interesting to check if the patient develops more diabetes-related autoantibodies in due course. This is an interesting and evolving field needing more studies investigating pancreatic antibody levels in patients with newly diagnosed T1DM following COVID-19 to better understand this phenomenon.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

RM and RR gathered patient data. RM, GE, LK, AJ, and RR contributed to the literature review and drafting of the manuscript. All authors approved the manuscript in its entirety.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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COVID-19 and diabetes—Two giants colliding: From pathophysiology to management

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Since December 2019, a new coronavirus, called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread around the world, causing the coronavirus 2019 (COVID-19) pandemic. From the beginning, SARS-CoV-2 has put a strain on the health system. In fact, many patients have had severe forms of the disease with the need for hospitalization due to respiratory failure. To contain the pandemic, the most widely used approach has been lockdowns. Social restrictions have been reduced thanks to the development of vaccines and targeted therapies. However, fatal events still occur among people at high risk of serious infection, such as patients with concomitant diabetes. Different mechanisms have been proposed to explain the poor prognosis of patients with diabetes and COVID-19, but the specific cause is unclear. It is now known that insulin resistance, inflammation, and cytokine storm are involved. Moreover, SARS-CoV-2 uses the angiotensin-converting enzyme 2 receptors to enter cells. This receptor is expressed on pancreatic beta cells and, during infection, it appears that receptor involvement may induce hyperglycemia in patients with or without diabetes. In this study, we discuss the mechanisms underlying the poor prognosis in people with COVID-19 and diabetes and what may improve the outcome in these patients.

KEYWORDS

blood glucose, SARS, lifestyle, diabetes mellitus, COVID-19

Introduction

On 31 December 2019, an outbreak of pulmonary infection due to an unknown coronavirus (later called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)) was first reported in Wuhan, China (1). After a few weeks, the new virus had already spread around the world, quickly reaching pandemic status. In more than 2 years since the onset, there have been about 340 million confirmed cases of coronavirus disease 2019 (COVID-19), and more than five million deaths worldwide. To date, several weapons to use against COVID-19, including vaccines, have been developed. However, fatal events still occur in a nonnegligible proportion of patients, especially among people at high risk of serious infection, such as the elderly or patients with concomitant diabetes mellitus (DM), hypertension, chronic obstructive pulmonary disease (COPD), obesity, and/or cardiovascular disease (2). Indeed, the clinical manifestations of COVID-19 span from completely asymptomatic cases up to severe cases of multiorgan dysfunction. Among the various risk factors that predict adverse outcomes after SARS-CoV-2 infection, DM plays an important role. According to the World Health Organization, about 422 million people worldwide have DM, and there are about 1.5 million DM-related deaths each year, especially in low-middle-income countries. These numbers are expected to increase over time (3). Thus, given the global dimension and burden of DM worldwide, it is important to understand and evaluate the impact of COVID-19 on this population.

DM and COVID-19 co-occurrence embody the perfect example of a syndemic status (4), namely a state in which two or more co-occurring diseases amplify each other in a synergistic manner (5). Generally, DM is a known risk factor for the onset of infections, and published data have shown a greater susceptibility of people with DM to other coronaviruses such as the Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-1. In addition, people with DM are often older; have other comorbidities; and have metabolic, pathophysiologic, and immunologic alterations that can create the perfect storm for the onset of severe COVID-19 (6). Considering the global prevalence of DM and the negative impact of COVID-19, in this review, we discuss the mechanisms underlying the poor prognosis in patients with COVID-19 and DM and what may improve the outcome in these patients.

Abbreviations: ACE2, angiotensin converting enzyme 2; ACEis, angiotensin-converting enzyme inhibitors; ADA, American Diabetes Association; ARDS, acute respiratory distress syndrome; ApoA-I, apolipoprotein A-I; ATP, adenosine triphosphate; BMI, body mass index; CHD, coronary heart disease; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease-19; CRP, C-reactive protein; DK, diabetic ketoacidosis; DM, diabetes mellitus; DPP4is, dipeptidyl peptidase-4 inhibitors; FPG, fasting plasma glucose; GLP-1Ras, glucagon-like peptide-1 receptor agonists; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; ICU, intensive care unit; IL, interleukin; IR, insulin resistance; LDL-C, low-density lipoprotein cholesterol; MERS-CoV, Middle East respiratory syndrome coronavirus; MV, mechanical ventilation; OR, odds ratio; CI, confidence interval; RAS, renin-angiotensin system; ROS, reactive oxygen species; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SGLT2is, sodium-glucose cotransporter 2 inhibitors; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TNF-α, tumor necrosis factor alpha.

COVID-19 and DM

DM is a chronic disease associated with microvascular and macrovascular complications. It is one of the main causes of morbidity and mortality in the world (7). Moreover, people with DM have an increased susceptibility to respiratory infections, particularly influenza and pneumonia (8). Consistently, several authors have highlighted that DM is a risk factor for the poor prognosis of COVID-19 (9–11).

Since the beginning of the COVID-19 pandemic, it has been reported that patients with DM have a poor prognosis. The first Chinese report from Wuhan, China, showed that compared with patients without DM, patients with DM had a higher risk of developing complicated infections with pneumonia and acute respiratory distress syndrome (ARDS) and a higher rate of in-hospital mortality (odds ratio (OR) 2.85, 95% confidence interval (CI) 1.35–6.05). As the COVID-19 pandemic continued, other evidence of the poor prognosis of diabetic subjects infected by the SARS-CoV-2 accumulated. For instance, the multicentric French Coronavirus SARS-CoV-2 and Diabetes Outcomes (CORONADO) study involved 1,317 participants with DM, most (88.5%) with type 2 diabetes mellitus (T2DM). The authors reported that 31.1% of participants were admitted to the ICU, including 20.3% who required mechanical ventilation, and 10.6% met the primary outcome, namely death within 7 days of admission. There were no differences between T2DM and type 1 diabetes mellitus (T1DM), likely due to low statistical power (there were only 39 patients with T1DM) (12). In a general population cohort from Sweden, subjects with T2DM were more likely to be hospitalized for COVID-19, as well as admitted to the ICU and die, compared with matched controls from the general population. The excess risk was reduced but persisted after adjustment for comorbidities and other factors only for T2DM (hazard ratio (HR) for COVID-19–related mortality was 1.53, 95% CI 1.39–1.63) (13). Guan et al. (14) reported that the prevalence of DM among Chinese patients with severe diseases was threefold higher than those with nonsevere diseases. In a meta-analysis by Li et al. (15), the prevalence of DM in patients admitted to the ICU was twofold higher compared with those

not admitted to the ICU. In a large general population cohort of Scotland (n = 5,463,300), McGurnaghan et al. (16) showed that people with T2DM or T1DM had an elevated risk of fatal or critical disease and admission to the ICU. This risk, adjusted for age, sex, and DM duration and type, was higher in those who were men, living in a residential care home, with microvascular complications such as retinopathy and nephropathy, or with poor glycemic control. Moreover, diabetic ketoacidosis (DK) or hypoglycemia that had required hospitalization in the past 5 years increased the risk in these subjects. The use of insulin or sulphonylureas was associated with the highest risks, likely due to the increased risk of hypoglycemia (16) induced by these treatments. A population-based cohort study conducted in the UK showed that mortality in people with T1DM or T2DM had increased during the COVID-19 pandemic (10,989 of 16,743 additional deaths (65.6%)) compared with mortality over the same period in the previous 3 years. Moreover, this study confirmed that beyond classic risk factors (i.e., cardiovascular disease, non-white ethnicity, impaired renal function, age, sex, socioeconomic deprivation, and smoking habit), hyperglycemia and body mass index (BMI) were associated with poor prognosis. In fact, mortality was associated with glycated hemoglobin (HbA1c) >86 mmol/mol (HR 2.23, 95% CI 1.50–3.30, $p < 0.0001$); socioeconomic deprivation (HR 1.93, 95% CI = 1.36–2.72, $p = 0.0002$); and a BMI of <20.0 kg/m^2 (HR 2.45, 95% CI 1.60–3.75, $p < 0.0001$), 35.0–39.9 kg/m^2 (HR 1.72, 95% CI 1.21–2.46, $p = 0.0028$), or >40.0 kg/m^2 (HR 2.33, 95% CI 1.53–3.56, $p < 0.0001$) (6). None of these studies have reported differences in risk by type of diabetes. Barron et al. investigated the relative and absolute risks of in-hospital deaths with COVID-19 by type of diabetes in a population-based study conducted in the UK (17). This study showed an increased risk of death in subjects with diabetes, with a third of all in-hospital deaths with COVID-19 occurring in patients with diabetes. Unadjusted mortality rates were significantly higher for patients with type 2 than for people with type 1 diabetes, with both being significantly higher than for people without diabetes. However, very interestingly, for the first time, adjusted for sex, age, region, and index of multiple deprivation ethnicity, the odds ratio for in-hospital deaths in patients with type 1 diabetes was 3.51 and for patients with type 2 diabetes was 2.03 compared with the population without known diabetes (17). This issue is very important in view of the need for specific advice for people with different types of diabetes and their families.

Several mechanisms have been highlighted to explain why patients with DM have higher mortality and risk of complications than the general population.

SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) receptor, which is involved in several molecular processes and glucose control (18). High plasma glucose levels, defined by two blood glucose measures of >180 mg/dl within a 24-h period, have been related to the risk of mortality in patients with COVID-19 (19). Bode et al. (20) reported a 41.7% mortality rate in their cohort. Zhang et al. (21) confirmed a higher composite outcome risk (mechanical ventilation (MV), admission to the ICU, and death; OR 5.47, 95% CI 1.51–19.82, $p = 0.010$) in patients with hyperglycemia and COVID-19 (defined as fasting plasma glucose (FPG) ≥7.0 mmol/L (≥126 mg/dl) but HbA1c <6.5%) compared to patients with normoglycemia. In addition, ACE2 is expressed in several organs, including the lungs, heart, kidneys, liver, and stomach (22); this could explain the severe disease that evolves into multiorgan failure. Normally, ACE2 is important in the control of inflammation—in fact, it degrades angiotensin II and angiotensin I into smaller peptides, angiotensin-(1–7) and angiotensin-(1–9), respectively. The first has an antioxidant and anti-inflammatory role, through the Mas receptor pathway; this process is altered in patients with DM (23). It seems that in nonsurvivors, there is an imbalance in this pathway with a decrease in angiotensin-(1-7) (24, 25). Moreover, in people with DM, ACE2 expression is increased (26, 27), and it could explain the propensity of these patients to develop severe illness, with ARDS, cardiac involvement, and acute kidney injury. Hyperglycemia is associated with glycation of proteins, microangiopathy of alveolar capillaries, and proteolysis of connective tissue, leading to the collapse of small airways during expiration (28, 29). DM is characterized by low-grade chronic inflammation; in particular, hyperglycemia activates inflammatory pathways and increases oxidative damage, leading to failure of the immune system (Figure 1).

In DM, there is an increase in some cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and IL-8, that facilitate infections (30). Moreover, there is an imbalance between anti-inflammatory and proinflammatory subsets of T cells, with overactivation of the Th_1 and Th_{17} subsets (31, 32). Moreover, compared with patients with COVID-19 but not DM admitted to the ICU, patients with COVID-19 and DM had significantly higher levels of C-reactive protein (CRP), procalcitonin, ferritin, and IL-6 (33), all of which are involved in a hyperimmune response called a cytokine storm (34, 35). Insulin resistance (IR) and hyperglycemia are involved in endothelial dysfunction and activation of platelet aggregation. Patients with severe COVID-19 and DM also have higher D-dimer blood concentrations (35) and hyperfibrinogenemia (36). This state of hypercoagulability is frequently reported in patients with a poor prognosis due to the formation of microemboli in the lungs and in large arterial vessels, leading to stroke (37).

Altered FPG as a predictor of poor outcome

When comparing patients with and without DM (38, 39), the FPG level (≥7.0 mmol/l) (40) is an independent predictor of mortality from COVID-19. Moreover, FPG is positively related to the prolonged duration of SARS-CoV-2 clearance (41).

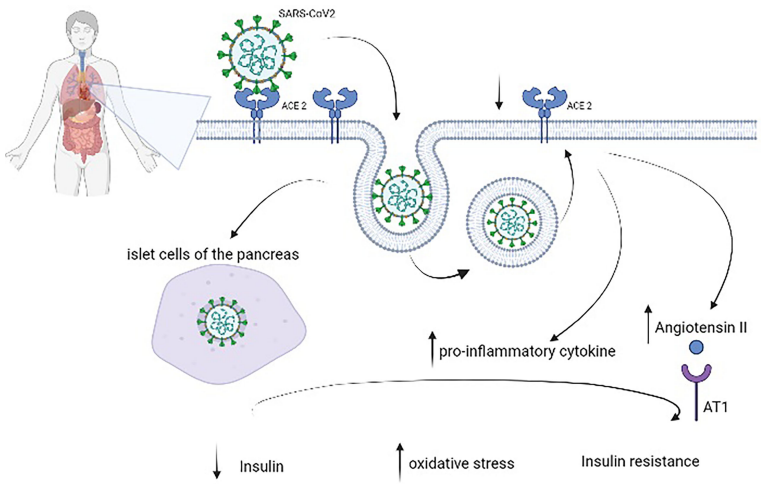

FIGURE 1
Pathophysiology of diabetes mellitus in patients with coronavirus disease 2019 (COVID-19). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds to angiotensin-converting enzyme 2 (ACE2), which is expressed in several organs, including the lungs, heart, kidneys, liver, and stomach, to enter cells. After endocytosis, SARS-CoV-2 could directly damage pancreatic beta cells as well as induce ACE2 downregulation, which leads to overexpression of angiotensin II with its harmful AT1-mediated effects and increases the levels of proinflammatory cytokines. These virus-induced alterations reduce insulin and augment oxidative stress and insulin resistance.

Additional studies have demonstrated a relationship between admission FPG level and the degree of severity in patients with COVID-19; it is also an independent predictor of poor prognosis at 28 days in patients with COVID-19 (42, 43). Chai et al. (44) reported that FPG ≥7.0 mmol/L at admission in patients with COVID-19 but not DM was related to chest tightness and slower recovery of lung abnormality 1 year after discharge. Indeed, hyperglycemia promotes the progression of COVID-19 and increases the risk of being admitted to the ICU and the incidence of clinical complications (use of vasoactive drugs or mechanical ventilation) (44). Several hypotheses have been advanced to explain the relationship between FPG and the poor outcome of patients with COVID-19. First, hyperglycemia alters innate cell-mediated immunity; inhibits neutrophil chemotaxis; and reduces phagocytosis by neutrophils, macrophages, and monocytes (41). Indeed, COVID-19 and hyperglycemia are tightly linked. If, as described above, SARS-CoV-2 infection impairs glucose metabolism, hyperglycemia could alter the antiviral interferon response, deferring activation of Th1/Th17 cells, inducing oxidative stress, and leading to endothelial dysfunction (44). Data have shown that myeloid cells (monocytes and macrophages) are the most documented in the lungs of patients with COVID-19 and are critical for the pathogenicity of the illness (45). In their *in vitro* study, Codo et al. (46) demonstrated that increased glucose concentrations affect viral replication. They proved that viral load and ACE2 and IL-1β expression increased in SARS-CoV-2–infected monocytes in a glucose-dependent manner. Moreover, they showed that aerobic glycolysis, necessary to produce adenosine triphosphate (ATP), is specifically upregulated in COVID-19-infected monocytes, and it is necessary and sufficient for SARS-CoV-2 replication (46).

Hyperglycemia and new-onset DM in COVID-19

In patients infected with SARS-CoV-2, it is common to experience "new-onset" hyperglycemia classified as "stress induced," "new-onset DM" in unknown prediabetes, or "secondary DM" following the use of corticosteroids (47). New-onset DM is defined by the American Diabetes Association (ADA) as two FPG measurements of ≥7.0 mmol/L (≥126 mg/dl) or HbA1c of ≥6.5% or a random glucose level of ≥11.1 mmol/L (≥200 mg/dl) with symptoms of hyperglycemia and without DM in the past. On the other hand, new-onset hyperglycemia without DM is defined as FPG between 5.6 and 6.9 mmol/L (100–125 mg/dl) and/or HbA1c between 5.7% and 6.4% (48). Previous studies have reported that DM is associated with poor prognosis in COVID-19, but COVID-19, in turn, leads to new-onset DM and acute metabolic decompensation of pre-existing DM (15, 40, 49–53).

Muller et al. (54) showed that SARS-CoV-2 infects cells of the human exocrine and endocrine pancreas. Indeed, they demonstrated that beta cells express viral entry proteins (ACE2 and TMPRSS2) and the pancreatic islets have a susceptibility to infection that could be inhibited by the use of

remdesivir. Furthermore, they demonstrated that impaired insulin secretion in pancreatic islets, due to infection, is mediated by a glucose-dependent mechanism. Thus, this evidence has supported the concept that the human pancreas is a target of SARS-CoV-2 infection and that this infection induces metabolic dysregulation detected in patients with COVID-19 (54).

Montefusco et al. (55) demonstrated that the COVID-19 inflammatory state, due to a cytokine storm, induced the presence of new-onset hyperglycemia, IR, and beta cell hyperstimulation in patients without a history of DM. They used continuous glucose monitoring to evaluate alterations in glycemic control and showed that normoglycemic patients with COVID-19 had an impaired glycemic profile and greater glycemic variability compared with healthy controls. Moreover, patients treated with tocilizumab showed a significant improvement in glycemic control compared with patients not treated with tocilizumab. Lastly, in the long term, these mechanisms led to beta cell deterioration and worsening of DM due to islet hyperstimulation and glucose toxicity (55).

Several hypotheses have been proposed to understand the pathophysiological mechanism underlying the onset of hyperglycemia or new-onset DM in patients with COVID-19. We will discuss some of these below.

Acute illness is characterized by a relative insulin deficit, increased lipolysis, and free fatty acids (56) that induce stress hyperglycemia due to a cytokine storm. In fact, a cytokine storm is characterized by higher levels of inflammatory markers such as CRP, a higher erythrocyte sedimentation rate, and more white blood cells (15). However, to date, few studies have determined whether stress hyperglycemia is transient or evolves into new-onset DM (57).

SARS-CoV-2 uses ACE2 as its receptor to enter human cells (49, 58, 59). After endocytosis, ACE2 is downregulated, leading to overexpression of angiotensin II. This change, along with direct entry of SARS-CoV-2 into the islet cells of the pancreas, damages beta cells by reducing blood flow. These alterations cause acute beta-cell dysfunction, leading to further impaired glucose homeostasis (60) and delaying insulin secretion (51, 61). SARS-CoV-2 infection promotes oxidative stress in the pancreatic cell (62), resulting in hypoxia and inflammation with impaired glucose metabolism (15). Moreover, SARS-CoV-2 directly damages crucial organs involved in glucose metabolism, such as the kidney and the liver, leading to altered glucose homeostasis (63).

Chronic hyperglycemia downregulates ACE2, which normally has an anti-inflammatory effect, promotes excessive secretion of proinflammatory cytokines such as IL-6 and TNF-α, and stimulates the renin–angiotensin system (RAS), leading to IR (64, 65). IR is distinguished by increased hepatic glucose release, reduced glucose utilization by muscle, and greater lipolysis. In this state, there is a reduced response to insulin (66).

Another hypothesis is that the common administration of dexamethasone in severe COVID-19 worsens the prevalence and severity of hyperglycemia in these patients by steroid-induced abnormalities with the attenuated recovery of beta cell damage (67). The phenomenon favors IR and impaired beta cell function, but the involvement of glucocorticoids to induce hyperglycemia in acute COVID-19 has not been fully elucidated (63).

Lastly, adipose dysfunction is an alternative factor considered to be the cause of hyperglycemia. Indeed, adiponectin and the adiponectin/leptin ratio are noticeably reduced in patients with severe COVID-19 (52).

The tight connection between COVID-19 and DM that has been well described by Mahrooz et al. (68) indicates that the interaction between DM and COVID-19 is a vicious cycle. SARS-CoV-2 infection induces the release of proinflammatory cytokines that, in turn, lead to IR and beta cell dysfunction with a consequent reduction in insulin secretion. Moreover, in patients with DM, SARS-CoV-2 can induce the release of catecholamines and glucocorticoids, which are hyperglycemic hormones that alter glycemic control during metabolic emergencies (DK and hyperosmolar hyperglycemia). All these mechanisms cause hyperglycemia that reduces the immune response and increases the virulence of SARS-CoV-2. Furthermore, the chronic inflammatory nature and the impaired glucose metabolism of DM dysregulate the immune system, including impaired T-cell and macrophage function and neutrophil chemotaxis, thus facilitating SARS-CoV-2 infection (68).

Lipid homeostasis and effects of SARS-CoV-2 in adipose tissue

Several studies have demonstrated alterations in the serum lipid levels in patients with COVID-19 compared with healthy people. Furthermore, dyslipidemia in these patients can worsen their prognosis. In particular, researchers have reported a significant decrease in the level of high-density lipoprotein cholesterol (HDL-C) only in critical cases and a significant decrease in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) in all patient groups (from mild to severe) (69–71). Li et al. (72) reported that patients with low HDL-C and apolipoprotein A-I (ApoA-I) concentrations at admission had high CRP concentrations, a protracted hospital stay, and augmented disease severity. Moreover, they showed that persistent hypolipidemia, including low TC, HDL-C, LDL-C, and ApoA-I concentrations, was mostly found in patients with COVID-19 who did not survive (72). The exact mechanism to explain these alterations is not well known, but several mechanisms have been proposed. First, in severe COVID-19, arachidonic acid is used to produce cytokines. A cytokine storm can, in turn, induce the release of unsaturated fatty acids as a

defense mechanism (73). Additionally, acute inflammation with a high release of proinflammatory cytokines, such as IL-1, IL-6, IL-12, interferon-gamma (IFN-γ), and TNF-α, can reduce the synthesis and/or secretion of apolipoproteins (74–76). Dias et al. (77) demonstrated that SARS-CoV-2 activates reprograming of lipid metabolism in monocytes, increasing the expression of CD36 (involved in lipogenesis), PPARγ, and SREBP-1 24 h after infection. Lastly, SARS-CoV-2 liver dysfunction can impair lipid metabolism, affecting the synthesis of apolipoproteins and lipoprotein in severe COVID-19 (78).

DM and cardiometabolic multimorbidity

A Finnish population-based study concluded that T2DM is a "coronary heart disease equivalent." The authors included 1,373 people without DM (638 men and 735 women) and 1,059 people with T2DM (581 men and 478 women). The follow-up period was 18 years, and they investigated the incidence of death due to coronary heart disease (CHD). The authors showed that T2DM without prior myocardial infarction had a similar risk for CHD death when compared with a group with prior myocardial infarction without DM (79). During the COVID-19 pandemic, several studies did not show independent associations between poor outcomes in hospitalized patients with T2DM and with a prior history of cardiovascular events (12, 80, 81). Infection with SARS-CoV-2 increased the risk of hospitalization in patients with cardiovascular disease (82, 83). Moreover, a previous history of CHD increases the risk of in-hospital death for COVID-19 (84). Two Italian studies (CoViDiab I and CoViDiab II) (85, 86) showed that the prevalence of previous cardiovascular events was similar in people with T2DM with and without COVID-19. Moreover, patients with T2DM with other cardiometabolic multimorbidity (defined as two or more DM, hypertension, and dyslipidemia) had a higher risk for ICU admission or death than those without DM. The authors hypothesized that cardiometabolic risk factors increased hypercoagulable and proinflammatory states in patients with DM.

What can improve the prognosis of patients with DM and COVID-19?

Understanding the distal and proximal causal pathways linking DM to severe COVID-19 is necessary to identify potential interventions and optimal treatments. Considering the close relationship between hyperglycemia and poor outcomes in patients with COVID-19, it is evident that good glycemic control is necessary for a proactive approach in these patients. For example, a recent cohort study underlined that well-controlled blood glucose levels are associated with sensibly lower mortality compared with individuals with poorly controlled blood glucose levels during hospitalization (87). Thus, standard prescriptions, such as a healthy diet, physical activity, and regular glucose and blood pressure monitoring, to prevent or reduce the burden of chronic hyperglycemia should play a primary role in reducing the risk of poor prognosis in patients with COVID-19 and DM. High-intensity exercise programs lower blood glucose, HbA1c, lipid levels (TC, HDL-C, and triglycerides), and blood pressure and improve body composition and endurance in individuals with T2DM (88). Hence, they should represent a key intervention to lower the risk of severe COVID-19 in the diabetic population.

Following a healthy diet is another crucial step to controlling HbA1c, FPG, and the metabolic profile in patients with T2DM. A recent study involving more than 500,000 adults found that following a healthy diet was associated with a 9% lower risk of getting infected by SARS-CoV-2 and a 41% lower risk of developing severe COVID-19 compared with people who reported eating the least fruits and vegetables (89). The authors quantified the diet quality by using the validated healthful plant-based diet index (hPDI score): healthy plant foods (nuts, whole grains, vegetables, fruits, legumes, tea/coffee, and vegetable oils) receive a positive score, while animal foods and fruit juices, sweets/desserts, sweetened beverages, potatoes, and refined grains receive a negative score. Plant-based diets are associated with a substantially lower risk of developing T2DM (90). The low risk of SARS-CoV-2 in people who consume a healthy diet is perhaps explained by the vitamin (A, B_6, B_{12}, C, D, and E), folate, omega 3 fatty acid (docosahexaenoic acid and eicosapentaenoic acid), and polyphenol contents. These components could change the immune response and improve glycemic control.

Therapeutic approaches in patients with COVID-19 and DM

All patients, particularly those with T1DM, should be instructed to increase the frequency of blood glucose measurements and to recognize the management of DK (91). To reach near-normal blood glucose levels, all available treatments must be put into practice: psychological approaches to a healthy lifestyle, intensive blood glucose monitoring, and reasonable drug treatment (92). Recent recommendations advise that drugs that may induce hypoglycemia should be avoided, and doses of oral medications may need to be reduced (93). Antidiabetic therapy must be started or strengthened for patients who repeatedly have preprandial blood glucose values of >180 mg/dl (>10 mmol/dl). The target preprandial glucose level is usually 140–180 mg/dl. When choosing the drug, it is important to remember that hypoglycemic drugs frequently used to treat DM could affect COVID-19 pathogenesis (94). In patients with T2DM, oral hypoglycemic drugs must be re-evaluated upon admission in relation to disease severity,

glucose level, chronic treatment, and other factors that could worsen adverse events such as dehydration and reduced oral intake (93). Below, we briefly explain the effects on inflammation of the various classes of drugs used in DM (Table 1).

Metformin

In addition to its known antihyperglycemic effect, metformin reduces the risk of death and hospitalization among patients with COVID-19 with DM (117). The mechanism is not clear, but several hypotheses have been proposed. First, it increases the expression and phosphorylation of ACE2, a change that decreases SARS-CoV-2 binding to ACE2 (95). Indeed, AMPK, a target of metformin, acts on the expression and stability of ACE2 (118). Furthermore, it leads to reduced production of reactive oxygen species (ROS), oxidative stress, and DNA damage (96). Finally, metformin diminishes TNF-α (95); reduction in this cytokine is associated with a decrease in mortality, as demonstrated by the use of TNF-α inhibitors (119). In a retrospective study, Li et al. reported that the use of metformin both prior to hospitalization and during treatment in hospital is significantly associated with lower mortality in COVID-19 patients with type 2 diabetes (97). Similarly, treatment with metformin at the time of hospitalization with COVID-19 infection appears to have better outcomes in terms of reduced need for intensive care (95). It is important to note that metformin presents several side effects

TABLE 1 Drugs to treat diabetes mellitus in patients with COVID-19: from mechanisms to indications for use.

Antidiabetic medications	Mechanisms	Conclusions from the literature	Indications for use/discontinuation
Metformin	1. Activation of AMPK, leading to: - Altered conformation of ACE2, decreasing SARS-CoV-2 binding (95) - Reduction of TNF-α and mTOR inhibition, with consequent anti-inflammatory and immunomodulatory activities (95) 2. Other actions: - Reduced production of ROS, oxidative stress, and DNA injury (96)	Reduced risk of death and hospitalization among patients with COVID-19 and DM (95, 97)	It may be continued in patients with milder forms of COVID-19 (95) Withdraw in patients with severe respiratory distress, renal impairment, or heart failure Risk of lactic acidosis and hypoxemia (95, 98)
DPP4is	- Reduction of proinflammatory cytokines (99) - Antifibrotic and immunomodulatory effects (100–105)	Nonhomogeneous data on the reduction of mortality in patients with DM (106)	Useful in patients with mild-to-moderate symptoms (106) Neutral effect on cardiovascular outcomes
GLP-1Ras	- Action on ACE2 and Mas receptor pathways (24) - Effect on inflammation and fibrosis (107, 108)	Available results indicate an advantageous effect on hospitalizations and mortality in patients with COVID-19 and DM (109, 110)	Useful in patients with mild-to-moderate symptoms (94)
SGLT2is	- Upregulation ACE2 (111) - Increase angiotensin (1-7) (112) - Anti-inflammatory, antioxidative, and antifibrotic effects (112) Reduction of cardiovascular risk factors	Heterogeneous and sparse evidence	In patients with cardiometabolic risk factors, evaluate the possible risk of dehydration, ketoacidosis, and acute kidney injury (98)
Thiazolidinedione	Increased ACE2 expression (106) Reduced secretion of proinflammatory cytokines (106) Increased secretion of anti-inflammatory cytokines (106)	Insufficient data	Withdraw in patients with acute diseases with specific contraindications (weight gain, edema, and worsening of heart failure) (113)
Insulin	- Downregulation of ACE2 receptors (27) - Positive effect on inflammation and coagulation (114)	- Several studies indicate insulin as the treatment of choice to optimize glycemic control in acutely serious hospitalized patients with COVID-19 (115) - T2DM treated with insulin had a decreased risk of COVID-19 infection requiring hospitalization (85)	Multi-injection insulin therapy or continuous intravenous infusion by a syringe pump in acutely serious hospitalized patients with COVID-19 (116)

ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019; DM, diabetes mellitus; mTOR, mammalian target of rapamycin; ROS, reactive oxygen species; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNF-α, tumor necrosis factor alpha.

(120) and potentially life-threatening adverse effects in acute illness, such as lactic acidosis (121) and acute renal disease. Therefore, metformin is not the proper therapy in patients with severe respiratory distress, renal impairment, or heart failure. The evidence supports the use of metformin when COVID-19 infection is not severe (95). In critically ill patients with COVID-19, metformin should be discontinued (95, 98).

Dipeptidyl peptidase-4 inhibitors and glucagon-like peptide-1 receptor agonists

Because other coronaviruses use DPP4 as a receptor to enter the host cell, researchers have suggested that dipeptidyl peptidase-4 inhibitors (DPP4is) may have a beneficial role against SARS-CoV-2 (122, 123). Based on this hypothesis, Fadini et al. (124) carried out a case-control study comparing DPP4is treatment among patients with COVID-19 and T2DM with the expected rate of DPP4is treatment in similar patients without COVID-19. The authors showed no evidence that DPP4is prevents people with T2DM from becoming infected by SARS-CoV-2. In the CORONADO study, the authors showed no evidence that treatment with DPP4is affects the severity of COVID-19; 21.6% of the population had been treated with DPP4is (12). Furthermore, some evidence suggests that incretin-based therapies may be helpful in people with COVID-19 and DM by improving the course of infection and reducing mortality in patients with DM (125). These hypotheses are based on the fact that DPP4 could affect the regulation of the immune system and increase inflammation (94, 95, 100–105, 117, 118). Moreover, it is unclear whether DPP4 could be a target receptor of SARS-CoV-2, similar to MERS-CoV (126). The use of DPP4is could promote anti-inflammatory activities *via* the reduction of proinflammatory cytokines (99). In a recent study, the administration of DPP4is induced neither harmful nor beneficial effects, so the authors did not advise the discontinuation of this class of drugs (127). In an Italian retrospective case-control study, however, the use of sitagliptin during hospitalization was associated with reduced mortality and better clinical outcomes (125). A retrospective study including patients with a moderate–severe SARS-COV-2 infection showed that treatment with DPP4is had no significant influence on clinical outcomes or mortality (106). To date, the published data do not contraindicate the use of DPP4is in patients with COVID-19 and T2DM (12, 125). However, further research is required to define the exact role of DPP4is in the course of COVID-19 in patients with diabetes.

Glucagon-like peptide-1 receptor agonists (GLP-1Ras) act on ACE2 and Mas receptor pathways, thus potentially preventing SARS-CoV-2 infection (24) and modulating inflammation (107) and fibrosis (108). The role of GLP-1RAs in the treatment of COVID-19 and T2DM is not clear. In a multinational retrospective cohort study, patients in treatment with GLP-1RAs showed a significant reduction in mortality (47%) and hospital admissions (40%), including a reduction in respiratory complications (46%) (109). A recent meta-analysis has also analyzed the impact of preadmission use of GLP-1Ras on the mortality outcomes of COVID-19 among patients with diabetes mellitus. Very interestingly, the data showed that preadmission usage of GLP-1RAs was associated with a reduction in mortality rate in patients with DM and COVID-19, independently of gender, age, gender, cardiovascular disease, hypertension, and the use of metformin and/or insulin (110). However, their initiation in critically ill patients is not fully recommended since they require titration, need time to become effective, and could be associated with several side effects (128). Recently, Lim et al. (94) advised using DPP4is and GLP-1Ras in patients with mild-to-moderate symptoms of COVID-19.

Sodium-glucose cotransporter 2 inhibitors

Sodium-glucose cotransporter 2 inhibitors (SGLT2is) are antidiabetic drugs that mainly act on the kidneys and inhibit renal glucose reuptake. Several studies have demonstrated that SGLT2is are also able to reduce renal and cardiovascular complications. SGLT2is have been shown to increase the expression of ACE in the kidney, and it is thought that they may increase susceptibility to COVID-19 infection (111). On the other hand, the upregulation of ACE2 leads to an increase in the production of angiotensin-(1-7), a vasodilator with both antioxidative and antifibrotic properties (112). One of the main indications in the therapeutic management in patients with COVID-19 and DM is that hypoglycemic drugs that could lead to volume depletion or hypoglycemia must be discontinued (98). Indeed, dehydration can predispose patients to lactic acidosis and DK during acute illness; thus, SGLT2is must be temporarily withdrawn in hospitalized patients (98, 129). Despite their cardiorenal beneficial effects in cardiovascular outcome trials, the use of SGLT2is might be complicated and even potentially dangerous in patients requiring critical care. However, a recent trial proved the efficacy and safety of dapagliflozin in hospitalized patients with COVID-19 with at least one cardiometabolic risk factor (DARE-19), and SGLT2is were well tolerated and fewer serious adverse events were observed in the dapagliflozin versus placebo group (130). The benefits of SGLT2is treatment during COVID-19 remain unknown, and the possible risk of diabetic ketoacidosis during treatment with gliflozin should not be ignored, and some cautions are necessary (106).

Sulphonylureas and thiazolidinediones

In current medical practice, hypoglycemic therapy must be at doses sufficient to reach glycemic control while avoiding hypoglycemia (131). In this view, sulphonylureas, which increase the hypoglycemic risk in the presence of reduced oral intake, must be discontinued during acute illness (113). Due to

its adverse effects, such as fluid retention, thiazolidinedione must be withdrawn in patients with acute diseases (113). Moreover, this drug class appears to increase the expression of ACE2, thus augmenting the susceptibility of cells to SARS-CoV-2 entry (106). Pioglitazone, an example of thiazolidinediones, has shown anti-inflammatory activity and may reduce the secretion of some proinflammatory cytokines (106).

Insulin

Several studies indicate insulin as the treatment of choice to optimize glycemic control in acutely serious hospitalized patients with COVID-19 (115). Insulin presents a significant anti-inflammatory effect in critically ill patients (27). Moreover, it seems that insulin could downregulate ACE2 (27). Treatment with insulin leads to optimal glycemia control in patients with T2DM and COVID-19 and seems to have a positive effect on inflammation and coagulation (114).

A case-control study (CoViDiab I) showed that people with T2DM treated with insulin had a decreased risk of COVID-19 infection requiring hospitalization (85). Some evidence has demonstrated that patients with severe COVID-19 and DM have a higher insulin requirement (92, 93), which may be explained by the dysfunction of beta-cells or the high inflammatory process induced by the virus. For these reasons, the best therapeutic choice for these patients is multi-injection insulin therapy or continuous intravenous infusion by syringe pump (116) with adequate enteral nutrition or regular oral food intake. Importantly, the use of insulin has some disadvantages, among which is the need to closely monitor the patient's glucose levels to avoid hypoglycemia (132, 133). Regarding the resumption of previous therapy, current guidelines suggest that it be resumed at the time of discharge (93).

Effectiveness and safety of COVID-19 vaccination in people with DM

In the context of the COVID-19 pandemic, vaccines have played a central role in protecting susceptible people (134). DM is known to impair the innate and adaptive immune systems by reducing antibody responses. However, there is conflicting evidence as to whether DM impairs seroconversion following COVID-19 vaccination. Some researchers have tried to compare the antibody response between patients with and without DM. In a retrospective study with a small sample of patients with nonsevere COVID-19 (n = 31), Pal et al. (135) showed that patients with DM had not reached seroconversion following COVID-19 vaccination 2 weeks after diagnosis. Data from two Italian studies that involved patients with or without DM revealed that the presence of DM and hyperglycemia did not impair the kinetics and durability of the neutralizing antibody response (136). Conversely, the CAVEAT study demonstrated a lower antibody titer in patients with DM and HbA1c of >7%, indicating that inadequate glycemic control during the postvaccination period could reduce the antibody response (137). On the other hand, in the COVAC-DM study, Sourij et al. (138) reported that in patients with DM, the age and estimated glomerular filtration rate are predictors for an immunological response after COVID-19 vaccination, while HbA1c levels are not. A recent systematic review demonstrated a lower seroconversion rate in patients with DM than in healthy controls after COVID-19 vaccination (134). Nevertheless, the antibody response was robust and persistent. Therefore, all patients with DM should be vaccinated given the high risk and poor prognosis for COVID-19.

Conclusions

COVID-19 and DM represent a dangerous combination in terms of mortality and hospitalization risk, in both general and high-risk populations. It is clear that these two entities influence each other and act synergistically, through molecular and clinical pathways, to affect the patient's prognosis. At the same time, and this is an intriguing point, there is ample evidence that some molecular and clinical factors act as prognostic factors (their presence influences a negative prognosis) as well as predictive factors (their control improves the prognosis). This is the case of hyperglycemia: it is a trigger of severe events among people with COVID-19 and DM but, when controlled appropriately, can reduce the risk of poor outcomes (139).

Moreover, patients with COVID-19 and DM provide an excellent opportunity to use a personalized medicine approach. For example, normalizing blood glucose in patients with COVID-19 and DM may require downtitration (or even suspension) of metformin and SGLT2is, which are, according to guidelines, the current first-line treatments aimed at reducing cardiovascular risk in patients with DM alone (140). All these concepts should be kept in mind to ensure the good impact and management of patients with COVID-19 and DM during the current pandemic—until it improves and, hopefully, resolves completely.

Author contributions

Conceptualization: MCP, IZ, MP, and GM. Methodology: MCP and IZ. Writing—original draft preparation: MCP, IZ, MP, and GM. Writing—review and editing: MP and GM. Supervision: FA. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Risk of severe COVID-19 infection in persons with diabetes during the first and second waves in Denmark: A nationwide cohort study

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Objective: Coronavirus disease-2019 (COVID-19) increases risk of hospitalization and death in diabetes and diabetes-related conditions. We examined the temporal trends in COVID-19-related hospitalization and mortality in the total Danish population by diabetes and diabetes-related conditions in the two first waves of COVID-19 in Denmark.

Materials and methods: We identified all persons with diabetes in the whole Danish population using national registries. COVID-19-related risks of hospitalization and death were assessed using Cox regression analysis in wave 1 (1 March-31 August 2020) and wave 2 (1 September 2020-28 February 2021) of the pandemic for persons with (n=321,933) and without diabetes (n=5,479,755). Analyses were stratified according to status of hypertension, obesity, cardiovascular and microvascular disease.

Results: The cumulative incidence of COVID-19 hospitalization increased from wave 1 to wave 2 in both persons without (from 4 to 10 in 10,000) and with diabetes (from 16 to 54 per 10,000). The relative risk of hospitalization, however, increased more in patients with diabetes compared to persons without (age-, sex- and co-morbidity-adjusted HR [aHR] 1.40 (95% CI 1.27, 1.55) versus 1.76 (1.65, 1.87), p<0.001 for interaction with wave). The mortality rate, according to the whole population, increased similarly in persons without and with diabetes from wave 1 to wave 2 (from 0.63 to 1.5 versus from 4.3 to 10 in 10,000; aHR 1.65; 1.34, 2.03 and 1.64; 1.43, 1.88). However, when mortality was restricted to the hospitalized population, the crude mortality fell from 26.8% to 19.6% in persons with diabetes, while only a minor decrease was seen in persons without diabetes (from 16.7% to 15.5%).

Conclusion: The risk of COVID-19-related hospitalization increased more in persons with than without diabetes from wave 1 to wave 2 of the COVID-19 pandemic in the Danish population. However, mortality according to the whole

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population did not change, due to reduced mortality among hospitalized persons with diabetes.

KEYWORDS

COVID-19, diabetes, cardiovascular disease, microvascular disease, hypertension, SARS-CoV-2, obesity, hospitalization

Introduction

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes severe pneumonia as well as other severe extrapulmonary conditions (1). By 8.June 2022 more than 6.3 mio deaths of coronavirus disease 2019 (COVID-19) had been reported globally (https://covid19.who.int/). Early in the pandemic it was established that high age and various co-morbidities conferred increased risk of hospitalization, treatment at an intensive care unit (ICU) and death due to COVID-19 (2, 3). Especially diabetes has been established as a strong risk factor for severe COVID-19 infections (4, 5), but also conditions overrepresented in individuals with diabetes, such as obesity (6), established cardiovascular disease (7) and hypertension (8), have been reported as risk factors.

Measures to protect the population against COVID-19 related hospitalization and adverse outcomes of COVID-19 involve restrictions on societal activity (9, 10), protective guidelines in general (11), and advances in treatment (12). Shielding of high risk population groups has been part of the protective strategy in some countries, including Denmark, but the effect of such measures are uncertain (13–17). Moreover, acceptance and implementation of these measures change over time (18). However, to our knowledge the combined effect of these factors on the risk of hospitalization with COVID-19 and death in persons with diabetes between the first two waves has not been established. We hypothesized that the risk of adverse COVID-19 outcomes in persons with diabetes would change during the first two waves of the pandemic in Denmark. The subsequent emergence of COVID-19 vaccines has decreased the risk (19), and competes with the effect of other protective measures.

Therefore, we investigated and compared the risk of hospitalization, need for intensive care and related death in the Danish population with and without diabetes in the first and second wave of the pandemic, before the introduction of COVID-19 vaccination. As shielding guidelines for specific groups, e.g. persons with diabetes, are dependent on precise risk estimation, we furthermore examined the combined effect of diabetes status and diabetes-related conditions such as hypertension, obesity, cardiovascular disease and microvascular disease on the risk of COVID-19 related outcomes.

Abbreviations: aHR, Adjusted hazard ratio; COVID-19, Coronavirus disease-2019; CRS, The Danish Civil Registration System; DNPR, Danish National Patient Registry; ICU, Intensive care unit; NPR, National Prescription Registry; MIBA, The Danish Microbiology Database; PCR, Polymerase Chain Replication; SARS-CoV-2, Acute respiratory syndrome coronavirus 2.

Materials and methods

Population and data sources

The Danish Civil Registration System includes data on citizenship, date of death (if any) and migration status for all Danish citizens (20). Using the unique civil registration number assigned to all Danish citizens, person-level linkage can be made between the content of all national health registries. All citizens of Denmark alive and not migrated by March 1st, 2020 were eligible for analysis of the first wave and by September 1st, 2020 for analysis of the second wave of the COVID-19 pandemic. Diagnosis codes from all hospital contacts, as well as codes for diagnostic and medical procedures and surgical interventions were obtained from the Danish National Patient Registry (DNPR) that has recorded all hospital contacts in Denmark since 1977 (outpatient contacts since 1995) (21). All medications redeemed at Danish community pharmacies were available from the National Prescription Registry (NPR) that has been in operation since 1995 (22). Results from Polymerase Chain Replication (PCR) tests for SARS-CoV-2 were obtained from The Danish Microbiology Database (MIBA) (23).

Outcomes

The primary outcomes were COVID-19 related hospitalization and COVID-19 related mortality. COVID-19 related death was defined as death within 30 days of the start of a COVID-19 related hospitalization. COVID-19 related hospitalization was defined as hospitalization for more than 12 hours within 30 days prior and 14 days after a positive test for SARS-CoV-2 and with a recorded COVID-19 diagnosis for the

hospital contact. In case multiple hospitalizations in this period were present, the first contact was used. The date of the outcome was defined by the first day of COVID-19 related hospitalization. Secondary outcomes were COVID-19 related admission to ICU. COVID-19 related admission to ICU was defined as intensive unit care within 30 days of COVID-19 related hospitalization. Precise definitions are given in electronic supplementary material (ESM) Table 1.

Variables

Diabetes was defined by any ICD-10 diagnosis codes of diabetes in DNPR or redemption of glucose-lowering medication in the whole time-span of the registries, except registrations coincident with a diagnosis of polycystic ovarian syndrome or gestational diabetes (ESM Table 2). Type 1 diabetes was defined by at least two redemptions of insulin, no redemption of non-insulin glucose-lowering treatment, and at least one ICD-10 diagnosis code for type 1 diabetes (ESM Table 2), while all others with diabetes were defined as having type 2 diabetes. Hypertension was defined by any ICD-10 diagnosis code for hypertension or redemption of any anti-hypertensive treatment in the whole timespan of the registries. Obesity was defined by ICD-10 diagnosis codes for obesity, operation codes for bariatric surgery or redemption of weight-lowering drugs (with other indication than diabetes). Cardiovascular disease was defined by diagnoses or operation codes defining ischemic heart disease, cerebrovascular disease, heart failure, peripheral and central ischemic disease and lower limb amputations. Microvascular disease was defined by diagnosis, procedure and operation codes defining chronic kidney disease, retinopathy or maculopathy and polyneuropathy. Other co-morbidity was defined by modified groups according to the Charlson Comorbidity Index (dementia, chronic pulmonary disease, connective tissue disease, ulcer disease, any liver disease, hemiplegia and cancer) as defined in ESM Table 3.

Statistical analysis

The analyses were restricted to two separate periods: The first wave from 1.3.2020-31.8.2020 and the second wave from 1.9.2020-28.2.2021. The index date was defined as 1.3.2020 for the first wave and as 1.9.2020 for the second wave. The definition of the time periods of the waves were based on the following considerations: 1) The waves had to be comparable in length. 2) The time periods should include one period with a rapidly increasing hospitalization rate, including the peak. 3) The waves should be free of any significant use of vaccines. 4) Significant changes in the nature of the variants should be as low as possible. Overall, the difference between the waves were therefore the difference in the enforcement of general societal measures. For each wave we constructed cumulative incidence curves for each outcome, for individuals with and without diabetes, censoring non-COVID-19 mortality and emigration. The COVID-19 related mortality and risk of hospitalization were assessed in the total Danish population. As the risk of COVID-19 mortality, as defined in our study, is determined by two components: The risk of COVID-19 hospitalization in the overall population and the risk of death in the admitted population, we also calculated the mortality according to the admitted population to illustrate these pathways. We further calculated the corresponding 6-months cumulative incidences, overall and stratified by age and sex. We used cox regression analysis to estimate adjusted hazard ratios of the endpoints for the first and second wave. Persons were followed until an outcome event, death, emigration or end of study period, whichever came first. Furthermore, admitted individuals who did not experience the endpoint was censored after 30 days for the events death and ICU admission. Besides the unadjusted model we adjusted for age and sex in a second models and for the main

TABLE 1 Basic characteristics of all persons with and without diabetes in Denmark.

	Persons with diabetes	Persons without diabetes
Total	321,933	5,479,755
Males, n (%)	170,374 (52.9%)	2,714,576 (49.5%)
Females, n (%)	151,559 (47.1%)	2,765,179 (50.5%)
0-69 years, n (%)	193,253 (60.0%)	4,768,399 (87.0%)
70-79 years, n (%)	85,587 (26.6%)	480,363 (8.8%)
80+ years, n (%)	43,093 (13.4%)	230,993 (4.2%)
Type 1 diabetes, n (%)	25,040 (7.8%)	
Type 2 diabetes, n (%)	296,893 (92.2%)	
Microvascular disease, n (%)	54,812 (17.0%)	66,845 (1.2%)
Cardiovascular disease, n (%)	82,018 (25.5%)	307,534 (5.6%)
Obesity, n (%)	63,490 (19.7%)	195,589 (3.6%)
Hypertension, n (%)	255,063 (79.2%)	1,295,980 (23.7%)

non-stratified analysis we further adjusted for co-morbidity (cardiovascular disease, microvascular disease, obesity, hypertension, dementia, chronic pulmonary disease, connective tissue disease, ulcer disease, any liver disease, hemiplegia and cancer) in a fully adjusted model. We explored the interaction between diabetes status and wave. We then performed stratified cox regression analysis for selected diabetes-related conditions and treatments: age, sex, diabetes type, hypertension, obesity, cardiovascular disease, microvascular disease and lipid-lowering treatment adjusted for age and sex. Each diabetes-related condition was stratified by diabetes status, yielding 4 subgroups for each analysis with persons without both diabetes and the diabetes-related conditions as the reference. Stratification is only presented for the second wave as the infection was more uniformly distributed compared to the first wave. To elucidate differences in treatment success, Cox regression for ICU admission was also explored as a secondary outcome, with both the hospitalized population and the total population as a base. The risk of a COVID-19 infection was assessed as the first positive SARS-CoV-2 PCR test in a COX regression model, adjusted as described for the main analyses above. In two sensitivity analyses we tested the risk of COVID-19 related hospitalization within 14 days of the first positive test and the risk of death within 30 days of the first positive test. All analyses including first positive PCR test were done for each wave separately and therefore the interaction between wave and diabetes was not assessed.

The Cox proportional hazards assumption was assessed by plotting the natural log of the cumulative hazard function for those with and without diabetes in each wave, against the natural log of time and then checked for parallelism. The proportional hazard assumption was not fulfilled for the Cox regression analyses in analyses when both waves were in the same model as the cumulative incidence cross and for these analyses the HR should be interpreted as averages over the entire waves. All analyses were performed with STATA version 17.

Results

In the total Danish population 321,933 had diabetes and 5,479,755 did not have diabetes at the start of the first wave. The prevalence of hypertension and obesity was higher among persons with diabetes as was diabetes-related complications, including cardiovascular and microvascular disease (Table 1).

Primary outcomes: Risk of hospitalization and mortality

Covid-19 related hospitalization was observed in a total of 2604 and 7347 persons in the first and second wave, respectively (Table 2). The crude 6-months cumulative incidence increased from wave 1 to 2 in persons without diabetes (from 4 to 10 in 10,000) and in persons with (from 16 to 54 in 10,000, ESM Figure 1, ESM Table 4). The risk of COVID-19 related hospitalization was higher in persons with diabetes in both waves after adjustment for age and sex (HR 2.33; 95% CI 2.11, 2.57 and 2.90; 95% CI 2.74, 3.07) and in the fully adjusted model (Table 3). Importantly, the relative risk in persons with diabetes compared to persons without diabetes increased from wave 1 to 2 (HR 1.40; 95% CI 1.27, 1.55 vs 1.76; 95% CI 1.65, 1.87; p<0.001 for interaction; Table 3).

We observed a total of 480 and 1173 deaths among persons hospitalized for COVID-19 in the first and second wave, respectively (Table 2). The crude 6-months mortality rate increased from 0.63 to 1.5 in 10,000 persons without diabetes and from 4.3 to 10 in 10,000 persons with diabetes from wave 1 to 2 (Figure 1, ESM Figure 2, ESM Table 4). HR for mortality, adjusted for sex and age, was 2.39 (95% CI 1.96, 2.92) and 2.36 (95% CI 2.07, 2.69) for persons with diabetes compared to persons without diabetes in the first and second wave, respectively (Table 3). In the fully adjusted model, the risk of death was also significantly increased in persons with diabetes

TABLE 2 Number of persons with COVID-19 infection, COVID-19 related hospitalization, death and intensive unit care (ICU) admission in the first and second wave of the pandemic, stratified by diabetes status.

	Persons with diabetes		Persons without diabetes		Hospitalized persons with diabetes		Hospitalized persons without diabetes	
	Wave 1 n=321,933	Wave 2 n=325,555	Wave 1 n=5,479,755	Wave 2 n=5,429,502	Wave 1 n=519	Wave 2 n=1758	Wave 1 n=2085	Wave 2 n=5589
COVID-19 infection*	1389 (0.43%)	9896 (3.05%)	15503 (0.28%)	181705 (3.36%)	–	–	–	–
Hospitalization	519 (0.16%)	1758 (0.54%)	2085 (0.04%)	5589 (0.10%)	–	–	–	–
Deaths	137 (0.04%)	334 (0.10%)	343 (0.01%)	839 (0.02%)	137 (26.4%)	334 (19.0%)	343 (16.5%)	839 (15.0%)
Admission to ICU	87 (0.03%)	217 (0.07%)	265 (0.00%)	554 (0.01%)	87 (16.8%)	217 (12.3%)	265 (12.7%)	554 (9.9%)

*Persons at risk was slightly different for this analysis as only the first positive test is present in the MIBA database. Positive tests before the start of the wave is therefore excluded. Persons with diabetes: wave 1 (n=321,933) and wave 2 (n=324,316). Persons without diabetes: wave 1 (n=5,479,752) and wave 2 (5,414,646)
COVID-19 infection was defined by the first positive PCR test.

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TABLE 3 Hazard ratio (HR) for persons with diabetes compared with persons without diabetes for COVID-19 related hospitalization, mortality, and admission to intensive care unit (ICU) in the whole Danish population, and COVID-19 related mortality and ICU admission in the hospitalized population only.

Outcome	HR (95% CI) crude		HR (95% CI) adjusted for age, sex		HR (95% CI) adjusted for age, sex and comorbidity		Interaction with wave, fully adjusted model
	Wave 1	Wave 2	Wave 1	Wave 2	Wave 1	Wave 2	
COVID-19 infection	1.53 (1.45, 1.62)	0.91 (0.89, 0.93)	1.58 (1.49, 1.67)	1.07 (1.05, 1.09)	1.36 (1.28, 1.45)	1.13 (1.11, 1.16)	p<0.001
Hospitalization	4.27 (3.87, 4.70)	5.29 (5.01, 5.58)	2.33 (2.11, 2.57)	2.90 (2.74, 3.07)	1.40 (1.27, 1.55)	1.76 (1.65, 1.87)	p<0.001
Mortality	6.84 (5.61, 8.34)	6.69 (5.89, 7.59)	2.39 (1.96, 2.92)	2.36 (2.07, 2.69)	1.65 (1.34, 2.03)	1.64 (1.43, 1.88)	p=0.95
Mortality in the hospitalized population	1.72 (1.41, 2.09)	1.29 (1.14, 1.47)	1.41 (1.16, 1.72)	1.19 (1.05, 1.35)	1.27 (1.03, 1.57)	1.07 (0.93, 1.23)	p=0.17
Admission to ICU unit	5.62 (4.41, 7.16)	6.58 (5.62, 7.70)	3.40 (2.64, 4.38)	3.99 (3.36, 4.75)	1.56 (1.20, 2.03)	1.85 (1.53, 2.23)	p=0.26
Admission to ICU in the hospitalized population	1.38 (1.09, 1.74)	1.26 (1.08, 1.47)	1.28 (1.01, 1.63)	1.18 (1.01, 1.39)	1.09 (0.84, 1.41)	1.02 (0.85, 1.21)	p=0.63

Mortality was defined as death within 30 days of the start of a COVID-19 related hospitalization, which was defined as hospitalization for more than 12 hours within 30 days prior and 14 days after a positive test for SARS CoV-2 and with a recorded COVID-19 diagnosis for the hospital contact.

compared to persons without diabetes, but the relative risk did not change between the two waves (HR 1.65; 95% CI 1.34, 2.03 and 1.64; 95% CI 1.43, 1.88; p=0.95 for interaction; Table 3).

When mortality was restricted to the hospitalized population only, the 6-months crude mortality rate decreased in persons with diabetes from 26.8% to 19.6% from the first to the second wave, while the corresponding mortality rate in persons without diabetes fell from 16.7% to 15.5% (ESM Figure 3, ESM Table 4). In the fully adjusted model, the mortality rate for persons with diabetes compared to persons without was increased in the first wave (HR 1.27; 95% CI 1.03, 1.57), while it was not increased in the second wave (HR 1.07; 95% CI 0.93, 1.23); p=0.17 for interaction with wave; Table 3).

As auxiliary analysis we estimated the rate of COVID-19 infection (Table 2). In wave 1 there was 16,892 COVID-19 cases, while there were 191,601 cases in wave 2. For persons with diabetes 1389 and 9896 had a COVID-19 infection in wave 1 and 2, respectively, corresponding to an infection rate of 0.43% and 3.05%. For persons without diabetes 15503 and 181705 persons experienced a COVID-19 infection, corresponding to an infection rate of 0.28% and 3.35% in wave 1 and 2, respectively. The risk of a COVID-19 infection was increased in persons with diabetes compared to persons without diabetes in both waves after adjustment for age and sex (HR 1.58; 95% CI 1.49, 1.67 and HR 1.07; 95% CI 1.05, 1.09). This was also seen in a fully adjusted model (HR 1.36; 95% CI 1.28, 1.45 and HR 1.13; 95% CI 1.11, 1.16). The risk was numerically higher in wave 1 compared to wave 2 (test for interaction not performed).

As a sensitivity analysis we also estimated the risk of a COVID-19 hospitalization given a positive COVID-19 PCR test (ESM Table 5). This validated our main analysis with an increased risk of being hospitalized in persons with diabetes in wave 1 and 2, in a fully adjusted model (HR 1.17; 95% CI 1.04, 1.32 and HR 1.57; 95% CI 1.47, 1.67). Mortality given a positive COVID-19 test was also increased in persons with diabetes in both wave 1 and 2 (HR 1.32; 95% CI 1.08, 1.60 and HR 1.28; 95% CI 1.15, 1.44), with no apparent difference between waves. The method used in the main analysis identified 480 deaths in wave 1 and 1173 in wave 2 compared to 624 and 1173 in the sensitivity analysis, corresponding to a difference of 144 and 582, respectively.

Secondary outcome: Risk of ICU admission

Admission to ICU during a COVID-19 related hospitalization was seen in 352 and 771 persons in the first and second wave, respectively (Table 2). The crude 6-months cumulative incidence increased from 0.5 to 1.0 in 10,000 persons without diabetes and from 3 to 7 in 10,000 persons with diabetes from wave 1 to 2 (ESM Figure 4, ESM Table 4). The risk of admission to ICU was increased in persons with diabetes compared with persons

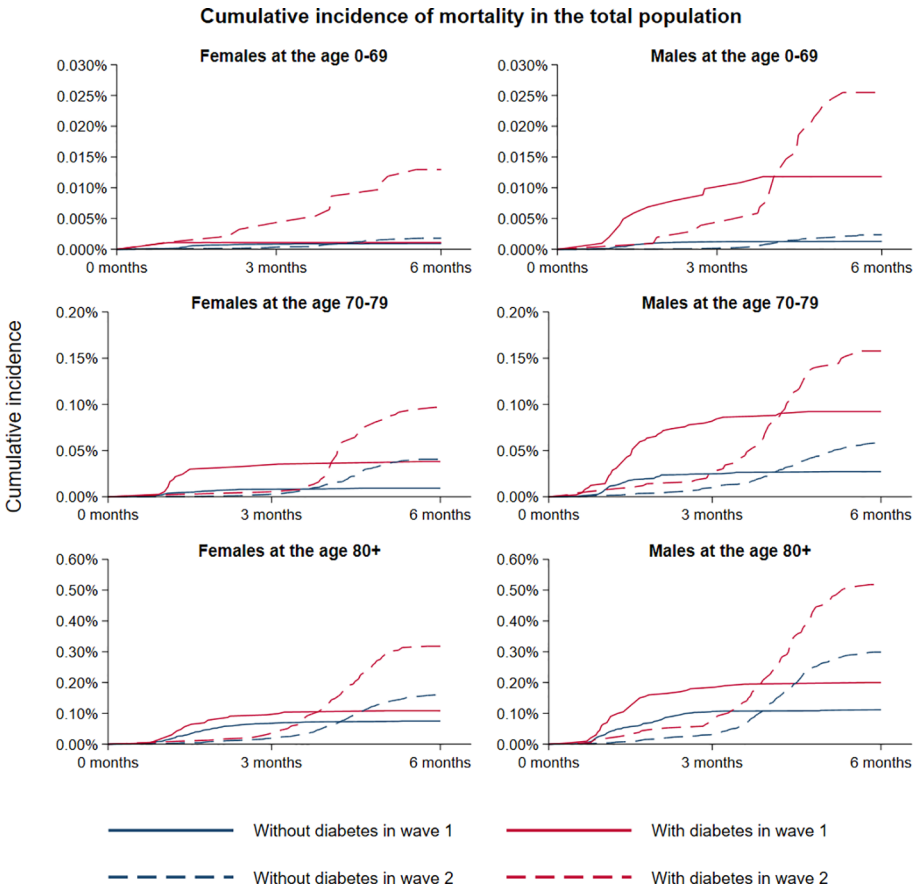

FIGURE 1
Crude mortality rates according to diabetes status during the first and second wave of COVID-19 in Denmark. Stratification were made according to females and males, and age 0-69, 70-79 and 80+ years, respectively. Time was defined according to the start date of the two waves.

without diabetes, and the relative risk numerically increased, from wave 1 to wave 2 (HR 1.56; 95% CI 1.20-2.03 and 1.85; 95% CI 1.53, 2.23, respectively; p=0.26 for interaction; Table 3). Restricting the analysis to the hospitalized population, the 6-months cumulative incidence of admission to ICU fell from 17.5% to 12.8% for persons with diabetes between the two waves, while it fell from 13.0% to 10.2% for persons without diabetes (ESM Figure 5, ESM Table 4). After full adjustment, the risk of ICU admission was not increased for persons with diabetes compared with persons without diabetes either in wave 1 or wave 2 when expressed according to the hospitalized population (HR 1.09; 95% CI 0.84, 1.41 and 1.02; 95% CI 0.85, 1.21; p=0.63 for interaction; Table 3)

Stratified analyses

All subgroup analyses presented here are from the second wave, whereas similar analyses for the first wave are given in ESM Table 6 only. In the total population, age and diabetes had a strong interaction with respect to hospitalization and mortality (p<0001) with diabetes inferring the highest risk in the youngest age group (HR 6.12; 95% CI 5.62, 6.66 and 9.30; 95% CI 6.40, 13.53, respectively; Figure 2 and ESM Table 7). However no significant overall interaction was present with respect to mortality in the hospitalized population, although younger persons with diabetes had an increased risk compared with persons without diabetes (HR 1.53; 95% CI 1.05, 2.23; Figure 2 and ESM Table 7). In the total population, diabetes significantly increased the risk of hospitalization in both females (ref=1.0 vs HR 2.73; 95% CI 2.50, 2.97) and in males (HR 1.33; 95% CI 1.26, 1.40 and 3.95; 95% CI 3.34, 4.68, respectively; p<0.001 for difference between males with and without diabetes), with males overall having the highest risk (Figure 2 and ESM Table 7). Diabetes also increased mortality risk both in females and males in the total population (Figure 2) as well as in the hospitalized population (females: ref=1.0 vs HR 1.21; 95% CI 0.99, 1.48 and males: HR 1.19; 95% CI 1.04, 1.36 vs HR 1.42; 95% CI 1.20, 1.68; p=0.04 for difference between males with and without diabetes).

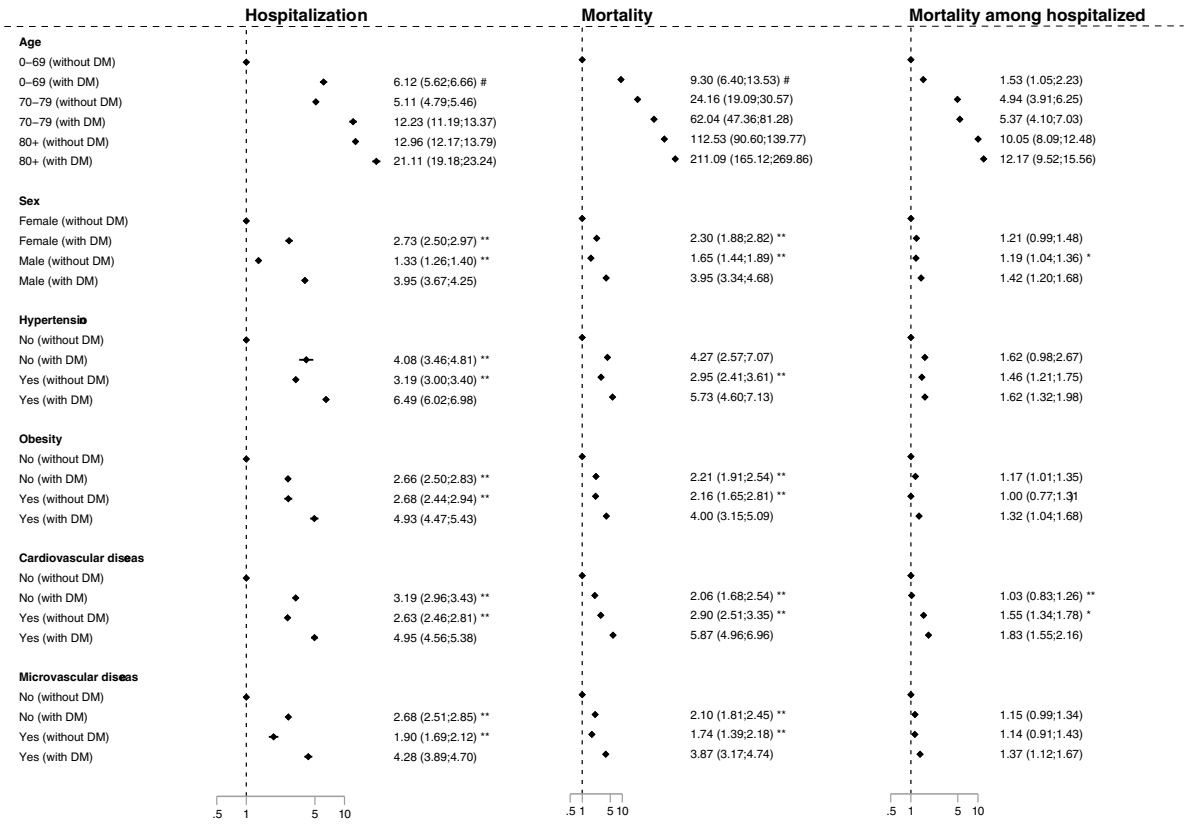

FIGURE 2
Forest plot of hazard ratios for the primary outcomes in persons with diabetes compared to persons without diabetes for stratified groups in wave 2, adjusted for age and sex. Mortality was defined as death within 30 days of the start of a COVID-19 related hospitalization, which was defined as hospitalization for more than 12 hours within 30 days prior and 14 days after a positive test for SARS-CoV-2 and with a recorded COVID-19 diagnosis for the hospital contact. Reference for the hazard ratio for the stratified groups were: 0-69 year without diabetes; females without diabetes; persons with neither hypertension nor diabetes; persons with neither obesity nor diabetes; persons with neither microvascular disease nor diabetes; persons with neither cardiovascular disease or diabetes, respectively. In addition the difference in HR was tested versus males with diabetes, hypertension with diabetes, obesity with diabetes, cardiovascular disease with diabetes, microvascular disease with diabetes, respectively: *p<0.05, **p<0.001. Difference in the risk between (the many) age groups and diabetes were tested overall by interaction analysis: # p<0.001.

Compared with persons without hypertension and without diabetes, persons with diabetes without hypertension had an increased risk of COVID-19 hospitalization (HR 4.08; 95% CI 3.46, 4.81), while persons with diabetes and hypertension had an even higher risk (HR 6.49; 95% CI 6.02, 6.96; p<0.001 versus diabetes without hypertension; Figure 2 and ESM Table 7). Mortality was similarly increased in persons with diabetes without hypertension (HR 4.27; 95% CI 2.57, 7.07) and further increased in persons with diabetes with hypertension (HR 5.73; 95% CI 4.60, 7.13: P=0.26, Figure 2 and ESM Table 7). However, when the mortality analysis was restricted to the hospitalized population, diabetes with hypertension did not increase the risk further compared to persons with diabetes without hypertension (HR 1.62; 95% CI 0.98, 2.67 vs HR 1.62; 95% CI 1.32, 1.98; p=0.99).

In the total population, diabetes without obesity conferred a higher risk of hospitalization compared to persons without diabetes and without obesity (HR 2.66; 95% CI 2.50, 2.83), while persons with both conditions had the highest risk (HR 4.93; 95% CI 4.47, 5.43; p<0.001 versus diabetes without obesity). The corresponding mortality was increased in persons with diabetes without obesity (HR 2.21; 95% CI 1.91, 2.54) and even higher in in persons with diabetes with obesity HR 4.00 (95% CI 3.15, 5.09; p<0.001 versus diabetes without obesity). Mortality in the hospitalized population was only increased in diabetes without obesity (HR 1.17; 95% CI 1.01, 1.35) and in persons with diabetes with obesity (HR 1.32; 95% CI 1.04, 1.68; p=0.11 versus diabetes without obesity).

In the total population, diabetes without cardiovascular disease also increased the risk of COVID-19 hospitalization

(HR 3.19; 95% CI 2.96, 3.43) and mortality (HR 2.06; 95% CI 1.68, 2.54), while diabetes with cardiovascular disease increased the risk even further (HR 4.95; 95% CI 4.56, 5.38 and HR 5.87; 95% CI 4.96, 6.96, respectively, both p<0.001 versus diabetes without cardiovascular disease; Figure 2 and ESM Table 7). However, in the hospitalized population, diabetes without cardiovascular disease did not increase mortality (HR 1.03; 95% CI 0.83, 1.26), whereas diabetes and cardiovascular disease still caused a higher mortality (HR 1.83; 95% CI 1.55, 2.16; p<0.001 versus diabetes without cardiovascular disease).

In the total population, diabetes without microvascular disease also increased the risk of COVID-19 hospitalization (HR 2.68; 95% CI 2.51, 2.85) and mortality (HR 2.10; 95% CI 1.81, 2.45), while diabetes with microvascular disease increased the risk even further (HR 4.28; 95% CI 3.89, 4.70 and HR 3.87; 95% CI 3.17, 4.74, respectively, both p<0.001 versus diabetes without microvascular disease; Figure 2 and ESM Table 7). However, in the hospitalized population, diabetes without microvascular disease might not increase mortality (HR 1.15; 95% CI 0.99, 1.34), whereas diabetes and microvascular disease caused a higher mortality, although might not higher than in diabetes without microvascular disease (HR 1.37; 95% CI 1.12, 1.67; p=0.13 versus diabetes without cardiovascular disease).

The risk of ICU in the presented subgroups did not differ from the main results (ESM Tables 8, 9, ESM Figure 4). However, when restricted to the hospitalized population patients with more co-morbidity or high age had the lowest risk of being submitted at the ICU (ESM Tables 8, 9, ESM Figure 5).

The risk of hospitalization and the risk of ICU admission were higher in patients with type 2 diabetes compared with patients with type 1 diabetes after adjustment for age and sex (ESM Table 10). However, the mortality did not differ among persons with type 1 and type 2 diabetes after adjustment for sex and age.

Discussion

As reported previously, diabetes increased the risk of COVID-19 related hospitalization and death compared to persons without diabetes. As a novel finding, we here report that the risk of COVID-19 hospitalization increased more in patients with diabetes than persons without diabetes from the first to the second wave, whereas the relative risk of mortality did not change due to a relative decrease in mortality among hospitalized patients with diabetes. Although the relative mortality risk did not change between the waves, the absolute increase in the cumulative incidence was much higher in persons with diabetes. Comparing individuals without hypertension, obesity, cardiovascular disease or microvascular disease, diabetes per se still conferred a higher risk of hospitalization and mortality, and these risks were further enhanced by the presence of each of these conditions. However, specifically, the risk of mortality in the hospitalized population was not increased in persons with diabetes without cardiovascular disease, while persons with both diabetes and cardiovascular disease had a particularly high mortality.

The ability to protect the population from severe COVID-19 infection and death is a primary concern in a pandemic. The risk of COVID-19 related death after hospitalization is determined by two components: 1) the risk of severe COVID-19 infection leading to hospitalization in the total population and 2) the risk of death among those hospitalized, as a marker of the effectiveness of the treatment of severe COVID-19. Despite the relative risk of COVID-19 related death was the same in wave 1 and 2 for persons with diabetes, this component analysis determined that the relative risk of hospitalization was further increased in wave 2. The absolute risk also increased substantially more in persons with diabetes compared to those without diabetes from wave 1 to wave 2. This suggests that the societal measures to prevent COVID-19 infection and hospitalization in susceptible individuals were more effective during wave 1. The specific reasons for this change cannot be completely ascertained. However, overall we consider the difference in societal measures to be most important for the reported change. During wave 1 a general and early lock-down on 11 March 2020, was effectuated in Denmark, including closing of schools, work at home for private and public employees in non-essential functions and a general minimization of societal activity (24). During wave 2 societal measures were more modest and selective and a national lock-down were enforced later in the COVID-19 second surge on 16 December 2020 (25, 26). The lockdown of the first wave were implemented on 11 March 2020 when the incidence of COVID-19 infections had risen from 10 per day on 7 March to 250 per day on 10 March and correspondingly the number of person in hospital due to COVID-19 were 19 on the 12 March (27). This was in contrast to the second wave were incident COVID-19 infection were 3692 per day on 16 December (28), while 404 were in hospital with COVID-19 and 57 were in the ICU (29). Other differences were that childcare services of preschool children were not included in the lockdown of wave 2 and that contact tracing and free access to testing were a part of the measures all through wave 2, a measure that was only part of wave 1 in later stages (30). An overview of the characteristics of the two waves are presented in ESM Table 11.

We have not identified any major changes in infectivity or virulence in the literature that are specific for persons with diabetes from wave 1 to wave 2, although a minor effect of the alpha variant cannot be ruled out as discussed below. We did not identify any changes in knowledge about the risk of people with diabetes, or in management of persons with diabetes within the first year of the pandemic. General information to high-risk

groups including persons with diabetes and their treating physicians about the increased risk of severe COVID-19 infection were promoted by the national Danish health authorities, also very early in the pandemic at 12 March 2020 (31). During the two first waves it consisted of intensification of the general advice of hand disinfection, limited physical contact, distance during social activities and cleaning, coupled with advice for relatives to persons at high risk (https://www.sst.dk/en/English/Corona-eng/Prevent-infection/People-at-higher-risk). Despite these measures, the risk of COVID-19 related hospitalization rose more in persons with than without diabetes, which indicate that this high risk population benefited more from an early and general lock-down and that selective advice for this group did not alleviate an increase in risk seen during less societal restriction.

We have showed that both the risk of COVID-19 infection and the risk of a severe COVID-19 infection leading to hospitalization, given a positive PCR test, was increased in persons with diabetes (13 and 57% excess, respectively in wave 2). This indicate that persons with diabetes have a higher susceptibility to COVID-19 infection and in addition more susceptible to develop severe complications once infected. The reported decrease in risk of COVID-19 infection in persons with diabetes from wave 1 to wave 2 must considered to be highly biased, due to the very high difference in test activity and any firm conclusions on the change over time cannot be given.

Our results confirm the increased risk of mortality among hospitalized patients with diabetes (32), but the relative decrease of this apparent risk in hospitalized patients with diabetes compared with persons without diabetes from one to a subsequent COVID-19 wave is new. This adds to prior reports of a decrease in the mortality among hospitalized persons between COVID-19 waves (33, 34), but our results indicate that this is especially pronounced in diabetes. The increased use of corticosteroids (35), prone positioning (36) and anti-viral treatment (37) during the pandemic could have had an impact on the risk of mortality in hospitalized persons. As the risk of severe COVID-19 infection is higher in persons with diabetes, a higher impact of advances in the treatment of severe COVID-19 would concomitantly be expected in persons with diabetes. On the other hand, an increased alertness towards increased susceptibility in diabetes patients perhaps increased the chance of being hospitalized with milder COVID-19 infection in wave 2. However, our data do not suggest that more mild cases of COVID-19 in persons with diabetes were hospitalized in wave 2, as the absolute and relative risk of ICU admission in the total population were numerically higher in wave 2 compared to wave 1 in persons with diabetes, indicating a higher burden of severe COVID-19 infection in wave 2.

Long term COVID-19 symptoms (so-called long COVID) are present in some patients and are associated to severity of the initial COVID-19 infection (38–40), although inflammatory biomarkers do not seem to be increased 6 months after a COVID-19 infection (41). However, the association between long COVID and diabetes are not clear, with some studies reporting a significant association (42–44) and others not (38, 40, 45, 46), with most studies done in patients with a prior COVID-19 hospitalization. However, our data would suggest that the higher risk of hospitalization for persons with diabetes, would also translate into a higher risk of having long COVID, at least when evaluated in the total population. Therefore, it could be hypothesized that the diabetes population would have a higher burden of long COVID in general. Further studies are needed in well-defined non-selected cohorts, with appropriate adjustment for competing risk by death, to clarify this question.

We did not observe any difference in COVID-19 mortality between type 1 and type 2 diabetes, which is in accordance with other studies (32). We did observe a higher risk of hospitalization in type 2 diabetes. However, the number of hospitalizations and deaths among persons with type 1 diabetes constituted only 2.8% and 2.7% of the events in persons with diabetes and the results should therefore be interpreted cautious.

The strength of our study is the complete nationwide coverage of the comprehensive national registries and a very high degree of registration of results of RT-PCR testing for SARS-CoV-2 (47). Furthermore, we analyzed multiple aspects of the risk of severe COVID-19 outcomes, thereby elucidating the complexity of progress or regress in persons with diabetes.

The study has some limitations. During the second wave the lineage B.1.1.7 emerged (the alpha strain), and gradually became more prevalent. In week 53 of 2020 only 2% of sequenced cases were B.1.1.7, while the prevalence was 81.3% in week 9 of 2021 (https://www.covid19genomics.dk/statistics). The B.1.1.7 lineage was associated with an increased risk of transmission (48), increased risk of hospitalization (25) and increased risk of mortality (49, 50), which could have had an impact on the differences seen between wave 1 and 2. The two waves were defined in Spring and Summer (wave 1) and Autumn and Winter (wave 2), which could also affect the risk of transmission (51, 52), however we do not expect that this would affect persons with and without diabetes differently. During the first year of the pandemics the PCR test capacity in Denmark gradually increased from 2000/day on 21.april 2020 to 170.000/day on 2. March 2021 (https://tcdk.ssi.dk/om-testcenter-danmark/testkapacitet-gennem-tiden). We did not express the risk according to SARS-CoV-2 RT-PCR positivity due to the large difference in test activity, which would have had a large impact on the risk estimates. We would suspect that a much more restrictive test strategy would favor testing of high risk persons with eg diabetes, which we would expect to falsely increase the risk of a positive test in persons with diabetes compared to persons without diabetes. This bias will be

lower for the hospitalized population, as their admission most considered based on more objective criteria. Therefore, we chose to define the population according to those hospitalized with COVID-19, as this population was expected to have close to complete coverage of screening for COVID-19. This strategy would possibly miss deaths from COVID-19 in persons not hospitalized or not tested for COVID-19, e.g. in persons in nursing homes. However, the proportion of persons which by clinical judgement are chosen not to be hospitalized, is expected to be rather constant. To assess the impact of these considerations, we also assessed the risk of hospitalization and death according to the test positive population. This sensitivity analysis confirmed the main results, indicating that these limitations did not impact the results. To further overcome these limitations, we both expressed the risk according to the whole population and according to the COVID-19 hospitalized population. As both mortality in the total and hospitalized population had a COVID-19 hospitalization as a prerequisite the two ways of assessing data are comparable. It is also important to recognize that at times of very high infection rates, many deaths, which would also had happened without COVID-19 being present (e.g accidents), by chance will happen after a positive COVID-19 test. When infection rates are very high, such deaths might constitute a high proportion of deaths within 30 days of a positive test, especially when the mortality of the infection at hand is relatively low. By restricting the analysis to persons being hospitalized with a COVID-19 diagnosis, this bias will be reduced.

Another limitation of the study is the lack of data on vaccination status which precludes assessing the impact of vaccination. Moreover, we did not assess the risk of long COVID-19, due to the difficult task of providing data of long COVID-19 from registries (53). Finally, the specific cause of mortality in COVID-19 related deaths in the hospitalized populations were unavailable.

In conclusion, we found that the risk of COVID-19 related hospitalization in the total Danish population increased more for persons with than without diabetes from wave 1 to 2 - but that the relative risk of mortality in hospitalized patients with diabetes actually decreased compared with persons without diabetes. Moreover, diabetes related co-morbidities as hypertension, obesity, microvascular disease and cardiovascular disease all further increased the risk of COVID-19 related hospitalization and death beyond diabetes. Any lessons learned from the first wave – about protective measures – did not reduce risk of hospitalization for persons with diabetes, but the reduced relative risk of mortality in hospitalized diabetes patients, shows that some improvement in treatment and care apparently equalized the differences. This implies that there is a need for better measures that specifically protect populations of high risk against COVID-19 hospitalization.

Data availability statement

The datasets presented in this article are not readily available because sharing of participant data from public registries are not allowed by Danish law. Requests to access the datasets should be directed to kontakt@sundhedsdata.dk.

Ethics statement

The study was approved by The Danish Data Protection Agency (Region of Southern Denmark, record no. 20/19971). Data were managed at The Danish Health Data Authority (project ID no. FSEID-00005015), who also approved the study and access to data. Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

JS, AG and KH conceived and designed study. JS prepared the first draft. LR performed the statistical analysis. All authors contributed to the interpretation of data and critically revised the manuscript. JS, AG and KH are the guarantors of the work and accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1025699/full#supplementary-material

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Metformin use and mortality and length of stay among hospitalized patients with type 2 diabetes and COVID-19: A multiracial, multiethnic, urban observational study

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Introduction: Diabetes mellitus is a common comorbidity among patients with coronavirus disease 2019 (COVID-19). Diabetic patients with COVID-19 have a two-fold increased risk of death and tend to have more severe infection compared to the general population. Metformin, a first-line medication for diabetes management, has anti-inflammatory and immunomodulatory effects. Previous studies focusing on metformin and COVID-19 clinical outcomes have had mixed results, with some showing a mortality benefit or decreased complications with metformin use. To date, few studies have analyzed such outcomes among a diverse, multiracial community.

Methods: This was a retrospective review of patients with Type 2 diabetes and a confirmed COVID-19 infection admitted to an urban academic medical center from January 1, 2020 to May 7, 2020. Baseline characteristics were collected. The primary outcomes of the study were in-hospital mortality and length of stay (LOS).

Results: A total of 4462 patients with Type 2 diabetes and confirmed COVID-19 were identified. 41.3% were Black, and 41.5% were Hispanic. There were 1021 patients in the metformin group and 3441 in the non-metformin group. Of note, more participants in the metformin group had comorbid disease and/or advanced diabetes. We found no statistically significant differences between the metformin and non-metformin group in in-hospital mortality (28.1% vs 25.3%, P=0.08) or length of hospital stay in days (7.3 vs. 7.5, P=0.59), even after matching patients on various factors (29.3% vs. 29.6%, P=0.87; 7.7 vs. 8.1, P=0.23).

Conclusion: While patients had more comorbid disease and advanced diabetes in the metformin group, there were no significant differences with regard to in-hospital mortality or length of stay due to COVID-19 compared to the non-metformin group. Prospective studies are needed to determine if there is clinical benefit for initiating, continuing, or re-initiating metformin in patients hospitalized with COVID-19.

KEYWORDS

diabetes, COVID-19, metformin, urban, multiracial, multiethnic

Introduction

Diabetes mellitus is a common comorbidity among patients with coronavirus disease 2019 (COVID-19). Diabetic patients with COVID-19 have a two-fold increased risk of death and tend to have more severe infection compared to the general population (1, 2). Early case-control studies have shown that COVID-19 infected patients with diabetes had a higher risk of severe pneumonia, as well as elevated tissue injury and inflammatory markers (3, 4). Hyperglycemia is known to interfere with control of viremia and inflammation, predisposing patients to poorer outcomes (3, 5).

The apparent association of glucose metabolism with COVID-19 prognosis calls for a better understanding of anti-diabetic agents that affect blood glucose control. Metformin is a widely used first-line agent for the management of diabetes with anti-inflammatory and immunomodulatory effects (6). It has been shown to decrease TNF alpha and IL-6, which are both proinflammatory adipokines associated with visceral obesity and also implicated in morbidity from COVID-19 infection (6). There have been systematic reviews examining metformin and COVID-19 outcomes (7, 8). Some studies show a mortality benefit (9, 10), while others show no statistically significant association (11, 12). Bramante et al., 2020 found an association of metformin with decreased mortality in women alone, possibly due to sex-specific reduction of TNF alpha (6). Jiang et al., 2020 did not find an effect on 30 day all-cause mortality but found a lower incidence of acute respiratory distress syndrome (ARDS), especially in women (13). A study by Gao et al., 2020 found that metformin users had a higher risk of severe COVID-19 illness, with severity defined in terms of life-threatening complications like ARDS, septic shock, and organ dysfunction requiring ICU admission (14).

In 2010, non-Hispanic Black populations were found to have the highest prevalence of diabetes at 12.6% (15). The racial and ethnic disparities in diabetes morbidity and mortality parallel those observed during the COVID-19 pandemic. A systematic review by Mackey et al., 2020 found with moderate-to-high strength evidence that Black and Hispanic populations experienced higher rates of COVID-19 infection, hospitalization, and mortality compared to non-Hispanic Whites (16). Therefore, it is important to reproduce the studies of COVID-19 and metformin use in a multiracial, multiethnic population. We conducted a retrospective study to compare in-hospital mortality and length-of-stay (LOS) at a major academic center in the Bronx, New York.

Materials and methods

Study design and participants

This is a retrospective analysis of patients with type II diabetes and a confirmed COVID-19 infection admitted to an urban academic medical center. COVID-19 was confirmed through polymerase chain reaction (PCR) testing. The study included patients admitted from January 1, 2020 to May 7, 2020 and was approved by the institution's institutional review board. Baseline characteristics including age, gender, race, ethnicity, comorbidities including hypertension, peripheral artery disease, coronary artery disease, chronic kidney disease, as well as number and type of anti-diabetic medications were collected. Additional data including HbA1c, anion gap at time of admission, creatinine, estimated glomerular filtration rate (eGFR), and body mass index (BMI) were also collected. The primary outcomes of the study were in-hospital mortality and length of stay (LOS).

Statistical analysis

Statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Patient demographics and clinical characteristics were summarized for the whole sample and compared between metformin and non-metformin groups using chi-squared or Fisher's exact tests for categorical variables,

and 2-sample t-tests for continuous variables. Univariate analysis was performed to assess the association between in-hospital mortality and length of stay with metformin treatment using chi-squared and 2-sample t-tests, respectively. Propensity score matching was performed on selected demographic and clinical variables by implementing a 1:1 matching algorithm, and the two outcomes were analyzed similarly using the matched data. Differences with a two-sided p-value less than 0.05 were considered to be statistically significant.

Results

Patient characteristics

A total of 4462 patients were identified. Patient demographics are shown in Table 1. The sample was 46.9% female, with a mean age (SD) of 64.4 (±16.2) years, 41.3% were Black and 41.5% were Hispanic. There were 1021 patients in the metformin group and 3441 in the non-metformin group. The prevalence of co-morbidities was higher in the metformin group when compared to the non-metformin group in such conditions as hypertension (58.2% vs. 37.5%, P<0.0001), coronary artery disease (13.4% vs. 6.7%, P<0.0001), and peripheral artery disease (1.7% vs. 1.0%, P=0.06). BMI (30.45 vs. 29.36, P<0.0001), HbA1c (8.21 vs. 6.52, P<0.0001) creatinine (1.62 vs. 2.14, P<0.0001), anion gap (17.06 vs. 16.62, P<0.01) and mean number of anti-diabetic agents (1.2 vs. 0.22, <0.0001) were also significantly higher in the metformin group. Patients in the metformin group were also significantly more likely to be on concomitant anti-diabetic agents including sulfonylureas (29.5% vs. 3.6%), dipeptidyl-4 inhibitors (36.4% vs. 4.6%), glucagon-like peptides (11.6% vs. 1.7%), sodium-glucose transport-2 inhibitors (7.0% vs. 0.4%), and insulin (36.6% vs. 11.8%) when compared to the non-metformin group (P<0.0001).

Outcomes

Overall, there were no statistically significant differences in in-hospital mortality or length of stay (LOS) between metformin and non-metformin groups. Results from univariate analysis and propensity score matching are shown in Table 2. Clinical outcomes of in-hospital mortality (28.1% vs 25.3%, P=0.08) and LOS in days (7.3 vs. 7.5, P=0.59) showed no significant difference between metformin and non-metformin groups. Similar analysis was completed after matching patients in the metformin and non-metformin group on factors noted to be significantly different between the two groups (Table 1). Using propensity score matching, participants were matched on factors including age, race, hypertension, coronary artery disease, insulin use, HbA1c, creatinine, BMI, and total number of anti-diabetic medications (not shown). Analysis of matched groups still showed no observable differences in either endpoint of mortality (29.3% vs. 29.6%, P=0.87) or LOS (7.7 vs. 8.1, P=0.23).

Discussion

While metformin may theoretically reduce inflammatory cytokines that are implicated in COVID-19 progression, there are mixed results regarding whether metformin decreases COVID-19 morbidity and mortality in diabetic patients. To our knowledge, this is one of few studies describing metformin use and COVID-19 outcomes in an urban, multiracial, multiethnic community.

We did not observe differences in in-hospital mortality or length of stay between the metformin and non-metformin groups. Additionally, propensity score matching analysis did not show a statistically significant difference in mortality between the groups. Chronic kidney disease (CKD) burden was comparable between groups, indicating that the non-metformin group did not have significant contraindications to being prescribed metformin. We noted a mean HbA1c of 6.52% (SD 1.79) in the non-metformin group, which suggests that some patients may initially have been diagnosed with diabetes, but lowered their HbA1c through successful treatment. Patients at the cutoff point with newly diagnosed diabetes may also have opted for aggressive diet and exercise rather than pharmacologic modalities. Additionally, despite metformin being a first-line agent for the treatment for diabetes, a significant proportion (n=3441) of patients were not on metformin. This could have been due to renal impairment, the intolerability of metformin's gastrointestinal side effects, patients being lost to outpatient follow-up after the initial diabetes diagnosis, or failure to pick up newly dispensed medications. Furthermore, while other studies have noted mortality benefits in women alone (6), we did not identify such gender-specific differences.

Of note, the metformin and non-metformin groups were imbalanced. The metformin group was more likely to have a higher comorbidity burden including hypertension (HTN), coronary artery disease (CAD), and peripheral artery disease (PAD). They had a higher mean body mass index (BMI), mean HbA1c, and mean number of anti-diabetic agents including insulin. Many of these conditions, including HTN, obesity, and cardiovascular disease have known associations with poorer COVID-19 outcomes (17). In contrast, the non-metformin group had a mean HbA1c close to the lower limit of a diagnosis of diabetes, suggesting that patients early on in the diagnosis or that have been successfully treated are disproportionately represented. Therefore, a mortality benefit from metformin, if any, may have been masked by a metformin group that had far more comorbid disease and advanced diabetes. While difficult to ascertain, metformin may even have played a role in offsetting the severity of COVID-19 disease and, subsequently, preventing patients with diabetes and COVID-19 from being admitted and

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TABLE 1 Patient demographics.

	Total (n = 4462)	Metformin (n = 1021)	No Metformin (n = 3441)	p-value
Age, mean (SD)	64.38 (16.19)	67.14 (12.47)	63.56 (17.01)	<0.0001
Gender, *n* (%)				0.53
Male	2367 (53.1)	533 (52.2)	1834 (53.3)	
Female	2094 (46.9)	488 (47.8)	1606 (46.7)	
Race, *n* (%)				<0.01
White	461 (11.3)	89 (9.5)	372 (11.9)	
Black	1681 (41.3)	395 (42.1)	1286 (41.0)	
Asian	111 (2.7)	39 (4.2)	72 (2.3)	
Other	1822 (44.7)	416 (44.3)	1406 (44.8)	
Ethnicity, *n* (%)				0.33
Hispanic	1659 (41.5)	366 (40.1)	1293 (41.9)	
Non-Hispanic	2341 (58.5)	547 (59.9)	1794 (58.1)	
Hypertension, *n* (%)				<0.0001
Yes	1886 (42.3)	594 (58.2)	1292 (37.5)	
No	2576 (57.7)	427 (41.8)	2149 (62.5)	
Peripheral Artery Disease, *n* (%)				0.06
Yes	50 (1.1)	17 (1.7)	33 (1.0)	
No	4412 (98.9)	1004 (98.3)	3408 (99.0)	
Coronary Artery Disease, *n* (%)				<0.0001
Yes	366 (8.2)	137 (13.4)	229 (6.7)	
No	4096 (91.8)	884 (86.6)	3212 (93.3)	
BMI, mean (SD)	29.62 (7.46)	30.45 (7.67)	29.36 (7.38)	<0.0001
Chronic Kidney Disease, *n* (%)				0.71
Yes	313 (7.0)	69 (6.8)	244 (7.1)	
No	4149 (93.0)	952 (93.2)	3197 (92.9)	
Sulfonylurea, *n* (%)				<0.0001
Yes	424 (9.5)	301 (29.5)	123 (3.6)	
No	4038 (90.5)	720 (70.5)	3318 (96.4)	
DPP4 Inhibitor, *n* (%)				<0.0001
Yes	529 (11.9)	372 (36.4)	157 (4.6)	
No	3933 (88.1)	649 (63.6)	3284 (95.4)	
GLP-1 Agonist, *n* (%) Yes	177 (4.0)	118 (11.6)	59 (1.7)	<0.0001
No	4285 (96.0)	903 (88.4)	3382 (98.3)	
SGLT-2 Inhibitor, *n* (%)				<0.0001
Yes	86 (1.9)	71 (7.0)	15 (0.4)	
No	4376 (98.1)	950 (93.0)	3426 (99.6)	
Insulin, *n* (%)				<0.0001
Yes	780 (17.5)	374 (36.6)	406 (11.8)	
No	3682 (82.5)	647 (63.4)	3035 (88.2)	
HbA1c, mean (SD)	7.02 (2.04)	8.21 (2.12)	6.52 (1.79)	<0.0001
Anion Gap, mean (SD)	16.72 (4.68)	17.06 (4.67)	16.62 (4.68)	<0.01
Creatinine, mean (SD)	2.02 (2.61)	1.62 (1.67)	2.14 (2.82)	<0.0001
eGFR, mean (SD)	56.00 (31.04)	57.37 (28.33)	55.59 (31.82)	0.09
Number of Medications, mean (SD)	0.45 (0.85)	1.21 (1.11)	0.22 (0.58)	<0.0001

hospitalized, being treated at home, and thus excluded from this study. Furthermore, it is possible that the metformin group would have had a higher mortality and a longer in-hospital length of stay, but did not due to the benefits of metformin.

There may be evidence that the overall health of the metformin group may be related to a detectable difference in study outcomes. The CORONADO study was a large European observational study (n = 2449) that evaluated mortality and

TABLE 2 COVID-19 Clinical outcomes and metformin use.

	Metformin (n = 1021)	No Metformin (n = 3441)	p-value*	Metformin (n = 796)	No Metformin (n = 796)	p-value**
In-Hospital Mortality, n (%)			0.08			0.87
Alive	734 (71.9)	2569 (74.7)		563 (70.7)	560 (70.4)	
Deceased	287 (28.1)	872 (25.3)		233 (29.3)	236 (29.6)	
Length of Stay, mean (SD)	7.3 (6.7)	7.5 (6.7)	0.59	7.7 (6.8)	8.1 (7.1)	0.23

*Comparison on original data.
**Comparison on PSM data matched on age, race, hypertension, coronary artery disease, insulin use, HbA1c, creatinine, BMI, and total number of anti-diabetic medications.

disease complications among patients with diabetes hospitalized with COVID-19 (18). In contrast to our study, their metformin group was overall younger and more racially and ethnically homogenous. They had a shorter duration of diabetes, fewer diabetic complications, and lower rates of comorbidities compared to the non-metformin group. They were more often using other oral agents; insulin therapy was two times less prevalent. The CORONADO study found that metformin users had significantly lower mortality rates on day 7 (8.2% vs. 16.1%, P<0.0001) and day 28 (16.0% vs. 28.6%, P<0.0001) compared to the non-metformin population (18).

In comparison, the TOGETHER trial (n = 418) is the first known randomized controlled trial to evaluate metformin in reducing time to hospitalization and mortality in an ambulatory setting (19). Symptomatic patients with a positive COVID-19 test were randomized to metformin vs. placebo for 10 days. The patient characteristics of the two groups were generally balanced with respect to age, BMI, and co-morbidities. Ultimately, the study did not find that metformin had a clinical benefit in the outpatient setting, and did not recommend repurposing of metformin as early treatment for COVID-19 disease (19). Further studies can examine metformin in COVID-19 recovered patients to assess when to re-initiate treatment. To evaluate the protective mechanism of metformin, these studies may choose to trend objective signs of disease recovery including inflammatory markers or viral load.

Most studies evaluating metformin use and COVID-19 clinical course have been conducted outside of the United States (U.S.), and fewer have evaluated clinical outcomes among a racially and ethnically diverse patient population (9). To the best of our knowledge, the only other study in the U.S. that has characterized patient demographics and clinical outcomes in an urban cohort was conducted by Crouse et al., 2020 (9). Although the study authors found metformin use to be associated with reduced mortality in patients with COVID-19 and diabetes, their sample size of metformin users was limited (n = 76) (9). An international study in the UK by Heald et al., 2022 found social disadvantage, as assessed by the Townsend score, to be associated with increased mortality from COVID-19 independent of diabetes status, and metformin use with decreased mortality (20). The intersectionality of race and economic status, however, calls for further consideration of such variables in a study population and healthcare setting more reflective of those communities with high disease burden in the U.S.

There are several strengths and limitations to this study. Strengths of this study include a large sample size and a focus on a highly diverse, at-risk population that is underrepresented in scientific literature. Given that Black and Hispanic populations experienced higher rates of COVID-19 infection, hospitalization, and mortality compared to non-Hispanic Whites (16), it is important to explore the utility of medications with proposed benefits in these specific populations. Our study builds a foundation for further investigations into the proposed benefits of medications that may decrease mortality in patients with diabetes and COVID-19. Our study adds to the observational work by Crouse et al., 2020, which was also conducted in a diverse urban cohort, but was limited by sample size of their metformin users (n = 76) (9). There are several limitations to our study. Due to the retrospective nature of our study, there may have been inconsistencies in provider medication reconciliation, limiting reliability of chart documentation. Information including metformin dose, formulation, adherence, and duration of use were unable to be obtained from chart review. Many variables including smoking status, diabetes duration, and mean blood glucose levels were not collected, which may have influenced the outcomes. For example, smoking is an independent risk factor for mortality in COVID-19 patients and therefore, could have been a confounding variable in our study (21).

Conclusion

While patients had more comorbid disease and advanced diabetes in the metformin group, there were no significant differences in in-hospital mortality or length of stay due to COVID-19 compared to the non-metformin group. Prospective studies in diverse populations are needed to determine if there is clinical benefit for initiating, continuing, or re-initiating metformin in patients with COVID-19.

Data availability statement

The de-identified data set is shared by individuals on the institutional IRB and is not publicly available. Requests to access these datasets should be directed to CG: email: clgeorge@montefiore.org.

Ethics statement

The studies involving human participants were reviewed and approved by Albert Einstein College of Medicine, Montefiore Medical Center Institutional Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

EM: Design, data management, interpretation of data, and preparation of manuscript; KZ: Design, data management, interpretation of data, and preparation of manuscript; JiL: Data management, analysis, interpretation of data, preparation of manuscript; JuL: Data management, analysis, interpretation of data, preparation of manuscript; DY: Interpretation of data, preparation of manuscript; CG: Design, acquisition of subjects, data management, analysis, interpretation of data, preparation of manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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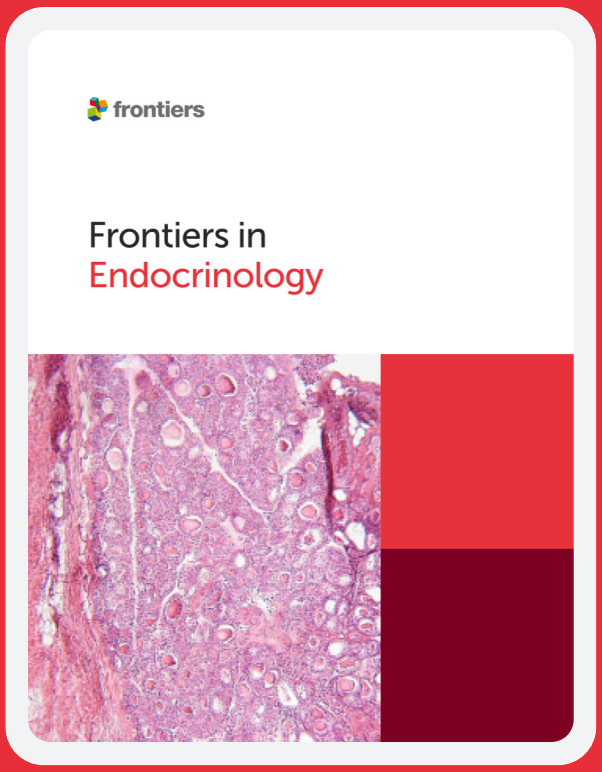

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