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Novel insights into the comorbidities and mortality in patients with diabetes

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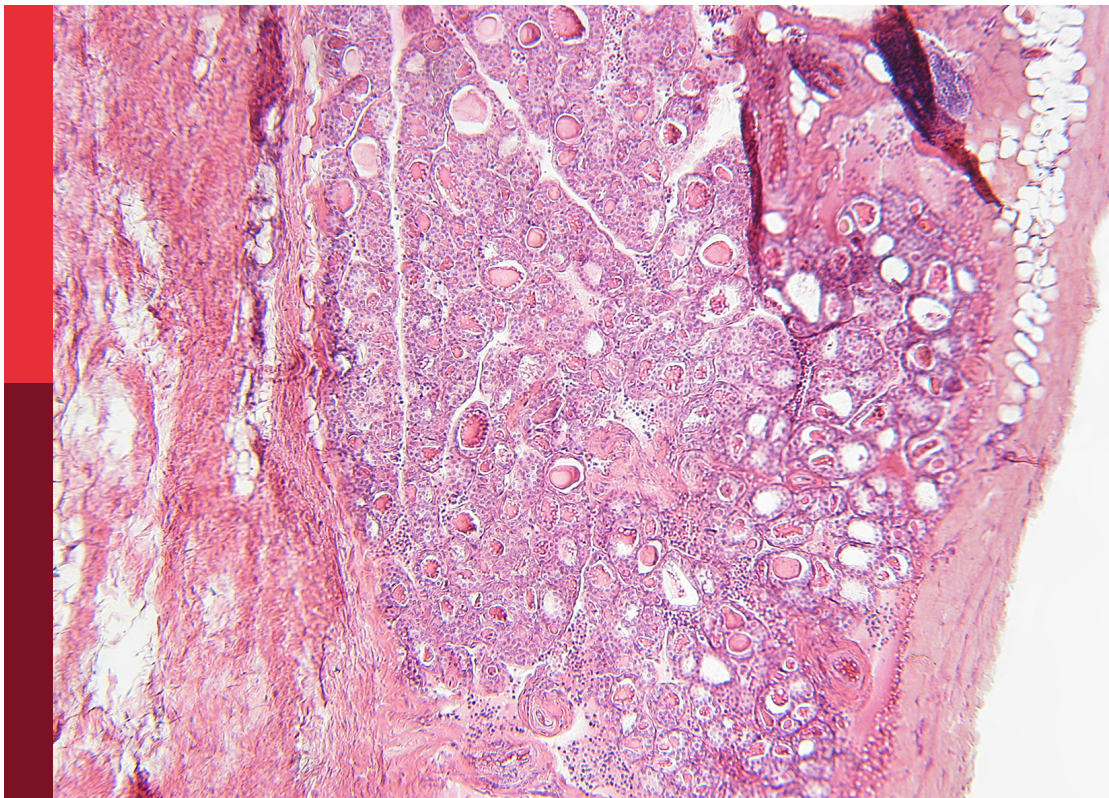

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Novel insights into the comorbidities and mortality in patients with diabetes

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Editorial: Novel insights into the comorbidities and mortality in patients with diabetes

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Editorial on the Research Topic:
Novel insights into the comorbidities and mortality in patients with diabetes

Diabetes is a persistent metabolic condition characterized by elevated blood sugar levels, leading to various complications. Around 537 million individuals globally were reported to have diabetes in 2021, and this number continues to rise (1). Diabetes and its associated complications substantially diminish the quality of life for patients and contribute to over 4 million deaths annually. Diabetes and its complications not only elevate premature mortality rates but also amplify the economic burden on society. Multiple studies indicate that diabetes can lead to chronic multisystem damage and dysfunction (2, 3). Moreover, diabetes frequently coincides with other illnesses that may share certain risk factors. For instance, individuals with diabetes have a higher likelihood of also experiencing hypertension, chronic obstructive pulmonary disease, or asthma (4, 5). Nevertheless, there remains a gap in understanding the correlation between diabetes and other potential comorbidities such as hepatitis infection and periodontal disease (PD). Moreover, the common risk factors that contribute to the development of diabetes and its comorbidities are not yet fully understood. Various comorbidities, such as cardiovascular and cerebrovascular diseases, significantly increase the risk of mortality in diabetes patients. However, the relationship between diabetes and the mortality rates of other diseases, like endometrial or kidney cancer, shows inconsistency. Additional research is required to uncover the heightened mortality linked with comorbidities in diabetic individuals.

This current Research Topic comprises a total of 12 studies. These studies specifically assess the prevalence and risk factors associated with diabetes complications, while also examining potential biological indicators linked to diabetes-related complications.

As commonly acknowledged, managing blood glucose levels serves as an efficient method for averting vascular complications in diabetes. Inadequate long-term management of blood sugar levels can lead to damage in nerves and blood vessels across the body, including those in the feet and eyes. In a review article, Waibel et al. examined research findings concerning the management, morbidity, mortality, and associated costs related to

diabetic foot disorder. In a cohort study reported by Zang et al., encompassing 800 participants, it was revealed that 71.2% of patients diagnosed with vision-threatening diabetic retinopathy (VTDR) were oblivious to their condition. These results underscore the insufficient screening endeavors for diabetic retinopathy (DR) within their study population. In a retrospective comparative investigation undertaken by Yan et al., they examined 2,961 individuals diagnosed with type 2 diabetes (T2D) to assess the occurrence rates of DR and VTDR categorized according to the duration of diabetes. The research revealed that while the likelihood of developing DR is relatively lower among individuals with ≤3 years' duration of T2D, the potential for VTDR should not be overlooked, particularly in patients with elevated glycated hemoglobin levels and/or diabetic nephropathy. Therefore, there is a need for more frequent retinal screening in individuals newly diagnosed with diabetes.

People with diabetes face a heightened risk of developing and experiencing PD. In a cross-sectional investigation, Gregorczyk-Maga et al. collected gingival crevicular fluid samples from adults with type 1 diabetes (T1D) undergoing continuous subcutaneous insulin infusion therapy and compared them with non-diabetic controls. They conducted a metagenomic/metabolomic analysis, leading to the conclusion that despite maintaining good metabolic control of diabetes, individuals with T1D remain vulnerable to the onset of PD. Additionally, Serón et al. undertook an extensive review of literature spanning from 2000 to 2023, examining the pathophysiological connections between periodontitis, diabetes, and atherosclerotic cardiovascular diseases. Their analysis indicates that integrating regular screening and treatment for periodontitis into national health programs is a cost-effective strategy to enhance metabolic management, decrease complications, and improve the overall well-being of individuals with diabetes.

It's crucial to comprehend and steer clear of the typical risk factors linked to the development and complications of diabetes for a favorable prognosis. In a systematic review and meta-analysis undertaken by Li et al., a cumulative total of 60 studies encompassing 5,960,224 participants were incorporated. The research findings indicated that hypoglycemia is correlated with an elevated risk of cardiac arrhythmia and mortality. Yang et al. performed a retrospective examination of 3,291 septic patients with diabetes from the extensive real-world database of the Medical Information Mart for Intensive Care. They pinpointed ten clinical variables, including respiratory failure, as prognostic factors for predicting the 28-day all-cause mortality in septic patients with diabetes. Zhao et al. utilized a Mendelian randomization (MR) approach to explore shared risk factors among T1D, T2D, and chronic kidney disease (CKD). They discovered that the eosinophil percentage may serve as a common risk factor for both T1D and CKD. Additionally, they identified various traits, including obesity and blood lipids, as shared risk factors for T2D and CKD. In another MR investigation carried out by Zhao et al., differing causal relationships were observed between T2D and chronic liver diseases in East Asians and Europeans.

Examining potential biological indicator in diabetic patients can assist in the early detection of diabetes complications, thereby enabling prompt intervention and treatment. Jin et al. performed a systematic review and meta-analysis of 42 studies, identifying a total of 68 serum/plasma biomarkers. They concluded that lipid metabolism biomarkers were the most reported factors to be associated with the risk of cardiometabolic multimorbidity. Su et al. studied 675 patients diagnosed with T2D who underwent percutaneous coronary intervention. Their findings suggested that fibrinogen levels have the potential to serve as a noninvasive biomarker for predicting coronary anatomical complexity in individuals with T2D, aiding in the early detection of those at elevated risk. In a cohort investigation reported by Ryu et al., which included 30,164 Korean individuals aged over 60 years, it was discovered that the metabolic score for insulin resistance index could serve as a valuable predictive indicator for all-cause mortality and cancer mortality within their study group. However, it did not exhibit predictive capability for cardiovascular mortality.

The studies encompassed within this Research Topic delve into the shared risk factors and potential biological markers associated with complications in diabetes. These findings carry substantial implications for the diagnosis, prevention, and treatment of diabetic complications. Through comprehension of these factors and biomarkers, there arises the opportunity to enhance the management of health conditions in diabetic patients and actively contribute to mitigating the risk of complications.

Author contributions

SL: Writing – original draft. HD: Writing – review & editing. PW: Writing – review & editing. HS: Writing – review & editing.

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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 predictive values of admission characteristics for 28-day all-cause mortality in septic patients with diabetes mellitus: a study from the MIMIC database

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Background: Septic patients with diabetes mellitus (DM) are more venerable to subsequent complications and the resultant increase in associated mortality. Therefore, it is important to make tailored clinical decisions for this subpopulation at admission.

Method: Data from large-scale real-world databases named the Medical Information Mart for Intensive Care Database (MIMIC) were reviewed. The least absolute selection and shrinkage operator (LASSO) was performed with 10 times cross-validation methods to select the optimal prognostic factors. Multivariate COX regression analysis was conducted to identify the independent prognostic factors and nomogram construction. The nomogram was internally validated *via* the bootstrapping method and externally validated by the MIMIC III database with receiver operating characteristic (ROC), calibration curves, decision curve analysis (DCA), and Kaplan-Meier curves for robustness check.

Results: A total of 3,291 septic patients with DM were included in this study, 2,227 in the MIMIC IV database and 1,064 in the MIMIC III database, respectively. In the training cohort, the 28-day all-cause mortality rate is 23.9% septic patients with DM. The multivariate Cox regression analysis reveals age (hazard ratio (HR) =1.023, 95%CI: 1.016-1.031, p<0.001), respiratory failure (HR=1.872, 95%CI: 1.554-2.254, p<0.001), Sequential Organ Failure Assessment score (HR=1.056, 95%CI: 1.018-1.094, p=0.004); base excess (HR=0.980, 95%CI: 0.967-0.992, p=0.002), anion gap (HR=1.100, 95%CI: 1.080-1.120, p<0.001), albumin (HR=0.679, 95%CI: 0.574-0.802, p<0.001), international normalized ratio (HR=1.087, 95%CI: 1.027-1.150, p=0.004), red cell distribution width (HR=1.056, 95%CI: 1.021-1.092, p=0.001), temperature (HR=0.857, 95% CI: 0.789-0.932, p<0.001), and glycosylated hemoglobin (HR=1.358, 95%CI: 1.320-1.401, p<0.001) at admission are independent prognostic factors for 28-day all-cause mortality of septic patients with DM. The established nomogram shows satisfied accuracy and clinical utility with AUCs of 0.870 in the internal validation and 0.830 in the external validation cohort as well as 0.820 in the

septic shock subpopulation, which is superior to the predictive value of the single SOFA score.

Conclusion: Our results suggest that admission characteristics show an optimal prediction value for short-term mortality in septic patients with DM. The established model can support intensive care unit physicians in making better initial clinical decisions for this subpopulation.

KEYWORDS

sepsis, diabetes mellitus, glycosylated hemoglobin, intensive care unit, all-cause mortality

Introduction

Sepsis is one of the leading life-threatening conditions caused by the dysregulated host response to infection (1–4). Due to the high incidence and subsequently, mortality risk, sepsis, and septic shock are the major medical problems, which affect millions of critically ill populations (3, 5). Sepsis is frequently observed in aging and cancer as well as immunosuppressive subpopulations (6). Despite recent improvements in diagnosis and treatment (including the use of organ support, antibiotics, and fluid resuscitation), sepsis remains a high hospitalization cost and fatal disease around the world (4, 7). How to make more precise clinical management decisions for septic patients with different comorbidities has raised wide concerns (8, 9).

Notably, diabetes mellitus (DM) is one of the most frequent comorbidities in critically ill patients (10). Globally, the prevalence of DM has quadrupled since the last 80s, which has become the ninth major cause of death (11, 12). The International Diabetes Federation (IDF) suggested that the number of DM would rise to almost 650 million by 2040 (11). To date, compelling evidence showed that patients with DM suffered from an increased risk of various infections (13–15) and represented the predominant population experiencing post-sepsis complications (16, 17). Moreover, septic patients with DM presented worse clinical outcomes during the hospitalization (10, 13, 17). The altered immune response and the hyperglycemia condition further assist the growth of microorganisms, which could lead to a more tough situation in septic patients with DM (15, 18). Therefore, identifying possible strategies to reduce in-hospital mortality and subsequent morbidity in such high-risk subpopulations would bring considerable clinical and social benefits. Several studies have explored the interaction between the blood glucose level or glycosylated hemoglobin or DM and sepsis (19–21). However, the results were inconsistent. Meanwhile, there was a persistent lack of studies on the evaluation of independent prognostic factors and models to guide the clinical decisions on septic patients with DM at initial admission.

To address the research gaps, we aim to determine the significant prognostic factors in septic patients with DM, based on a large-scale intensive care database involving hospitalized patients between 2001 and 2019. Furthermore, we aim to further establish and validate a feasible individualized model to assess ICU physicians to predict short-term mortality in septic patients with DM.

Materials and methods

Database

Two cohorts of septic patients admitted to the ICU coexisted with DM, from a publicly available real-world clinical database named Medical Information Mart for Intensive Care Database III (MIMIC III, version 1.4) and IV (MIMIC IV, version 2.2), and maintained by Beth Israel Deaconess Medical Center in Boston, MA, USA from 2001 to 2019 was included (22). The description of this large-scale database has been published in the previous literature in chapters (23–26). The first author was permitted to extract data from the database after passing the related examination. The reporting of this study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (27).

Ethical approval

The MIMIC-III and MIMIC-IV databases used in our study were approved by the Institutional Review Boards (IRB) of the Massachusetts Institute of Technology and do not contain protected health information. As the clinical data were extracted from the MIMIC-III and MIMIC-IV databases, they did not contain any individually identifiable information. Informed consent and ethical approval were not required from the Ethics Committee of the West China Fourth Hospital.

Study population

Initially, the study population was focused on septic patients with DM. Additionally, the medical records of all adult patients aged at least 18 years admitted to the ICU were analyzed. To reduce

selection bias, we only use first-round ICU admission records for patients who were enrolled in the ICU more than once. Patients who were discharged <24 hours and encountered missing variable data (medication information), as well as outcome data (28-day in-hospital mortality), were excluded. The flow chart of the patient selection process is shown in Figure 1.

Definition of sepsis, septic shock, and DM

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection (3). The diagnoses of sepsis and septic shock were based on the 'Third International Consensus Definitions for Sepsis and Septic Shock (sepsis-3)' (3). The organ dysfunction can be identified as an acute change in total SOFA score ≥2 points consequent to the infection. The SQL language method and the dictionary of codes for the International Classification of Diseases and Ninth Revision (ICD-9) and International Classification of Diseases and Tenth Revision (ICD-10) codes dictionary were used to screen and extract septic and septic shock patients with DM from the MIMIC III and MIMIC IV databases, respectively.

Definition and selection of variables

The clinical characteristics of each patient were collected after admission to the ICU. Variables were divided into categorical and continuous variables. Categorical variables included sex (female and male), race (white, black, and other), weight, comorbidities (heart failure, respiratory failure, liver disease including hepatitis and cirrhosis, renal diseases including chronic kidney disease (CKD) and acute kidney injury (AKI), and malignancy), the medical usage records of continuous renal replacement therapy (CRRT), oxygen, ventilation, antibiotic, dopa, norepinephrine, and fluid input during the ICU stay.

Continuous variables included the age at admission, SOFA score, length of hospital stay, length of ICU stay, total fluids administrations, peripheral blood test data (red blood cell (RBC), platelets (PTL), white blood cell (WBC), albumin (Alb), alanine transaminase (ALT), glutamic transaminase (AST), hemoglobin (Hb), lactate (Lac), lymphocyte, neutrophil, international normalized ratio (INR), blood urea nitrogen (BUN), creatinine, potassium ion (K), chloride ion (Cl), sodium ion (Na), bicarbonate ($HCO3^-$), prothrombin time (PT), mean erythrocyte volume (MCV), hematocrit (HCT), red cell distribution width (RDW)), arterial blood gas analysis (base excess (BE), total calcium (TCa), anion gap (AG)), glycosylated hemoglobin (HbA1c), fasting glucose, and general examination (heart rate (HR), mean blood pressure (MBP), temperature, respiration rate). There were thirty-eight variables at admission were selected for further analysis. The unit of the selected variable was summarized in Table S1.

Primary outcome

The primary endpoint of this study was the 28-day all-cause mortality of septic patients with DM.

Model construction and validation

Nomograms are widely used as clinical prognostic models in different fields of diseases (28). It can generate an individual probability of a clinical event by integrating diverse prognostic and determinant variables (29, 30). In this study, the patients from

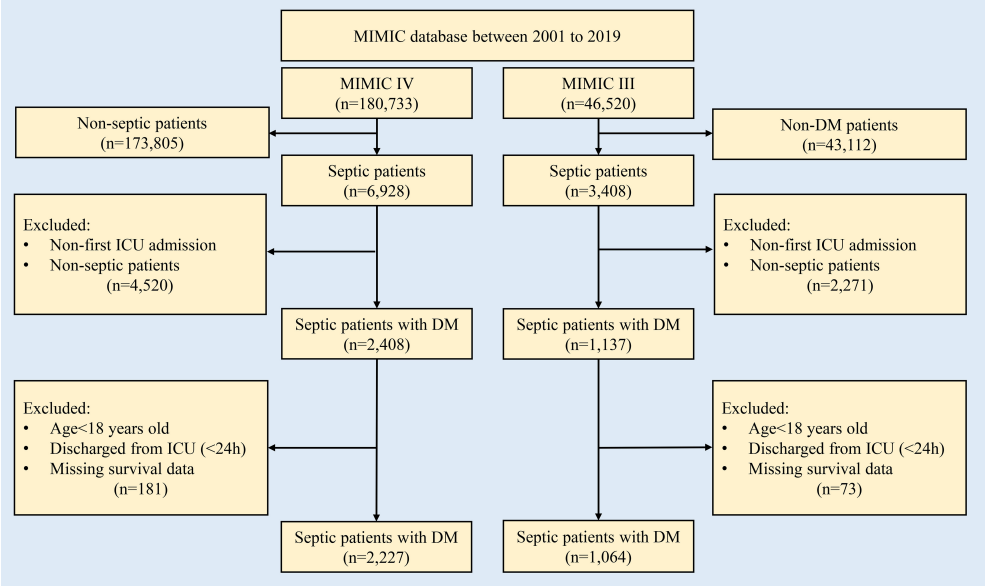

FIGURE 1
Flow chart for patient selection in this study. DM, diabetes mellitus; MIMIC, Medical Information Mart for Intensive Care Database.

the MIMIC IV database were used for nomogram construction due to the larger sample size. Bootstrap analysis was used for internal validation. Moreover, patients from the MIMIC III database were further settled as the validation cohort.

Statistical analysis

The variables with a missing value (<20%) were filled out using the median interpolation method (31). Categorical variables were presented as the number of the subgroup population with the percentage (%). Continuous variables were expressed as the mean and standard deviation (*Mean* ± *SD*). LASSO regression was used to remove less important variables and reduce the potential overfitting between included variables *via* the regression coefficients penalizing the size of the parameters. The LASSO regression curtailed the coefficient estimates toward zero, with the degree of shrinkage dependent on an additional parameter (λ) (32). To determine the optimal values for λ, 10-time cross-validation was used, and the minimum criteria λ was selected for further analysis (32). The multivariate Cox regression analysis was conducted to determine the independent short-term prognostic factors in septic patients with DM. The comparisons of selected variables among survivor and non-survivor subpopulations were conducted by using the Pearson-Chi square test or Student's t-text as appropriate. The LASSO and stepwise multivariate Cox regression analyses were conducted by using the "rms" package from the "R" software (http://www.r-project.org, R Foundation, Vienna, Austria, version 4.1.2). The nomogram of prognostic factors associated with 28-day mortality in septic patients with DM was based on the statistically significant different variables during the multivariate Cox regression analysis.

The C-index (33) and the area under the receiver operating characteristic (ROC) curves (AUCs) were calculated for evaluating the discrimination of the nomogram. Furthermore, the calibration curves and decision curve analysis (DCA), and Kaplan-Meier (KM) curves were performed to assess the utility of the nomogram. A two-tailed *P*-value < 0.05 was considered statistically significant.

Results

The clinical features of septic patients with DM

In this study, 2,227 patients in the MIMIC IV database and 1,064 in the MIMIC III database who met the inclusion criteria were enrolled. In the training data, the overall 28-day all-cause mortality rate of septic patients with DM was 23.9% (n=533) and most of the patients were white in race (61.1%) and the male subpopulation showed a relatively higher proportion (58.3%). The septic shock was identified in 57.6% of septic patients from the MIMIC IV database and 51.2% of septic patients from the MIMIC III database, respectively. Septic patients with DM were accompanied by various degrees of comorbidities at admission, with a more pronounced in renal diseases (82.4%), respiratory failure (46.9%), and heart failure (39.6%) in the training cohort. During ICU hospitalization, the majority of the septic patients with DM received ventilation (83.0%) and antibiotic (98.5%) therapeutic interventions but only 11.3% of the patients received CRRT management. In the external validation cohort from the MIMIC III database, several differences were noticed in the proportions of heart and liver comorbidities, ventilation intervention, dopa usage, total fluids administrations, and length of ICU stay, compared with the training cohort (p<0.001). In addition, the mean proportion of HbA1c was 7.27% in the training cohort and 8.38% in the validation cohort, respectively. The clinical characteristics of the entire study population are shown in Table 1.

LASSO regression analysis

A total of 38 variables were initially elected to the LASSO regression analysis with 10-fold cross-validation (Figures 2A, B; Table S1). Ultimately, ten variables including age, respiratory failure, SOFA score, Alb, BE, AG, INR, RDW, temperature, and HbA1c were selected for the stepwise multivariate Cox regression analysis (Table S2).

The comparison of clinical variables between survivor and non-survivor

Based on the selected variables, we compared the clinical characteristics between the survivor and non-survivor subpopulations in 28-day all-cause mortality (Table 2). Regarding the age at admission, non-survivors were significantly older than the survivors (71.43 years *vs.* 66.89 years, p<0.001). Similarly, non-survivors showed a higher proportion of respiratory failure (691 cases (40.8%) *vs.* 354 (66.4%), p<0.001), SOFA score (4.08 *vs.* 3.13, p<0.001), RDW (16.53% *vs.* 15.45%, p<0.001), AG (18.44mEq/L *vs.* 14.17mEq/L, p<0.001) and prolonged INR (1.90s *vs.* 1.50s, p<0.001) as well as Hb1Ac (9.50% *vs.* 7.27%, p<0.001), when compared with the survivors. On the contrary, the non-survivors showed a decrease in Alb (2.67g/dL *vs.* 2.77g/dL, p<0.001), BE (-5.69mEq/L *vs.* -2.68mEq/L, p<0.001), and body temperature (36.61°C *vs.* 36.98° C, p<0.001) at admission, compared to the survivors. During the hospitalization, survivors showed longer hospitalization than non-survivors (length of hospital stay: 16.83 *vs.* 8.38 days, p<0.001; length of ICU stay: 5.57 *vs.* 5.21 days, p<0.001). There was a significant difference in total amounts of fluid input between survivors and non-survivors during the ICU stay (46720.13 ml *vs.* 59784.26 ml, p=0.004).

Multivariate Cox regression analyses

Multivariate Cox regression analysis revealed that age at admission (hazard ratio (HR)=1.029 *per year*, 95%CI: 1.022-1.036, p<0.001), respiratory failure (HR=1.872, 95%CI: 1.554-2.254, p<0.001), SOFA (HR=1.056, 95%CI: 1.018-1.094, p=0.004), levels of BE (HR=0.980, 95%CI: 0.967-0.992, p=0.002), AG (HR=1.100,

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TABLE 1 The clinical characteristics of the whole study population.

Variable	Training cohort [a] (n=2,227)	Validation cohort [b] (n=1,064)	*P* [c]
Baseline			
Age	67.98 ± 13.55 *	69.09 ± 13.77 *	**<0.001**
Race (%)			
White	1,361 (61.1)	729 (68.5)	**<0.001**
Black	299 (13.4)	126 (11.8)	
other	567 (25.5)	209 (19.6)	
Sex (%)			
Female	928 (41.7)	442 (41.5)	0.944
male	1,299 (58.3)	622 (58.5)	
Weight	88.08 ± 27.01 *	88.70 ± 27.90 *	0.542
SOFA	3.36 ± 2.07 *	7.04 ± 3.76 *	**<0.001**
Comorbidity			
COPD (%)			
Yes	212 (9.5)	18 (1.7)	**<0.001**
No	2015 (90.5)	1046 (98.3)	
Respiratory failure (%)			
Yes	1045 (46.9)	468 (44.0)	**<0.001**
No	1182 (53.1)	596 (56.0)	
Hypertension (%)			
Yes	545 (24.5)	467 (43.9)	**<0.001**
No	1682 (75.5)	597 (56.1)	
Atrial fibrillation (%)			
Yes	774 (34.7)	708 (66.5)	**<0.001**
No	1453 (65.2)	356 (33.5)	
Heart Failure (%)			
Yes	882 (39.6)	429 (40.3)	0.704
No	1345 (60.4)	635 (59.7)	
Renal failure (%)			
Yes	1835 (82.4)	896 (84.2)	0.198
No	392 (17.6)	168 (15.7)	
CKD (%)			
Yes	814 (36.5)	290 (27.3)	**<0.001**
No	1413 (63.5)	774 (72.7)	
Malignancies			
Yes	573 (25.7)	220 (20.7)	**0.002**
No	1654 (74.3)	844 (79.3)	
Liver diseases			
Yes	645 (29.0)	139 (13.1)	**<0.001**

(Continued)

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TABLE 1 Continued

Variable	Training cohort [a] (n=2,227)	Validation cohort [b] (n=1,064)	*P* [c]
No	1582 (71.0)	925 (86.9)	
Intervention			
CRRT (%)			
Yes	252 (11.3)	98 (9.2)	0.067
No	1,975 (88.7)	966 (90.8)	
Ventilation (%)			
Yes	2,194 (98.5)	1004 (94.4)	**<0.001**
No	33 (1.5)	60 (5.6)	
Antibiotic (%)			
Yes	2,194 (98.5)	1004 (94.4)	**<0.001**
No	33 (1.5)	60 (5.6)	
Dopa (%)			
Yes	139 (6.2)	159 (14.9)	**<0.001**
No	2,088 (93.8)	905 (85.1)	
NE (%)			
Yes	1,156 (51.9)	568 (53.4)	0.428
No	1,071 (48.1)	496 (46.6)	
Total fluid input	49,846.84 ± 92,181.40	59,717.59 ± 111,866.20	**0.007**
Length of hospital stay	14.81 ± 16.37	15.42 ± 15.98	0.313
Length of ICU stay	5.48 ± 7.18	7.86 ± 10.36	**<0.001**
Blood test			
Alb	2.75 ± 0.51 *	2.71 ± 0.52 *	**0.045**
ALT	108.87 ± 379.29 *	113.39 ± 383.04 *	0.750
AST	158.73 ± 563.50 *	220.47 ± 1,041.81 *	**<0.001**
BE	-3.40 ± 5.71 *	-3.62 ± 5.51 *	0.744
Hb	10.21 ± 2.10 *	10.39 ± 2.02	**0.024**
Lac	2.74 ± 2.47 *	2.60 ± 2.21 *	0.114
Lymphocyte	9.50 ± 9.41 *	8.93 ± 8.70 *	0.096
Neutrophil	79.69 ± 12.95 *	80.19 ± 13.39 *	0.309
PCO_2	41.54 ± 11.34 *	41.03 ± 12.78 *	0.250
PO_2	95.23 ± 75.40 *	123.81 ± 89.13 *	**<0.001**
RBC	3.47 ± 0.74 *	3.49 ± 0.70 *	0.434
tCa	8.03 ± 0.88 *	7.91 ± 0.91 *	**<0.001**
AG	15.19 ± 5.01 *	16.02 ± 4.60 *	**<0.001**
INR	1.60 ± 0.87 *	1.72 ± 1.08 *	**<0.001**
BUN	39.12 ± 27.72 *	39.49 ± 26.27 *	0.715
WBC	15.35 ± 11.25 *	15.31 ± 11.28 *	0.910
Creatinine	2.10 ± 1.89 *	2.08 ± 1.71 *	0.696

(Continued)

TABLE 1 Continued

Variable	Training cohort [a] (n=2,227)	Validation cohort [b] (n=1,064)	*P* [c]
K^+	4.24 ± 0.85 *	4.19 ± 0.81 *	0.128
Cl^-	103.28 ± 7.58 *	105.54 ± 7.31 *	**<0.001**
Na^+	137.64 ± 6.28 *	138.37 ± 6.05 *	**0.002**
HCO_3^-	21.74 ± 11.99 *	21.27 ± 5.32 *	0.223
Pt	18.75 ± 12.58 *	17.93 ± 9.28 *	0.058
PLT	206.56 ± 120.49 *	219.40 ± 125.29 *	**0.005**
MCV	91.44 ± 7.88 *	90.75 ± 7.63 *	**0.018**
HCT	31.51 ± 6.24 *	31.41 ± 6.01 *	0.687
RDW	15.71 ± 2.40 *	15.87 ± 2.29 *	0.068
Glucose	190.06 ± 102.15 *	178.70 ± 100.22 *	**0.002**
HbA1c	7.27 ± 1.81 *	8.38 ± 1.74 *	**<0.001**
Admission condition			
Temperature	36.89 ± 0.95 *	36.82 ± 0.83 *	**0.039**
Respiration	21.35 ± 6.36 *	20.60 ± 4.32 *	**0.001**
HR	95.94 ± 20.95 *	89.65 ± 16.52 *	**<0.001**
MBP	78.82 ± 18.77 *	72.25 ± 9.86 *	**<0.001**
Septic shock			
No	944 (42.4)	519 (48.8)	**0.001**
Yes	1283 (57.6)	545 (51.2)	

[a]it contains the study population from the MIMIC IV database.
[b]it contains the study population from the MIMIC III database.
[c]Student's t-test/Chi-square test.
* Mean± standard deviation (SD).
COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; CRRT, continuous renal replacement therapy; NE, norepinephrine; SOFA, Sequential Organ Failure Assessment; Alb, albumin; ALT, alanine transaminase AST, aspartate aminotransferase; BE, base excess; Hb, hemoglobulin; Lac, lactate; RBC, red blood cell; tCa, total calcium; AG, anion gap; INR, international normalized ratio; BUN, blood urea nitrogen; WBC, white blood cell; K^+, potassium; Cl^-, chlorine; Na, sodium; HCO_3^-, hydrocarbonate; Pt, prothrombin time; PLT, platelets; MCV, mean corpuscular volume; HCT, hematocrit; RDW, red blood cell distribution width; HbA1c, glycosylated hemoglobin; HR, heart rate; MBP, mean blood pressure.
Bold value means statistically significant (p<0.05).

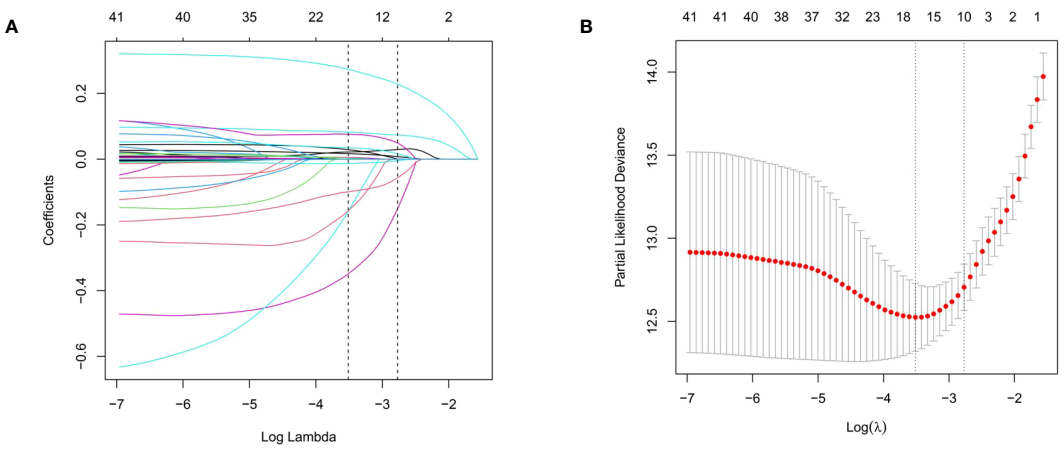

FIGURE 2
The LASSO regression analysis to select the potential variables. A total of thirty-eight variables are initially included and ten variables are finally selected for further analysis. **(A)** The LASSO coefficient analysis of the clinical features. **(B)** Tuning parameter selection in the LASSO Cox regression model.

TABLE 2 Comparisons of clinical characteristics between survivors and non-survivors of the training cohort.

Variable	Subgroup	Survivors (n=1694)	Non-survivors (n=533)	*P*
Age	/	66.89 ± 13.56*	71.43 ± 12.92*	**<0.001**[a]
Respiratory failure (%)	Yes	691 (40.8)	354 (66.4)	**<0.001**[b]
	No	1003 (59.2)	179 (33.6)	
SOFA score	/	3.13 ± 1.82	4.08 ± 2.57	**<0.001**[a]
BE	/	-2.68 ± 4.88*	-5.69 ± 7.33*	**<0.001**[a]
Alb	/	2.77 ± 0.48*	2.67 ± 0.57 *	**<0.001**[a]
AG	/	14.17 ± 3.88*	18.44 ± 6.58*	**<0.001**[a]
INR	/	1.50 ± 0.70*	1.90 ± 1.24*	**<0.001**[a]
RDW	/	15.45 ± 2.25*	16.53 ± 2.67*	**<0.001**[a]
HbA1c	/	7.27 ± 1.81*	9.50 ± 2.57*	**<0.001**[a]
Length of hospital stay	/	16.83 ± 17.89	8.38 ± 7.01	**<0.001**[a]
Length of ICU stay	/	5.57 ± 7.69	5.21 ± 5.24	**<0.001**[a]
Total fluid input	/	46,720.13 ± 95,902.51	59,784.26 ± 78,460.59	**0.004**
Temperature	/	36.98 ± 0.88*	36.61 ± 1.11*	**<0.001**[a]

* Mean± standard deviation (SD).
[a]Student's t-test.
[b]Pearson-Chi square test.
SOFA, Sequential Organ Failure Assessment; BE, base excess; Alb, albumin; AG, anion gap; ICU, intensive care unit; INR, international normalized ratio; RDW, red blood cell distribution width; HbA1c, glycosylated hemoglobin.
Bold value means statistically significant (p<0.05).

95%CI: 1.080-1.120, p<0.001), Alb (HR=0.679, 95%CI: 0.574-0.802, p<0.001), INR (HR=1.087, 95%CI: 1.027-1.150, p=0.004), RDW (HR=1.056, 95%CI: 1.021-1.092, p=0.001), and body temperature (HR=0.857, 95%CI: 0.789-0.932, p<0.001), as well as HbA1c (HR=1.358, 95%CI: 1.320-1.401, p<0.001), were the independent prognostic factors in 28-day mortality of septic patients with DM (Figure 3).

Nomogram construction and validation

Depending on the prognostic factors determined by the multivariate Cox regression analysis, an individualized nomogram was subsequently established with the selected ten variables (Figure 4A). Each admitted septic patient with DM could get a total score and the corresponding risk for 28-day hospitalization mortality by adding the scores derived from the ten variables (Figure 4B).

To validate the clinical utility of the nomogram, both internal bootstrap analysis and external cohort validation were performed. The C-indexes, which were consistent with the AUC of the ROCs, were all above 0.80, with 0.870 (95%CI: 0.850-0.880) in the internal bootstrap analysis and 0.830 (95%CI:0.810-0.860) in the external validation cohort, respectively (Figures 5A, B). Compared with the single SOFA score, our model was internally and externally validated to have better discrimination and accuracy in predicting the 28-day all-cause mortality of septic patients with DM (Figures 5A, B).

Besides, we applied the nomogram to the septic shock subpopulation to further validate the generalization of the model. The AUC of the ROC for predicting the 28-day mortality of the septic shock subpopulation reached 0.82 (95%CI: 0.790-0.850), which indicated the identified prognostic indicators were also pivotal in more severe subpopulations at admission (Figure S1).

Robustness check

To further evaluate the utility of the nomogram, calibration curves were applied. Detailly, the calibration curves showed promising agreement in the ideal and observation events in both training (Figure 5C) and validation cohorts (Figure 5D) as well as the septic shock cohorts (Figure S2).

Similarly, the DCA curves showed that the scores derived from the nomogram could be more accurate than a treat-none or treat-all strategy when the threshold probability was above 5% in the training cohort (Figure 6A) and 10% in the septic shock subpopulation (Figure S3), respectively. Meanwhile, the external validation cohort also supported an optimal clinical utility of the model at the threshold probability intervals greater than 5% (Figure 6C), which was also better than the prediction value of SOFA scores (Figures 6B, D) in the study population.

Based on the risk model, the KM curves showed good discrimination in identifying the real high-risk subpopulation (cutoff value: 186) in both the internal (Figures 7A, B) and external validating cohorts (Figures 7C, D).

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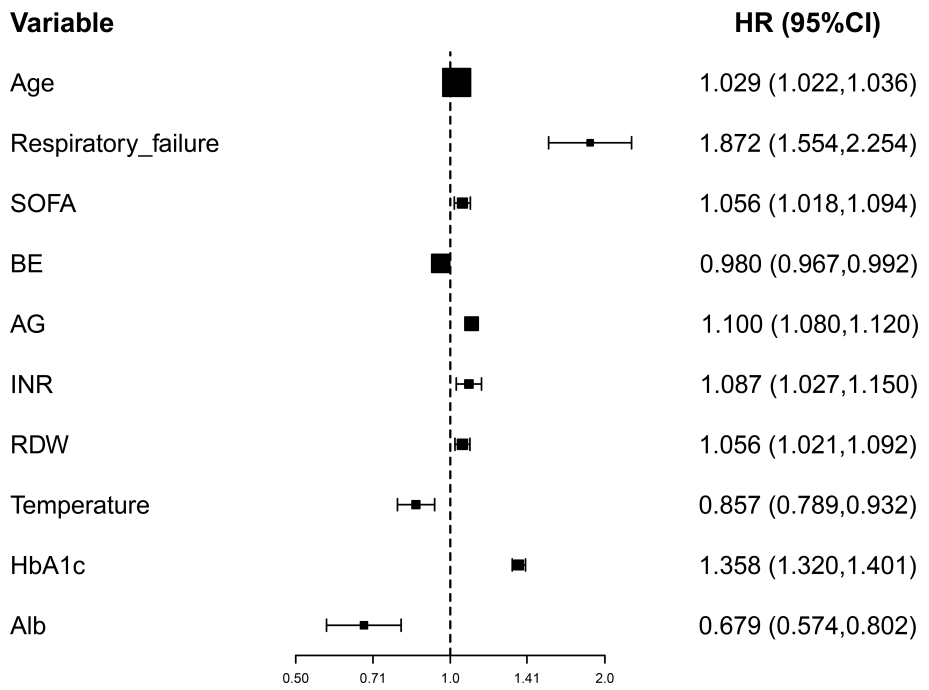

FIGURE 3
Forest plot for the multivariate Cox regression analysis. BE, base excess (mEq/L); SOFA, Sequential Organ Failure Assessment; AG, anion gap (mEq/L); INR, international normalized ratio (second); RDW, red blood cell distribution width (%), HbA1c, glycosylated hemoglobin (%).

Discussion

Currently, prognostic factors for septic patients with DM remain limited. In this study, we fill this research gap and identify that various admission characteristics were significantly associated with the short-term mortality risk in septic patients with DM. Additionally, we developed an individualized prediction nomogram for the clinical management of septic patients with DM at the initial round evaluation. Robustness analyses showed good discrimination, calibration ability, and clinical usefulness of this predictive model.

Sepsis is still the leading cause of death in patients elected to the ICU (15, 18). In the present study, the 28-day mortality rate of septic patients with DM was 23.9% (n=533), which was higher than in general septic patients (10%-15%) (34–36). Regarding the hospitalization characteristics of the study population, we observed that survivors showed longer hospital and ICU stays than non-survivors, which was consistent with previous studies (24, 37). Besides, our study also determined that non-survivors received a higher volume of fluids input during the ICU stay than survivors. Interestingly, the optimal amount of fluid administration for septic and septic shock patients is still uncertain (24, 38–40). Notably, one meta-analysis included 13 trials that suggested that lower standard intravenous (IV) fluid volumes showed no significant difference in all-cause mortality compared with higher IV fluid volumes (41). Furthermore, the recent Conservative versus Liberal Approach to Fluid Therapy in Septic Shock in Intensive Care (CLASSIC) study also revealed that adult patients with septic shock might not get more survival or health-related quality of life benefits from restrictive fluid therapy when compared with IV fluid therapy (38). Thus, clinicians should trade off the risks and benefits of fluid administration in each stage of critical illness. Further works are needed to help clinicians to make tailored fluid management.

Among septic patients with DM, recent basic and clinical studies revealed that systemic immune dysfunctions including, but not limited to, deficiencies in neutrophil function and declining T-cell responses triggered by the hyperglycemia condition were associated with the infection development and sepsis mortality (10, 42). As one of the representative indicators in DM, HbA1c was mainly interpreted as a biomarker to reflect the short and intermediate terms of glycemic control. Of note, recent studies demonstrated the clinical predictive value of HbA1c in the development and prognosis of critically ill diseases, particularly sepsis. However, the findings were not always consistent. Notably, Anca et al. determined the nonlinear relationship between HbA1c and the risk of sepsis (43). However, HbA1c was not identified significantly associated with mortality in septic patients (43). On the contrary, the study by Guo et al. showed that the levels of HbA1c at admission were remarkably associated with morbidity and mortality in septic patients (44). In our analysis, high proportions of HbA1c were strongly associated with an increased risk of short-term mortality in septic patients with DM. Preclinical studies showed that chronic dysglycemia could impair the vascular endothelial glycocalyx and further cause complications and the mortality of sepsis (45). Especially, the lung was one of the most vulnerable organs in DM and septic patients. In our study, we

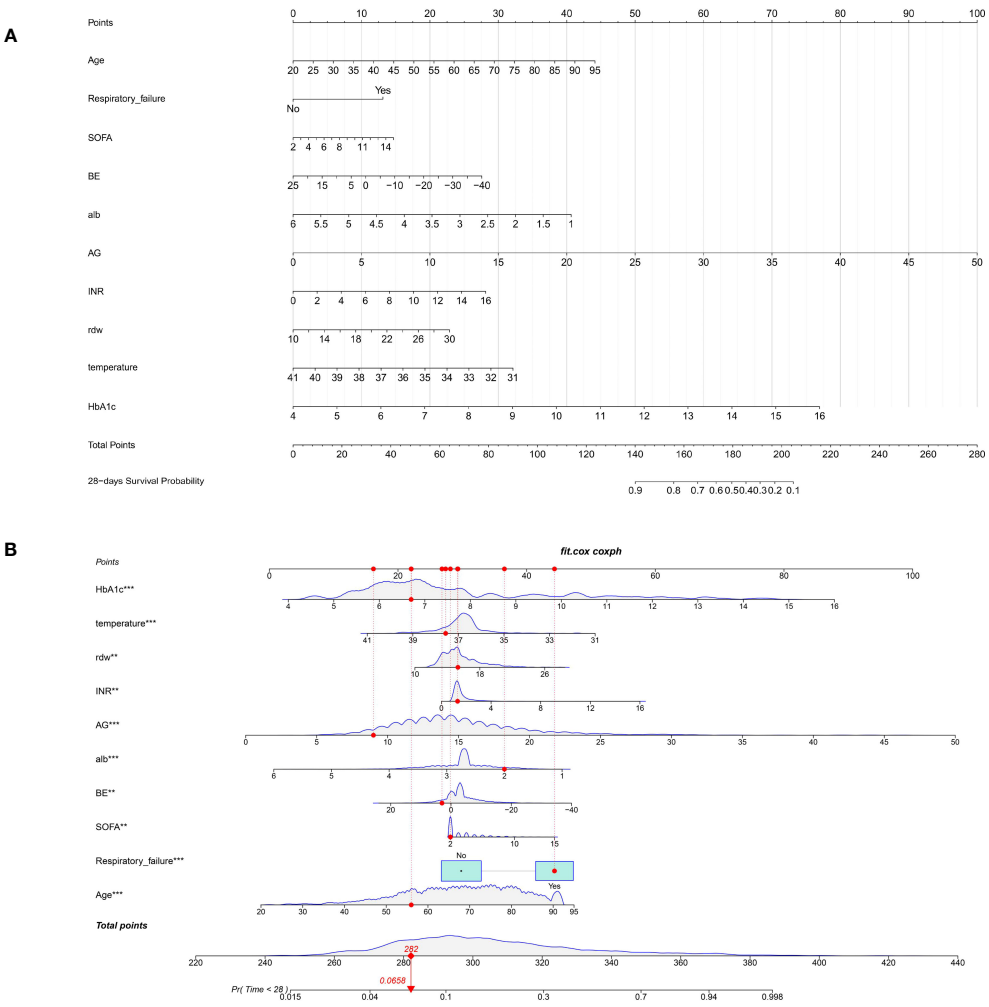

FIGURE 4
The nomogram for predicting the 28-day all-cause mortality in septic patients with diabetes mellitus. **(A)**. The nomogram is constructed by ten admission clinical variables; **(B)**. The example for explaining the clinical utility of the nomogram. The admitted patient would get an individualized score for each variable. By adding all scores, the clinicians could calculate the potential risk of 28-day all-cause mortality for each patient (red dot). BE, base excess (mEq/L); SOFA, Sequential Organ Failure Assessment; AG, anion gap (mEq/L); INR, international normalized ratio (second); RDW, red blood cell distribution width (%), HbA1c, glycosylated hemoglobin (%).

observed that patients with respiratory failure showed a nearly two-fold risk (HR=1.872) of mortality, compared with patients without respiratory failure. Notably, several previous studies suggested that DM was associated with an increased risk of respiratory failure. For example, Gulcan et al. reported that half of the patients with DM exhibited respiratory failure during sleep, which might be due to restrictive impairment of respiratory function secondary to DM (46). Besides, the preclinical data also demonstrated that hyperglycemia could activate sodium-potassium-chloride cotransporter 1(NKCC1) related pathways and adversely affect alveolar fluid regulation and lung function (47). Therefore, septic patients with DM are expected to receive more active respiratory function surveillance during the ICU stay.

Compared with recent studies on general sepsis, we validated several clinical indicators in the subpopulation of patients with DM. Initially, we identified that age was a strong prognostic indicator of short-term mortality in septic patients with DM. There is a significant increase in the prevalence of DM in the elderly population (48–50), leading to exacerbation, poor prognosis, and increased mortality risk in sepsis (51). Additionally, serum biomarkers were important predictors in the surveillance of critically ill patients (34, 36, 52–55). Septic patients were often accompanied by hypoproteinemia due to capillary leakage, reperfusion injury, tissue ischemia, and inflammation (56, 57). More importantly, septic patients with hypoproteinemia showed worse clinical outcomes during hospitalization. Similarly, hypoproteinemia was also associated with multiple adverse complications and increased risk of adverse outcomes in DM (58). Therefore, septic patients with DM may suffer from a higher risk of mortality in the condition of hypoproteinemia. Furthermore, acid-base disorders are frequently observed in critically ill patients (59), especially in patients with DM (60). Of note, serum lac level

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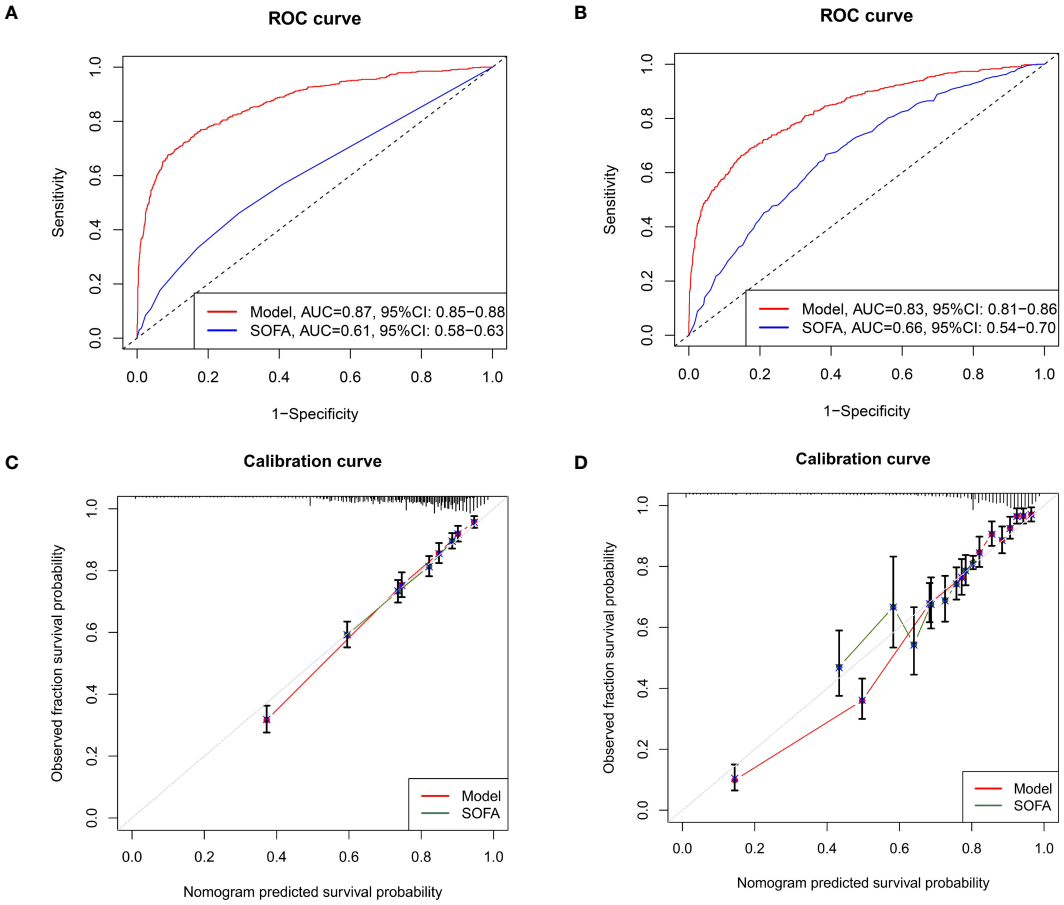

FIGURE 5
Methods to evaluate the accuracy and discrimination of the nomogram. **(A)** The ROC curves for evaluating the accuracy of the nomogram through internal bootstrapping analysis. **(B)** The ROC curves for evaluating the accuracy of the nomogram *via* external cohort analysis; **(C)** The calibration curves for evaluating the discrimination of the nomogram in the training cohort; **(D)** The calibration curves for evaluating the discrimination of the nomogram in the external validating cohort. ROC, receiver operating characteristic.

was a tight biomarker for predicting the clinical outcome of patients with sepsis (61, 62). Interestingly, hyperlactatemia was a frequent metabolic condition in DM patients with ketoacidosis (63, 64). However, compared with other critically ill diseases, the elevated lac level was not determined to be associated with increased length of ICU stay or mortality in DM patients (65). Furthermore, some researchers even queried whether the standard hyperlactatemia cut-off value was also adapted for the survival prediction in DM patients (66). For these reasons, the value of lactate levels might be undermined in predicting short-term mortality in septic patients with DM, particularly when they were concurrent with diabetic ketoacidosis.

As a common biomarker for evaluating the acid-base balance, the AG levels were frequently used to evaluate the type of metabolic acidosis. However, acid-base imbalance, especially metabolic acidosis, frequently occurred in seriously ill patients and was significantly related to mortality. In our analysis, the levels of BE and AG were determined to be significantly associated with short-term mortality among septic patients with DM. Recent two studies from different regions also highlighted the predictive role of BE in the diagnosis and prognosis of sepsis (67, 68). Moreover, AG was recently identified to be an optimal serum biomarker for reflecting systemic dysfunctions in critically ill patients (69–71). In particular, high levels of AG were not only associated with impaired cardiorespiratory fitness (72) but also mediated more severe insulin resistance conditions (73). This could partially explain the underlying mechanism that AG played an important role in the prognosis of septic patients with DM. Currently, whether AG can accurately predict the prognosis of critically ill patients is still debatable. These discrepancies may be due to differences in varied study populations and measuring methods as well as other factors that influence AG values may also influence the exact correlations. Further studies are warranted to explore the underlying pathophysiological mechanism.

Consistent with the study by Liu et al. (74), we observed that prolonged INR was positively associated with short-term mortality in septic patients with DM. Some possible mechanisms might explain this finding. It is noteworthy that the activated

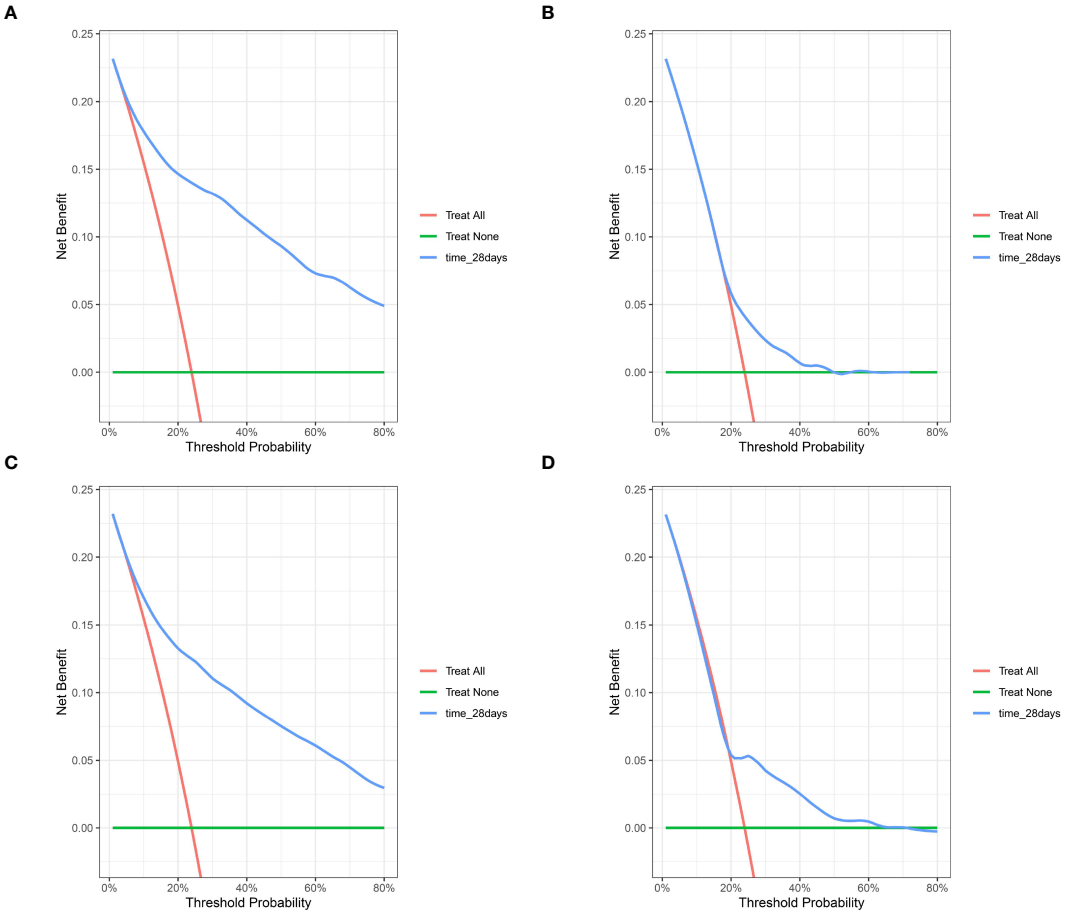

FIGURE 6
The DCA for evaluating the clinical utility of the nomogram. **(A)** the DCA of internal bootstrapping analysis; **(B)** The DCA of the training SOFA score; **(C)** The DCA of external validation cohort; **(D)** The DCA of the external SOFA score. DCA, decision curve analysis; SOFA, Sequential Organ Failure Assessment.

coagulation system was regarded as the primary response of host systemic defense in sepsis (75). However, chronic septic conditions could adversely mediate the coagulation dysfunction with subsequent disseminated intravascular coagulation (DIC) (76). Besides, we also determined that a high percentage of RDW was an adverse prognostic factor for septic patients with DM. Most recently, the latest evidence showed that RDW could predict poor outcomes in various chronic diseases (77–81). In the DM population, Nada found that RDW was markedly higher in DM patients than in healthy subjects and particularly higher in uncontrolled glycemia (82). And in the population with sepsis, a meta-analysis of three studies revealed that a high level of RDW was associated with septic death (83). Thus, the percentage of RDW, affected by the hyperglycemia condition, showed a unique clinical value in critically ill patients, which could be a supplement indicator for assessing the prognosis of septic patients with DM.

Interestingly, the admitting body temperature was observed to be associated with the severity and prognosis of septic patients with DM. The latest two large-scale multi-center studies yielded the distinguished role of body temperature in the prognosis of critically ill patients, especially in terms of septic patients (84, 85). Similarly, our study supported that the patients with relatively higher body temperatures presented a lower risk for short-term mortality.

Based on identified prognostic factors, we established a new individualized nomogram to evaluate the condition of septic patients with DM at admission. The optimal C-index and AUCs in both the internal and external validation cohorts suggested the good discrimination and accuracy of the model in detecting high-risk patients. Furthermore, compared with the separate SOFA evaluation system, our model showed better predictive accuracy combined with various clinical characteristics. Additionally, we explored the utility of the nomogram in septic shock patients with DM, the subpopulation which was much more related to mortality. As expected, the model also presented promising predictive ability in detecting high-risk septic shock patients. Therefore, by using the predictive model, clinicians could better stratify risk for septic patients with DM at the initial evaluation.

Nevertheless, our study has some limitations. First, some factors, including laboratory indicators (such as fast plasma glucose, fasting insulin, fasting C-peptide, and C-reactive protein),

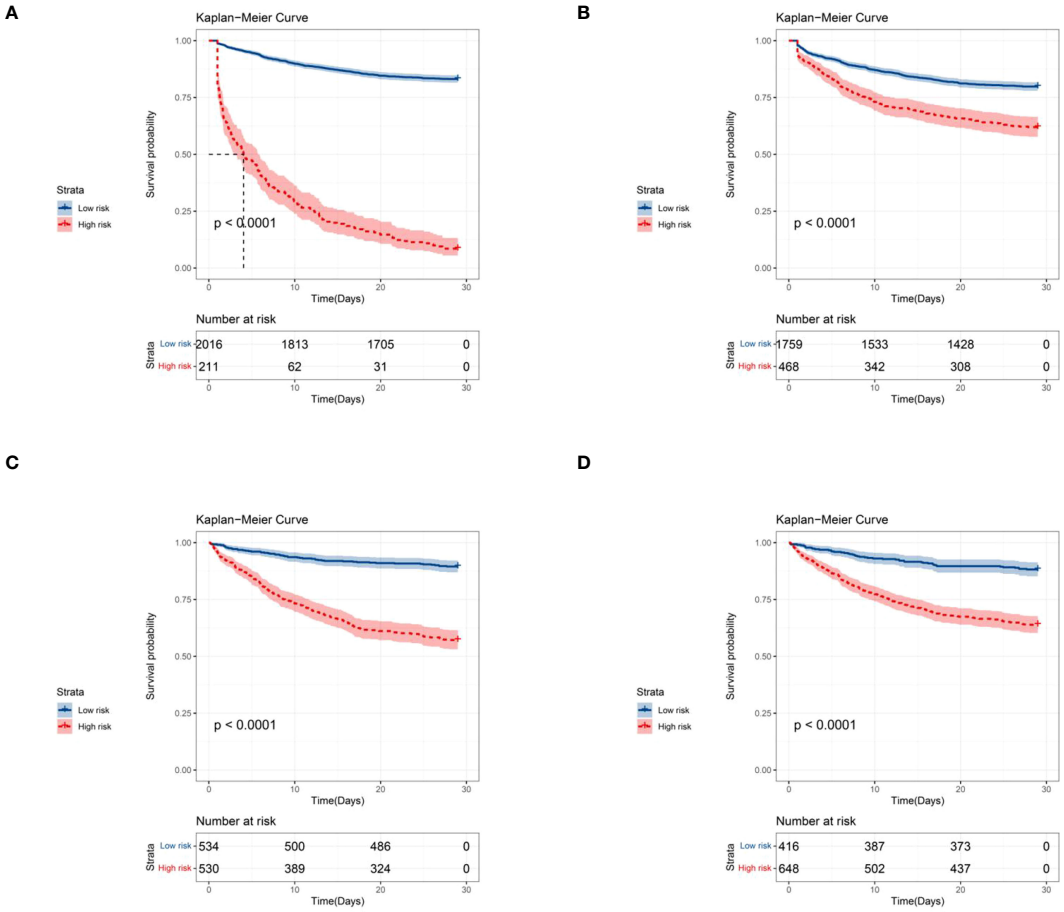

FIGURE 7
Kaplan-Meier curves for predicting the 28-day all-cause mortality in septic patients with DM by stratifying the different risk subgroups (the cut-off point was 186). **(A)** Kaplan-Meier curves based on the nomogram; **(B)** Kaplan-Meier curves based on the training SOFA score; **(C)** Kaplan-Meier curves based on the external validation cohort; **(D)** Kaplan-Meier curves based on the external validation SOFA score.

site of infection, drugs, and interventions that could be related to prognosis, were not included in this study. Second, although this is a large-scale cohort study with over 3000 patients involved, the nature of the retrospective study design could inevitably lead to selection bias. Third, the MIMIC database was constructed from the medical records of admitted patients during the past two decades. However, intensive care medicine has developed substantially during the same period. Whether our findings would also be adapted to the current clinical practice needs further validation. Last, although we have successfully developed and validated a short-term mortality risk stratification model, data from other countries or regions are needed to verify this model in the future with more useful variables added.

Conclusions

In this study, ten clinical variables including age, respiratory failure, SOFA, BE, Alb, AG, INR, RDW, HbA1c, and temperature at admission were identified as independent prognostic factors in predicting the 28-day all-cause mortality in septic patients with DM. Based on these factors, we developed and externally validated a predictive nomogram, with optimal discrimination and accuracy to detect the high-risk subgroup. This model can be implemented for ICU physicians to quickly make the initial clinical decision for septic patients with DM in clinical practice.

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

(I) Conception and design: XH and CY. (II) Administrative support: XH. (III) Provision of study materials or patients: CY, YJ, and YM. (IV) Collection and assembly of data: CY, YJ, and YM. (V)

Data analysis and interpretation: XH, CY, and CZ. (VI) Manuscript writing: All authors. (VII) Final approval of manuscript: All authors.

Acknowledgments

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1237866/full#supplementary-material

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Association of hypoglycaemia with the risks of arrhythmia and mortality in individuals with diabetes - a systematic review and meta-analysis

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Background: Hypoglycaemia has been linked to an increased risk of cardiac arrhythmias by causing autonomic and metabolic alterations, which may be associated with detrimental outcomes in individuals with diabetes(IWD), such as cardiovascular diseases (CVDs) and mortality, especially in multimorbid or frail people. However, such relationships in this population have not been thoroughly investigated. For this reason, we conducted a systematic review and meta-analysis.

Methods: Relevant papers published on PubMed, Embase, Cochrane, Web of Knowledge, Scopus, and CINHAL complete from inception to December 22, 2022 were routinely searched without regard for language. All of the selected articles included odds ratio, hazard ratio, or relative risk statistics, as well as data for estimating the connection of hypoglycaemia with cardiac arrhythmia, CVD-induced death, or total death in IWD. Regardless of the heterogeneity assessed by the I^2 statistic, pooled relative risks (RRs) and 95% confidence intervals (CI) were obtained using random-effects models.

Results: After deleting duplicates and closely evaluating all screened citations, we chose 60 studies with totally 5,960,224 participants for this analysis. Fourteen studies were included in the arrhythmia risk analysis, and 50 in the analysis of all-cause mortality. Hypoglycaemic patients had significantly higher risks of arrhythmia occurrence (RR 1.42, 95%CI 1.21-1.68), CVD-induced death (RR 1.59, 95% CI 1.24-2.04), and all-cause mortality (RR 1.68, 95% CI 1.49-1.90) compared to euglycaemic patients with significant heterogeneity.

Conclusion: Hypoglycaemic individuals are more susceptible to develop cardiac arrhythmias and die, but evidence of potential causal linkages beyond statistical associations must await proof by additional specifically well planned research that controls for all potential remaining confounding factors.

KEYWORDS

hypoglycaemia, diabetes, arrhythmia, mortality, risk, systematic review, meta-analysis

Introduction

Diabetes, a non-communicable disease, is a serious public health concern worldwide. More than half a billion people (536.6 million) aged 20-79 were predicted to develop diabetes mellitus (DM) in 2021, with roughly 90% having type 2 DM (T2DM) (1). Diabetes is a serious, chronic disease that profoundly affects the lives and well-being of individuals, families, and communities throughout the world. Diabetes is one of the leading ten causes of adult mortality, with a total estimated global healthcare expenditure of 966 billion USD (1).

Cardiovascular diseases (CVDs) are the most common cause of morbidity and mortality in this populace (2). Numerous large epidemiological studies demonstrate that maintaining target glycemic control is the only proven strategy for preventing diabetic vascular complications (3). To accomplish this goal, active use of oral diabetes medications or insulin as well as lifestyle modifications in accordance with the individual situation shall be initiated after the diagnosis of diabetes (4–6). However, intensive glycaemic control with antidiabetic drugs inevitably exposes IWD to the common side effect of hypoglycaemia (7–10). Recent evidence confirms that a hypoglycaemic episode, irrespective of severity, is clinically important because of its link to increased arrhythmias (via effects on cardiac repolarisation and alterations in cardiac autonomic activity, hypokalemia due to excess of insulin and increased secretion of catecholamines, which might drive potassium into the cell during hypoglycemia, and fuel energy shortage at the level of the cardiomyocyte due to low availability of glucose despite stress and increased demand as well (11, 12)) and other cardiovascular (CV) events and mortality (13–18). Similarly, a recent systematic review (19) reports that hypoglycaemia leads to electrocardiogram changes associated with a greater likelihood of cardiac arrhythmias, which are related to increased CV events and mortality. Another systematic review (20) concludes that hypoglycaemia is a risk factor for adverse vascular events and death. Nevertheless, contradictory evidence exists regarding the association between hypoglycaemia and the risks of cardiac arrhythmias and mortality (21–24). The aforementioned reviews have numerous limitations, including a lack of subgroup analysis, and a failure to recognise heterogeneity. Specifically, some undetected confounders (e.g. study design, hypoglycaemia criteria, ignorance of model adjustment, and dissimilar comorbidity profile) that are ignored by model adjustment can result in bias. Meanwhile, some studies (25) show that only spontaneous hypoglycaemia is associated with increased mortality, while others (26, 27) show no effect. Based on the available evidences, several authors (28, 29) even suggest that hypoglycaemia is a marker of disease severity and/or comorbidity burden in hospitalised patients.

For these reasons, our aim was to assess if hypoglycaemia affects the risk of arrhythmia or mortality in IWD.

Methods

Search strategy and criteria for inclusion

Two separate reviewers searched PubMed, EMBASE, Wiley Cochrane Central Register of Controlled Trials(CENTRAL), Web of knowledge, Scopus and CINAHL complete up to December 22, 2022 as per applicable recommendations (30). Any disagreement between them was resolved through consensus. In addition to keywords, we used Medical Subject Headings terms in PubMed, EMTREE terms in EMBASE, CINAHL headings in CINAHL, and keywords in all included databases as a part of our strategy. The search terms were "hypoglycaemia", "hypoglycemia" or "hypogly*" and "diabetes" or "diabet*", and "arrhythmias" or "arrhythmia*" or "dysrhythmia*" or "mortality" or "mortalit*" or "death" (search technique shown in Additional file 1). The grey literature was found by manually searching reference lists from eligible research and associated reviews. Authors were contacted to obtain necessary data. Prior to the start of this investigation, no review protocol for this systematic review has been published or registered.

The inclusion criteria were: (i) observational studies (OSs) [cohort, case-control, cross-sectional, and longitudinal studies], *post-hoc* analysis or sub-analysis of randomised controlled trials (p-h/sa of RCTs); (ii) focusing on the relationship of any hypoglycaemia with arrhythmias or CVD-induced death or overall death in IWD, and reporting risk or prevalence or incidence of arrhythmias (mortality) for IWD with hypoglycaemia as compared with euglycaemia, and (iii) standardized mortality or incidence ratio (SMR or SIR), or incidence rate ratio (IRR), or odds ratios (OR), or hazard ratio (HR), or relative risk (RR), as well as pertinent relevant raw data for recalculation are all used to report effect estimates. Exclusion criteria were as follows: (I) emphasizing the connection of hypoglycaemia with adverse outcomes such as arrhythmias- or CVD-caused death or all-cause mortality in non-diabetic people; (II) case report, quasiexperiment (in which subjects are not assigned at random), editorial, remark, review, letter or unpublished study; (III) only published as abstract or conference proceeding. Diabetes studies that did not provide such estimations were also omitted. When a site-specific dataset was published multiple times, the study with the newest t publication or the largest sample size was generally picked.

Data extraction and quality evaluation

Data were collected about the research (design, first author's name, title, year of publication, data source, country/region, baseline years, sample size, follow-up duration, diabetes clarity, endpoints, measure of relationship, number of observed and expected events), participants (mean age and the gender), analysis strategy (statistical models, adjustment factors), effect size (e.g., SMR or IRR, or SIR, or HRs, RRs or ORs), as well as pertinent raw data for recalculation.

Two researchers independently assessed the quality of each p-h/sa of RCT and enrolled study based on cohort or case-control design utilizing the 9-star Newcastle-Ottawa Scale (NOS) (31). A rating of above six stars (31) indicates high quality. The 11-item Agency for Healthcare Research and Quality (AHRQ) checklist is recommended for assessing the quality of cross-sectional or longitudinal studies (32, 33). Each item's responses are "yes," "no," and "unclear". If the response to each question is "yes", one

point is awarded; otherwise, no points are given. Studies with a total score of 0–5, 6–7, 8–11, respectively, were deemed to be of low, moderate and high quality. Disagreements were resolved through dialogue.

Statistical analysis

The main and second goals were the hazards of arrhythmias and overall death (CVD death) whereas the analysis period, respectively. OR, or RR with 95% confidence interval (CI) was used to summarize dichotomous outcomes. ORs are comparable to RRs since the absolute risk of arrhythmias or death is minimal in these populations and the two indices provide equivalent RR estimates (34). The I^2 statistic was used to determine the fraction of variability between studies because of heterogeneity between studies, and with I^2 values greater than 50%, 25-50%, and less than 25%, was classified as high, medium, and low respectively (35). The iterative non-central Chi-2 test was used to find a CI for I^2 (36).

Concerning the antecedent discrepancy of p-h/sa of RCT and OSs, we performed subgroup analyses for arrhythmia by sample size (<1000, and ≥1000), diurnal differences (nocturnal hypoglycaemia, and daytime hypoglycaemia), severity of hypoglycaemia (severe, and total), clarity of diabetes (T1DM, T2DM, and total), and type of arrhythmia (incidence of QTc interval prolongation, and other arrhythmia). For all-cause mortality analysis, subgroup analyses were conducted with sample size, country/region (developed, developing, and total), study population (simple diabetes, and diabetes with other disease or high risk for CV disease), hypoglycaemia episode (1, and ≥2), clarity of diabetes, study design (OSs, and p-h/sa of RCT), severity of hypoglycaemia, follow-up duration (≤1 year [including in-hospital mortality, and ICU mortality], and >1 year), and methods for effect estimate extraction (reported and calculated). Any quantifiable source of heterogeneity was identified through sensitivity analysis by excluding each study individually.

Publication bias was examined using Begg's and Egger's tests when at least five studies were available for analysis, as well as by visually inspecting the asymmetry of funnel plots of estimated effects against standard errors (37). Any publication bias (P<0.10) was corrected using Duval & Tweedie's trim-and-fill approach. All other analyses were conducted using STATA 14.0 (US) at P < 0.05 significance level.

Results

Study identification

From the 51,794 articles discovered through a systematic search, 137 were selected for further analysis (Figure 1). Two articles (24, 38) from one data source presented conflicting findings, and thus were both included. Four studies (22, 39–41) each two from the same teams/institutions and each reporting different outcomes for analysis were included. Finally, 60 articles provided data on the relationship between hypoglycaemia and arrhythmias or mortality (Table 1). The 60 studies included 11 p-h/sa RCTs (13, 21, 22, 41, 51, 75–77, 82, 86, 91), and 50 OSs (41 cohort studies (14, 23, 24, 39, 40, 43–45, 47–50, 52, 53, 55–65, 67–70, 72, 73, 78–81, 83, 85, 87–90) 3 (nested) case-control studies (54, 71, 84), 3 cross-sectional studies (38, 42, 74) 1

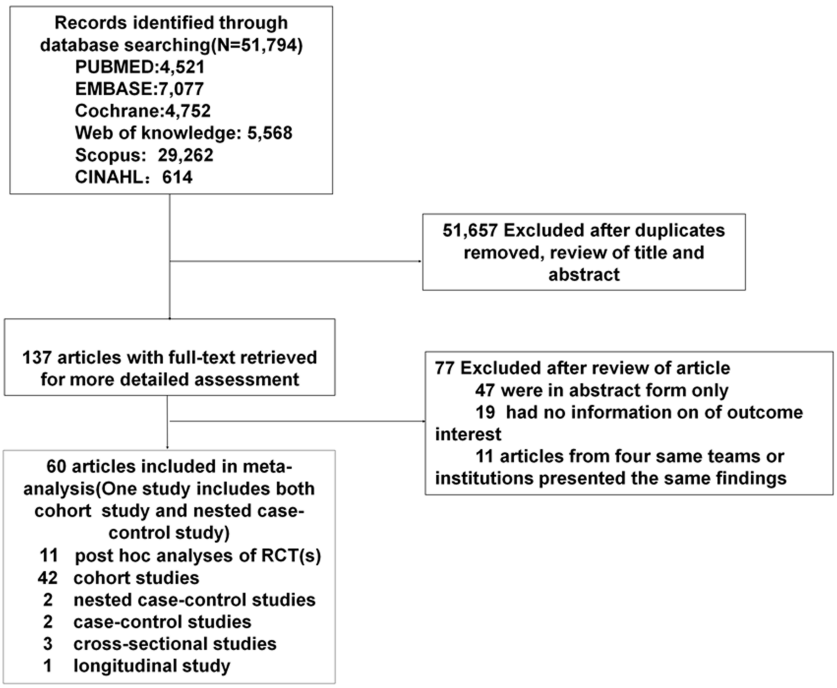

FIGURE 1
Flow diagram of study selection.

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

Study	Data source /Country; Region	Study design	Population	Sample Size	Baseline years/ Duration of follow-up	Mean age (years)	Female (% Total)	Mean DM duration (years)	Severity of hypoglycemia	Hypoglycaemia definition[a]	Outcomes assessment:/ Effect Estimate
GRUDEN et al. (38)	EURODIAB Prospective Complications Study /Multicenter from 16 European countries	Cross-sectional	T1DM	3,248	NR/NA	NR	NR	14.7	Severe	1	Incidence of QTc interval prolongation/OR
Pistrosch et al. (42)	One outpatient department for metabolic diseases/Germany	Cross-sectional	T2DM with a proven cardiovascular event	94	2012-2014/5 days	67.68	21.28	17.07	Any	6	Ventricular tachycardia/RR (calc)
Chow et al. (23)	Sheffield Teaching Hospitals diabetes outpatient clinics /The United Kindom	Cohort	T2DM	25	NR/5 days	64	48.0	17.0	Any	5	Bradycardia, Atrial ectopic, VPB, Complex VPB/IRR
Tsujimoto et al. (43)	The National Center for Global Health and Medicine/Japan	Cohort	Total	192	2006-2012/NR	72.06	33.87	15.17	Severe	1	Incidence of QTc interval prolongation/aOR
Amione et al. (24)	EURODIAB Prospective Complications Study/Multicenter from 16 European countries	Cohort	T1DM	1,415	1989-1991/7.0 years	32.1	NR	14.2	Severe	1	Incidence of QTc interval prolongation/aOR
Ko et al. (44)	The Korean NHIS/Korea	Cohort	T2DM	1,509, 280	2005-2008/8.6 years	54.9	36.2	NR	Severe	1	AF, All-cause mortality/aHR
Novodvorsky et al. (45)	Sheffield Teaching Hospitals outpatient clinics/The United Kindom	Cohort	T1DM	37	NR/96 hours	34.0	48.6	19.3	Any	5	Bradycardia, Atrial ectopic, VPB/IRR
Lee et al. (39)	The ARIC study/The United States	Cohort	T2DM	1,209	1996-1998/3.0 years	63.60	54.37	NR	Severe	3	AF, All-cause mortality, CVD mortality/aHR
Zhang et al (46)	UEBMI claims of Tianjin/China	Cohort; Nested case-control	T2DM	8,466	2008-2015/ 2.58 years	58.97	47.64	NR	Severe	1	Arrhythmias, All-cause mortality/ aHR, aOR
Mylona et al. (47)	A multicentre study from eight hospitals (nine clinics)/Greece	Cohort	Total	249	NR/16 months	72.25	50.20	15.06	Any	8	Incidence of QTc interval prolongation/RR (calc)
Echouffo-Tcheugui et al. (40)	The ARIC study/The United States	Cohort	T2DM	2,193	2011-2013/6.1 years	75.85	57.27	10.44	Severe	3	AF, All-cause mortality /aHR
Abdelhamid et al. (48)	Mixed medical-surgical ICUs in two geographically distinct university-	Cohort	T2DM	31	2016-2019/5 days	65.0	39.0	21.16	Any	5	Bradycardia, Atrial ectopic, VPBs/IRR

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TABLE 1 Continued

Study	Data source /Country; Region	Study design	Population	Sample Size	Baseline years/ Duration of follow-up	Mean age (years)	Female (% Total)	Mean DM duration (years)	Severity of hypoglycemia	Hypoglycaemia definition[a]	Outcomes assessment:/ Effect Estimate
	affiliated hospitals/Australia, The United Kindom										
Andersen et al. (49)	The diabetes outpatient clinics at Steno Diabetes Center Copenhagen, and Nordsj.llands Hospital Hillerod/Denmark	Cohort	T2DM	21	NR/1.0 years	66.8	28.6	18.2	Any	4	Arrhythmia/IRR
Kaze et al. (41)	ACCORD study/The United States, Canada	*Post-hoc* analyses	T2DM	8,277	NR/5.0 years	62.6	38.7	9.0	Severe	2	Incidence of QTc interval prolongation/aRR
Mellbin et al. (21)	DIGAMI 2 trial/Sweden	*Post-hoc* analyses	T2DM with AMI	1,253	1998-2003/2.1 years	68.39	33.2	7.90	Any/severe	6/11	All-cause mortality, CVD mortality/ aHR
Curkendall et al. (50)	HIPAA-compliant operating policies and procedures/The United States	Cohort	Total	107,736	2000-2006/NR	65.93	51.25	NR	Any/Severe	4/7	In-hospital mortality/aOR
Bonds et al. (22)	ACCORD study/The United States	*Post-hoc* analyses	T2DM	10194	NR/3.5 years	NR	NR	NR	Symptomatic severe	2	All-cause mortalit/aHR
Zoungas et al. (51)	ADVANCE study /Multicenter from 20 countries	*Post-hoc* analyses	T2DM	11,140	2001-2003/3 months, 6 months,5.0 years	NR	NR	NR	Severe/Minor	2/10	All-cause mortality, CVD mortality/aHR
Li et al. (52)	The Department of Cardiology, Xuanwu Hospital, Capital Medical University/ China	Cohort	Total with AMI	246	1995-2005/NR	NR	NR	NR	Any	8	In-hospital mortality/RR (calc)
Nirantharakumar et al. (53)	University Hospital Birmingham/The United Kindom	Cohort	Total	6,374	2007–2010/NR	66.30	42.48	NR	Mild~moderate/ Severe	4/7	Inpatient mortality/RR (calc)
MCCOY et al. (54)	Mayo diabetes clinic/The United States	Case-control	Total	1,013	2005-2006/5.0 years	60.5	45.2	1.36	Mild/Severe	9/1	All-cause mortality/aOR
ZHAO et al. (55)	VISN 16/The United States	Cohort	T2DM	1,522	2004-2010/ 3.93 years	62.58	3.9	NR	Any	3	All-cause mortality /aHR
ORIGIN Trial Investigators et al. (13)	ORIGIN Trial/Multicenter from 40 countries	*Post-hoc* analyses	Total	12,537	2003-2005/6.2 years	63.53	34.98	NR	Severe	7	All-cause mortality, CVD mortality/aHR
Tan et al. (56)	Derriford Hospital medical assessment unit/The United Kindom	Cohort	Total	1,457	2010 -2011/ NR	71.0	NR	NR	Any	4	In-hospital mortality/RR (calc)

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TABLE 1 Continued

Study	Data source /Country; Region	Study design	Population	Sample Size	Baseline years/ Duration of follow-up	Mean age (years)	Female (% Total)	Mean DM duration (years)	Severity of hypoglycemia	Hypoglycaemia definition[a]	Outcomes assessment:/ Effect Estimate
HSU et al. (14)	The NHIRD/Taiwan	Cohort	T2DM	9,220	1998–2009/ 11.0 years	63.30	56.29	4.96	Mild/Severe	The outpatient/ hospital claims dataset	All-cause mortality/HR
Sechterberger et al. (57)	a 24-bed mixed surgical/medical ICU in a teaching hospital/Netherlands	Cohort	Total	1,638	2004-2011/NR	68.0	37.0	NR	Any	7	ICU mortality/ aOR
Cooper et al. (58)	the Western Australia Children's Diabetes Database/Australia	Cohort	T1DM	1,309	1987-2012/7.6 years	25.6	49.60	NR	Severe	1	All-cause mortality/aHR
Lee et al. (59)	The KAMIR and the KorMI/Korea	Cohort	T2DM with AMI	20,714	2005–2008, 2008–2012/30 days	64.09	37.25	NR	Any	4	30-day mortality/ aHR
Kong et al. (60)	Hong Kong Diabetes Registry/Hong Kong, China	Cohort	T2DM with or without CKD	8,767	1995- 2007/ 6.66 years	58.46	53.02	5.49	Severe	7	All-cause mortality /aHR
Lung et al. (61)	The Swedish National Diabetes Register/ Sweden	Cohort	T1DM with a major cardiovascular complication	1,839	2002–2010/28 days	59.46	45.0	44.25	Any	3	All-cause mortality/HR, OR
Elwen et al. (62)	an emergency services call-out for hypoglycemia/The United Kindom	Cohort	Total	1,156	2005-2013/12 months	61.0	40.0	NR	Any	4	All-cause mortality /OR
Khunti et al. (63)	The CPRD database/The United Kindom; the HES data/England	Cohort	T1DM/T2DM with or without History of CVD	13,682	2001-2007/5 years	62.28	44.0	4.85	Severe or nonsevere /Severe	7	All-cause mortality /aHR
Gómez-Huelgas et al. (64)	The BMDS registry/Spain	Cohort	Total	309,008	1997-2010/NR	72.0	49.1	NR	Any	3	In-hospital mortality /OR
Escalada et al. (65)	Medicare Advantage claims database/The United States	Cohort	T2DM	31,035	2007-2012/2.9 years	72.0	53.0	NR	Any	1	All-cause mortality, CVD mortality /HR
Freemantle et al. (66)	The CREDIT study/Multicenter from 12 countries(10 in Europe, one in Canada and one in Japan)	Longitudinal	T2DM	2,999	NR/54 months	61.0	48.8	9.0	Any/Severe	4/1	All-cause mortality /aHR
Rauh et al. (67)	the Hoorn Diabetes Care System Cohort/ Netherlands	Cohort	T2DM	1,667	2000-2002/1.9 years	67.2	47.0	11.5	Mild/Severe	11/1	All-cause mortality /aOR

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TABLE 1 Continued

Study	Data source /Country; Region	Study design	Population	Sample Size	Baseline years/ Duration of follow-up	Mean age (years)	Female (% Total)	Mean DM duration (years)	Severity of hypoglycemia	Hypoglycaemia definition[a]	Outcomes assessment:/ Effect Estimate
Takeishi et al. (68)	Ichinomiyanishi Hospital/Japan	Cohort	Total with various infections	620	2009- 2014/5 years	79.0	39.19	NR	Any	4	In-hospital mortality /aOR
Cha et al. (69)	VDR/Korea	Cohort	T2DM	906	2000 -2010/ 10.4 years	55.76	59.27	8.17	Severe	1	All-cause mortality, CVD mortality/aHR
Sejling et al. (70)	Nordsjællands Hospital Hillerød/ Denmark; The Radboud University Medical Centre/ Netherlands	Cohort	T1DM	751	Denmark: 1999–2001/12 years; Netherlands: 2006–2008/6.5 years	45.61	49.04	23.85	Severe	1	All-cause mortality, CVD mortality/HR
Lu et al. (71)	Taiwan's NHI research database/Taiwan	nested case-control	T1DM	10,314	1997- 2011/<1 year, 3-5 years	41.42	42.18	NR	Severe	1	All-cause mortality /aOR
Chevalier et al. (72)	IMS HDD/Belgium	Cohort	Total	30,710	2011-2014/NR	NR	NR	NR	Any	3	In-hospital mortality /aOR
CHI et al. (73)	Taiwan's NHI research database/Taiwan	Cohort	Total	10,623	2001-2011/7.0 years	74.1	49.2	NR	Any	3	All-cause mortality /aRR
Zhao et al. (74)	the China PEACE–Retrospective AMI study/China	Cross-sectional	Total with AMI	2,280	2001, 2006, and 2011/NR	NR	NR	NR	Any	4	In-hospital mortality /aOR
Standl et al. (75)	TECOS/Multicenter from 38 countries	*Post-hoc* analyses	T2DM	14,671	2008-2012/3.0 years	65.04	29.29	10.08	Severe	1	All-cause mortality, CVD mortality/aHR
Zinman et al. (76)	LEADER cardiovascular (CV) outcomes trial /Multicenter from 32 countries	*Post-hoc* analyses	T2DM with high risk for CV disease	9,340	2010-2012/3.8 years	64.26	36.37	12.79	Any	6	All-cause mortality, CVD mortality/aHR
Pieber et al. (77)	DEVOTE 3/Multicenter from 20 countries	*Post-hoc* analyses	T2DM with high risk of cardiovascular events	7,637	2013-2014/≤1 year	64.97	37.44	16.43	Severe	1	All-cause mortality/HR
Leung et al. (78)	MacKay Memorial Hospital/Taiwan	Cohort	Total with carbapenem-resistant Acinetobacter baumannii complex bacteremia	146	2010-2015/5 years	69.23	41.1	NR	Any	4	All-cause mortality /RR(calc)

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TABLE 1 Continued

Study	Data source /Country; Region	Study design	Population	Sample Size	Baseline years/ Duration of follow-up	Mean age (years)	Female (% Total)	Mean DM duration (years)	Severity of hypoglycemia	Hypoglycaemia definition[a]	Outcomes assessment:/ Effect Estimate
Lo et al. (79)	NHIRD/Taiwan	Cohort	T1DM/T2DM	30,471	1999-2001/≤1 year; ≥1 year	64.63	48.80	NR	Severe	3	All-cause mortality /aHR
Wei et al. (80)	The Central Hospital of Wuhan/China	Cohort	T2DM	1,520	2013-2017/31 months	59.43	48.49	6.79	Any	4	All-cause mortality, CVD mortality/aHR
Yun et al. (81)	The Korean NHI System database/Korea	Cohort	T2DM	1,568,097	2007-2009/ ≤12months; >24-months	58.60	45.30	NR	Severe	3	All-cause mortality/aHR
Davis et al. (82)	VADT/The United States	Post -hoc analysis	T2DM	1,791	2000-2000/3 months	60.40	2.90	11.5	Severe	1	All-cause mortality, CVD mortality/aHR
Wernly et al. (83)	The Jena University Hospital/German	Cohort	T2DM	685	2004 -2009/4-7 years	70.11	NR	NR	Any	4	Intra-ICU mortality, Long-term mortality/ OR, HR
Ferreira et al. (84)	Centro Hospitalar do Porto/Portugal	Case-control	Total with community-acquired pneumonia or chronic obstructive pulmonary disease	242	2016-2016/NR	77.0	42.1	NR	Any	4	In-hospital mortality/RR (calc)
Mattishent et al. (85)	The CPRD database/The United Kindom	Cohort	Total with or without dementia	19,993	1997-2016/1 year, 12-60months	78.88	53.0	NR	Any	7	All-cause mortality /aHR
Standl et al. (86)	EXSCEL/Multicenter from 35 countries	Post -hoc analysis	T2DM	14,752	2010-2015/3.2 years	62.03	37.98	12.06	Severe	1	All-cause mortality, CVD mortality /aHR
Jensen et al. (87)	The Danish National Patient Register or the National Pharmacological Database/ Denmark	Cohort	T1DM/T2DM	27,746	1996 - 2017/ T1DM:8.9-year; T2DM:2.3-year	57.49	41.66	11.81	Severe	3	All-cause mortality/aHR
Zaccardi et al. (88)	The CPRD database/The United Kindom	Cohort	T2DM	74,610	1998- 2011/7.1 years	67.73	45.22	NR	Severe	1	All-cause mortality, CVD mortality/RR (calc)

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TABLE 1 Continued

Study	Data source /Country; Region	Study design	Population	Sample Size	Baseline years/ Duration of follow-up	Mean age (years)	Female (% Total)	Mean DM duration (years)	Severity of hypoglycemia	Hypoglycaemia definition[a]	Outcomes assessment:/ Effect Estimate
Han et al. (89)	The Korean NHIS/Korea	Cohort	T2DM with or without dementia	2,032,689	2009-2015/6.9 years	59.85	42.33	6.89	Severe	1	All-cause mortality /aHR
Cha et al. (90)	St. Vincent's Hospital/Korea	Cohort	T2DM with heart failure	397	2016-2018/25 months	73.08	52.64	12.00	Any	4	All-cause mortality, CVD mortality /RR(calc)
Heller et al. (91)	LEADER study/Multicenter from 32 countries	*Post-hoc* analyses	T2DM	9,340	2010-2012/3.8 years	64.30	35.70	12.80	Any/Severe	6	All-cause mortality, CVD mortality /RR(calc)

DM, diabetes mellitus; T1DM, Type 1 diabetes; T2DM, Type 2 diabetes; CVD, Cardiovascular disease; QTc, corrected QT interval; VPB, Ventricular Premature Beats; AF, Atrial fibrillation; AMI, Acute myocardial infarction; NHI, National Health Insurance; NHIS, National Health Insurance Service; ARIC, Atherosclerosis Risk in Communities; UEBMI, Urban Employee Basic Medical Insurance; ACCORD, Action to Control Cardiovascular Risk in Diabetes; DIGAMI 2,The second Diabetes Glucose and Myocardial Infarction; HIPAA, Health Insurance Portability and Accountability Act; ADVANCE, Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation; ORIGIN, Outcomes Reduction with an Initial Glargine Intervention; NHIRD, The National Health Insurance Research Database released; KAMIR, The Korea Acute Myocardial Infarction Registry; KorMI, The Korea Working Group on Myocardial Infarction; CPRD, The Clinical Practice Research Datalink; HES, Hospital Episode Statistics; BMDS, The Basic Minimum Data Set; VDR, The Vincent Type 2 Diabetes Registry; HDD, Hospital Disease Database; LEADER, Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results; DEVOTE, Trial Comparing Cardiovascular Safety of Insulin Degludec vs Insulin Glargine in Patients with Type 2 Diabetes at High Risk of Cardiovascular Even; NHIRD, The National Health Insurance Research Database; CPRD, The Clinical Practice Research Datalink; VADT, The Veterans Affairs Diabetes Trial; VISN 16, Veterans Integrated Service Network 16; CREDIT, The Cardiovascular Risk Evaluation in people with type 2 Diabetes on Insulin Therapy; PEACE–Retrospective AMI, Patient–centered Evaluative Assessment of Cardiac Events–Retrospective acute myocardial infarction study; TECOS, Trial Evaluating Cardiovascular Outcomes With Sitagliptin; EXCEL, Exenatide Study of Cardiovascular Event Lowering; IG, interstitial glucose; HR, Hazard ratio; OR, odds ratio; IRR, incident rate ratio; aHR, Adjusted hazard ratio; aOR, Adjusted odds ratio; aRR, Adjusted risk ratio; NR, not reported; NA, not appliable.

a[1] denoted as an attack serious enough to require the help of another person or an episode resulting in hospital admission or loss of consciousness; a[2] denoted as either a blood glucose concentration <2.7~2.8mmol/l (<50 mg/dl) or symptoms that resolved with treatment and that required either the assistance of another person or medical assistance; a[3] denoted as definition with ICD-9 codes/ICD-10 codes/Read codes; a[4] denoted as a blood glucose concentration ≤ 3.9~4.0 mmol per liter (70~72 mg per deciliter); a[5] denoted as a blood glucose concentration ≤ 3.5 mmol per liter (63 mg per deciliter); a[6] denoted as a blood glucose concentration ≤ 3.0 mmol per liter (54 mg per deciliter); a[7] denoted as a blood glucose concentration < 2.0~2.8 mmol per liter (36~50 mg per deciliter) or the presence of typical symptoms and signs of hypoglycemia without other apparent cause; a[8] denoted as a blood glucose concentration ≤ 5 mmol per liter (70 mg per deciliter); a[9] denoted as symptoms of dizziness, blurry vision, confusion, and/or sweating that the patient was able to terminate without assistance; a[10] denoted as either a blood glucose concentration < 2.0~2.8 mmol per liter (36~50 mg per deciliter) or symptoms that resolved with themselves; a[11] denoted as either a blood glucose concentration ≤ 3.0 mmol per liter (54 mg per deciliter) or the presence of typical symptoms.

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longitudinal study (66) and one study (46) including both cohort and nested case-control studies). The sample sizes ranged from 25 to 2,032,689 patients, and the mean age of patients ranged between 25.6 and 81.86 years. The female proportion ranged from 2.90% to 59.27%, and duration of DM was from 1.36 to 44.25 years.

Apart from 10 studies on multinational origins, other origins were mentioned developed countries or regions in 46 articles, and developing countries in 4 articles. More than 50% studies (34/60) reported severe hypoglycaemia. Sixteen articles reported IWD with comorbidities or at high risk of CVD. Definitions of hypoglycaemia varied widely and were mostly based on blood glucose concentration or the presence of symptoms necessitating the assistance of another individual or medical help. Ten, 46, and 15 articles presented data on arrhythmia risk only, all-cause mortality only, and CVD death only, respectively, and four studies reported data on both arrhythmia and all-cause mortality risk.

Quality evaluation

For cardiac arrhythmia analysis, quality analysis showed about 7 of 11 cohort studies and one case-control study were of high methodological quality, with ≥7 NOS scores (mean= 7.58; Table S1). One p-h/sa of RCT showed high quality according to NOS scores. Two cross-sectional studies were of moderate quality according to AHRQ (Table S1). When analysing the risk of all-cause mortality in 46 studies, 24 cohort studies and 2 case-control studies were of high methodological quality, with ≥7 NOS scores (mean= 7.73; Table S1). According to AHRQ, one cross-sectional study and one longitudinal research were of moderate quality. All 10 p-h/sas of RCTs were of high quality (mean NOS score= 8.50; Table S1).

Risk of cardiac arrhythmia

The analysis of the connection between hypoglycemia and the risk of arrhythmia involved fourteen relevant studies. Overall, the pooled RR showed a 42% greater incidence of cardiac arrhythmia in IWD with versus without hypoglycemia (RR,1.42; 95%CI,1.21-1.68; p<0.001), but there was evident heterogeneity between studies (I^2 = 71.7%, p<0.001) (Figure 2). Sensitivity analysis revealed that heterogeneity did not vanish after single studies were removed. In the arrhythmia risk analysis, Funnel plots revealed no evidence of systematic bias (Begg's test, P=0.443; Egger's test, P=0.245) (Figure S1).

The pooled RRs were essentially uniform regardless of the sample size (<1000 patients, P=0.001; ≥1000 patients, P=0.001), severity of hypoglycaemia (severe hypoglycaemia P=0.001; total hypoglycaemia, p=0.014), noctural hypoglycaemia (P=0.012), type of arrhythmia (incidence of QTc interval prolongation, P=0.004; other arrhythmia, P=0.007), T2DM (P =0.001), or total diabetes (P<0.001) (Figure 3).

Risk of all-cause mortality

Fifty studies reported a link between hypoglycemia and overall death in IWD. In a random-effects model, the pooled RR was 1.68 (95%CI, 1.49 to 1.90; P<0.001) with severe heterogeneity (I^2 = 97.1%; P<0.001; Figure 4).

A sensitivity analysis revealed that heterogeneity did not vanish after removing single studies. Neither Egger's test (P =

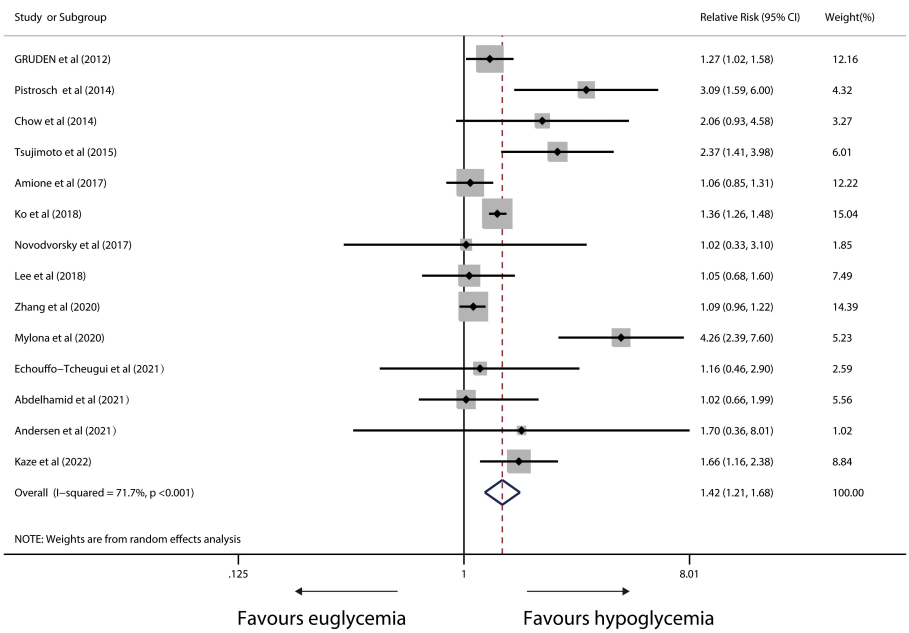

FIGURE 2
Forest plot for the association between hypoglycaemia with risk of cardiac arrhythmia in diabetic patients (X-axis: log scale; solid square: relative risk; horizontal lines: 95% CIs. The same in other figures).

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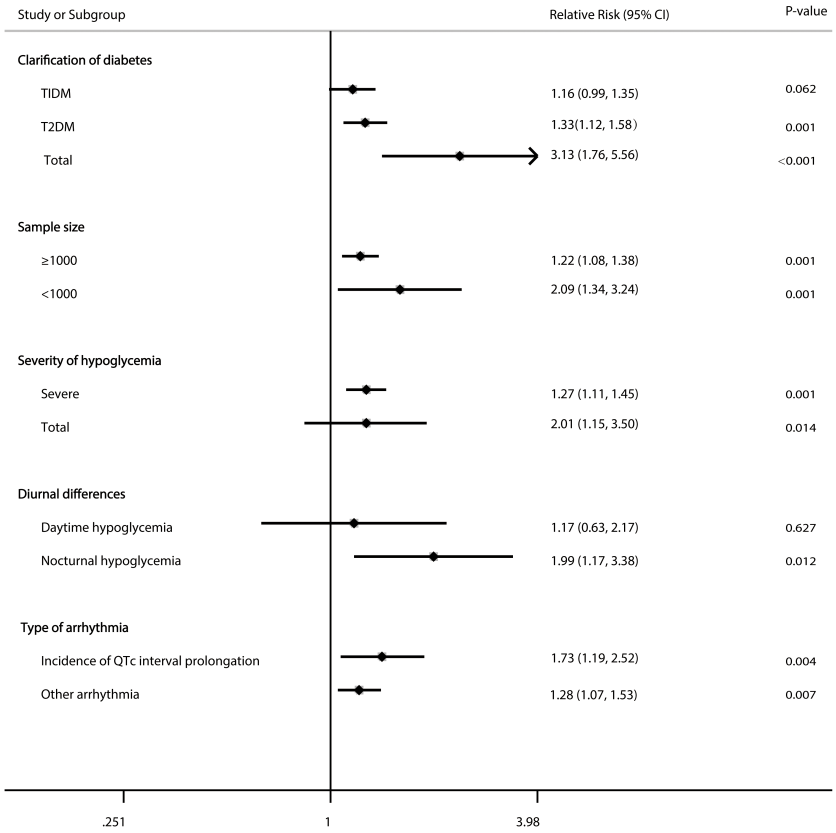

FIGURE 3
Forest plot for the association between hypoglycaemia with risk of cardiac arrhythmia in diabetic patients according to some clinically important variables.

0.514) nor visual inspection revealed significant publication bias (Figure S2).

Estimated sample size, follow-up duration, country/region, study population, clarity of diabetes, study design, severity of hypoglycaemia, hypoglycaemia episode and methodologies for effect estimate extraction yielded similar results (Figure 5).

Risk of CVD death

A random-effects model exhibiting substantial heterogeneity (I^2= 80.8%; P<0.001) from 15 relevant studies found that hypoglycemic versus euglycaemic IWD had a 59% significantly higher risk (pooled RR, 1.59; 95%CI, 1.24-2.04; P<0.001) in susceptibility to CVD-caused death. Figure 6 depicts meta-analysis forest plots. A sensitivity analysis revealed that heterogeneity did not vanish after single studies were removed. Tests by Begg's and Egger's tests showed no clear systematic bias in the CVD-induced death risk analyses (both P > 0.1; Figure S3).

Discussion

All current studies with 5,960,224 individuals were reviewed to find out evidence of a link between hypoglycemia and negative consequences, including cardiac arrhythmia, overall death, and CVD-induced death. We did not aim to establish a direct relationship, but did highlight the importance of hypoglycemia in relation to CV events and death. It is generally recognised that hypoglycemia, particularly severe hypoglycemia (SH), carries a greater proarrhythmic risk than euglycemia or hyperglycemia, as also observed in this study. The proarrhythmic mechanisms of hypoglycaemia, especially SH, may be various (92). Low glucose directly affects the human Ether-à-go-go Related Gene ion channel (93). Hypokalemia, and catecholamine both delay cardiac repolarisation, increasing the risk of early afterdepolarisations and ventricular arrhythmias. QT prolongation throughout the day may further trigger early afterdepolarisations. In the same way, activation of the sympathetic nervous system and an increase in calcium in the cytosol can lead to delayed afterdepolarisation and early heartbeats, which contribute to more ventricular ectopic activity during the day. Nevertheless, cardiac arrhythmias induced by hypoglycemia exhibit diurnal changes, which were also discovered in our study by subgroup analyses. Arrhythmia rates are even higher at night or early morning, when sympathoadrenal responses are suppressed (94). A sluggish sinus rate at a state of vagal dominance may disclose latent pacemakers (especially under situations of heightened automaticity), which causes excessive atrial and ventricular ectopic activity during nocturnal hypoglycaemia (45).

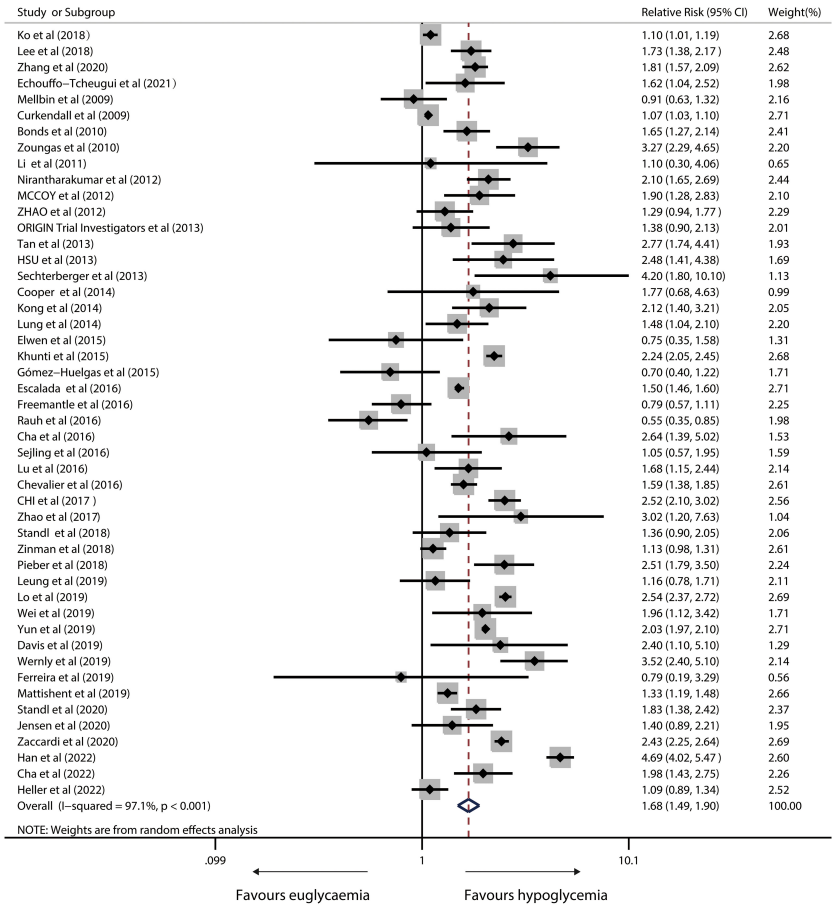

FIGURE 4
Forest plot for the association between hypoglycaemia with risk of all-cause mortality in diabetic patients.

Even though the overall pooled RR suggests a 42% substantially higher likelihood of cardiac arrhythmia in hypoglycemic patients over euglycaemia patients, around 57% (8/14) recruited studies report no significant link between hypoglycemia and cardiac arrhythmia. There can be several reasons for this null result. First, the antagonistic autonomic nerve responses are a fundamental mechanism underlying the harmful effect of SH, but can be diminished in patients with long-standing diabetes, long follow-up, or established cardiovascular risks (95–97). Considering the involved patients here have relatively longer follow-up time, the effect of hypoglycemia is most likely obscured by the highly frequent cardiovascular and cerebrovascular comorbidities at baseline. Amione et al. (24) investigated the possible impact of repeated bouts of SH as a marker of QTc prolonged QTc interval over a seven-year period, and concluded that the impact of prediction could be due to adaptive mechanisms (98, 99). Second, earlier research revealed a dose-response or linear relationship between hypoglycemia and various unfavorable outcomes (71, 100), though only two studies (24, 41) were included. Third, individuals with repeated hypoglycemic episodes typically have a diminished awareness of hypoglycemia (101), which explains why there is no apparent temporal link between hypoglycemia and arrhythmia.

As for the mortality analysis, we did not aim to prove causality, but did highlight the associations between hypoglycemia and death. Due to pre-existing comorbidities, CVD-induced death usually accounted for the majority of deaths in the diabetes group. The relationship between hypoglycaemia and cardiovascular events is bidirectional in accordance with previous studies (75, 86). Firstly, we discovered a link between hypoglycemia and all-cause mortality in IWD, with a higher all-cause mortality in individuals with hypoglycemia compared to those without. The underlying biological mechanisms directly linking hypoglycaemia to death have yet to be elucidated. Nevertheless, some established scientific hypotheses suggest the fundamental mechanisms are primarily sympathoadrenal activation, cardiac or cerebral ischemia, abnormal cardiac repolarisation, QT prolongation, or fatal arrhythmia or autonomic impairment, increased thrombogenesis, inflammation, endothelial dysfunction, and vasoconstriction caused by aberrant endocrine or nervous reactions during recognised or unrecognised hypoglycaemic episodes (11, 102–113). All of these alterations can affect heart anatomy, cardiac stress, vascular hemodynamics, and function (13, 114–118). Episodes of asymptomatic or unreported symptomatic hypoglycaemia probably occur after the initial occurrence of SH, increasing the likelihood of recurrent incidents. Although these effects are true,

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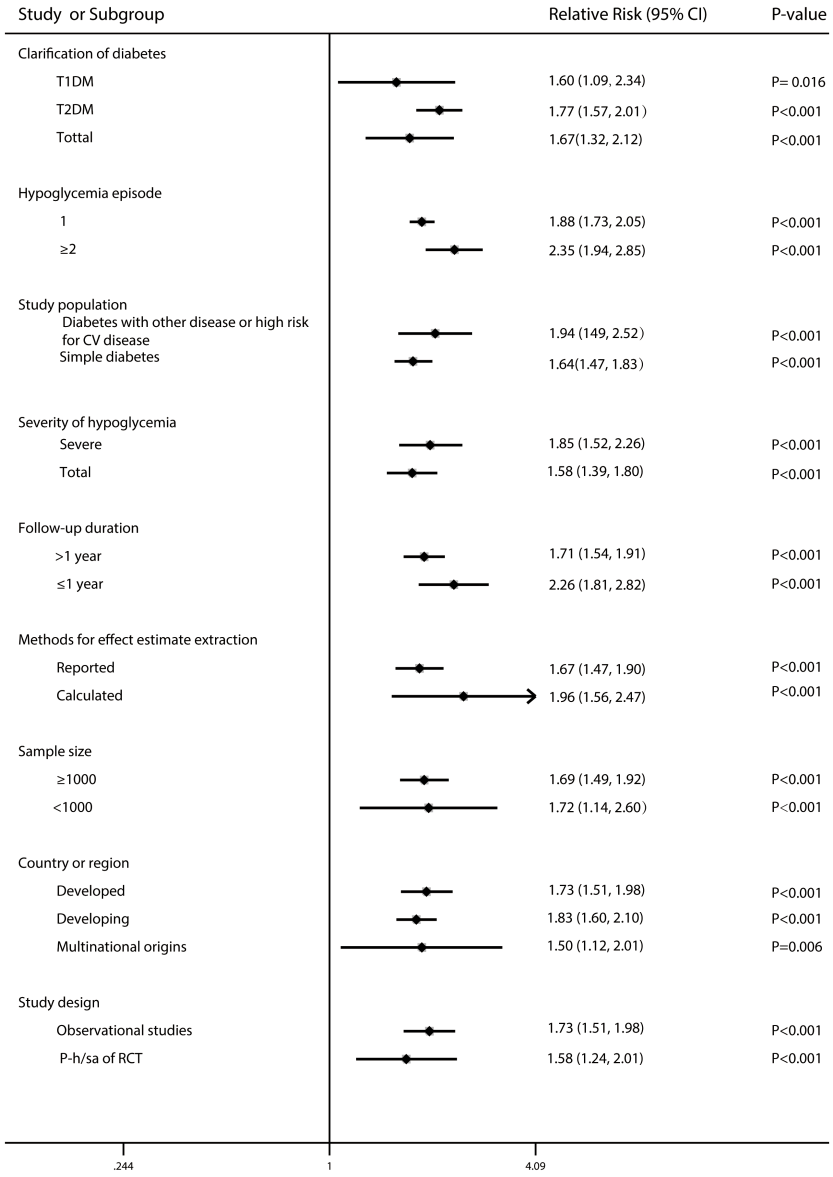

FIGURE 5
Forest plot for the association between hypoglycaemia with risk of all-cause mortality in diabetic patients according to some clinically important variables.

they are more likely to explain a temporary increase in CV risk at the acute phase of hypoglycaemia rather than the long-term connection observed in another study (63).

Secondly, hypoglycaemia or SH is a plausible indicator instead of a direct cause of an elevated risk of unfavorable clinical outcomes, which reflects the influence of concomitant illnesses in addition to unmeasured or incompletely defined confounding variables (119) that independently increase the risk of mortality as opposed to being a risk factor causing these events. Other studies suggest that SH is only associated with cardiovascular events in individuals who already have a high cardiovascular risk (120, 121). Nonetheless, our subgroup analyses reveal a quantitative insignificant difference in the RR of all-cause mortality for those with low versus high cardiovascular risk (RR 1.62 vs. 1.94, respectively). This result suggests the effect may be more pronounced in in those with a greater cardiovascular risk.

The mechanisms that relate SH to non-cardiovascular fatalities remain unknown (22, 54). SH may be a signal for greater provider monitoring in clinical settings, or a sign of quickly deteriorating health. SH may be appropriate for clinicians to review the mental and physical states of a patient to see whether any therapy adjustment is required. Such actions may minimize the risks of future hypoglycaemia and CVDs.

Subgroup analyses were performed so as to clarify whether the incidence of cardiac arrhythmia or all-cause death differed among relevant characteristics. In the trials with T2DM patients, preliminary subgroup analysis revealed a substantial increase in overall death risk among hypoglycemic versus euglycaemic individuals. Because T1DM patients are frequently younger than T2DM patients, they have less risk factors for death. Despite this, SH is more likely to attack T1DM

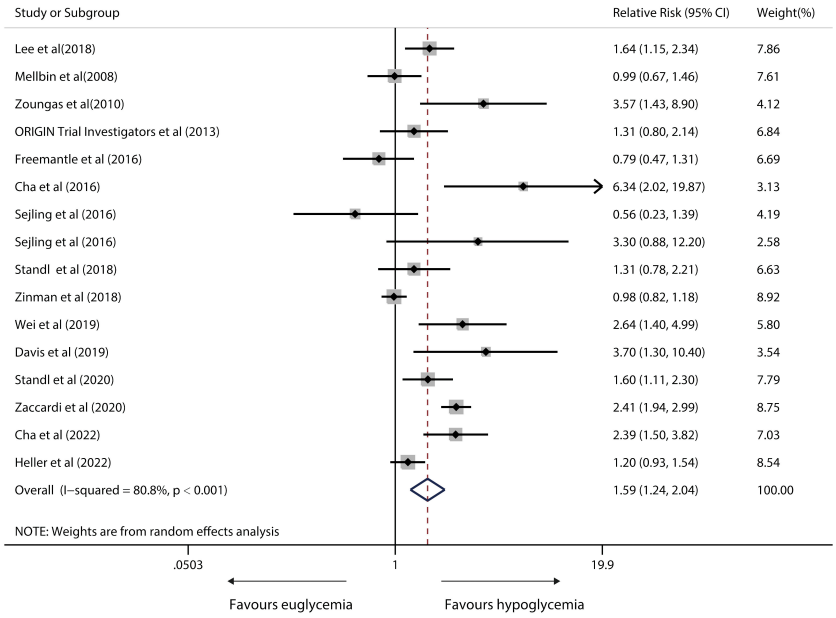

FIGURE 6
Forest plot for the association between hypoglycaemia with risk of CVD death in diabetic patients according to some clinically important variables.

patients, raising the risk of arrhythmia. SH is more likely to assault T1DM patients, increasing the risk of arrhythmia. This is not the case, and the underlying reason is unknown (87). In terms of hypoglycemia episodes and severity, more episodes and severity lead to higher risks of arrhythmia and all-cause mortality rate, and may indicate quickly failing health. Shorter follow-up period is associated with a greater increase in all-cause mortality in hypoglycemic patients compared euglycaemic patients. It might be that the population with the shortest follow-up duration has the most co-morbidities or frailty, in whom severe hypoglycemia episodes may be a surrogate for the underlying severity of the overall advanced and complex illness condition. Subgroup analysis based on study design methodologies for extracting effect estimates calls for more well-designed research in the future.

No review or meta-analysis can avoid heterogeneity of OS due to the lack of standardisation of method for clarifying cases, study design and time, classification of endpoints, and the amount of inter-study confounding that has been adjusted. For example, definitions of hypoglycaemia differ greatly. In spite of the sensitivity testing, we were unable to account for the greatest between-study heterogeneity in the outcome of cardiac arrhythmia and death. Therefore, these results need confirmation from more research. Furthermore, the statistical methodologies used in p-h/sa of RCTs and OSs did not adequately address the influence of unmeasured confounding components regarding overall effect estimation. The origins of variability were attributed to differences in study populations and exposure, according to sensitivity analyses.

Some of the significant strengths of this meta-analysis include the detailed retrieval plan with Cochrane procedures, the broad search approach, the relatively large sample size, and the capacity to investigate the link between hypoglycemia and cardiac arrhythmia or death. Nevertheless, this study has some limitations. First, the avoidance of unreported reports can have influenced our findings. Second, there is also a wide range of exposure, including severe hypoglycemia and any hypoglycemia. Third, the analyses employed for arrhythmia source research have drawbacks as well. Some studies have presented incident rate ratios for arrhythmias, implying that an increased risk applies to the whole research group whereas, in fact, only a few individuals who are extremely vulnerable may be impacted (45). Fourth, because we did not test autonomic function, we were unable to investigate its potential contributions.

In summary, hypoglycemia, as opposed to euglycaemia, is associated with a higher risk of cardiac arrhythmia and mortality. Nevertheless, evidence of potential causal linkages beyond statistical associations must await proof by additional specifically well planned research that controls for all potential remaining confounding factors, including a unified definition of hypoglycemia (122) as recommended by the International Hypoglycaemia Study Group.

Data availability statement

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

Author contributions

XW and GL conceived the conception and design of the study, the search of the relevant literature, the extraction and analysis of data, and the drafting and revision of the final manuscript. SZ and FZ interpreted the analysed data, reviewed the manuscript critically, and contributed to its drafting. The final manuscript was read and approved by all authors.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1222409/full#supplementary-material

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Assessment of common risk factors of diabetes and chronic kidney disease: a Mendelian randomization study

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Background: The increasing prevalence of diabetes and its significant impact on mortality and morbidity rates worldwide has led to a growing interest in understanding its common risk factors, particularly in relation to chronic kidney disease (CKD). This research article aims to investigate the shared risk factors between type 1 diabetes (T1D), type 2 diabetes (T2D), and CKD using a Mendelian randomization (MR) design.

Methods: The study utilized genome-wide association study (GWAS) datasets for T1D, T2D, and CKD from the FinnGen research project. GWAS summary statistics datasets for 118 exposure traits were obtained from the IEU OpenGWAS database. MR analyses were conducted to examine the causal relationships between exposure traits and each of the three outcomes. Multiple methods, including inverse-variance weighted, weighted median, and MR-Egger, were employed for the MR studies.

Results: Phenome-wide MR analyses revealed that eosinophil percentage exhibited a significant and suggestive causal association with T1D and CKD, respectively, suggesting its potential as a shared risk factor for T1D and CKD. For T2D, 34 traits demonstrated significant associations. Among these 34 traits, 14 were also significantly associated with CKD, indicating the presence of common risk factors between T2D and CKD, primarily related to obesity, height, blood lipids and sex hormone binding globulin, blood pressure, and walking pace.

Conclusion: This research has uncovered the eosinophil percentage as a potential common risk factor for both T1D and CKD, while also identifying several traits, such as obesity and blood lipids, as shared risk factors for T2D and CKD. This study contributes to the understanding of the common risk factors between diabetes and CKD, emphasizing the need for targeted interventions to reduce the risk of these diseases.

KEYWORDS

type 1 diabetes, type 2 diabetes, chronic kidney disease, risk factors, Mendelian randomization

Introduction

The prevalence of diabetes has been raised worldwide and is recognized as a leading cause of high morbidity rates (1). The global number of individuals with diabetes is projected to reach 642 million by 2040, up from 415 million in 2015 (2). Furthermore, diabetes imposes a significant economic burden due to management costs and escalating complications (3). Type 1 diabetes (T1D) is featured by the failure of insulin production due to the destruction of pancreatic β-cells caused by autoimmunity mediated by T-cells (4). In contrast, type 2 diabetes (T2D) is featured by insulin resistance and decreased insulin production (5). Chronic kidney disease (CKD) is a condition related to the progressive malfunction of kidney over time (6), which can occur due to physical injury or conditions such as high blood pressure (7). Patients with CKD face a higher risk of kidney failure compared to individuals with normal kidney function (8). Early detection and treatment can help prevent or delay many complications associated with CKD (9).

Diabetes mellitus (DM) is a major cause of CKD (10). Additionally, diabetes and CKD, share common risk factors like obesity and high blood pressure (11, 12). Extensive research has established the linkage between clinical and lifestyle risk factors and the enhanced risk of noncommunicable diseases like DM and CKD. However, there has been limited investigation into the overlapping risk factors for these main noncommunicable diseases. Mendelian randomization (MR) is an analytical approach where genetic variations serve as instrumental tools (IVs) to represent exposure variables. MR analyses can be used to determine causation and minimize bias resulting from reverse causality and confounding factors (13). In this study, MR analyses were employed to investigate the common risk factors for diabetes and CKD, with the aim of exploring potential strategies for effectively lowering the risks associated with these diseases through focused efforts.

Methods

All three genome-wide association study (GWAS) datasets for T1D, T2D, and CKD were obtained from the FinnGen research project to enable a better comparison of the common risk factors of these three diseases. For the exposures, this study utilized GWAS summary statistics datasets from the IEU OpenGWAS, which includes a compilation of comprehensive GWAS summary datasets that are accessible as open-source files for downloading or through querying the complete dataset repository. A total of 118 traits were included as exposure variables in the current study, and the selection procedure closely resembled that employed in a recent study by Walker et al. (14). The 118 exposure traits contain variables from various categories, such as anthropometric measurements, biochemistry markers and lifestyle behaviors. Supplementary Tables 1-3 contain the trait information. MR analyses were conducted to examine the causal relationships between exposure traits and each of the three outcomes (T1D, T2D, and CKD). The status of common risk factors of these three diseases was illustrated by a Venn diagram.

In the MR investigations, the selection of IVs for exposures took into account several considerations. Firstly, the selected genetic variations should exhibit a strong and robust association with the exposure, and the P-value threshold was set at $P < 5\times10^{-8}$. Secondly, a linkage disequilibrium threshold of $R^2 < 0.001$ was applied, along with clumping within a 10-Mb window. The traits with less than 5 IVs in the IV preparation step were excluded from the analyses. For the MR studies, three methods were employed: the inverse-variance weighted (IVW) method, weighted median (WM) method, and MR-Egger. The IVW method was used as primary method in MR analyses because IVW method has the highest effectiveness when all IVs are deemed valid. The MR-Egger intercept test was applied to assess potential horizontal pleiotropy which is a potential limitation of MR analyses because it contradicts the fundamental assumption of MR study. Multiple comparison correction was performed using a 5% false-discovery rate (FDR). The MR studies utilized modified code from a recent publication (14), employing the R package TwoSampleMR for the MR analyses.

Results

Phenome-wide MR analyses were conducted to identify potential risk factors for T1D, T2D, and CKD separately (Supplementary Tables 1-3). The GWAS summary data of 118 exposures from the IEU OpenGWAS database were incorporated to achieve this. For T1D as the outcome, 15 traits among the 118 exhibited suggestive evidence of association ($P < 0.05$) (Figure 1; Supplementary Table 1). Among these 15 traits, only one trait (eosinophil percentage) remained significant after correcting for multiple comparisons using a 5% FDR. Moreover, the associations of eosinophil percentage with T1D were consistent across three different MR methods (IVW, WM, and MR-Egger). For T2D as the

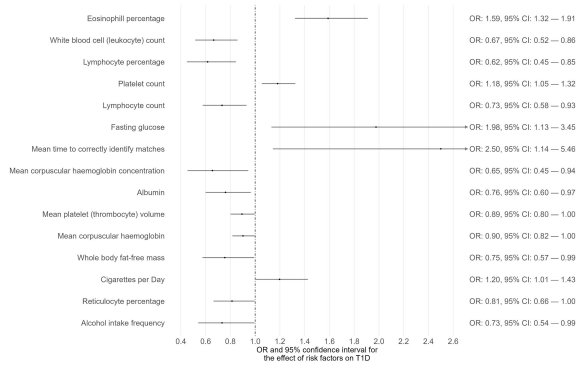

FIGURE 1
The causal associations between exposures and T1D with suggestive level of significance ($P < 0.05$).

Abbreviations: CKD, Chronic kidney disease; T1D, Type 1 diabetes; T2D, Type 2 diabetes; MR, Mendelian randomization; GWAS, Genome-wide association study; IVW, Inverse-variance weighted; WM, Weighted median; SHBG, Sex hormone binding globulin; FDR, False-discovery rate; SNP, Single-nucleotide polymorphism; FFA, Free fatty acid; HDL, High-density lipoprotein; eGFR, Estimated glomerular filtration rate.

outcome, 40 traits among the 118 showed suggestive evidence of association ($P < 0.05$) (Supplementary Table 2). Among these 40 traits, 34 traits (e.g., body mass index, waist circumference, glycated hemoglobin) remained significant after multiple comparison correction with a 5% FDR (Figure 2). For CKD as the outcome, 35 traits among the 118 exhibited suggestive evidence of association ($P < 0.05$) (Supplementary Table 3). Among these 35 traits, 19 traits (e.g., creatinine, body mass index, whole-body fat mass) remained significant after correcting for multiple comparisons using a 5% FDR (Figure 3).

To demonstrate the shared risk factors between diabetes and CKD, a Venn diagram was created to illustrate the common risk factors of T1D, T2D, and CKD that survived 5% FDR correction (Figure 4A) or showed suggestive significance (Figure 4B). After correcting for multiple comparisons using a 5% FDR, no common risk factor between T1D and CKD was identified, while 14 common risk factors between T2D and CKD were found, including body mass index, waist circumference, and glycated hemoglobin (Figure 4A). If the P-value threshold was relaxed to 0.05, eosinophil percentage and "cigarettes per day" were identified as common risk factors for both T1D and CKD, and 19 exposure traits were found to be common risk factors for both T2D and CKD (Figure 4B). However, no risk factor was common to all three disease outcomes (Figure 4B).

Discussion

In the present study, we conducted phenome-wide MR analyses incorporating 118 exposure traits. Our findings revealed that eosinophil percentage exhibited a significant causal association with T1D following correction for multiple comparisons. Additionally, eosinophil percentage showed suggestive evidence of association with CKD, suggesting its potential as a shared risk factor for T1D and CKD. In the case of T2D, 34 traits demonstrated significant associations after correcting for multiple comparisons. Among these 34 traits, 14 were also associated with CKD, indicating the presence of common risk factors between T2D and CKD, predominantly related to obesity, height, blood lipids and sex hormone binding globulin (SHBG), blood pressure, and walking pace.

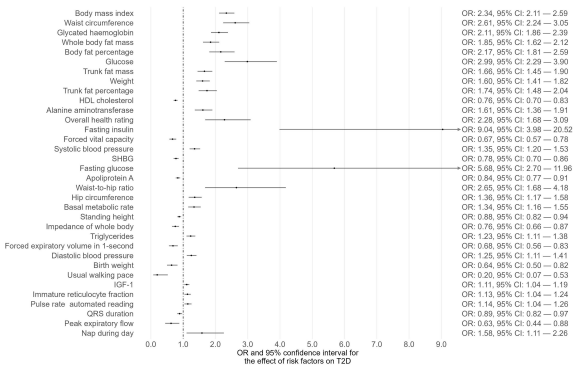

FIGURE 2
The causal associations between exposures and T2D that survived FDR correction.

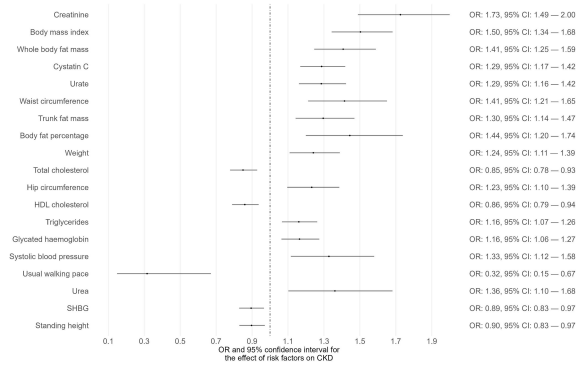

FIGURE 3
The causal associations between exposures and CKD that survived FDR correction.

High eosinophil levels as a shared risk factor for T1D and CKD

Eosinophils play an important role in the immune response (15). Traditionally, eosinophils have been associated with certain allergic diseases (15). In individuals with T1D, eosinophils are found in pancreatic tissue, and significant differences in eosinophil levels have been observed between T1D patients and healthy individuals (16). Notably, T1D patients exhibit higher percentages of immature eosinophils in circulation compared to healthy individuals (16). Transcriptionally active eosinophils in patients with diabetes indicates their involvement in the complex network of innate immune cells associated with the development of diabetes (17). Eosinophils from individuals with T1D have elevated levels of myeloid alpha-defensins and myeloperoxidase, which play a role in inflammatory and autoimmune diseases (17, 18). Moreover, a study using animal model of diabetes demonstrated a relationship between the expression of defensins in specific tissues and the occurrence of diabetes (17).

Increased levels of eosinophils have also been linked to a higher risk of CKD (19). Eosinophils release a range of inflammatory signaling molecules, which can contribute to chronic inflammatory infiltration in the kidneys (20, 21). Eosinophil presence in the kidneys can promote oxidative stress and contribute to the release of pro-fibrotic factors, causing renal fibrosis (15, 22). These findings support the notion that peripheral eosinophilia and the accumulation of eosinophil-derived cytokines stimulate fibroblast proliferation and contribute to tissue damage, particularly in the kidneys.

Obesity as a shared risk factor for T2D and CKD

Obesity, characterized by excessive accumulation of body fat that impairs physical and psychosocial health, is a well-known risk

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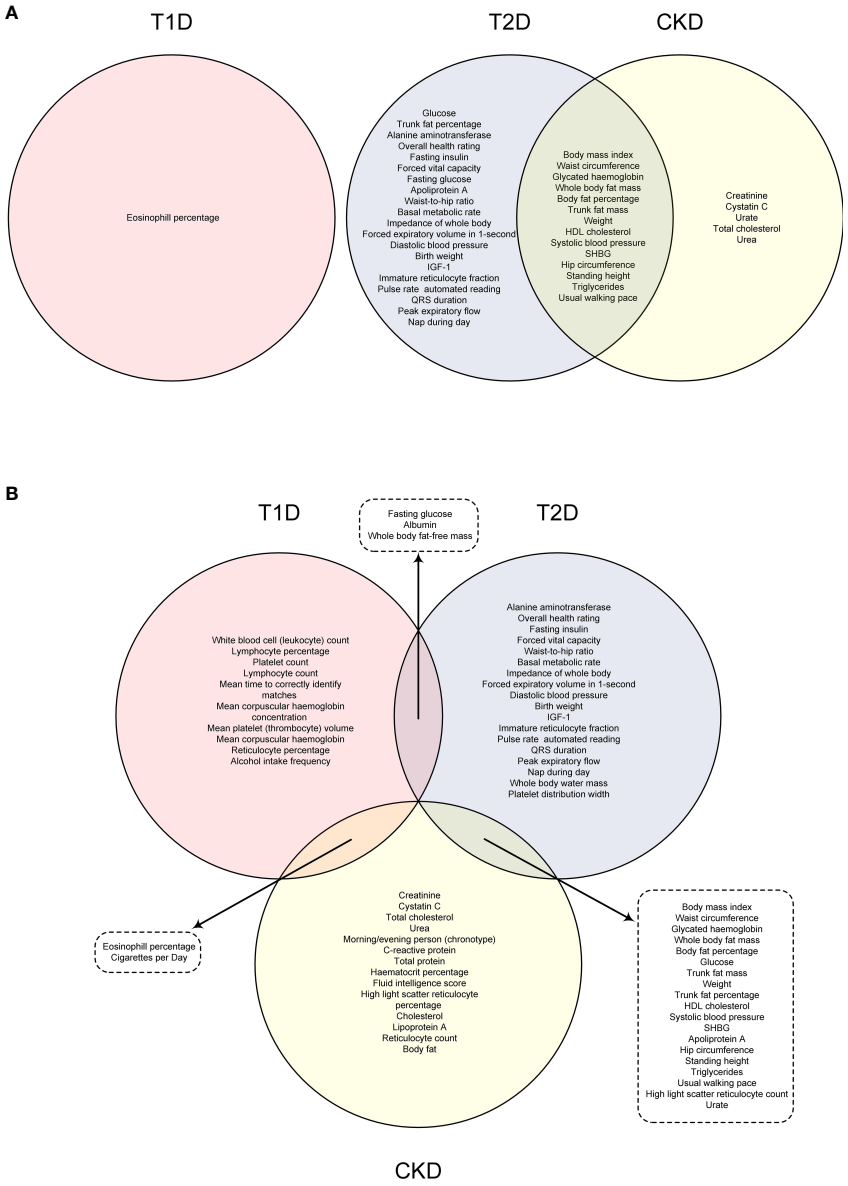

FIGURE 4
Venn graph indicating the common risk factors among T1D, T2D and CKD under FDR correction **(A)** or with suggestive level of significance ($P < 0.05$) **(B)**.

factor for various non-communicable diseases (23). T2D is highly associated with obesity, and the prevalence of diabetes which is related to obesity is projected to double by 2025 (24). Adipose tissues in obese individuals increase the release of free fatty acids (FFAs) through enhanced lipolysis, resulting in elevated levels of circulating FFAs. This process promotes muscle and hepatic insulin resistance and impairs insulin secretion by pancreatic β-cells (25, 26).

Obesity has also been identified as a major cause of CKD, independent of body mass index (27). A high BMI is a strong risk factor for the onset and progression of CKD and end-stage renal disease (ESRD) (28, 29). Obesity is associated with several risk factors that contribute to the increased incidence and prevalence of nephrolithiasis, such as abnormal urinary composition and renal hyperfiltration (28, 30). Additionally, obesity-related insulin resistance exacerbates the effects of angiotensin-II, leading to increased proteinuria and the production of inflammatory cytokines, all of which contribute to kidney damage (31). The increased enteral oxalate resulting from insulin resistance in obesity may also predispose individuals to nephrolithiasis (32).

Central obesity as a shared risk factor for T2D and CKD

Central obesity, featured by an accumulation of fat beyond the normal level in the abdominal region, is a specific type of obesity measured using indices like waist circumference. Individuals with central obesity have a significantly higher likelihood of developing diabetes (33). Excess body fat, especially visceral adipose tissue, is a

known risk factor for T2D (34). Central obesity has also been correlated with an increased risk of CKD, regardless of BMI (35). The adipose tissue in the central region secretes various adipokines, including leptin and adiponectin, which influence insulin sensitivity and the regulation of glucose levels. Leptin can promote insulin resistance and downregulate insulin signaling in various cell models (36). Visceral adipose tissue, which is characteristic of central obesity, produces more pro-inflammatory cytokines like interleukin-6 (37). Chronic low-grade inflammation associated with visceral fat accumulation contributes to the development of both T2D and CKD.

Dyslipidemia as a shared risk factor for T2D and CKD

Dyslipidemia refers to abnormal lipid profiles, and is closely associated with diabetes. Hyperglycemia causes apoptosis of β-cells in the pancreas, and affects the accumulation of oxidized LDLs. Dyslipidemia has a significant impact on the adverse outcomes of diabetes (38). High-density lipoprotein (HDL) exerts several anti-atherogenic effects, including anti-inflammatory, antioxidant, and anti-thrombotic properties (39). More than 75% of patients with T2D have mixed dyslipidemia, featured by low HDL cholesterol levels and high triglycerides levels (40). Low HDL-C levels have been related to impaired β-cell function in individuals with an altered state of fasting glucose levels or glucose tolerance (41). Decreased β-cell survival and secretory function may contribute to the increased risk of T2DM associated with low HDL levels (42).

In the development of CKD, reduced lecithin-cholesterol acyltransferase activity hinders the maturation of lipid-poor precursors of HDL (pre-β HDL) into spherical HDL particles (43). The degraded pre-β HDLs are cleared by the kidneys, leading to decreased apolipoprotein A-I level, which is a component of HDL (44). Thus, altered lipid metabolism and decreased HDL function contribute to the development of CKD.

Low levels of SHBG as a shared risk factor for T2D and CKD

Reduced levels of SHBG are often seen in insulin-resistant conditions and have been investigated as a predictor of the T2D risk in overweight populations (45). Variants of certain SHBG single-nucleotide polymorphisms (SNPs) are associated with altered SHBG levels and an increased risk of T2D (46). Clinical studies have linked low circulating levels of SHBG to impaired glucose control, suggesting a role for SHBG in maintaining glucose homeostasis (47).

SHBG also potentially reduces sex hormone bioactivity and plays a role in CKD (48). Men with lower SHBG levels have a higher risk of low estimated glomerular filtration rate (eGFR), an indicator of reduced kidney function (49). *In vitro* experiments have shown that SHBG suppresses inflammation, which could be relevant to the association between SHBG and CKD (50). Inflammation and insulin resistance may mediate the link between SHBG and CKD.

Short stature as a shared risk factor for T2D and CKD

Height plays a role in determining overall health, with associations observed between height and mortality as well as various diseases such as cancers and cardiovascular diseases (51). Numerous studies have indicated a significant link between shorter stature and an elevated risk of developing diabetes. The hazard ratio for developing diabetes gradually increases from the 5th quintile (reference) to the 1st quintile group based on height measurements (52). Furthermore, a comprehensive meta-analysis utilizing random-effects models indicated an inverse relationship between adult height and T2D (51).

There is an negative association of adult height with the incidence of newly diagnosed ESRD as well as all-cause mortality (53). Causal estimates based on eGFR and CKD indicate that taller individuals genetically predisposed to greater height have a lower log-eGFR and a higher risk of developing CKD (54). Short stature may serve as an indicator of insufficient fetal growth during childhood, potentially influencing the development of certain metabolic diseases in adulthood. Further work is necessary to study the biological mechanisms underlying the potential impact of height on the risk of T2D and CKD.

High blood pressure as a shared risk factor for T2D and CKD

Individuals with T2D have a higher prevalence of elevated blood pressure compared to the general population (55). Hypertension is a known risk factor for individuals with diabetes (56). While high blood pressure is related with an enhanced risk of developing T2D, its independent association with new-onset diabetes is less apparent (57). Resistant hypertension is usually seen in CKD, and increased blood pressure can raise glomerular capillary pressure and the filtration rate (58). Thus, high blood pressure contributes to the development of both T2D and CKD.

Slow self-reported walking pace as a shared risk factor for T2D and CKD

Walking is the most commonly chosen form of physical activity among older adults (59). Numerous studies have shown that indicators of physical capability, such as walking pace, are correlated with a range of health consequences (60). Gait speed has been identified as a strong indicator of the extent of functional changes in the olders (61). Combining walking pace with grip strength could be a practical method for identifying individuals at a higher risk of developing T2D (62).

Numerous studies have also provided evidence that the gait speed test is a reliable measure for assessing the risk of all-cause mortality in individuals with CKD (63). Additionally, impaired ability to engage in physical exercise has been revealed to be an indicator of survival among ambulatory patients with ESRD (64).

Participants who exhibited lower walking speed or were unable to walk demonstrated higher level of muscle mass deterioration, leading to a decreased function and heightened vulnerability to harmful conditions related to CKD (63).

Strengths and limitations

The current study benefits from the use of a MR design, which helps reduce biases stemming from residual confounding and reverse causality. To account for potential issues like horizontal pleiotropy and instrument strength, various methods of MR, including the WM method and MR-Egger, were utilized for sensitivity analyses. However, there are several limitations to consider in this study. Firstly, the inclusion of a relatively large number of traits increased the challenge of correcting for multiple comparisons. Secondly, horizontal pleiotropy, which is commonly observed in MR analyses, may have introduced bias into the findings of this study.

Conclusion

This research has uncovered the eosinophil percentage as a potential common risk factor for both T1D and CKD, while also identifying several traits, such as obesity and blood lipids, as shared risk factors for T2D and CKD. Focusing on precise risk reduction initiatives holds the potential to simultaneously impact both T2D and CKD. This strategy has the capability to bring about substantial advantages for the overall well-being of the public and enhance the quality of life for individuals who are affected by these diseases. Understanding the shared risk factors and their interplay is also crucial for the effective development of public health interventions.

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

SZ: Writing – original draft, Formal Analysis, Methodology, Software. YL: Writing – original draft, Conceptualization, Data curation. CS: Writing – original draft, Investigation, Project administration, Supervision, Writing – review & editing.

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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.2023.1265719/full#supplementary-material

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Serum/plasma biomarkers and the progression of cardiometabolic multimorbidity: a systematic review and meta-analysis

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Background: The role of certain biomarkers in the development of single cardiometabolic disease (CMD) has been intensively investigated. Less is known about the association of biomarkers with multiple CMDs (cardiometabolic multimorbidity, CMM), which is essential for the exploration of molecular targets for the prevention and treatment of CMM. We aimed to systematically synthesize the current evidence on CMM-related biomarkers.

Methods: We searched PubMed, Embase, Web of Science, and Ebsco for relevant studies from inception until August 31st, 2022. Studies reported the association of serum/plasma biomarkers with CMM, and relevant effect sizes were included. The outcomes were five progression patterns of CMM: (1) no CMD to CMM; (2) type 2 diabetes mellitus (T2DM) followed by stroke; (3) T2DM followed by coronary heart disease (CHD); (4) T2DM followed by stroke or CHD; and (5) CHD followed by T2DM. Newcastle-Ottawa Quality Assessment Scale (NOS) was used to assess the quality of the included studies. A meta-analysis was conducted to quantify the association of biomarkers and CMM.

Results: A total of 68 biomarkers were identified from 42 studies, which could be categorized into five groups: lipid metabolism, glycometabolism, liver function, immunity, and others. Lipid metabolism biomarkers were most reported to associate with CMM, including TC, TGs, HDL-C, LDL-C, and Lp(a). Fasting plasma glucose was also reported by several studies, and it was particularly associated with coexisting T2DM with vascular diseases. According to the quantitative meta-analysis, HDL-C was negatively associated with CHD risk among patients with T2DM (pooled OR for per 1 mmol/L increase = 0.79, 95% CI = 0.77–0.82), whereas a higher TGs level (pooled OR for higher than 150 mg/dL = 1.39, 95% CI = 1.10–1.75) was positively associated with CHD risk among female patients with T2DM.

Conclusion: Certain serum/plasma biomarkers were associated with the progression of CMM, in particular for those related to lipid metabolism, but heterogeneity and inconsistent findings still existed among included studies. There is a need for future research to explore more relevant biomarkers associated with the occurrence and progression of CMM, targeted at which is important for the early identification and prevention of CMM.

KEYWORDS

biomarker, cardiometabolic multimorbidity, type 2 diabetes mellitus, coronary heart disease, stroke, systematic review

1 Introduction

With the growth of the aging population, non-communicable diseases (NCDs) have become the major global disease burden and the leading cause of death worldwide (1). Some NCDs may share similar pathogenesis or identical risk factors (2, 3), leading to their simultaneous coexistence in individuals and resulting in multimorbidity (i.e., the coexistence of two or more NCDs) (4). Cardiometabolic multimorbidity (CMM) is one of the most studied patterns of multimorbidity (5), defined as the co-occurrence of two or more cardiometabolic diseases (CMDs), including coronary heart disease (CHD), stroke, and type 2 diabetes mellitus (T2DM) (2, 6, 7). The prevalence of CMM has increased rapidly in the past few decades (8), potentially resulting in worse quality of life, excess morbidity, and mortality (8–11).

Current studies on the risk factors of CMM mainly focused on some macro or external factors, such as lifestyle factors (12), dietary factors (6, 13), and environmental factors (14). Limited studies have explored the role of micro risk factors, such as serum/plasma biomarkers, on CMM. Biomarkers are the most objective and quantifiable medical markers that can be measured and are commonly used as clinical and diagnostic tools (15). Changes in biomarker levels can reflect the interaction effects among genetic factors, lifestyle factors, environmental factors, and health conditions, which can be used to explore new routes of disease occurrence, improve the accuracy of risk prediction, and achieve stratifying prevention and management of these diseases (16, 17). The relationship between specific biomarkers and single CMD was extensively investigated. For example, high levels of triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C) and fasting plasma glucose (FPG), low levels of high-density lipoprotein cholesterol (HDL-C) have been reported to be risk factors of cardiovascular complications in T2DM patients (18–20). A 12-year follow-up longitudinal analysis revealed that high homocysteine (tHcy) and low Methionine (Met) levels were associated with a higher risk of cardiovascular multimorbidity in older age (21). However, there remains a lack of studies investigating the relationship between biomarkers and CMM. Moreover, no systematic review and meta-analysis has yet synthesized existing evidence on the association between biomarkers and the progression of CMM, which is critical for the clinical practice of identifying high-risk populations early via laboratory blood testing. We aimed to synthesize the available scattered evidence on the role of serum/plasma biomarkers in the development and progression of CMM.

2 Methods

2.1 Search strategy

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (22). Databases, including PubMed, Embase, Web of Science, and Ebsco, were searched for eligible articles published in English from January 1st, 1900, to August 31st, 2022. Details of search strategies are presented in Supplementary Table S1. Reference lists and Google were also searched to identify any additional or gray literature that met the inclusion criteria.

2.2 Selection criteria

After removing the duplicate records, three authors (JY, XZ, ZY) independently screened a third of the total records for titles and abstracts and evaluated the full text to select eligible articles with exclusion reasons recorded. A total of 5% of the records were randomly selected and cross-checked for verification. Inter-examiner agreements across the three authors were calculated using Cohen κ statistics, with ranges of 0.01–0.20 representing slight agreement, 0.21–0.40 representing fair agreement, 0.41–0.60 representing moderate agreement, 0.61–0.80 representing substantial agreement, and 0.81–0.99 representing almost perfect agreement (23). Discrepancies were discussed with a fourth author (XX) in regular group meetings.

Inclusion criteria were: (1) observational studies such as cohort studies and nested case-control studies; (2) the primary outcome of the study was CMM, which was defined as the co-occurrence of at least two of the following CMDs: CHD, T2DM, and stroke. Myocardial infarction (MI), ischemic heart disease (IHD), angina pectoris, coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, coronary revascularization procedures, and CHD-related death are all examples of CHD occurrences; (3) biomarkers include all detectable and quantifiable biochemical parameters found in plasma or serum, except for gene regulatory molecules such as microRNA. Laboratory examinations were conducted at baseline or before the occurrence of CMM; (4) participants were free of CMM at baseline; (5) effect sizes [e.g., hazard ratio (HR), odds ratio (OR), relative risk (RR), and 95% confidence interval (CI)] of the biomarkers on CMM were reported; and (6) written in English.

2.3 Quality assessment and data extraction

Three authors (JY, XZ, ZY) independently extracted data from all included articles using a pre-designed standardized data extraction form, including title, first author, year of publication, country, sex distribution and age of participants, sample size, follow-up duration, biomarkers, definition of CMM, number (percentage) of participants developing CMM, comparison, adjusted effect sizes of biomarkers on CMM, and covariates. Disagreements on the extraction were discussed with a fourth author (XX).

Three authors independently (JY, XZ, ZY) assessed the methodological quality of each study using Newcastle-Ottawa Quality Assessment Scale (NOS) (24), with discrepancies discussed with a fourth author (XX). Studies scored ≥7 (out of 9) were

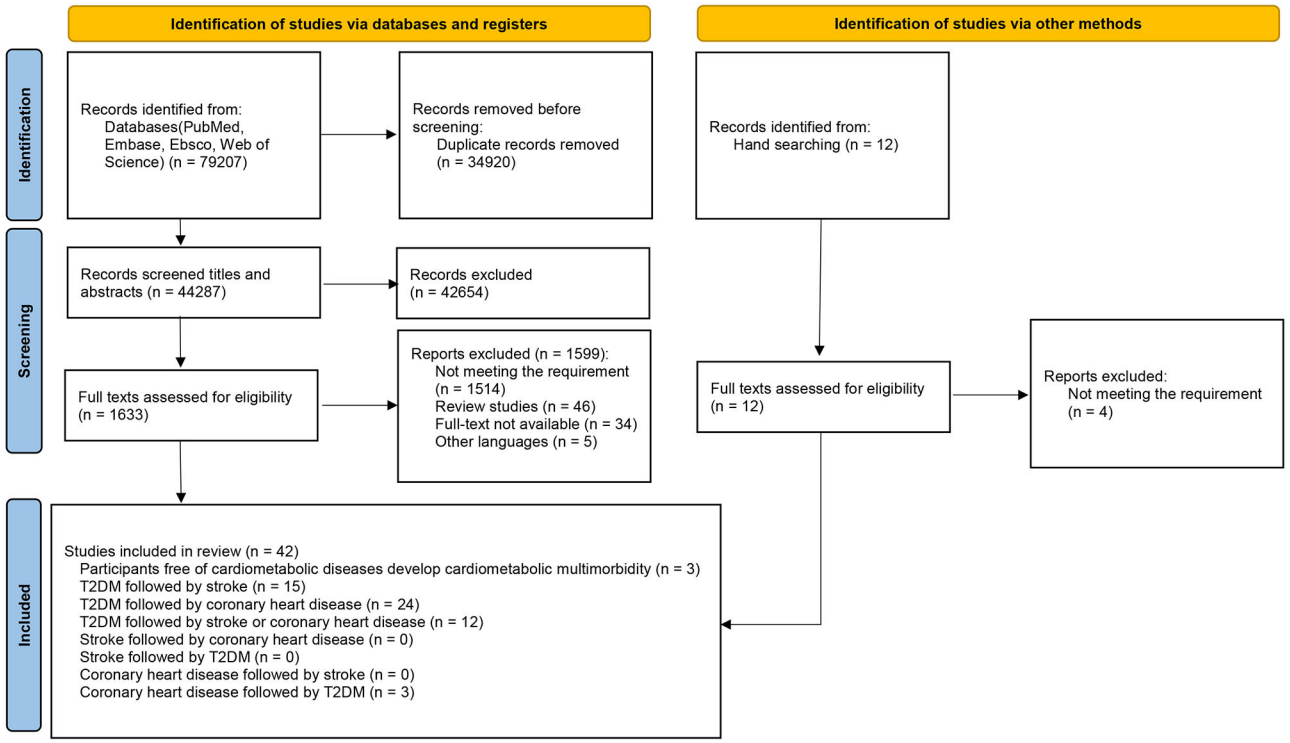

FIGURE 1
Flowchart of study selection according to the PRISMA guideline.

considered as high quality, and those scored ≤3 were of low quality (25).

2.4 Evidence synthesis

A narrative synthesis approach was used to summarize the effect sizes of specific biomarkers on different progression patterns of CMM, which was visualized through a heatmap. However, due to the heterogeneity in the cut-off points or measurement units of biomarkers among included articles, we could not pool the effect sizes of the majority of biomarkers on CMM through meta-analysis. For eligible biomarkers with enough data for meta-analysis, different measurement levels of specific biomarkers were converted into a consistent standard. Categorical variables were converted into binary forms, while effect sizes of continuous variables were transformed into ORs according to the following formulas (26):

$$\mathrm{OR}_{(\mathrm{standardized})} = OR_{(original)}^{Increment\ (standardized)/Increment\ (original)} \quad (1)$$

The heterogeneity was assessed using Cochran's *Q*-statistic and I^2 statistics, with thresholds of 25%, 50%, and 75% for low, moderate, and high heterogeneity (23). Depending on the degree of heterogeneity, either a fixed effect model or a random effect model was used to estimate the pooled ORs (95% CIs). We weighted studies using the inverse-variance approach. The meta-analysis was conducted using Review Manager (RevMan) Version 5.4. (Copenhagen, Denmark: The Nordic Cochrane Center, The Cochrane Collaboration).

3 Results

3.1 Characteristics of included studies

A total of 79,207 publications were initially identified through a database search. After screening for titles and abstracts, 1,633 were selected for full-text review (inter-reader agreement κ = 0.81/0.85/0.83). A total of 42 studies (7, 27–67) that met the inclusion criteria were finally included in our review after the full-text screen (inter-reader agreement κ =0.86/0.88/0.82). The selection process is shown in Figure 1, and the basic characteristics of included studies are presented in Supplementary Table S2. Among the 42 studies included in our review, we selected five of them for the meta-analysis (40, 43, 48, 49, 62) (Table 1), which were prospective studies focusing on the associations of FPG, LDL-C, HDL-C, and TGs with the progression of CMM among T2DM patients based on populations from Japan, Greece, and Italy. These 42 studies were grouped into five categories by different progression patterns of CMM: (1) participants were free of CMD at baseline and developed CMM during follow-up ($n = 3$); (2) T2DM followed by stroke ($n = 15$); (3) T2DM followed by CHD ($n = 24$); (4) T2DM followed by stroke or CHD ($n = 12$); and (5) CHD followed by T2DM ($n = 3$).

A total of 8 studies were from the USA, followed by Japan ($n = 7$) and the UK ($n = 6$). The sample size varied considerably

TABLE 1 Characteristics of the studies included in the meta-analysis.

Study	Country	Mean age	*n* (female, %)	Follow-up years	Definition of CMM	No. of events	Comparison	HR (95% CI)	Covariates
FPG									
Hayashi et al. (48)	Japan	67.4	4,014 (48.2)	5.5	Patients with T2DM developed IHD (Definite fatal and nonfatal MI or angina pectoris);	153	Per 10 mg/dL higher	1.00 (0.99–1.01);	NA
					Ischemic stroke or primary intracerebral hemorrhage	104		1.01 (0.99–1.01)	
Sone et al. (62)	Japan	58.2	1,771 (46.9)	7.86 (median)	Patients with T2DM developed CHD (angina pectoris and MI);	109	Per 1 mmol/L higher	0.99 (0.91–1.09);	Gender, age, diabetes duration, body mass index, systolic blood pressure, HbA1c, LDL cholesterol, HDL cholesterol, triglycerides, smoking status, and alcohol intake
					Stroke	85		1.02 (0.91–1.13)	
LDL-C									
Protopsaltis et al. (49)	Greece	60.4	599 (46.0)	10.1 (median)	Patients with T2DM developed ischemic stroke	78	Per 1 mg/dL	1.01 (0.99–1.02)	Gender, age, smoking, body mass index, HbA1C, lipids, and diabetes duration
Sone et al. (62)	Japan	58.2	1,771 (46.9)	7.86 (median)	Patients with T2DM developed stroke	85	Per 1 mmol/L higher	1.00 (0.76–1.32)	Gender, age, diabetes duration, body mass index, systolic blood pressure, HbA1c, HDL cholesterol, triglycerides, smoking status, and alcohol intake

(Continued)

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TABLE 1 (Continued)

Study	Country	Mean age	*n* (female, %)	Follow-up years	Definition of CMM	No. of events	Comparison	HR (95% CI)	Covariates
HDL-C									
Avogaro et al. (40)	Italy	65	9,979 (51.7)	4	Patients with T2DM developed CHD events (MI, coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, and electrocardiogram-proven angina)	881	Per 5 mg/dL higher	M: 0.98 (0.94–1.02) F: 0.96 (0.92–1.00)	Age, disease duration, serum triglycerides, microangiopathy, antihypertensive therapy, and insulin treatment, waist girth, glycemic control, total cholesterol, blood pressure, and geographic area and lipid-lowering
Sone et al. (62)	Japan	58.2	1,771 (46.9)	7.86 (median)	Patients with T2DM developed CHD (angina pectoris and MI)	109	Per 1 mmol/L	0.99 (0.56–1.74)	Gender, age, diabetes duration, body mass index, systolic blood pressure, HbA1c, LDL cholesterol, triglycerides, smoking status, and alcohol intake
TGs									
Protopsaltis et al. (49)	Greece	60.4	599 (46.0)	10.1 (median)	Patients with T2DM developed ischemic stroke	78	≥150 vs. <150 mg/dL	1.03 (0.90–1.15)	Gender, age, smoking, body mass index, HbA1c, lipids, and diabetes duration
Sone et al. (43)	Japan	58.4	1,424 (45.9)	8	Patients with T2DM developed stroke;	59	≥150 vs. <150 mg/dL	M: 1.10 (0.50–2.40) F: 0.70 (0.20–1.90);	NA
					Patients with T2DM developed CHD (MI, angina pectoris)	62		M: 2.90 (1.60–5.30) F: 1.70 (0.60–4.40)	

(Continued)

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TABLE 1 (Continued)

Study	Country	Mean age	*n* (female, %)	Follow-up years	Definition of CMM	No. of events	Comparison	HR (95% CI)	Covariates
Avogaro et al. (40)	Italy	65	9,979 (51.7)	4	Patients with T2DM developed CHD events (MI, coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, and electrocardiogram-proven angina)	881	≥150 vs. <150 mg/dL	M: 1.19 (0.94–1.50) F: 1.33 (1.05–1.68)	Age, disease duration, microangiopathy, antihypertensive therapy, and insulin treatment, waist girth, glycemic control, total cholesterol, blood pressure, and geographic area, HDL cholesterol and lipid-lowering

CMM, cardiometabolic multimorbidity; FPG, fasting plasma glucose; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TGs, triglycerides; T2DM, type 2 diabetes mellitus; IHD, ischemic heart disease; MI, myocardial infarction; CHD, coronary heart disease; HbA1c, hemoglobin A1c; HR, hazard ratio; CI, confidence interval; M, male; F, female; NA, not available.

among included studies, ranging from 224 to 891,095 participants. The mean age of participants ranged from 46.9 to 72.5 years across included studies. Most studies were of high quality ($n = 41$), and one study was of moderate quality (Supplementary Figure S1A). The items "Ascertainment of exposure" and "Demonstration that outcome of interest was not present at start of study" were rated as low risk of bias in all included studies, and the item "Adequacy of follow up of cohorts" was rated as high risk in the most of studies ($n = 28$) (Supplementary Figure S1B).

3.2 Overview of biomarkers

Among the included studies, a total of 68 serum/plasma biomarkers were identified (Figure 2). They are categorized into five groups: lipid metabolism, glycometabolism, liver function, immunity, and others. The most frequently studied biomarkers were HDL-C ($n = 19$), TGs ($n = 18$), FPG ($n = 15$), LDL-C ($n = 15$), and lipoprotein-A [Lp(a)] ($n = 7$). Effects of some biomarkers reported in more than two studies were also presented according to races (Supplementary Figure S2). The directions of the association for most biomarkers with CMM were similar among different populations, except for HDL-C, which showed positive association with CMM in Chinese populations and negative association in Japanese. Four biomarkers (i.e., FPG, HDL-C, LDL-C, and TGs) were available for meta-analysis.

3.3 The role of biomarkers in different progression patterns of CMM

3.3.1 Healthy participants progressed to CMM

Three studies (7, 66, 67) reported the association of lipid metabolism related biomarkers with the progression of CMM, including total cholesterol (TC), TGs, HDL-C, LDL-C, VLDL-C, NHDL-C, Apo A1, and ApoB. According to these studies, a higher level of TGs or VLDL-C was reported to positively associate with an increased risk of CMM, while HDL-C level was reported to inversely associate with CMM. FPG was the only glycometabolism biomarker associated with CMM, and a higher FPG level was positively associated with an increased risk of CMM.

3.3.2 T2DM followed by CHD

A total of 15 biomarkers were reported to be positively associated with a higher risk of CHD among T2DM patients, including six lipid metabolism biomarkers [i.e., TC, TGs, LDL-C, NHDL-C, Lp(a), and Apo B], three glycometabolism biomarkers (i.e., FPG, 2h PG and fasting insulin), one immunity biomarkers (i.e., hs-CRP), and five other biomarkers [i.e., N-terminal pro-B type natriuretic peptide (NT-proBNP), sRAGE, esRAGE, five inflammation-sensitive plasma proteins (ISPs) and TnT]. Five biomarkers were reported to negatively associate with the risk of CHD following T2DM, including HDL-C, direct bilirubin, indirect bilirubin, bicarbonate, and 25(OH)D. Four articles (40, 43, 48, 62) were available for the meta-analyses, which showed no significant associations of FPG (pooled OR for per 10 mg/dL increase = 1.00,

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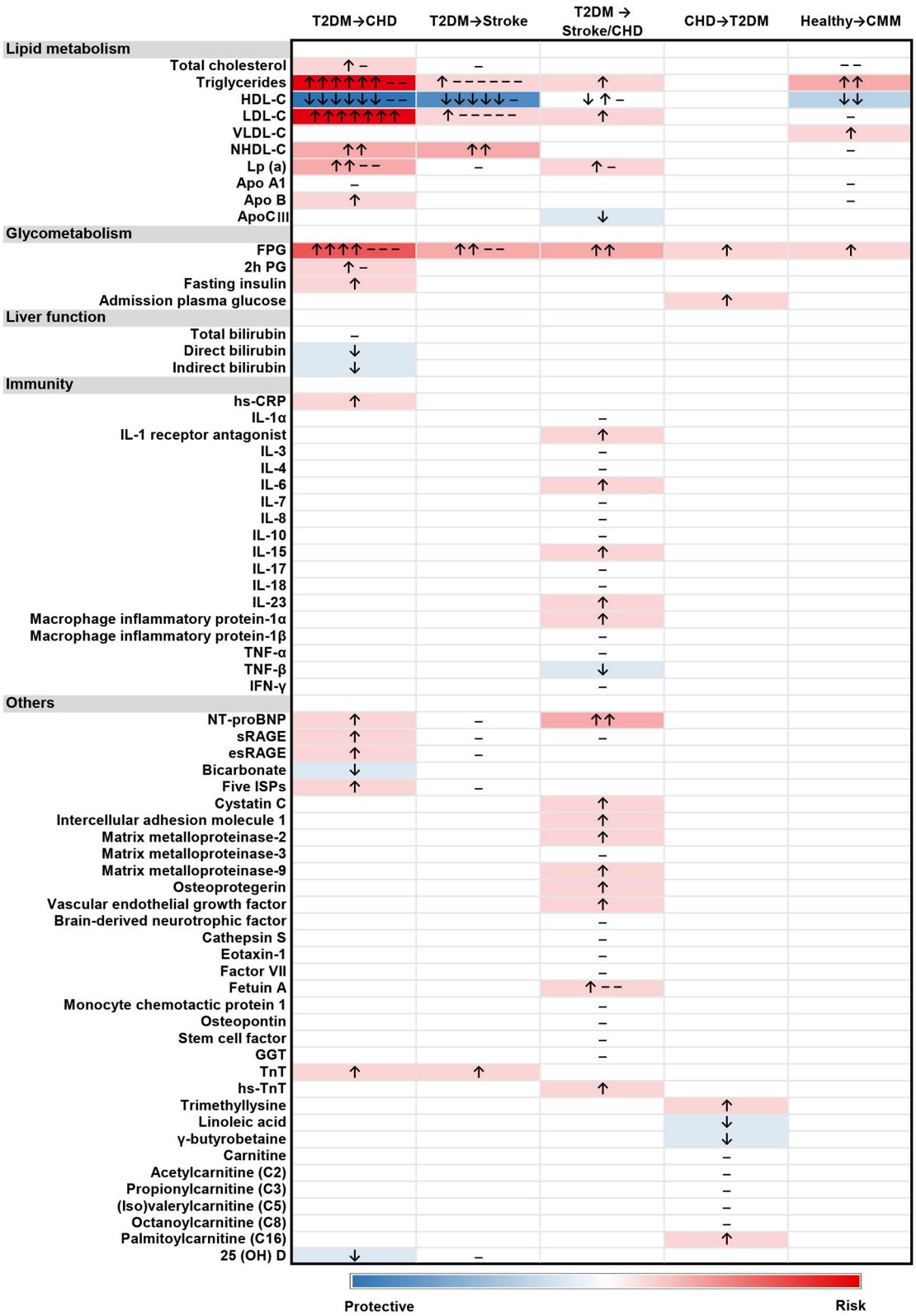

FIGURE 2
Heatmap of the associations between 68 biomarkers and 5 types of CMM progression. "↑", positive association; "↓", negative association; "–", no significant association. The studies reporting different associations were counted and presented in the heatmap. The intensity of the color depended on the strength of overall association between biomarker level and the outcome.

95% CI = 1.00–1.01) with CHD among T2DM patients. A higher level of TGs was positively associated with CHD risk among female T2DM patients (pooled OR for higher than 150 mg/dL among male T2DM patients = 1.80, 95% CI = 0.76–4.22, pooled OR for higher than 150 mg/dL among female T2DM patients = 1.39, 95%CI: = 1.10–1.75). Besides, an inverse association of HDL-C (pooled OR for per 1 mmol/L increase = 0.79, 95% CI = 0.77–0.82) with CHD risk among T2DM patients was found (Figure 3).

3.3.3 T2DM followed by stroke

A total of five biomarkers were reported to be positively associated with a higher risk of stroke among T2DM patients, including three lipid metabolism biomarkers (i.e., LDL-C, TGs, and NHDL-C), one glycometabolism biomarker (i.e., FPG), and one other biomarker (i.e., TnT). Besides, HDL-C was reported to be inversely associated with the risk of stroke among the population with T2DM. Four articles (43, 48, 49, 62) were available for the meta-analyses: FPG (pooled OR for 10 mg/dL increase = 1.01, 95% CI = 1.00–1.01), LDL-C (pooled OR for 1 mg/dL increase = 1.00, 95% CI = 0.99–1.01), and TGs (pooled OR for higher than 150 mg/dL cutoff = 1.03, 95% CI = 0.92–1.16) showed no significant association with stroke among T2DM patients (Figure 4).

3.3.4 T2DM followed by stroke or CHD

A total of 18 biomarkers were identified to be positively associated with a higher risk of stroke or CHD among T2DM patients, including three lipid metabolism biomarkers (i.e., Lp(a), LDL-C, and TGs), one glycometabolism biomarker (i.e., FPG), five immunity biomarkers (i.e., IL-1 receptor antagonist, IL-6, IL-15, IL-23, and macrophage inflammatory protein-1α) and nine other biomarkers [i.e., NT-proBNP, cystatin C, intercellular adhesion molecule 1 (ICAM-1), matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), osteoprotegerin (OPG), vascular endothelial growth factor (VEGF), fetuin-A, and hs-TnT]. Besides, two biomarkers were inversely associated with the risk of stroke or CHD in T2DM patients, including apolipoprotein C III (Apo CIII) and tumor necrosis factor-β (TNF-β).

3.3.5 CHD followed by T2DM

Two glycometabolism biomarkers (i.e., FPG and admission plasma glucose) and two additional biomarkers [trimethyllysine and palmitoylcarnitine (C16)] were reported to positively associated with a greater risk of T2DM in CHD patients. In addition, linoleic acid and γ-butyrobetaine were revealed to be inversely related to a greater risk of T2DM among CHD patients.

4 Discussion

4.1 Principle findings

We obtained 68 types of serum/plasma biomarkers from 42 included studies and summarized the evidence of associations between these biomarkers and five progression patterns of CMM. Lipid metabolism biomarkers were most reported to associate with the risk of CMM, including TC, TGs, HDL-C, LDL-C, and Lp(a). Only four biomarkers (FPG, HDL-C, LDL-C, TGs) were available for meta-analysis due to methodological heterogeneity among studies. A higher level of HDL-C was shown to significantly associate with lower risks of CHD among T2DM patients; on the other hand, a higher level of TGs was positively associated with CHD risk among female T2DM patients.

4.2 Comparison with previous studies

Lipid metabolism biomarkers were the most studied type of biomarkers. The meta-analysis showed that HDL-C was negative associated with CHD risk among patients with T2DM, and a higher TGs level was positively associated with CHD risk among female patients with T2DM.

Among all included studies, our heatmap suggested that elevated levels of TGs, LDL-C, NHDL-C, and Lp(a) were associated with a higher risk of CMM, while HDL-C presented a significant inverse association with the progression of CMM. In line with our findings, strong associations between lipid metabolism biomarkers (e.g., elevated levels of LDL-C, TGs and Lp(a), decreased level of HDL-C) and CMM have been found, especially in patients with T2DM (18, 19, 68–70). Also, it is worth noting that T2DM patients with high HDL-cholesterol levels had paradoxically higher risk of composite CVD outcomes in an included study (33). Previous studies (71, 72) also suggested that the association between HDL-C concentrations and CVD events might be a U-shaped curve, indicating that abnormally low or high HDL-C levels were both inversely associated with health status. However, due to the limited number and heterogeneity of included studies, we could not identify such an association in our analysis. There also existed sex differences in the effect of lipid metabolism biomarkers on the risk of coronary heart disease in patients with T2DM. One included study (40) reported the risk effect of TGs, and the protective effect of HDL-C on CHD only existed in female participants. Another study (43) showed a significant association between high levels of LDL-C and CHD outcome only in males. It can be explained that women with T2DM were more susceptible to the atherogenic effects of non-LDL-C factors (i.e., high level of TGs and low level of HDL-C) (73, 74). Future studies are warranted to validate such associations.

Several glycometabolism biomarkers were also reported to associate with CMM, especially for FPG. Hyperglycemia and uncontrolled glycemia have been extensively discussed as risk factors for the development of stroke and CHD in previous studies (20, 75, 76) in both the general population and T2DM patients. Hyperglycemia can detrimentally affect normal endothelial function, contributing to plaque formation and rupture, and finally, thrombosis (76, 77), thus correlating with a higher risk of atherosclerotic cardiovascular diseases (ASCVD). In addition, a J-shaped relationship between FPG and adverse cardiovascular events has been reported in people with T2DM (78, 79), especially in older patients with high comorbidity load, consistent with our results (54, 63). As a traditional diagnostic biomarker for T2DM, the level of FPG may provide more information for health status

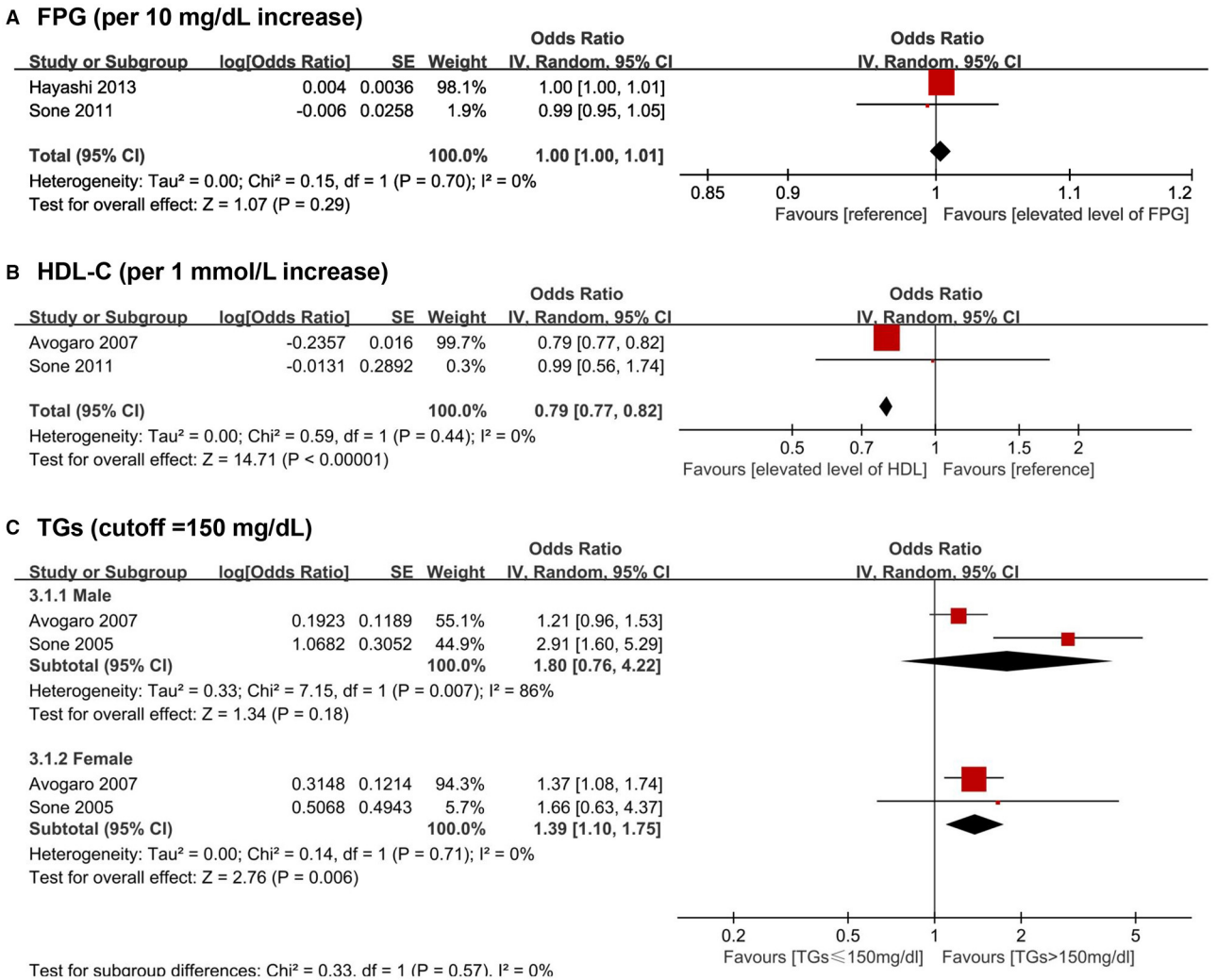

FIGURE 3
Forest plot of prospective studies examining FPG **(A)**, HDL-C **(B)**, TGs **(C)** levels and risk of CHD in subjects with T2DM. **(A)** Pooled analysis of increase risk of CHD in subjects with T2DM for per 10 mg/dL increase of FPG level. **(B)** Pooled analysis of increase risk of CHD in subjects with T2DM for per 1 mmol/L increase of HDL-C level. **(C)** 3.1.1 Pooled analysis of increase risk of CHD in male T2DM patients with TGs level higher than 150 mg/dL. **(C)** 3.1.2 Pooled analysis of increase risk of CHD in female T2DM patients with TGs level higher than 150 mg/dL. CHD, coronary heart disease; CI, confidence interval; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; T2DM, type 2 diabetes mellitus; TGs, triglycerides.

that could also serve as an indicator of the deterioration of T2DM and a potential biomarker for CMM.

Several inflammatory factors were also identified to associate with CMM in our study. In addition to what we found, many previous studies reported that an elevated concentration of inflammatory factors (e.g., IL-6, hs-CRP, TNF-α) were positively correlated with ASCVD outcomes (80–82) due to their effects on plaque development and rupture, endothelial dysfunction, and coronary thrombosis (82). However, the role of some other immunity factors in the progression of CVD is controversial, and there remains a lack of evidence to assess the association between immunity biomarkers and the development of CMM.

The majority of the immunity biomarkers in our research were collected from one study (57), which intended to choose predictive biomarkers for CVD in T2DM patients. More research is needed to determine the relevance of immunity biomarkers as potential indications of CMM progression.

Liver function related biomarkers were also crucial in the progression of CMM, but current findings of their impacts on CMM were scarce and inconsistent. For example, bilirubin, an essential marker of liver function, has been indicated as an antioxidant with anti-inflammatory and antiapoptotic effects (83–85). One included study (60) revealed that higher levels of serum direct and indirect bilirubin were related to decreased CHD risk in T2DM patients. But the evidence from prospective studies was limited. Another study (86) suggested that serum bilirubin could add predictive value to future cardiovascular deaths in patients with T2DM. Besides, NT-proBNP was widely used in detecting heart failure and was also a prognostic marker in patients with acute decompensated heart failure (87, 88). Another review (89) regarded

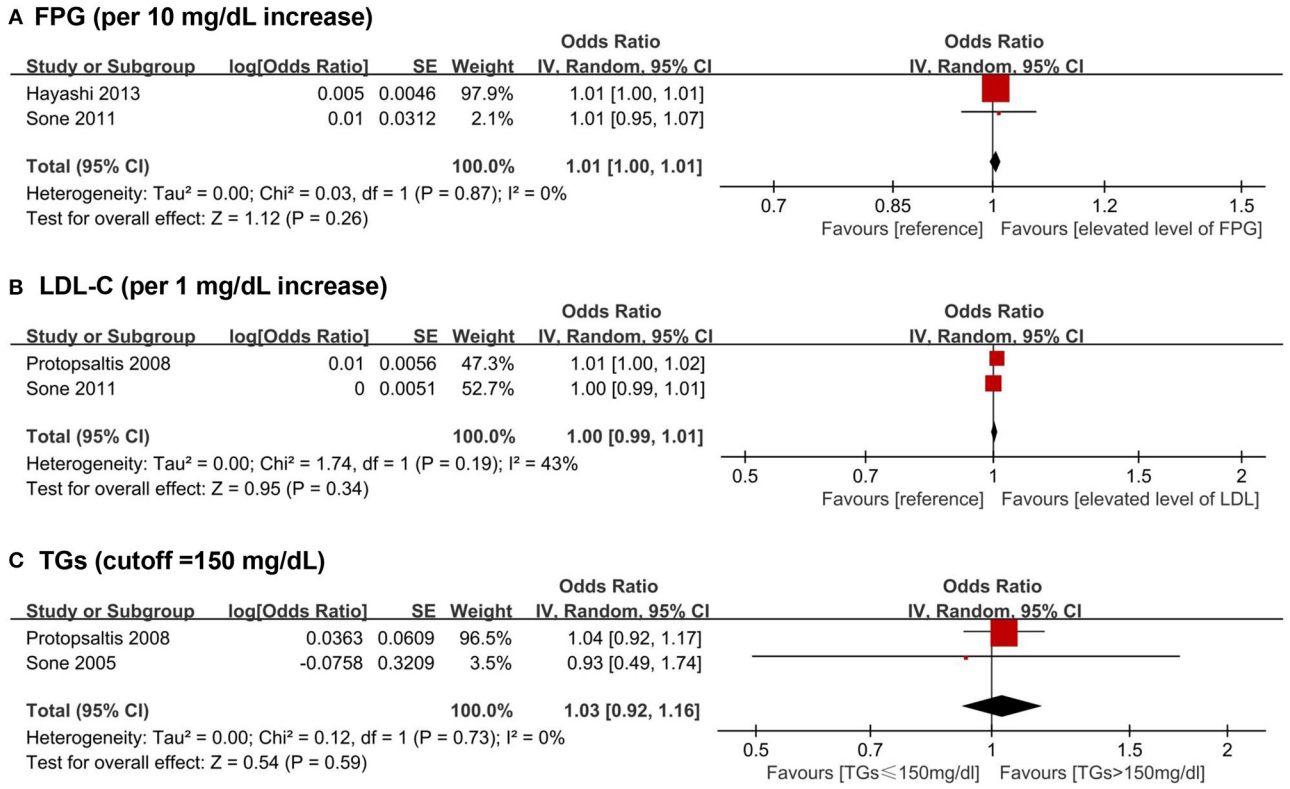

FIGURE 4
Forest plot of prospective studies examining FPG **(A)**, LDL-C **(B)**, TGs **(C)** levels and risk of stroke in subjects with T2DM. **(A)** Pooled analysis of increase risk of stroke in subjects with T2DM for per 10 mg/dL increase of FPG level. **(B)** Pooled analysis of increase risk of stroke in subjects with T2DM for per 1 mg/dL increase of LDL-C level. **(C)** Pooled analysis of increase risk of stroke in subjects with T2DM with TGs level higher than 150 mg/dL. CI, confidence interval; FPG, fasting plasma glucose; LDL-C, low-density lipoprotein cholesterol; T2DM, type 2 diabetes mellitus; TGs, triglycerides.

NT-proBNP as a predictor of CVD events in T2DM patients, which was consistent with our findings.

Many included studies recruited patients with one initial CMD at baseline and most had T2DM. It has been proved that hypertension and dyslipidemia were quite common in patients with T2DM, even in prediabetic individuals (90–92). Many studies reported using current diabetes treatments, antihypertensive therapy, and lipid-lowering treatments at baseline, which could interfere with the biomarker measurements and the association between biomarkers and CMD (93, 94). Several studies addressed the influence of medication and incorporated it into the multivariate model. Some studies compared the risk effect before and after further adjustment for treatment and observed similar results. However, the impact of treatment and drugs was not accounted for in some studies due to the complexity of potential interactions. The role of medication in the association between biomarkers and CMM requires further investigation to prevent the development of CMM in people who already have one basis disease.

Lifestyle and diet habits are also important factors associated with certain biomarkers levels and CMM progression according to previous studies (6, 12, 95–97). Many included studies adjusted alcohol intake and physical activity as covariates in the multivariate analysis. However, few of them estimated the effect sizes of these factors with biomarkers or further discuss other lifestyle or diet factors. The interaction between lifestyle, diet habits, biomarkers, and CMM awaits more investigations and explorations in the future.

4.3 Implications

For future research, there remains a lack of observational studies, especially longitudinal cohort studies, to provide more evidence on the associations between biomarkers and the progression of CMM. Evidence on individuals from an apparently healthy state to CMM and various CMM patterns is needed since the majority of included studies in our review focused on populations with T2DM at baseline. Also, the role and effect of medications, treatment, lifestyle, and diet factors in the association between biomarkers and cardiometabolic diseases await more evidence and further exploration. Furthermore, it is also worth exploring the variation of biomarkers over time in the progression of CMM. Finally, a few included studies reported the joint assessments of multiple biomarkers, but the prediction performance of multiple biomarkers in comparison with a single biomarker still needs more evidence.

Our results highlight the value of serum/plasma biomarkers in the primary prevention of CMM among

healthy people and secondary prevention in people who already have CMD. Lowering levels of risk biomarkers could be considered potential preventive targets of lifestyle and therapeutic interventions. Our finding may also provide important clinical implications for the early screening and prediction of CMM through targeted biomarkers. Measurements of serum biomarkers in the general population may help to identify individuals at high risk and maximize healthcare resources.

4.4 Limitations

Some limitations of our current review should be addressed. First, because of the limited number of available studies, several biomarkers were only reported once or twice, suggesting that there may be publication bias. Additionally, most included studies were conducted in specific regions or countries, recruiting participants of specific race. Previous studies have shown the various clinical impacts of some metabolic characteristics [e.g., metabolic syndrome (43), serum triglyceride levels (62)] on diabetes across different races. The results could not be fully generalized to the overall population. Third, although many studies included potential confounders in the multivariate models, the adjustments differed in the originally included studies, and some studies' confounders were unavailable. Finally, as mentioned above, integrative analysis of some biomarkers failed due to methodological heterogeneity across studies, which may lead to unconvincing results of the meta-analysis.

5 Conclusion

Our systematic review and meta-analysis summarized the evidence on the role of a broad number of biomarkers in the development and progression of CMM. However, studies focusing on the association of biomarkers and CMM were scarce, requesting more evidence on this topic to provide implications for early prevention, detection, and intervention of CMM.

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

XX: Conceptualization, Methodology, Project administration, Supervision, Validation, Writing—review & editing. YJ: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing—original draft. ZX: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing—original draft. YutZ: Data curation, Investigation, Methodology, Validation, Writing—original draft. YueZ: Writing—review & editing. DW: Methodology, Visualization, Writing—review & editing. YC: Writing—review & editing. YZho: Writing—review & editing. MF: Writing—review & editing.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1280185/full#supplementary-material

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The association between fibrinogen levels and severity of coronary artery disease and long-term prognosis following percutaneous coronary intervention in patients with type 2 diabetes mellitus

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Background: Fibrinogen is a potential risk factor for the prognosis of CAD and is associated with the complexity of CAD. There is limited research specifically investigating the predictive role of fibrinogen in determining the severity of CAD among patients with T2DM, as well as its impact on the prognosis following PCI.

Methods: The study included 675 T2DM patients who underwent PCI at the Third People's Hospital of Chengdu between April 27, 2018, and February 5, 2021, with 540 of them remaining after exclusions. The complexity of CAD was assessed using the SYNTAX score. The primary endpoint of the study was the incidence of MACCEs.

Results: After adjusting for multiple confounding factors, fibrinogen remained a significant independent risk factor for mid/high SYNTAX scores (SYNTAX score > 22, OR 1.184, 95% CI 1.022-1.373, P = 0.025). Additionally, a dose-response relationship between fibrinogen and the risk of complicated CAD was observed (SYNTAX score > 22; nonlinear P = 0.0043). The area under the receiver operating characteristic curve(AUROC) of fibrinogen for predicting mid/high SYNTAX score was 0.610 (95% CI 0.567–0.651, P = 0.0002). The high fibrinogen group (fibrinogen > 3.79 g/L) had a higher incidence of calcified lesions and an elevated trend of more multivessel disease and chronic total occlusion. A total of 116 patients (21.5%) experienced MACCEs during the median follow-up time of 18.5 months. After adjustment, multivariate Cox regression analysis confirmed that fibrinogen (HR, 1.138; 95% CI 1.010-1.284, P = 0.034) remained a significant independent risk factor for MACCEs. The AUROC of fibrinogen for predicting MACCEs was 0.609 (95% CI 0.566-0.650, P = 0.0002). Individuals with high fibrinogen levels (fibrinogen > 4.28 g/L) had a higher incidence of acute myocardial infarction (P < 0.001), MACCEs (P < 0.001),

all-cause death (P < 0.001), stroke (P = 0.030), and cardiac death (P = 0.002). Kaplan-Meier analysis revealed a higher incidence of MACCEs in the high fibrinogen group (Log-Rank test: P < 0.001).

Conclusions: Elevated fibrinogen levels were associated with increased coronary anatomical complexity (as quantified by the SYNTAX score) and a higher incidence of MACCEs after PCI in patients with T2DM.

KEYWORDS

fibrinogen, Percutaneous coronary intervention, prognosis, The SYNTAX score, type 2 diabetes mellitus

1 Introduction

Coronary artery disease (CAD) is among the leading causes of mortality worldwide (1). Hyperglycemia, abnormal lipid metabolism, insulin resistance, and oxidative stress reactions caused by diabetes can exacerbate the development of atherosclerosis in patients (2), which negatively impacts their clinical prognosis. The SYNTAX score is commonly used to assess the complexity of coronary artery lesions and guide the selection of revascularization strategies between coronary artery bypass grafting surgery(CABG) and percutaneous coronary intervention (PCI) in patients with complex CAD (3–5). Previous studies have shown that it holds substantial predictive value in assessing the prognoses of patients undergoing percutaneous coronary intervention (6, 7). Patients can be categorized into different risk groups based on their SYNTAX scores: low risk (≤22), intermediate risk (23-32), and high risk (≥33). Higher scores indicate a greater complexity of coronary artery lesions and suggest a poorer prognosis (8, 9). The SYNTAX score derives from invasive coronary angiography, non-invasive assessments for determining the complexity of CAD might have potential benefits, as they can aid in patient stratification prior to invasive coronary angiography.

Underlying processes such as inflammation, endothelial dysfunction and enhanced coagulant activity are closely associated with the initiation and progression of atherosclerosis (10). Fibrinogen is a key component that drives blood coagulation and functions as an inflammatory factor, promoting the onset and growth of thrombosis and atherosclerosis (11, 12). Fibrinogen levels have been linked to the incidence and advancement of CAD (13–16). Moreover, it can predict the short-term and long-term risks of death and adverse cardiovascular events in patients with CAD, even those who have undergone PCI (17–22). Previous studies have established a connection between fibrinogen and cardiovascular events in patients with CAD and type 2 diabetic mellitus (T2DM) patients (23). Moreover, studies revealed that fibrinogen levels can act as an index of the severity of coronary artery lesions in patients with stable angina pectoris (SAP) (24) and acute coronary syndrome (ACS) (25). However, the ability of fibrinogen to assess the complexity of CAD in patients with T2DM remains unclear, and limited research has investigated the correlation between fibrinogen and the prognosis of patients with T2DM who undergo PCI. Therefore, the aim of this study is to investigate the association between fibrinogen levels and the complexity of CAD, as well as the prognosis after PCI in patients with T2DM.

Abbreviations: CAD, Coronary artery disease; CABG, Coronary artery bypass grafting surgery; PCI, Percutaneous coronary intervention; T2DM, Type 2 diabetic mellitus; SAP, Stable angina pectoris; ACS, Acute coronary syndrome; MACCEs, Major cardiovascular and cerebrovascular adverse events; ADA, American Diabetes Association; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; HDL-C, High-Density lipoprotein cholesterol; LDL-C, Low-Density lipoprotein cholesterol; LEVF, Left ventricular ejection fraction; ORs, Odds ratios; CIs, Confidence intervals; RCS, Restricted cubic splines; ROC, Receiver operating characteristic; AUROC, Area under the receiver operating characteristic curve; AMI, Acute myocardial infarction; MVD, Multivessel disease; CTO, Chronic total occlusion; AUC, Area under the curve; IL-6, Interleukin-6.

2 Manuscript

2.1 Materials and methods

2.1.1 Study design and participants

This study is a single-center, retrospective, observational cohort study. A total of 675 patients with T2DM who had undergone PCI between April 27, 2018, and February 5, 2021, at the Third People's Hospital of Chengdu (Sichuan, China), were included in the study (Figure 1). After applying the inclusion and exclusion criteria, the analysis included a total of 540 patients. The cohort comprised 364 (67.4%) males and 176 (32.6%) females, with ages ranging from 27 to 97 years. The inclusion criteria encompassed individuals older than 18 years, afflicted with T2DM and CAD who had undergone PCI. Additionally, comprehensive hospitalization records, examination data, interventional surgery details, and relevant imaging data should be readily available. The exclusion criteria comprised the absence of fibrinogen levels, SYNTAX scores, or follow-up data, along with hematological, tumorous, severe liver or renal diseases, pulmonary embolism, lower limb deep vein

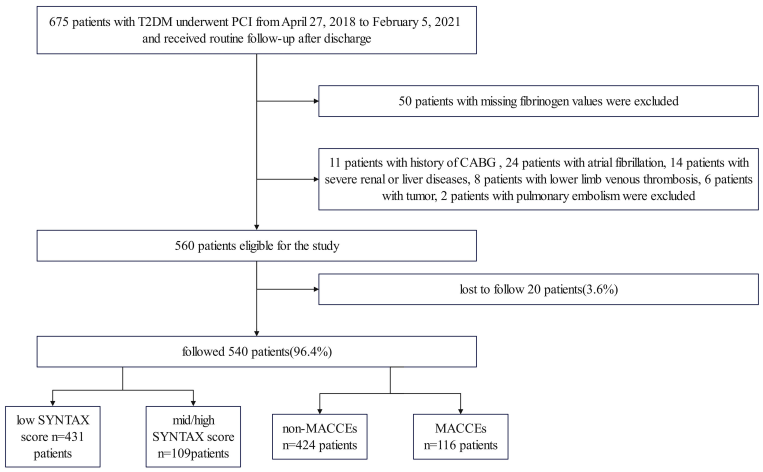

FIGURE 1
Study flowchart.

thrombosis, atrial fibrillation, and previous coronary artery bypass grafting. The study received approval from the local ethics committee and followed the Declaration of Helsinki guidelines, including obtaining informed consent from participants.

Data on medical history, smoking status, sociodemographic information, laboratory results and procedural details of patients were extracted from their electronic medical records. The patients were followed up at 3, 6, and 12 months, and subsequently annually thereafter, via outpatient visits or phone interviews which were conducted to collect follow-up data. For patients who adhered to their prescribed outpatient clinic visits, clinical outcomes were recorded during these appointments. Any patients not present at these scheduled visits were contacted via telephone, at the corresponding time intervals, to determine whether they had experienced any serious incidents such as death, stroke, recurrent myocardial infarction, or revascularization events. In cases of patients encountering severe events — such as a recurrence of myocardial infarction or repeat revascularization — and admitted to the Third People's Hospital of Chengdu, their pertinent data during this subsequent hospitalization were gathered to uphold the precision of the study outcomes. The median follow-up duration was recorded as 18.5 months, while the interquartile range for the follow-up period was found to be 14.4-22.6 months. The primary endpoint is major cardiovascular and cerebrovascular adverse events (MACCEs), defined as a composite of all-cause death (cardiac or non-cardiac), recurrent myocardial infarction, unplanned revascularization, and stroke. The secondary endpoints included all-cause death, cardiac death, recurrent myocardial infarction, unplanned revascularization, and stroke.

T2DM was defined according to the criteria established by the American Diabetes Association (ADA) (26). Hypertension was defined as systolic blood pressure (SBP) above 140 mmHg and/or diastolic blood pressure (DBP) above 90 mmHg or the use of antihypertensive medications (27). Medical history data included prior occurrences of hypertension, percutaneous coronary intervention, heart failure, chronic obstructive pulmonary disease, stroke, peripheral arterial disease, and chronic kidney disease. Standard biochemical techniques were employed at the Clinical Laboratory of the Third People's Hospital of Chengdu, China to measure laboratory parameters. Residual cholesterol levels (mmol/L) were determined by subtracting the sum of High-Density Lipoprotein Cholesterol (HDL-C) and Low-Density Lipoprotein Cholesterol (LDL-C) from the total cholesterol concentration (28). The left ventricular ejection fraction (LVEF) (29) was determined using the two-dimensional modified Simpson's method. A web-based tool named http://syntaxscore.com/ was used to compute the SYNTAX score (3). Two independent cardiologists blinded to the study protocol and baseline clinical characteristics performed this task using the procedural angiograms.

2.1.2 Statistical analysis

The normality of the samples was assessed using the Shapiro-Wilk test. Due to deviation from normal distribution, continuous variables were reported as median and interquartile range. Inter-group comparisons were performed using the non-parametric rank sum test. Categorical variables were presented as frequencies and percentages. Group differences were assessed using either the Chi-square test or Fisher's exact test.

Univariate and multivariate logistic regression analysis were adopted to determine the correlation between fibrinogen and the angiographic severity of CAD, classified as a SYNTAX score of ≤22 versus >22. Multivariate regression analyses included variables that had an unadjusted P of <0.05 after checking for collinearity. Odds ratios (ORs) with 95% confidence intervals (CIs) are used to describe the results. Restricted cubic splines (RCS) were employed to determine the potential dose-response relationship between the baseline fibrinogen level and CAD severity.

Univariate and multivariate COX survival analyses were used to identify the risk factors linked with MACCEs. Hazard ratios (ORs) with 95% confidence intervals (CIs) are used to describe the results. The Kaplan-Meier curve was constructed with end follow-up time of 1287 days used to construct survival curves and compared them

through log-rank tests for time-to-event analyses of clinical endpoints.

To determine the diagnostic performance of fibrinogen in detecting the severity of CAD and MACCEs in patients with T2DM, we calculated the area under the receiver operating characteristic (ROC) curve (AUROC). The Delong test was utilized to determine the statistical significance between fibrinogen and SYNTAX score in predicting MACCEs.

All statistical analyses were conducted using SPSS version 28.0 software (IBM Corporation, New York, NY, USA), MedCalc 20.100 and R version 4.2.3 software (R Foundation for Statistical Computing, Vienna, Austria). A $P < 0.05$ was considered statistically significant.

2.2 Results

2.2.1 Baseline characteristics between low (SYNTAX score ≤ 22) and mid/high risk (SYNTAX score > 22) groups

The baseline characteristics of 540 patients based on of the SYNTAX score are shown in Table 1. Compared to patients in the low SYNTAX score group, though in the mid/high SYNTAX score group had higher levels of heart rate, troponin-T, B-type natriuretic peptide, cystatin C, cholesterol, residual cholesterol, apolipoprotein B, homocysteine, fibrinogen, and D-dimer, and lower levels of albumin, direct bilirubin, indirect bilirubin, red blood cell count, hemoglobin, and LVEF. Patients with mid/high SYNTAX score also had a larger number and length of stents. Furthermore, these

TABLE 1 Baseline characteristics between low (SYNTAX score ≤ 22) and mid/high risk (SYNTAX score > 22) groups [Median (IQR)].

Variables	Syntax scores ≤ 22 (n=431)	Syntax scores > 22 (n=109)	P
Male, n (%)	290(67.3)	73(67.0)	0.950
Age, years	69(62,76)	69(61,78)	0.653
BMI, kg/m^2	24.52 (22.76,26.82)	24.22(22.74,26.38)	0.514
Hypertension, n (%)	324(75.2)	77(70.6)	0.334
Previous PCI, n (%)	47(10.9)	7(6.4)	0.163
Previous heart failure, n (%)	19(4.4)	7(6.4)	0.380
COPD, n (%)	13(3.0)	2(1.8)	0.731
Previous stroke, n (%)	27(6.3)	5(4.6)	0.508
Peripheral arterial disease, n (%)	7(1.6)	0	0.180
Chronic kidney disease, n (%)	16(3.7)	9(8.3)	0.044
Smoking, n (%)	200(46.5)	45(40.9)	0.950
SBP, mmHg	133 (119.75,147.00)	132(117.75,150.00)	0.705
DBP, mmHg	77(67.00,85.00)	77(69.75,87.00)	0.243
Heart rate, bpm	77.50 (69.00,88.25)	80(71.00,90.00)	0.017
Troponin-T, pg/ml	29.12 (12.16,402.88)	137.50 (18.06,1256.00)	<0.001
BNP, pg/ml	97.40 (40.70,291.00)	210.10(53.20,724.95)	<0.001
Creatinine, umol/l	76.80 (64.00,95.70)	80.75(64.63,108.40)	0.117
Uric acid, umol/l	367.10 (295.35,439.85)	372.00 (302.00,450.10)	0.542
Cystatin c, mg/l	1.18(0.98,1.47)	1.30(1.04,1.71)	0.019
FBG, mmol/l	7.90(6.11,10.60)	7.91(6.15,10.88)	0.531
HbA1c, mmol/l	7.50(6.70,8.70)	7.70(6.70,9.00)	0.546
Triglycerides, mmol/l	1.60(1.13,2.30)	1.70(1.16,2.79)	0.345
Cholesterol, mmol/l	4,18(3.46,4.97)	4.31(3.69,5.59)	0.047
HDL-C, mmol/l	1.10(0.91,1.27)	1.11(0.94,1.31)	0.269
LDL-C, mmol/l	2.53(1.95,3.13)	2.65(2.11,3.46)	0.231
Residual cholesterol, mmol/l	0.52(0.35,0.75)	0.62(0.43,1.31)	<0.001
Lipoprotein(a), mg/l	94.30 (48.10,265.80)	112.40(51.95,265.50)	0.577
Apo(A), mmol/l	1.21(1.04,1.37)	1.18(1.02,1.42)	0.834
Apo(B), mmol/l	0.80(0.61,1.01)	0.87(0.70,1.12)	0.027
Homocysteine, umol/l	13.30 (10.50,17.85)	14.25(10.68,18.98)	0.318
Albumin, g/l	40.00 (37.10,42.52)	37.45(35.10,40.93)	<0.001
AST, IU/l	25.05 (18.23,42.33)	24.45(17.53,42.23)	0.739
ALT, IU/l	25.70 (17.90,40.60)	23.75(15.25,41.98)	0.191
Direct bilirubin, umol/l	2.69(2.04,3.78)	2.37(1.75,3.26)	0.021
Indirect bilirubin, umol/l	9.92(7.48,13.24)	8.91(6.77,11.71)	0.030
Fibrinogen, g/l	3.52(3.00,4.34)	3.99(3.32,4.72)	<0.001
D-dimer, mg/l	0.34(0.20,0.65)	0.50(0.28,0.93)	<0.001
PT, s	13.70 (12.50,16.50)	14.20(12.50,16.85)	0.421
INR	0.95(0.91,1.01)	0.95(1.91,1.01)	0.892
APTT, s	37.90 (34.93,41.10)	36.45(33.93,40.33)	0.112

(Continued)

TABLE 1 Continued

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TABLE 1 Continued

Variables	Syntax scores ≤ 22 (n=431)	Syntax scores > 22 (n=109)	P
RBC count, *10^12/l	4.37(3.97,4.75)	4.30(3.63,4.58)	0.009
Hemoglobin, g/l	135.00 (123.00,147.00)	127.00 (108.75,140.75)	<0.001
WBC count, *10^9/l	6.98(5.72,8.89)	7.11(5.92,9.27)	0.396
Platelet count, *10^9/l	161.00 (131.00,200.00)	173.50 (138.75,207.00)	0.067
LVEF, n (%)	58.00 (53.00,62.00)	55.00(43.00,60.00)	<0.001
AMI, n (%)	174(40.4)	65(59.6)	<0.001
Diagnosis, n%			<0.001
SAP	69(16.0)	10(9.2)	
UA	188(43.6)	34(31.2)	
NSTEMI	79(18.3)	38(34.9)	
STEMI	95(22.0)	27(24.8)	
Angiographic data			
LM, n (%)	10(2.3)	14(12.8)	<0.001
MVD, n (%)	298(69.1)	99(90.8)	<0.001
Calcified lesions, n (%)	54(12.7)	41(39.4)	<0.001
Thrombosis, n (%)	39(9.2)	11(10.6)	0.667
Long lesion, n (%)	86(20.0)	36(33.0)	0.004
CTO, n (%)	67(15.8)	47(45.2)	<0.001
Number of stents	1(1,2)	2(1,3)	<0.001
Length of stents, mm	32.00 (20.00,49.50)	52.00(33.00,79.00)	<0.001

Data are presented as median (IQR) or n (%). BMI, body mass index; PCI, percutaneous coronary intervention; COPD, chronic obstructive pulmonary disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; BNP, B-type natriuretic peptide; FBG, fasting blood glucose; HbA1C, glycosylated hemoglobin A1c; HDL-C, High-Density Lipoprotein Cholesterol, LDL-C, Low-Density Lipoprotein Cholesterol; Apo(A), Apolipoprotein A; Apo (B), Apolipoprotein B; AST, aspartate transaminase; ALT, alanine transaminase; PT, prothrombin time; INR, international normalized ratio; APTT, activated partial thromboplastin time; RBC count, red blood cell count; WBC count, white blood cell count; LVEF, left ventricular ejection fraction; AMI, acute myocardial infarction; SAP, stable angina pectoris, UA, unstable angina; NSTEMI, non-ST-segment elevation myocardial infarction; STEMI, ST-segment elevation myocardial infarction; LM, left main disease; MVD, multivessel disease; CTO, chronic total occlusion.

patients had higher prevalence rates of chronic kidney disease, acute myocardial infarction (AMI), left main lesion, multivessel disease (MVD), calcified lesions, thrombosis, long lesion, and chronic total occlusion (CTO).

2.2.2 The correlation between fibrinogen and severity of CAD inT2DM patients

Univariate and multivariate logistic regression analysis of the association between the multiple characteristics and mid/high SYNTAX score are shown in Table 2. As noted in Table 2, the univariate logistic regression analysis indicated that heart rate, troponin-T, B-type natriuretic peptide, cholesterol, residual cholesterol, albumin, indirect bilirubin, fibrinogen, hemoglobin and LVEF were identified as potential risk factors for having a mid/high SYNTAX score (SYNTAX score > 22). Multivariate analysis was performed to assess the significant predictors identified through univariate screening (univariate $P < 0.05$). Cholesterol, as a component of the residual cholesterol, was not included in the multivariable logistic regression model in order to avoid any potential interactions. Furthermore, chronic kidney disease (OR, 2.329; 95%CI 1.000-5.433, $p = 0.050$) also was included in the multivariable logistic regression model. After checking for collinearity, the multivariate logistic regression analysis revealed that fibrinogen was an independent predictor of a mid/high SYNTAX score (SYNTAX score > 22, OR,1.184; 95% CI 1.022-1.373, $P = 0.025$).

The spearman's correlation analysis revealed an extremely weak positive correlation between fibrinogen and SYNTAX scores ($r = 0.109$, $P = 0.011$). However, the RCS results indicated that there was a potential dose-response relationship between fibrinogen and the risk of a mid/high SYNTAX score, as shown in Figure 2. Further testing uncovered a non-linear correlation between fibrinogen and SYNTAX score (overall model validity: total: $X^{2\ =}$ 12.23, $P = 0.0022$, nonlinear: $X^{2\ =}$ 8.15, $P = 0.0043$).

The receiver operating characteristic (ROC) curve analysis exhibited that fibrinogen predicted the mid/high SYNTAX score, with an area under the curve (AUC) of 0.610, 95% confidence interval (CI) 0.567-0.651, and $p = 0.0002$ (Figure 3). Fibrinogen's optimal cut-off value for predicting the mid/high SYNTAX score, with a maximum sensitivity of 57.8% and specificity of 61.7%, was 3.79g/L. This cut-off value divided patients into two groups: those with fibrinogen levels ≤ 3.79g/L and those with levels > 3.79g/L (Supplementary Material Table S1). Patients with fibrinogen levels > 3.79g/L exhibited significantly greater prevalence of previous stroke and chronic kidney disease when compared to those with fibrinogen levels ≤ 3.79g/L. Moreover, the fibrinogen > 3.79g/L group showed significantly higher levels of heart rate, troponin-T, B-type natriuretic peptide, creatinine, cystatin C, fasting blood glucose, lipoprotein(a), apolipoprotein B, D-dimer, white blood cell count, platelet count, the SYNTAX score, number of stents, and stent length, but significantly lower levels of albumin, RBC count, hemoglobin, and LVEF. Additionally, patients in the higher fibrinogen group had more frequent cases of AMI, calcified lesions, and MACCEs.

2.2.3 Baseline characteristics between MACCEs and non-MACCEs

During the median follow-up time 18.5 months, 116 (21.5%) out of 540 patients reached a clinical endpoint - including all-cause deaths in 35 patients (6.5%), recurrent myocardial infarction in 15 patients (2.8%), unplanned revascularization in 60 patients (11.1%), and stroke in 23 patients (4.3%). Furthermore, 17 of the patients demonstrated several endpoints as classified in the MACCEs. The

TABLE 2 Univariate and multivariate logistic regression analysis of syntax.

Variables	Univariate analysis		Multivariate analysis	
	OR (95%CI)	P	OR (95%CI)	P
Chronic kidney disease	2.329(1.000-5.433)	0.050	1.049(0.379-2.899)	0.927
Heart rate	1.0180(1.004-1.033)	0.012	1.010(0.994-1.027)	0.214
Troponin-T	1.000(1.000-1.000)	0.031	1.000(1.000-1.000)	0.952
BNP	1.001 (1.000-1.001)	0.029	1.001(1.000-1.001)	<0.001
Cystatin c	0.994(0.957-1.031)	0.734		
Cholesterol	1.262(1.079-1.477)	0.004	/	/
Residual cholesterol	2.254(1.417-3.584)	<0.001	2.293(1.423-3.696)	<0.001
Apo(B)	1.026(0.871-1.209)	0.759		
Albumin	0.946(0.906-0.988)	0.027	1.006(0.956-1.006)	0.121
Direct bilirubin	0.886(0.776-1.010)	0.071		
Indirect bilirubin	0.947(0.903-0.993)	0.034	0.954(0.905-1.006)	0.080
Fibrinogen	1.167(1.020-1.335)	0.025	1.184(1.022-1.373)	0.025
D-dimer	0.994(0.962-1.027)	0.709		
RBC count	1.009(0.985-1.033)	0.477		
Hemoglobin	0.986(0.977-0.994)	0.001	0.990(0.978-1.002)	0.105
LVEF	0.963(0.943-0.997)	<0.001	0.958(0.936-0.981)	<0.001

Data are presented as OR (95%CI). Abbreviations as shown in Table 1.

baseline characteristics of the patients are provided in Table 3. Among the MACCEs group, there was a higher occurrence of chronic kidney disease, MVD, calcified lesions, and CTO. The MACCEs group also exhibited elevated levels of heart rate, creatinine, uric acid, cystatin C, lipoprotein (a), homocysteine, fibrinogen, and D-dimer, in addition to a high SYNTAX score. In contrast, the MACCEs group had a low level of alanine aminotransferase, aspartate aminotransferase and LVEF ($P < 0.05$).

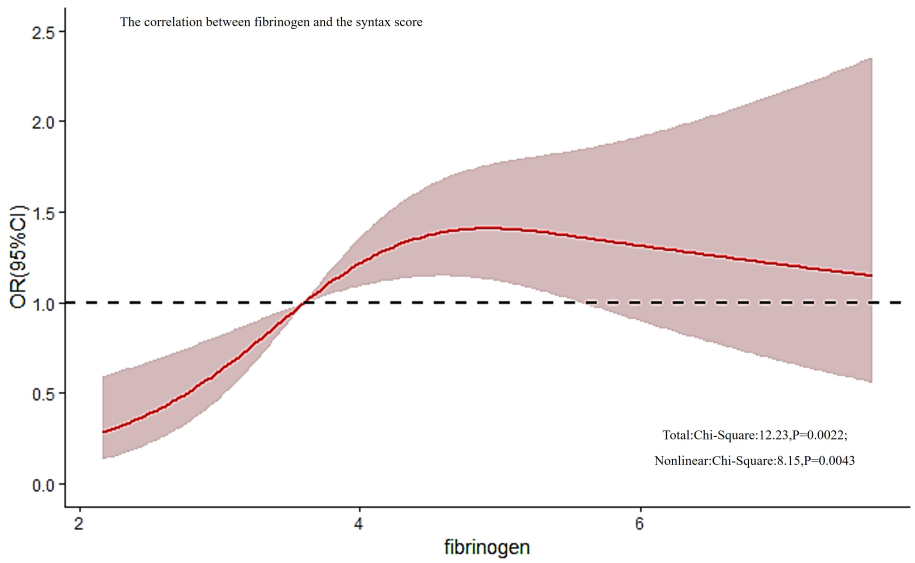

FIGURE 2
The correlation between fibrinogen and the SYNTAX score. RCS analysis found that there was a non-linear correlation between fibrinogen and SYNTAX score (overall model validity: total: $X^2 = 12.23$, P = 0.0022, nonlinear: $X^2 = 8.15$, P = 0.0043). RCS, restricted cubic spline; SYNTAX, score Synergy Between Percutaneous Coronary Intervention score.

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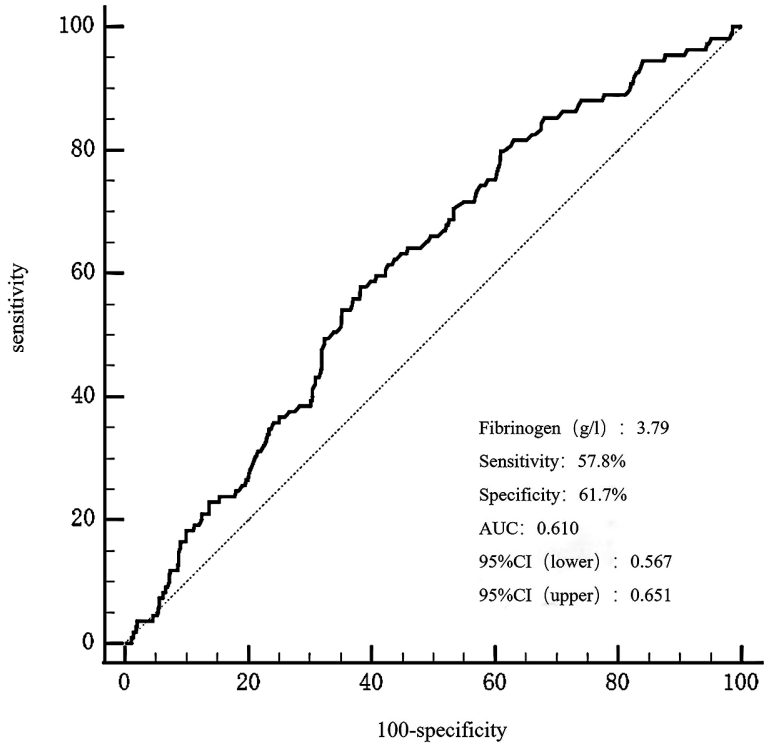

FIGURE 3
The ROC curves for predicting a mid/high SYNTAX score by fibrinogen. The area under the ROC curve of the fibrinogen for predicting a mid/high SYNTAX score (> 22) was 0.610 (95% CI 0.567–0.651, P = 0.0002), respectively. ROC, curve receiver operating characteristic curve; SYNTAX, score Synergy Between Percutaneous Coronary Intervention score.

TABLE 3 Baseline characteristics between MACCEs and non-MACCEs [Median (IQR)].

Variables	Non-MACCEs (n=424)	MACCEs (n=116)	P
Male, n (%)	288(67.9)	76(65.5)	0.624
Age, years	69(61,76)	69(61.25,78)	0.653
Hypertension, n (%)	313(75.8)	88(75.9)	0.656
Previous PCI, n (%)	41(9.7)	13(11.2)	0.625
Previous heart failure, n (%)	17(4.0)	9(7.8)	0.095
COPD, n (%)	12(2.8)	3(2.6)	0.887
Previous stroke, n (%)	29(6.8)	3(2.6)	0.086
Peripheral arterial disease, n (%)	7(1.7)	0	0.164
Chronic kidney disease, n (%)	15(3.5)	10(8.7)	0.020
Smoking, n (%)	190(44.5)	55(46.6)	0.791
SBP, mmHg	132(119,148)	138(120,149.25)	0.168
DBP, mmHg	77(67.50,85)	76(68.75,84)	0.882
Heart rate, bpm	78(67.75,87)	80(72,90)	0.010
Troponin-T, pg/ml	29.13(12.11,458.30)	48.91 (16.47,402.88)	0.217
BNP, pg/ml	100.85(42.05,348.73)	151.35 (48.68,656.20)	0.072
Creatinine, umol/l	76.60(63.70,94.80)	82.20 (69.00,116.10)	0.006
Uric acid, umol/l	362.40(293.45,435.15)	392.40 (313.58,469.48)	0.014
Cystatin c, mg/l	1.16(0.98,1.44)	1.34(1.05,1.82)	<0.001
FBG, mmol/l	7.96(6.12,10.99)	7.51(6.05,10.20)	0.231
HbA1c, mmol/l	7.60(6.70,8.70)	7.50(6.60,9.30)	0.586
Triglycerides, mmol/l	1.61(1.12,2.39)	1.58(1.19,2.40)	0.792
Cholesterol, mmol/l	4.26(3.45,5.16)	4.12(3.53,5.03)	0.784
HDL-C, mmol/l	1.10(0.92,1.28)	1.09(0.93,1.28)	0.934
LDL-C, mmol/l	2.56(1.95,3.21)	2.49(2.03,3.11)	0.618
Residual cholesterol, mmol/l	0.55(0.36,0.78)	0.56(0.40,0.79)	0.712
Lipoprotein(a), mg/l	89.95(45.73,264.03)	132.15 (63.90,299.45)	0.022

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TABLE 3 Continued

Variables	Non-MACCEs (n=424)	MACCEs (n=116)	P
Apo(A), mmol/l	1.22(1.04,1.37)	1.19(1.03,1.41)	0.973
Apo(B), mmol/l	0.82(0.62,1.04)	0.82(0.67,1.03)	0.955
Homocysteine, umol/l	13.00(10.30,17.60)	15.10 (11.25,20.73)	0.005
Albumin, g/l	39.80(36.80,42.43)	39.05 (36.00,41.30)	0.085
AST, IU/l	25.40(18.40,44.85)	22.45 (16.60,31.33)	0.014
ALT, IU/l	25.70(17.90,41.98)	21.30 (15.40,35.60)	0.018
Direct bilirubin, umol/l	2.55(1.92,3.77)	2.78(2.06,3.50)	0.605
Indirect bilirubin, umol/l	9.85(7.39,12.86)	9.20(7.39,11.75)	0.285
Fibrinogen, g/l	3.52(3.01,4.27)	3.94(3.28,4.97)	<0.001
D-dimer, mg/l	0.41(0.26,0.77)	0.46(0.30,0.97)	0.041
PT, s	13.80(12.50,16.70)	13.60 (12.52,16.30)	0.890
INR	0.95(0.91,1.00)	0.95(0.92,1.01)	0.332
APTT, s	37.60(34.80,40.75)	37.95 (34.73,42.28)	0.550
RBC count, *10^12/l	4.37(3.97,4.70)	4.31(3.72,4.68)	0.170
Hemoglobin, g/l	133.00(121.50,146.00)	131.50 (111.75,143.25)	0.085
WBC count, *10^9/l	6.97(5.64,9.01)	7.15(5.86,8.92)	0.526
Platelet count, *10^9/l	163.00(131.00,204.00)	170.00 (135.00,204.00)	0.463
LVEF, %	58(52,62)	56(45,61)	0.006
Syntax score	14(8,20)	19(12,27)	<0.001
AMI, n%	186(43.9)	53(45.7)	0.726
Diagnosis, n%			0.854
SAP	64(15.1)	15(12.9)	
UA	174(41.0)	48(41.4)	
NSTEMI	89(21.0)	28(24.1)	
STEMI	97(22.9)	25(21.6)	
Angiographic data			
LM, n (%)	16(3.8)	8(6.9)	0.148
MVD, n (%)	294(69.3)	103(88.8)	<0.001
Calcified lesions, n (%)	64(15.1)	31(29.5)	<0.001
Thrombosis, n (%)	41(9.7)	9(8.6)	0.725
Long lesion, n (%)	95(22.4)	27(23.3)	0.843
CTO, n (%)	79(18.7)	35(33.3)	<0.001
Number of stents	1(1,2)	1(1,2)	0.117
Length of stents, mm	33.00(20.00,56.00)	33(23.00,56.00)	0.261

(Continued)

TABLE 3 Continued

Data are presented as median (IQR) or n (%). Abbreviations as shown in Table 1.

2.2.4 The correlation between fibrinogen and MACCEs

Univariate Cox regression analysis was conducted on numerous variables, including chronic kidney disease, heart rate, creatinine, uric acid, cystatin C, lipoprotein (a), homocysteine, AST, ALT, fibrinogen, D-dimer, LVEF, the SYNTAX score, MVD, calcified lesions, and CTO ($P < 0.05$). Age and female gender were included in the analysis as potential risk factors for MACCEs. The results are shown in Table 4. Based on the outcomes of the univariate Cox regression analysis, multiple factors, including chronic kidney disease, creatinine, uric acid, alanine aminotransferase, aspartate aminotransferase, fibrinogen, LVEF, the SYNTAX score, MVD, calcified lesions, and CTO, were included in the multivariate model ($P < 0.05$). Following adjustment for various confounding variables, fibrinogen (HR, 1.138; 95% CI 1.010-1.284, P= 0.034) was established as an independent risk factor for MACCEs.

The area under the receiver operating characteristic curve (AUROC) of fibrinogen in predicting MACCEs was 0.609 (95% CI 0.566-0.650, $P = 0.0002$), as presented in Figure 4. The optimal cut-off value for predicting MACCEs with maximal sensitivity (42.24%) and specificity (75.9%) was 4.28g/L. Patients were divided into two groups based on the optimal cutoff value of fibrinogen to predict MACCEs: low fibrinogen levels ≤ 4.28g/L and high fibrinogen levels > 4.28g/L (Supplementary Material Table S2). A greater occurrence of patients with a history of stroke and chronic kidney disease was observed in the high fibrinogen group. Comparatively, patients with high fibrinogen levels recorded increased levels of heart rate, troponin T, B-type natriuretic peptide, creatinine, cystatin C, fasting blood glucose, lipoprotein (a), D-dimer, international normalized ratio, activated partial thromboplastin time, white blood cell count, platelet count, the SYNTAX score, number of stents, and stent length. In contrast, this group had lower levels of apolipoprotein A, albumin, red blood cell count, hemoglobin, and LVEF.

Apparently, the higher fibrinogen group had a higher incidence of AMI and MACCEs ($P < 0.001$) (Supplementary Material Table S2). Patients with high fibrinogen levels had a significantly greater incidence of all-cause death ($P < 0.001$) and stroke ($P = 0.030$). Moreover, this group had a higher probability of cardiac death ($P = 0.002$). However, there was no apparent difference in unplanned revascularization and recurrent myocardial infarction occurrence between the two groups during the follow-up ($P > 0.05$). As illustrated in Kaplan-Meier analysis (Figure 5), the high-fibrinogen group recorded a much higher MACCEs rate (Log-Rank test: $P < 0.001$).

TABLE 4 Univariate and multivariate COX regression analysis of MACCEs.

Variables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P	HR (95%CI)	P
Chronic kidney disease	2.519(1.163-5.457)	0.019	1.202(0.388-3.724)	0.750
Age	1.000(0.982-1.019)	0.974		
Female	1.031(0.687-1.549)	0.882		
Heart rate	1.017(0.997-1.037)	0.632		
Creatinine	1.001(1.000-1.002)	0.013	1.000(0.999-1.002)	0.511
Uric acid	1.002(1.001-1.004)	0.006	1.002(1.000-1.004)	0.020
Cystatin c	1.008(0.992-1.024)	0.340		
Lipoprotein(a)	1.001(1.000-1.001)	0.069		
Homocysteine	1.013(0.997-1.029)	0.101		
AST	1.000(1.000-1.001)	0.037	0.999(0.998-1.002)	0.456
ALT	1.000(1.000-1.001)	0.030	0.999(0.997-1.002)	0.609
Fibrinogen	1.147(1.033–1.274)	0.010	1.138(1.010-1.284)	0.034
D-dimer	1.031(0.945-1.123)	0.495		
LVEF	0.976(0.959-0.994)	0.009	0.998(0.976-1.019)	0.834
Syntax score	1.073(1.048-1.099)	<0.001	1.051(1.023-1.081)	<0.001
MVD	2.938(1.608-5.366)	<0.001	1.874(0.995-3.530)	0.052
Calcified lesions	2.411(1.571-3.698)	<0.001	1.887(1.177-3.025)	0.008
CTO	1.891(1.253-2.8522)	0.002	1.283(0.800-2.057)	0.301

Data are presented as HR (95%CI). Abbreviations as shown in Table 1.

2.2.5 Subgroup analysis

Subgroup analysis showed that fibrinogen was independently associated with mid/high SYNTAX risk and MACCEs in patients with ACS (Supplementary Material Tables S3-S4). In the total patient study, fibrinogen was an independent predictor of MACCEs, all-cause death, and cardiac death in patients with diabetes and CAD after PCI. Further analysis in subgroup showed that the predictive value of fibrinogen for poor prognosis in ACS patients mainly came from patients with unstable angina pectoris. The results showed that after adjustment, fibrinogen was an independent predictor of MACCEs, all-cause death, and repeat revascularization in patients with unstable angina pectoris (Supplementary Material Table S4), and fibrinogen can predict the risk of mid/high SYNTAX in UA patients, as well as the risk of MACCEs, all-cause death, and cardiac death (Supplementary Material Table S5).

2.3 Discussion

The present study demonstrates that elevated fibrinogen levels are independently associated with higher coronary anatomical complexity (assessed using a SYNTAX score > 22) and an increased risk of major adverse cardiac and cerebrovascular events in patients with T2DM who undergo PCI.

Clinical guidelines recommend the utilization of the SYNTAX score as a reliable tool for evaluating the complexity of CAD in patients with multi-vessel lesions (30). Previous studies have indicated the predictive capability of certain biomarkers, such as the Triglyceride glucose index (31) and C-reactive protein (32), in relation to the SYNTAX score.

The pathogenesis of coronary atherosclerosis involves various local inflammatory mechanisms such as endothelial dysfunction, leukocyte migration, extracellular matrix degradation, and platelet activation (10). Fibrinogen, primarily synthesized by the liver, not only serves as a crucial factor in blood coagulation but also as an acute inflammatory mediator (33). It is observed that elevated fibrinogen levels are present in various chronic inflammatory conditions, including diabetes and atherosclerosis (34, 35). Fibrinogen plays an essential role in all stages of atherosclerosis development, from initial leukocyte recruitment to eventual formation of atherosclerotic plaques (36), leading to CAD or adverse cardiovascular events through various mechanisms, including increasing plasma viscosity, inducing reversible red blood cell aggregation, binding with receptors on platelet membranes to induce platelet aggregation, and the formation of fibrin and fibrin degradation products that stimulate smooth muscle cell proliferation and migration (37). Elevated fibrinogen levels may be due to acute complications of vascular disease caused by serious events, including acute thrombosis and enhanced

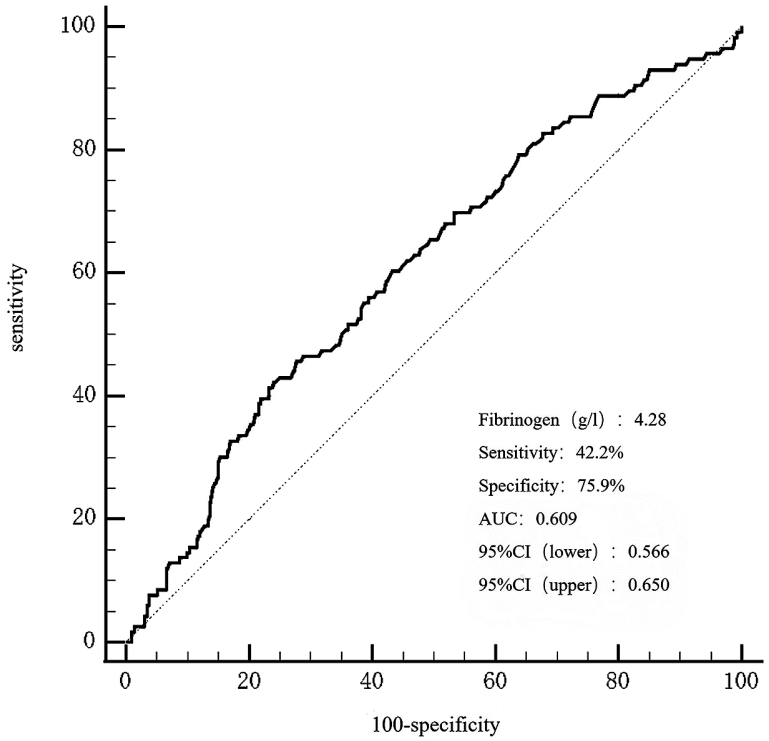

FIGURE 4
The ROC curves for predicting MACCEs. The area under the ROC curve of the fibrinogen for predicting MACCEs was 0.609 (95% CI 0.566–0.650, P < 0.001), respectively. ROC, curve receiver operating characteristic curve; MACCEs, major adverse cardiovascular and cerebrovascular events.

clotting activity resulting from impaired fibrinolytic function. In our study, high fibrinogen level group had a higher incidence of calcified lesions and an elevated trend of more multivessel disease and chronic total occlusion. It follows that high fibrinogen level may be a manifestation of more complex coronary atherosclerotic lesions. In addition, our findings showed an extremely weak positive correlation that was statistically significant (Spearman's correlation analysis: r = 0.109, P = 0.011) and a non-linear

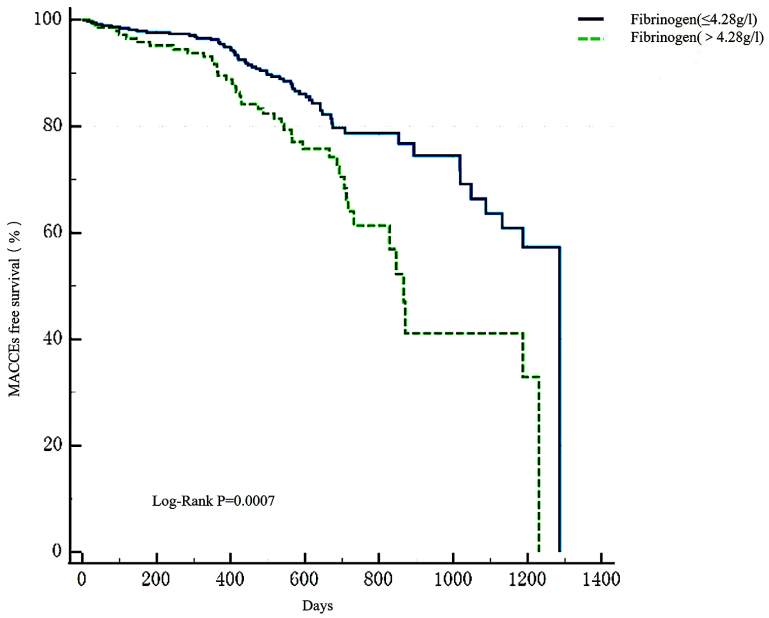

FIGURE 5
Kaplan-Meier survival analysis of MACCEs. Kaplan-Meier survival analysis found that there was a higher occurrence of MACCEs in the high-fibrinogen group (Log-Rank test: P = 0.0002). MACCEs, main adverse cardiovascular and cerebrovascular events.

correlation between fibrinogen and the SYNTAX scores (X^2 = 8.15, and P = 0.0043). It confirmed that fibrinogen can predict the risk of mid/high SYNTAX scores in patients with type 2 diabetes (OR, 1.184; 95% CI 1.022-1.373, P = 0.025). This point is supported by previous researches. Kurtul A et al. (24) showed plasma fibrinogen to be an independent predictor of intermediate-high syntax scores (OR 1.008, 95% CI: 1.005-1.010, p < 0.001). The ROC curve analysis showed that fibrinogen predicted the intermediate-high syntax scores of patients with diagnosis of ACS with an area under the curve of 0.812 (95% CI: 0.778-0.846). Tabakcı MM et al. (25) observed a positive linear correlation between plasma fibrinogen levels and the syntax score (Spearman's correlation analysis: r = 0.535, P <.001). They confirmed that fibrinogen could predict the complexity of coronary lesions in patients with stable angina pectoris (AUROC: 0.72, 95% CI 0.61-0.82, P < 0.001), even though merely 134 patients with stable angina pectoris were included. Contrarily, our subgroup analysis yielded a disparate result that might stem from differing study populations. In addition, while our study enlisted patients afflicted with both T2DM and CAD, it comprised only 79 patients with stable angina. Such a limited participant size might have restricted sample representativeness. In the context of ACS patients, fibrinogen's capability to forecast a mid/high SYNTAX score was predominantly observed in patients with unstable angina pectoris, rather than in patients suffering from myocardial infarction (Supplementary Material Tables S3, S5). This can be attributed to the significant correlation found between fibrinogen and the SYNTAX score in patients with ACS(P=0.005) and unstable angina pectoris(P=0.031), and this correlation was not significant in patients with myocardial infarction(P=0.071).

Earlier studies have established an association between fibrinogen levels and the occurrence (38) or adverse clinical outcomes of CAD, encompassing both stable CAD (39, 40) and ACS (22). Additionally, increased fibrinogen levels have been associated with adverse clinical results in patients undergoing percutaneous coronary intervention (PCI) (18, 22, 41). Despite Ferraro S et al. discovering no discernible correlation between fibrinogen levels and adverse clinical outcomes post-PCI in patients with ST-segment elevation myocardial infarction (STEMI) (42), Wasilewski J et al. (43) have demonstrated that high baseline fibrinogen concentration is an independently risk factor of no tissue reperfusion in STEMI treated with successful primary PCI. This phenomenon could potentially be attributed to changes in rheology caused by increased fibrinogen, including increased blood viscosity and enhanced platelet aggregation leading to heightened microcirculatory resistance.

In patients with stable CAD, fibrinogen has been independently associated with an increased occurrence of cardiovascular events in those with T2DM (23). The absence of this phenomenon in our study could potentially be attributed to an insufficient sample size(79 patients vs 1422 patients). Another notable observational study has reported that fibrinogen is associated with an increased risk of MACCEs, especially in patients with diabetes and prediabetes (39). In terms of patients who underwent PCI, Zhang L et al. (22) demonstrated a positive association between elevated fibrinogen levels and the occurrence of MACCEs in patients with ACS, particularly those with diabetes mellitus. Another large cohort study in CAD patients after PCI showed similar results (44), assessing long-term mortality rates for both all-cause and cardiac events. In this study, we observed a significant association between elevated fibrinogen levels and a high risk of MACCEs following PCI in patients with T2DM at a median follow-up time of 18.5 months, particularly a high risk of all-cause mortality. Stroke incidence was higher in the elevated fibrinogen group compared to the low fibrinogen group (P = 0.03); however, this difference may be due to baseline distinctions in stroke events (P = 0.04). Though there was a tendency towards an increase in unplanned revascularization and recurrent myocardial infarction in the elevated fibrinogen group, no statistically significant difference was observed in the incidence of unplanned revascularization (P = 0.318) or recurrent myocardial infarction (P = 0.102) between the two groups (Supplementary Material Table S1, Figure F1). Concurrently, the subgroup analysis indicated that fibrinogen's predictive capability for adverse prognosis predominantly originated from patients diagnosed with unstable angina pectoris (Supplementary Material Tables S4, S5). However, it bore no significant relation to adverse events like MACCEs found in patients with myocardial infarction. These findings are consistent with a previous large single-center study that focused on CAD patients who receive PCI (41) and another research study involving STEMI patients (42). However, the ADVANCE study, a case-cohort investigation involving 3,865 patients with T2DM and cardiovascular diseases or risk factors, indicated that only interleukin-6 presented a statistical significance in predicting macrovascular events and mortality following adjustment for IL-6, CRP, and fibrinogen (45). This difference may be attributed to the inclusion of patients with T2DM and one or more additional cardiovascular risk factors instead of T2DM and cardiovascular diseases. Moreover, Chinese and Indian subjects were excluded from the ADVANCE study, which could be another reason for the results disparity.

Studies have demonstrated the benefits of anti-inflammatory therapy in both chronic coronary disease (46) and ACS (47). In addition, in the ECAT Angina Pectoris Study (48), a prospective study for investigating the associations between base-line level of hemostatic factors and coronary events, coronary events were associated with higher fibrinogen levels, and in the case of high cholesterol levels, the risk of coronary events was still low as long as the fibrinogen level was controlled at a low level. The result is consistent with a pathogenetic role of impaired fibrinolysis, endothelial-cell injury, and inflammatory activity in the progression of coronary artery disease. Therefore, the role of fibrinogen in the pathogenesis of CAD and the promotion of atherosclerosis should be taken into account. Fibrinogen has been linked to mid/high SYNTAX risk and increased MACCEs risk among patients with T2DM after PCI. However, fibrinogen's predictive ability for MACCEs risk is not entirely dependent on the SYNTAX score's predictive capability. Firstly, we conducted a comparison between fibrinogen and the SYNTAX score to assess their ability in predicting MACCEs within this study population. The Delong test revealed no statistically significant difference (Z=1.162, P=0.245) in the predictive capacity of MACCEs between fibrinogen and the SYNTAX score. It suggested that fibrinogen possesses similar predictive power to the SYNTAX

score in forecasting MACCEs among patients with T2DM. And in our study, no significant difference was observed in residual SYNTAX score levels among patients with T2DM after revascularization, regardless of the fibrinogen-level groups (3.79g/l or 4.28g/l) (Supplementary Material Tables S1, S2) ($P > 0.05$). But the results demonstrated a higher incidence of MACCEs in the high fibrinogen level group. This shows fibrinogen could independently predict a high risk of MACCEs in patients with T2DM, even if the SYNTAX score was excluded. Given that the fibrinogen test is relatively simple and inexpensive, it could serve as a biomarker to identify high-risk patients for MACCEs among individuals with T2DM after undergoing PCI.

Research has demonstrated that smoking (49), sedentary (50), and an unhealthy diet (51) can raise fibrinogen levels, while exercise training can decrease levels (52). High-density lipoprotein cholesterol has been shown to have a negative relationship with fibrinogen (53, 54). Furthermore, studies have exhibited that both statins and fibrates can effectively diminish plasma fibrinogen levels, with fibrates appearing to be more successful (55). Previous studies have indicated that fibrinogen levels are higher in diabetic patients than in non-diabetic patients (34), therefore, it is worthwhile to investigate the potential benefits of lowering fibrinogen levels for patients withT2DM.

2.4 Limitations

This study has several limitations. Firstly, it is a single-center study with a small sample size, making it challenging to completely eliminate selection bias and confounding factors. To obtain more precise results, it is necessary to increase the sample size and design a prospective multicenter study. Secondly, the majority of patients in this study were only monitored via telephone. As such, the resultant follow-up findings may be influenced by variables such as the working status of the follow-up staff, the degree of patient collaboration, and awareness of the disease. Thirdly, it is important to note that this study focused solely on the baseline fibrinogen levels and did not consider the potential impact of antiplatelet or lipid-lowering medications on fibrinogen levels in the post-PCI period being studied. Finally, our study specifically focused on patients with T2DM who underwent PCI. Therefore, it may not be possible to generalize these findings to the wider population.

2.5 Conclusion

Elevated fibrinogen levels were associated with increased coronary anatomical complexity and a higher incidence of MACCEs after PCI in patients with T2DM. Therefore, fibrinogen levels hold potential as a noninvasive biomarker for predicting both coronary anatomical complexity and clinical prognosis in patients with T2DM, facilitating the early identification of individuals at high risk.

Data availability statement

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

Author contributions

HS: Writing – original draft. YC: Writing – original draft. QC: Writing – original draft. TY: Writing – original draft. CC: Writing – original draft. XC: Writing – original draft. SY: Writing – original draft. LQ: Writing – original draft. YL: Writing – original draft. SX: Writing – review & editing. LC: Writing – review & editing.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1287855/full#supplementary-material

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Undiagnosed diabetic retinopathy in Northeast China: prevalence and determinants

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Objective: To report the prevalence and contributing factors of undiagnosed diabetic retinopathy (DR) in a population from Northeastern China.

Subjects/Methods: A total of 800 subjects from the Fushun Diabetic Retinopathy Cohort Study were enrolled. A questionnaire assessing incentives and barriers to diagnosis of DR was administered. Logistic regression was used to identify clinical and sociodemographic factors associated with undiagnosed DR. In a prespecified subgroup analysis, we divided patients into vision-threatening diabetic retinopathy (VTDR) and non-VTDR (NVTDR) subgroups.

Results: Among 800 participants with DR, 712 (89.0%) were undiagnosed. Among 601 with NVTDR, 566 (94.2%) were undiagnosed. Among 199 with VTDR, 146 (73.4%) were undiagnosed. The risk factors affecting the timely diagnosis of NVTDR and VTDR exhibit significant disparities. In multivariate models, factors associated with undiagnosed VTDR were age over 60 years (*OR* = 2.966; 95% *CI* = 1.205-7.299; *P* = 0.018), duration of diabetes over 10 years (*OR* = 0.299; 95% *CI* = 0.118-0753; *P* = 0.010), visual impairment or blindness (*OR* = 0.310; 95% *CI* = 0.117-0.820; *P* = 0.018), receiving a reminder to schedule an eye examination (*OR* = 0.380; 95% *CI* = 0.163-0.883; *P* = 0.025), and the belief that "people with diabetes are unlikely to develop an eye disease" (*OR* = 4.691; 95% *CI* = 1.116-19.724; *P* = 0.035). However, none of the factors were associated with undiagnosed NVTDR (all $P \geq 0.145$).

Conclusion: Our research has uncovered a disconcerting trend of underdiagnosis in cases of DR within our population. Addressing determinants of undiagnosed DR may facilitate early detection.

KEYWORDS

diabetic retinopathy, epidemiological investigation, retinal screening, public health, vision

1 Introduction

Diabetic retinopathy (DR) stands as a predominant etiology of visual impairment among adults within the working age population who are afflicted with diabetes (1). Timely detection and judicious therapeutic interventions, such as pan-retinal photocoagulation (PRP) and anti-vascular endothelial growth factor (VEGF) injections, have been demonstrated to mitigate the risk of vision loss attributable to diabetes by 50% to 70% (2–4). However, the insidious nature of undiagnosed DR poses a significant clinical challenge, exacerbating the risk of adverse outcomes and complicating long-term management (5–7).

The efficacy of DR diagnosis is intrinsically linked to adherence to regular ophthalmic screenings. For individuals diagnosed with Type 2 Diabetes Mellitus (T2DM), immediate DR screening is imperative due to the likelihood of a pre-existing condition. Follow-up screenings are recommended annually for those with no detectable retinopathy, semi-annually to annually for mild to moderate non-proliferative diabetic retinopathy (NPDR), and quarterly for severe NPDR or proliferative diabetic retinopathy (PDR), with the latter necessitating potential expedited specialist consultation (8). In China, the burgeoning diabetes epidemic has catalyzed the initiation of regional DR screening initiatives, often orchestrated through synergistic collaborations among hospitals, community healthcare centers, and governmental agencies (9). These programs employ a spectrum of diagnostic approaches, from conventional clinical assessments to cutting-edge telemedicine platforms. Despite these efforts, the diagnostic yield remains suboptimal due to factors such as patient unawareness (10, 11), subpar screening protocols (12), limited access to healthcare resources (13), and socioeconomic and demographic disparities (13, 14).

In real-world clinical scenarios, the rate of early DR detection is disconcertingly low; an estimated 75% of DR cases in developed countries go undiagnosed (5, 15). The economic ramifications of this diagnostic gap are substantial, extending beyond the direct healthcare costs of managing advanced DR and its sequelae to include indirect costs related to productivity loss and compromised quality of life. Moreover, there is a conspicuous dearth of population-based epidemiological data on undiagnosed DR from developing nations. Hence, this study aims to elucidate the demographic, clinical, and behavioral determinants of undiagnosed DR in Northeast China, thereby offering actionable insights for enhancing the region's DR diagnostic and management strategies.

2 Materials and methods

2.1 Participants

This study was conducted as part of the Fushun Diabetic Retinopathy Cohort Study (FS-DIRECT) from July 2012 to May 2013. Detailed information on the study design, methodology, and baseline results can be found in previous publications (16). Succinctly, the FS-DIRECT included individuals with T2DM residing in the communities of Jiangjun Street, Fushun, Liaoning Province, China. Rigorous clinical diabetes mellitus evaluations of participants were meticulously sourced from the community health center prior to the study's initiation. Prospective candidates meeting the stipulated criteria were formally solicited for participation. Every individual diagnosed with DR from the FS-DIRECT was incorporated into our research. All participants provided signed consent forms, and the study was approved by the Institutional Review Board of Fushun Eye Hospital, adhering to the principles of the Declaration of Helsinki.

2.2 Socio-demographic and clinical data

All participants underwent clinical eye examinations, which involved assessments of presenting visual acuity (PVA), intraocular pressure (IOP), slit lamp examination, and fundus photography. Color fundus photography was performed on all subjects diagnosed with diabetes using a 45° nonmydriatic retinal camera (Kowa, VK-2, Tokyo, Japan). A stereoscopic macula image of each eye was captured by certified photographers after pupil dilation. The six fields of fundus photos were taken and defined as follows: Field 1 - center of the optic disc, Field 2 - center of the macula, Field 3 - temporal to the macula, Field 4 - temporal superior, Field 5 - temporal inferior, Field 6 - nasal to the optic disc (16). Individuals without DR, with ungradable retinal photographs, or with uncompleted questionnaires were excluded from the analysis.

2.3 Questionnaire

Interviews were conducted face-to-face in Chinese with all enrolled patients with T2DM to collect comprehensive socio-demographic data and medical conditions (16). A brief questionnaire, based on previous surveys (12, 17, 18), was utilized to gather information on the incentives and barriers by participants in attending regular retinopathy screenings conducted by certified ophthalmologists (Supplementary Table 1).

2.4 Assessment of DM

In accordance with the guidelines established by the American Diabetes Association (19), diabetes mellitus (DM) was diagnosed under the following criteria: a fasting plasma glucose (FPG) level of 7.0 mmol/L or greater; a value equal to or surpassing 11.1 mmol/L in the 2-hour oral glucose tolerance test (2-h OGTT); or the self-disclosure of prescribed diabetes medication utilization by participants.

2.5 Assessment of DR

We differentiate between two pivotal aspects concerning the diagnosis of DR: the objective clinical diagnosis and the subjective patient's awareness of their DR status.

The clinical diagnosis of DR was conducted via 6-field fundus photography during the enrolment phase. Photographs were independently reviewed in detail by two graders. In cases where there was a discrepancy in the assigned levels for each eye, a consensus was reached with a third grader. The grading protocols for DR were based on the Early Treatment Diabetic Retinopathy Study (ETDRS) adaptation of the modified Airlie House classification of DR (20). The following criteria were used for grading the eyes: mild to moderate non-proliferative DR (NPDR) was characterized as levels 31-47; severe NPDR (levels 53) and proliferative DR (PDR) encompassed levels 60-85. Vision-threatening diabetic retinopathy (VTDR) was defined as the presence of severe NPDR, PDR, or clinically significant macular edema (CSME), according to the definition by the Eye Diseases Prevalence Research Group (21). Non-VTDR (NVTDR) was defined as mild or moderate NPDR or diabetic macular edema (DME) that did not meet the threshold for CSME. CSME was defined according to the ETDRS definition as thickening of the retina at or within 500 μm of the center of the macula, hard exudate at or within 500 μm of the center of the macula if associated with adjacent retinal thickening, or a zone or zones of retinal thickening of at least 1 disc area that is located at least 1 disc diameter from the center of the macula (18).

2.6 Patient awareness of having DR

To clarify the participants' self-awareness of their condition, we categorized DR patients into two groups: 1) Diagnosed DR: Participants cognizant of their DR diagnosis; and 2) Undiagnosed DR: Participants oblivious to their DR condition (Supplementary Table 2).

The participants were asked the following two questions:

1. Have they ever received a diagnosis of DR or been informed by a doctor about eye diseases or eye problems related to their diabetes?
2. Have they ever undergone laser treatment for their diabetic eye disease?

Participants were considered undiagnosed for DR if they did not answer 'yes' to both questions. Participants with VTDR, were considered undiagnosed if they answered "no" to the first question and had no laser scars visible in retinal photography.

2.7 Assessment of visual acuity

PVA was measured for each participant using their current correction, such as glasses or contact lenses, at the time of the examination. The measurement was performed following the protocol of the ETDRS, using the logMAR visual acuity chart (Precision Vision, USA) at a distance of 4 meters for both eyes. The modified World Health Organization (WHO) definition of visual impairment (VI) was used, with LogMAR > 0.48 (20/60) to ≤1.30 (20/400) indicating VI, and LogMAR > 1.30 (20/400) indicating blindness (22). Unilateral VI or blind was categorized using the worse-seeing eye.

2.8 Statistical analyses

The baseline characteristics of participants in the FS-DIRECT were summarized using proportions to represent categorical factors. Chi-square tests were utilized to compare the characteristics of participants across different DR diagnosis statuses. Pairwise comparisons among multiple groups were conducted using Bonferroni correction.

Logistic regression models were then constructed to assess the associations between different classifications of PVA, specifically pertaining to the better-seeing eye and the worse-seeing eye, and the diagnosis status of DR. These associations were examined in both crude models and models adjusted for age, gender, and duration of diabetes. To determine the statistical significance of the variations in odds ratio estimates between the two PVA exposures and diagnosed DR, a cluster sandwich estimator was utilized.

To identify which items are associated with undiagnosed DR, we conducted a series of analyses. Firstly, we employed univariate analyses to explore the relationships between a range of demographic variables, clinical characteristics, barriers to attendance, and diagnosed DR. We then incorporated items demonstrating significant univariate associations into a multivariate logistic regression model. These analyses were performed for the overall population, as well as for two specific subgroups: non-VTDR (NVTDR) and VTDR. We also investigated whether there was a multiplicative interaction between each item correlated with the diagnosis status of NVTDR/VTDR and the predetermined variables such as age, sex, education level, income, occupation, duration of diabetes, and HBA1c levels. Analyses within subgroups defined by these variables were performed if the *P* value for interaction was <0.05. All statistical analyses were conducted using R software (version 4.0.4). A two-sided *P*-value of less than 0.05 was considered statistically significant.

3 Results

3.1 Patients demographics and diagnosis status by DR severity

This study included a total of 800 participants, of which 88 were diagnosed with DR and 712 were undiagnosed (Tables 1, 2). When stratified by DR severity, 53 (26.6%) of those with VTDR were previously diagnosed, whereas 38 (28.8%) of those with CSME and 43 (55.8%) of individuals with PDR were previously diagnosed. Notably, only 55 (5.8%) of those with NVTDR and 5 (6.5%) of those with severe NPDR had a prior DR diagnosis (Table 1).

3.2 Demographic factors and DR diagnosis

The majority of participants in both the diagnosed DR and undiagnosed DR groups were of Han Chinese ethnicity, had no religious affiliation, and were retired (Table 2). There were no statistically significant differences between diagnosed and undiagnosed DR groups in terms of age (P = 0.415), sex (P = 0.413), ethnicity (P = 0.930), religion (P = 0.966), education (P =

TABLE 1 Diagnosis status of different severity of diabetic retinopathy.

	N	Diagnosed	Undiagnosed
ALL DR	800	88.0 (11.0)	712.0 (89.0)
NVTDR	601	55.0 (5.8)	566.0 (94.2)
VTDR	199	53.0 (26.6)	146.0 (73.4)
CSME	132	38.0 (28.8)	94.0 (71.2)
Severe NPDR	77	5.0 (6.5)	72.0 (93.5)
PDR	77	43.0 (55.8)	34.0 (44.2)

Data are presented as frequency (%).
DR, diabetic retinopathy; NVTDR, non-vision-threatening diabetic retinopathy diabetic retinopathy; VTDR, vision-threatening diabetic retinopathy; CSME, clinically significant macular edema; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

0.568), marital status ($P = 0.258$), income ($P = 0.870$), glycosylated haemoglobin A1c ($P = 0.960$), low-density lipoprotein ($P = 0.102$) and total cholesterol ($P = 0.562$).

3.3 Duration of diabetes and DR diagnosis

In subsequent analysis, statistically significant differences were observed among the groups in terms of duration of diabetes ($P < 0.001$) and PVA ($P < 0.001$). Participants with a duration of DR for ≥ 15 years had a significantly higher proportion of diagnosed cases (21.0%) compared to those with a diagnosis of DR for < 5 years (6.8%) and those with DR for 6-10 years (7.1%).

3.4 Visual Impairment and DR Diagnosis

Based on unilateral classifications of PVA, participants without VI had a lower proportion of diagnosed DR (7.4%) compared to those with VI (16.1%, $P = 0.002$) or blindness (24.3%, $P < 0.001$). Upon employing bilateral classifications, no discernible difference was observed in the proportion of individuals diagnosed with DR between patients experiencing unilateral VI or blindness and those with bilateral VI or blindness. Table 3 demonstrated that VI or blindness, when based on the better-seeing eye, was associated with a reduced prevalence of undiagnosed DR (*OR* = 0.376, 95%*CI*: 0.227, 0.624), and an even more attenuated proportion when estimated upon the worse-seeing eye (*OR* = 0.358, 95%*CI*: 0.229, 0.561). Moreover, the difference between the ORs calculated from worse-seeing or better-seeing eyes was not statistically significant ($P = 0.978$) (Table 3). And these associations remained unaltered after adjusting for age, sex, and duration of diabetes.

3.5 Factors associated with undiagnosed DR

Items that were significantly associated with undiagnosed DR were analyzed in all DR patients and several subgroups. Figure 1 presented the items related to undiagnosed DR, stratified by the severity of DR. Among all DR patients, individuals who only sought eye care when experiencing vision problems were associated with a higher prevalence of undiagnosed DR (*OR* = 2.224; 95% *CI* = 1.015-4.877; $P = 0.046$). Conversely, patients with a diabetes duration of over 10 years (*OR* = 0.409; 95% *CI* = 0.244-0.688; $P = 0.001$), VI or blindness in the worse eye (*OR* = 0.265; 95% *CI* = 0.146-0.483; $P < 0.001$), and those who received a reminder to schedule an eye examination (*OR* = 0.440; 95% *CI* = 0.267-0.726; $P = 0.001$) were associated with a lower likelihood of undiagnosed DR. In the subgroup with VTDR, these relationships were attenuated but remained statistically significant for the duration of diabetes over 10 years (*OR* = 0.299; 95% *CI* = 0.118-0753; $P = 0.010$), VI or blindness in the worse eye (*OR* = 0.310; 95% *CI* = 0.117-0.820; $P = 0.018$), and receive a reminder to schedule an eye examination (*OR* = 0.380; 95% *CI* = 0.163-0.883; $P = 0.025$). Age over 60 years (*OR* = 2.968; 95% *CI* = 1.197-7.357; $P = 0.019$) and the belief that "people with diabetes are unlikely to develop an eye disease" (*OR* = 4.691; 95% *CI* = 1.116-19.724; $P = 0.035$) were associated with a reduced probability of receiving a diagnosis in patients with VTDR. In the subgroup with NVTDR, none of the factors were associated with undiagnosed NVTDR.

3.6 Interaction effects

We did not observe any clinical characteristics that modified the associations between risk factors and undiagnosed VTDR. However, we did identify a significant interaction between education level and duration of diabetes (P interaction = 0.025), with the inverse associations with undiagnosed NVTDR being stronger in patients with a middle school education or below (*OR* = 0.242; 95% *CI* = 0.081-0.723; $P = 0.011$; Figure 2).

4 Discussion

Our community-based study provides the prevalence and contributing factors of undiagnosed DR in Northeast China. Our study revealed that 89.0% of DR patients remained undiagnosed. This finding aligns with the Singapore Epidemiology of Eye Diseases Survey (SEED) (83.3%) (5) and the National Health and Nutrition Examination Survey (NHANES) in the USA (70.1%) (23). Furthermore, we identified an even higher proportion of undiagnosed cases among patients with NVTDR (93.5%). 71.2% of patients with VTDR were unaware of their condition, which significantly exceeds the 27.3% of undiagnosed VTDR cases reported by the SEED (5) and the 19% reported by the Diabetic Retinopathy Inpatient Study (DRIPS) (6). These findings highlight the inadequate screening efforts for DR in our population.

Our research sought to uncover the reasons behind the high rates of undiagnosed DR. We found that patients without VI were more likely to be undiagnosed, which is consistent with the findings of the SEED (5). The asymptomatic nature of DR often results in individuals presenting with advanced stages of the disease when they first consult an ophthalmologist, which is a concerning trend (15, 24–26). Another noteworthy finding is that subjective visual

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TABLE 2 Baseline characteristics of participants.

	Total (N = 800)	Diagnosed DR (N = 88)	Undiagnosed DR (N = 712)	*P* Value*
Age				0.415
<50	97 (12.1)	16 (18.2)	81 (11.4)	
50-60	279 (34.9)	31 (35.2)	248 (34.8)	
60-70	298 (37.3)	29 (33.0)	269 (37.8)	
≥70	126 (15.8)	12 (13.6)	114 (16.0)	
Sex				0.413
male	314 (39.3)	31 (35.2)	283 (39.7)	
female	486 (60.8)	57 (64.8)	429 (60.3)	
Ethnicity				0.930
Han	751 (94.1)	83 (94.3)	668 (94.1)	
Other	47 (5.9)	5 (5.7)	42 (5.9)	
Occupation				0.335
Employed	90 (11.3)	14 (15.9)	76 (10.7)	
Unemployed	72 (9.0)	8 (9.1)	64 (9.0)	
Retired	638 (79.8)	66 (75.0)	572 (80.3)	
Religion				0.966
No	698 (87.4)	77 (87.5)	621 (87.3)	
Yes	101 (12.6)	11 (12.5)	90 (12.7)	
Education				0.568
Illiteracy or primary school	518 (64.8)	54 (62.1)	464 (65.2)	
High school or above	281 (35.2)	33 (37.9)	248 (34.8)	
Marital status				0.258
Without a partner	134 (16.8)	11 (12.5)	123 (17.3)	
With a partner	666 (83.3)	77 (87.5)	589 (82.7)	
Income (Yuan/Month)				0.870
<3000	327 (41.2)	37 (42.0)	290 (41.1)	
≥3000	466 (58.8)	51 (58.0)	415 (58.9)	
Duration of diabetes (years)				<0.001
<5	205 (25.6)	14 (15.9)	191 (26.8)	
6-10	254 (31.8)	18 (20.5)	236 (33.1)	
11-15	184 (23.0)	23 (26.1)	161 (22.6)	
≥15	157 (19.6)	33 (37.5)	124 (17.4)	
Mean HbA1c (%)				0.960
<7.0	241 (30.3)	27 (31.0)	214 (30.2)	
7.0-9.0	288 (36.2)	32 (36.8)	256 (36.1)	
≥9.0	267 (33.5)	28 (32.2)	239 (33.7)	
LDL (mmol/L)				0.102
<2.6	188 (23.4)	26 (29.9)	154 (21.7)	

(Continued)

TABLE 2 Continued

	Total (N = 800)	Diagnosed DR (N = 88)	Undiagnosed DR (N = 712)	*P* Value*
≥2.6	616 (76.6)	61 (70.1)	555 (78.3)	
TC (mmol/L)				0.562
<5.18	314 (39.4)	37 (42.5)	277 (39.1)	
≥5.18	482 (60.6)	50 (57.5)	432 (60.9)	
Unilateral Visual status				<0.001
No VI	538 (67.3)	40 (45.5)	498 (69.9)	
VI	192 (24.0)	31 (35.2)	161 (22.6)	
Blind	70 (8.8)	17 (19.3)	53 (7.4)	
Bilateral Visual status				<0.001
No VI	538 (67.3)	40 (45.5)	498 (69.9)	
Unilateral VI or blind	139 (17.4)	22 (25.0)	117 (16.4)	
Bilateral VI or blind	123 (15.4)	26 (29.5)	97 (13.6)	

Data are presented as frequency (%).
*P value with Chi-square tests.
DR, diabetic retinopathy; HbA1c, glycosylated haemoglobin A1c; LDL, low-density lipoprotein; TC, total cholesterol; VI, visual impairment.

disturbance was not included in the final model, indicating that objective visual impairment is more of a predictor of diagnosed DR than subjective visual function. This could be due to the fact that in the early stage of DR, patients may already experience changes in contrast sensitivity (27), color discrimination (28), and low luminance VA (29). However, these symptoms may not prompt patients to seek medical care until their central vision is affected. Therefore, these findings emphasize the importance of educating individuals about diabetes and its potential eye complications, regardless of the exemption from VI. One reassuring finding is that we observed no difference in the proportion of undiagnosed cases between patients with bilateral VI and those with unilateral VI. Additionally, regression analysis showed undiagnosed cases were more strongly associated with the worse-seeing eye than the better-seeing eye. This suggests that patients tend to seek medical attention when one eye experiences VI rather than waiting until both eyes are affected.

TABLE 3 Associations between different classification of visual acuity and undiagnosed diabetic retinopathy.

	VA classification	*OR* (95%*CI*)	*P* value‡
Model 1*			0.960
	Worse eye VI or blind	0.358 (0.229, 0.561)	
	Better eye VI or blind	0.376 (0.227, 0.624)	
Model 2†			0.978
	Worse eye VI or blind	0.380 (0.239, 0.604)	
	Better eye VI or blind	0.417 (0.248, 0.703)	

*Crude regression model without any correction.
†Age, sex, and duration of diabetes adjusted.
‡Odd ratio estimate comparison using cluster sandwich estimator for estimating cross-model covariance matrices.
VA, visual acuity; VI, visual impairment; OR, odd ratio.

In terms of demographic variables, our results were coupled with the previous research (5, 30), indicating that a longer duration of T2DM is associated with a lower proportion of undiagnosed DR. Over time, patients with diabetes are more likely to receive regular medical supervision, education about potential complications, and understand the importance of regular check-ups, including eye examinations. Consequently, they may be more proactive in monitoring their health, leading to earlier detection of DR.

Moreover, we found that advanced age was associated with a higher proportion of undiagnosed VTDR. As individuals age, they may experience other co-existing age-related conditions, and other health conditions take priority, resulting in delayed diagnosis of DR.

Although it is known that individuals living with close family members, particularly a partner, tend to exhibit better attendance at medical examinations (31), our study failed to find a significant association in this regard. Additionally, in line with earlier studies, we did not observe any associations between undiagnosed DR and factors such as gender (24, 32), ethnicity (24), or marital status (32).

Our study shed light on patient-reported incentives and barriers for undiagnosed DR. Since our study initiated the attempt to address this issue, direct comparisons with other studies were not possible. However, there is circumstantial evidence from studies examining the reasons for low adherence to screening guidelines that support our findings (12, 18, 33, 34).

Our study reveals that a considerable proportion (69.3%) of patients with DR reported not receiving information from their internists regarding the need for an eye examination. Additionally, we found 77.9% of participants stated that they only felt the need to seek such examinations when they experienced problems with their eyesight. Furthermore, only 22.3% of participants had visited an

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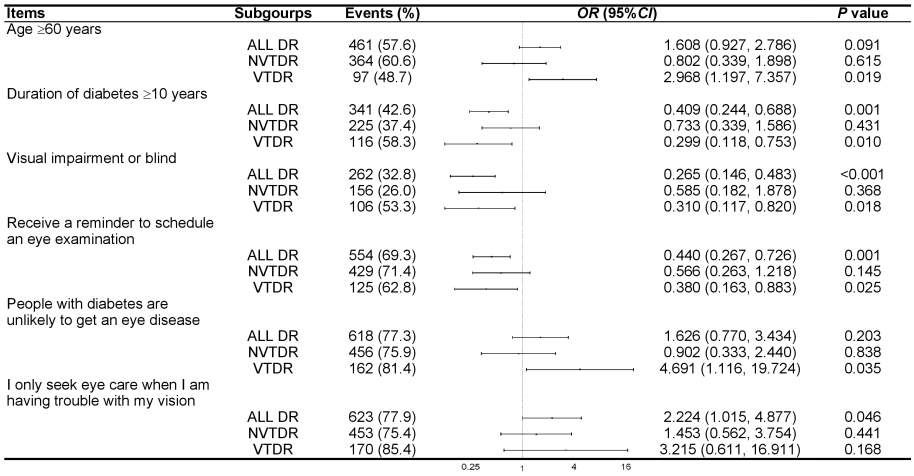

FIGURE 1
Subgroup analysis of factors associated with undiagnosed diabetic retinopathy. OR, odd ratio; CI, confidence interval; DR, diabetic retinopathy; NVTDR, non-vision-threatening diabetic retinopathy; VTDR, vision-threatening diabetic retinopathy.

ophthalmologist. An alarming 75.1% of T2DM patients expressed unawareness of the recommended frequency of eye checks. In contrast, a study conducted in Saudi Arabia revealed that 73.3% of diabetic patients were aware of the need for regular eye check-ups (35). The significant knowledge gap observed in our study regarding screening for DR may be attributed to a lack of understanding about the importance of eye screening. These findings highlight the critical importance of effective inter-specialty communication in the comprehensive management of complex chronic diseases like diabetes. Healthcare providers, particularly general practices and endocrinologists, should adopt a proactive role in disseminating this crucial information, including the necessity and frequency of diabetic eye screening, addressing misconceptions, and promoting regular ophthalmological examinations (36).

The fact that over 77% of patients demonstrated awareness of the potential link between DM and DR is noteworthy. However, it is concerning that only 28.5% of patients are aware that DR could lead to blindness, which aligns with previous studies (37). These findings emphasize the necessity for targeted patient education initiatives that focus on raising awareness about the risks and potential consequences of DR, specifically highlighting the risk of blindness. Such a proactive approach has the potential to facilitate earlier detection of DR, enhance patient outcomes, and ultimately reduce vision loss associated with diabetes (2–4).

As the duration of diabetes increases, individuals with lower levels of education are more likely to have NVTDR, while this association is not observed among individuals with higher levels of education. These findings imply the existence of potential communication gaps between these patients and healthcare providers. These patients may have difficulty fully comprehending the health information provided, leading to challenges in making informed decisions about their healthcare. This, in turn, increases the risk of undiagnosed NVTDR. Additionally, despite the similar proportion of patients with NVTDR and VTDR attending regular screenings (21.0% vs. 19.1%), we observed a significantly lower diagnosis rate of DR in the former group (5.5% vs. 26.6%). It is important to note that the research was conducted in a third-tier city, which typically has limited healthcare infrastructure and a scarcity of ophthalmologists specialized in retinal compared to larger urban centers. Even if patients undergo regular follow-up visits, the mild manifestations of NVTDR pose challenges in achieving accurate diagnoses (36). This could be a major contributing factor to the underdiagnosis of NVTDR in our population.

While traditional methods such as direct and indirect ophthalmoscopy have served as foundational tools in DR screening, their limitations in sensitivity and specificity, especially in non-dilated pupils, are evident. The advent of advanced imaging techniques, such as Fluorescein Angiography (FA) and Optical Coherence Tomography (OCT), has revolutionized the precision with which DR can be detected and monitored. With the rapid advancements in technology, the future holds promise for even more efficient and accessible DR screening methods. Artificial intelligence and machine learning algorithms, for instance, are being integrated into retinal imaging tools to provide instant, automated assessments of DR severity (38). Such innovations

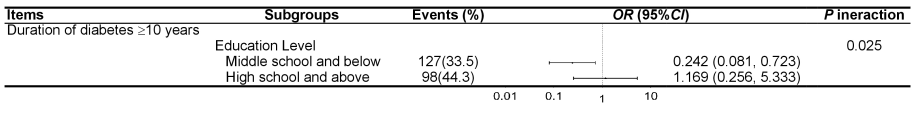

FIGURE 2
Subgroup analysis of factors associated with undiagnosed non-vision-threatening diabetic retinopathy. OR, odd ratio; CI, confidence interval.

could significantly reduce the time and expertise required for DR screenings, facilitate the early diagnosis of NVTDR, making it more widely available and potentially more cost-effective.

The strengths of our study lie in the inclusion of a large community-based sample, the utilization of six-field retinal photographs for assessing DR, and the adoption of masked grading to ensure accurate evaluation. In order to obtain a precise and comprehensive understanding, it is imperative to delineate the limitations inherent to this study. Primarily, the data utilized is circumscribed to the FS-DIRECT, executed within a distinct region of China. Consequently, extrapolating the findings of this study to more heterogeneous populations or disparate regions may be constrained. It is salient to recognize that our study specifically targeted individuals diagnosed with diabetes, who could have been predisposed to ocular examinations, potentially attenuating the true prevalence of undiagnosed diabetic retinopathy (DR). Moreover, the study's exclusionary criteria, specifically omitting participants with ungradable retinal images or incomplete questionnaires, introduce a potential selection bias, thereby influencing the study's outcomes. The reliance on self-reported DR diagnoses further augments the potential for response biases and data inaccuracies. Furthermore, the cross-sectional design of this investigation precludes the establishment of causative relationships, accentuating the exigency for prospective longitudinal studies to corroborate our findings. It is also pivotal to underscore that the analysis did not encompass salient determinants, such as the health insurance status of patients, which could significantly modulate the study's conclusions.

In conclusion, our study reveals the concerning prevalence of underdiagnosis in cases of DR. Considering the critical significance of early detection, it is imperative to undertake concerted efforts aimed at improving the timely diagnosis of DR. To this end, addressing potential barriers and misconceptions surrounding DR screening and its severity is of paramount importance. Moreover, a key aspect of our approach should involve enhancing patient education, with particular emphasis on older individuals and those with lower educational attainment. By prioritizing these areas, we can actively work towards achieving early detection of DR, thus leading to improved management outcomes.

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 humans were approved by Institutional Review Board of Fushun Eye Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

BZ: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft. SR: Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. YW: Funding acquisition, Writing – original draft. DL: Conceptualization, Writing – original draft. XD: Conceptualization, Writing – original draft. DZ: Data curation, Writing – original draft. FW: Writing – review & editing. YL: Writing – review & editing. GZ: Writing – review & editing. KF: Formal analysis, Writing – original draft. ZZ: Writing – original draft.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1263508/full#supplementary-material

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Prevalence and predictors of developing vision-threatening diabetic retinopathy within the first three years of type 2 diabetes

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Purpose: To investigate the prevalence of diabetic retinopathy (DR) and vision-threatening DR (VTDR) in patients with type 2 diabetes mellitus (T2DM) stratified by the duration of diabetes and to identify the clinical variations and risk factors for VTDR occurring at different stages of T2DM.

Methods: This was a retrospective comparative study. Patients were divided into short- (≤3 years), intermediate- (3–7 years), and long-duration (>7 years) groups. All patients were followed-up for DR and VTDR development. Risk factors were explored using logistic regression analysis.

Results: A total of,2961 patients were included; among them, 1,036 (35.0%) patients developed DR, and 293 (9.9%) had VTDR. The frequency of VTDR in patients who developed DR in the short-duration group was significantly higher than that in the intermediate-duration group (25.7% vs. 15.0%; p = 0.019), but comparable with that of the long-duration group (25.7% vs. 31.8%; p = 0.138). Patients who developed VTDR within the first 3 years of T2DM were more likely to have a family history of diabetes (p = 0.024), had higher glycated hemoglobin (p = 0.025), were males (p = 0.042), and were notably older at the onset of diabetes (p <0.001) but younger when diagnosed with DR (p <0.001). Moreover, higher glycated hemoglobin (OR = 1.14; 95% CI: 1.00–1.29; p = 0.043) and diabetic nephropathy (DN) (OR = 2.31; 95% CI: 1.08–4.91; p = 0.030) were independent risk factors for developing VTDR during the first 3 years of T2DM.

Conclusion: The risk of DR is not high in persons with ≤3 years' duration of T2DM, however, if afflicted, the risk of VTDR should never be neglected. More frequent retinal screening is warranted in patients with newly diagnosed T2DM.

KEYWORDS

type 2 diabetes, diabetic retinopathy, vision-threatening diabetic retinopathy, risk factor, early-onset retinopathy

Introduction

Diabetes mellitus is a global epidemic with remarkable morbidity and mortality rates. Globally, the number of people with diabetes is predicted to reach 629 million by 2045, accounting for 9.9% of the global population (1). Diabetic retinopathy (DR), a major microvascular complication of diabetes, is the leading cause of blindness in working-age adults (2). A pooled analysis of 35 population-based studies worldwide (22,896 participants) reported an overall prevalence of 34.6% for any DR and 10.2% for vision-threatening DR (VTDR) (3), including severe non-proliferative DR (NPDR), proliferative DR (PDR), and clinically significant diabetic macular edema (DME) (2). Early detection and intervention of VTDR can prevent up to 98% of visual loss caused by diabetic complications (4). Therefore, the identification of the risk factors for VTDR may assist in the early detection of individuals with the greatest risk.

The risk of DR increases with diabetes duration (5–7). Up to 77.8% of individuals with diabetes for 15 years or more were afflicted with DR (5). The 2000 American Diabetes Association statement suggested that VTDR generally does not affect individuals with type 1 diabetes during the first 3–5 years after diagnosis (8); therefore, an initial retinal examination should be performed within 3–5 years of the initial diagnosis. However, a subsequent study indicated that individuals in whom retinopathy developed during the first 5 years of diabetes had a more rapid progression of retinal pathology, suggesting that dilated eye examinations and retinal photography should be performed from the onset of type 1 diabetes to identify individuals with a high risk of vision-threatening problems (9).

In type 2 diabetes, the thresholds for the prevalence of DR with regard to diabetes duration indicate that the risk of having DR is not linearly associated with exposure to various influencing factors, but the risk relationship may be cumulative, such that the chances of having DR may increase after certain periods of exposure (10). Notably, 20.8% of individuals with type 2 diabetes have DR at the initial diagnosis of diabetes for approximately 4–7 years of undiagnosed diabetes (6). In type 2 diabetes, the first peak for vision-threatening problems caused by retinopathy may occur within the first three years after the diagnosis of type 2 diabetes.

In this study, we analyzed a hospital-based cohort of 2,961 Chinese individuals with type 2 diabetes. Participants were divided into short- (≤3 years), intermediate- (4–7 years), and long-duration (>7 years) groups and were followed up for the development of DR and VTDR. We then focused on the VTDR occurring at each stage of type 2 diabetes regarding its clinical variations and distinct risk factors.

Materials and methods

Data collection

The medical charts of patients diagnosed with type 2 diabetes at Shanghai General Hospital from January 2007 to December 2012 were reviewed, and 7,034 patients were identified. Among them, 6,768 underwent standardized eye examinations at baseline. In addition, 311 patients were excluded for severe visual impairments other than DR, such as neovascular age-related macular degeneration, ischemic retinal vein occlusion, uveitis, primary glaucoma, and no light perception in one or both eyes. DR was established according to an outpatient diagnosis based on two or more follow-up visits or a one-time inpatient diagnosis during the exposure period. A total of 3,267 patients were matched in our database, and they had type 2 diabetes for 1.0–46.0 years. However, 306 individuals were excluded because of incomplete data collection, leaving 2,961 individuals eligible for this study.

We explained the purpose of this study to patients with type 2 diabetes and suggested follow-up time points. All patients voluntarily participated in this study without any additional compensation. Oral informed consent was obtained from all patients. The Declaration of Helsinki was followed in this study. Our study was approved by the institutional review board of the Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine.

Screening tests for DR included slit-lamp examinations and color fundus photography through a dilated pupil (Carl Zeiss Meditec AG, Jena, Germany) and optical coherence tomography (OCT) (Heidelberg Engineering, Heidelberg, Germany), if necessary. DR was considered as the presence of any lesion defined by the Early Treatment Diabetic Retinopathy Study (ETDRS). (11) :retinal microaneurysms, blot hemorrhages, hard or soft exudates, venous beading, intraretinal microvascular abnormalities, retinal neovascularization, laser scatter photocoagulation scars, preretinal or vitreous hemorrhage, proliferative membrane and tractional retinal elevation. The primary outcome was the initial occurrence of DR, and the severity of DR was scaled according to the ETDRS grading

standards: mild-moderate NPDR (ETDRS level 20–47), severe NPDR (ETDRS level 53), and PDR (ETDRS level ≥60) (11). DME was graded according to the International Clinical Diabetic Retinopathy/Macular Edema Severity Scale (12). Clinically significant DME was defined as retinal edema or hard exudates approaching or involving the fovea. The patients were grouped based on their worse eye into three classes: no DR, mild DR, and VTDR. Mild DR was considered as mild-moderate NPDR, whereas VTDR was defined as the presence of severe NPDR, PDR and/or clinically significant DME. At least two vitreoretinal specialists assessed the retinal lesions per patient. The consistency between the first-round graders was 94.29%. A third-grader, who was not involved in the initial assessment, was asked to deliberate on discrepancies.

The baseline was set as the date of the first type 2 diabetes registration. Clinical details before referral were retrieved for review if the patient had established this diagnosis elsewhere. The date of the initial DR diagnosis was documented. The period between the diagnosis of diabetes and DR was calculated. Individuals with DR were stratified into three groups according to the period clinically free of DR after the diagnosis of type 2 diabetes: early- (≤3 years), intermediate- (4–7 years), and late-onset (>7 years). Individuals who did not have DR during the exposure time were considered censored, and the date of the last visit was recorded.

The data collected included patient demographics, clinical characteristics, and final outcomes at follow-up. The demographics included sex, age at onset of type 2 diabetes and DR, and a family history of type 2 diabetes. The clinical characteristics obtained from the electronic chart records included systolic and diastolic blood pressure, body mass index (BMI), insulin use, and biochemical laboratory information on glycated hemoglobin (HbA1c), fasting glycemia, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, total cholesterol, triglycerides, serum creatinine, and uric acid. The included parameters were assessed every 6–12 months. For individuals with DR, we used the last information before the diagnosis of DR. The last information was carried forward for those who were free of retinopathy.

Apart from DR, all individuals underwent a valid assessment of two other microvascular complications: diabetic nephropathy (DN) and diabetic peripheral neuropathy (DPN). The diagnosis of DN requires at least two albumin excretions of >30 μg/mg creatinine or an excretion rate of >30 mg/24 h on different occasions within one year. DPN was ascertained using biothesiometry measurements with bilateral testing of the big toes. Vibration perception testing (0–50 V) was performed and abnormal readings (on both sides) above age-specific thresholds were recorded (13). After excluding patients with other conditions explaining this neural or renal deficiency, a diagnosis of DN or DPN was established. Only concurrent DN or DPN status may have an impact on the development of DR. Therefore, DN or DPN occurring after the debut of DR is not considered a potential correlate of DR.

Statistical analysis

Categorical and continuous variables are presented as frequencies (percentages) and medians (interquartile ranges [IQRs]), respectively. Univariate analyses, either ANOVA (continuous factors) or a chi-square test (categorical factors), were used to identify the possible correlates. The significant parameters were then entered into a multivariate logistic regression model as independent variables to explore the independent predictors. Odds ratios (ORs) with 95% confidence intervals (CIs) were also calculated. All analyses were conducted using SPSS (version 21.0; IBM Corp., New York, NY, USA) and two-sided tests with a significance threshold of 0.05.

Results

In total, 2,961 individuals were included, and the median duration of diabetes was 10.0 years (IQR, 4.0–15.0 years). Among these participants, 660 (22.3%) had diabetes for ≤3 years (short duration group), 529 (17.9%) had diabetes for 4–7 years (intermediate duration group), and 1,772 (59.8%) had diabetes for >7 years (long duration group). There were 1,661 (56.1%) men and 1,300 (43.9%) women, with a median age of 50.0 (IQR, 43.0–57.0) years at diabetes diagnosis. Of 1,036 (35.0%) individuals with DR, 743 (25.1%) had mild DR and 293 (9.9%) had VTDR. The median time interval to DR was 11.0 years (IQR, 6.0–16.0 years), and this period for mild DR and VTDR was 10.0 years (IQR, 6.0–15.0 years) and 13.0 years (IQR, 8.0–17.0 years), respectively. The numbers of individuals with DR were 152 (23.0%), 160 (30.2%), and 724 (40.9%) in the short-, intermediate-, and long-duration groups, respectively. The demographics and concurrent clinical characteristics are presented in Table 1.

The distribution of the different severities of DR stratified by diabetes duration is shown in Table 2; Figure 1. Notably, a significantly larger proportion of individuals who developed DR in the short duration group had VTDR (25.7%) than those in the intermediate duration group (15.0%) ($p = 0.019$). Among those who were diagnosed with DR after 7 years of type 2 diabetes, up to 31.8% of individuals were afflicted with VTDR; however, no significant difference was observed when compared with in the short duration group ($p = 0.138$).

In subsequent analyses, we investigated the clinical variations among the VTDR subgroups (Table 3). Those persons who developed VTDR within the first three years were more likely to have a family history of diabetes (66.7% vs. 37.5%, $p = 0.024$) and higher glycated hemoglobin levels (9.7% vs. 8.3%, $p = 0.025$) than those who developed VTDR 4–7 years after type 2 diabetes. Furthermore, the persons who developed VTDR within the first three years were notably older at the onset of diabetes (51.8 years vs. 44.0 years, $p < 0.001$) but younger when diagnosed with DR (52.0 years vs. 60.0 years, $p < 0.001$), and were more likely to be males

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TABLE 1 Clinical characteristics for 2,961 Chinses patients with type 2 diabetes.

Variables	Total (n = 2,961)	No DR (n = 1,925)	Any DR (n = 1,036)	DR developed in ≤3 years of T2DM (n = 152)	DR developed in 4–7 years of T2DM (n = 160)	DR developed in >7 years of T2DM (n = 724)
Age at diabetes (y)	50.0 (43.0–57.0)	51.0 (44.0–58.8)	47.0 (41.0–54.0)	51.0 (43.3–57.8)	49.0 (40.3–58.0)	46.0 (40.0–52.0)
Age at DR (y)	/	/	59.0 (52.0–66.0)	52.0 (45.3–59.0)	54.0 (45.2–63.0)	61.0 (55.0–68.0)
Gender						
Male	1,661 (56.1)	1,090 (56.6)	571 (55.1)	98 (64.5)	107 (66.9)	366 (50.6)
Female	1,300 (43.9)	835 (43.4)	465 (44.9)	54 (35.5)	53 (33.1)	358 (49.4)
Family history of diabetes	1,623 (54.8)	1,020 (53.3)	603 (58.2)	92 (60.5)	77 (48.1)	434 (59.9)
Body mass index (kg/m^2)	24.7 (22.6–27.3)	24.7 (22.6–27.2)	24.7 (22.7–27.3)	24.3 (22.2–27.0)	25.5 (23.5–27.7)	24.6 (22.6–27.2)
SBP (mmHg)	130 (120–140)	130 (120–140)	130 (120–150)	130 (120–140)	130 (120–142)	135 (120–150)
DBP (mmHg)	80 (72–86)	80 (70–85)	80 (75–88)	80 (70–90)	80 (78–90)	80 (75–86)
Glycated hemoglobin (%)	8.4 (7.1–10.1)	8.2 (7.0–9.9)	8.7 (7.4–10.3)	8.6 (7.0–10.5)	8.4 (7.1–10.3)	8.8 (7.6–10.3)
Insulin use	1,648 (55.7)	1,000 (51.9)	648 (62.5)	82 (53.9)	87 (54.4)	479 (66.2)
Total cholesterol (mg/dL)	4.7 (4.0–5.4)	4.7 (4.0–5.3)	4.8 (4.0–5.5)	4.6 (4.0–5.3)	4.7 (4.1–5.5)	4.8 (4.0–5.6)
HDL cholesterol (mg/dL)	1.0 (0.9–1.3)	1.0 (0.9–1.3)	1.1 (0.9–1.3)	1.0 (0.9–1.2)	0.8 (1.0–1.2)	1.1 (0.9–1.3)
LDL cholesterol (mg/dL)	2.8 (2.2–3.4)	2.8 (2.2–3.4)	2.8 (2.3–3.5)	2.7 (2.2–3.3)	2.8 (2.4–3.4)	2.9 (2.3–3.5)
Triglycerides (mg/dL)	1.4 (1.0–2.0)	1.4 (1.0–2.0)	1.4 (0.9–2.0)	1.4 (0.9–2.2)	1.4 (1.0–2.1)	1.3 (0.9–1.9)
Serum creatinine	67.0 (56.0–79.0)	67.0 (56.0–79.0)	66.0 (55.0–79.0)	66.0 (52.5–75.5)	65.0 (58.0–73.0)	66.0 (55.0–81.0)
Uric acid	314 (260–378)	316 (262–380)	312 (258–375)	307 (241–382)	314 (262–383)	313 (259–368)
Urine microalbumin	16.4 (8.4–56.6)	13.9 (7.8–37.9)	23.6 (10.3–131.3)	18.0 (8.8–77.4)	21.9 (10.3–90.5)	25.9 (10.6–151.0)
Diabetic nephropathy	664 (22.4)	371 (19.3)	293 (28.3)	27 (17.8)	45 (28.1)	221 (30.5)
Diabetic peripheral neuropathy	603 (20.4)	318 (16.5)	285 (27.5)	24 (15.8)	33 (20.6)	228 (31.5)

Data are presented as median (interquartile range)/n (%). Symbol "/" means "not applicable".
DR, diabetic retinopathy; T2DM, type 2 diabetes; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein.

TABLE 2 The distribution of different severities of diabetic retinopathy stratified by the diabetes duration.

Any DR (n = 1,036)	DR developed in ≤3 years of T2DM	DR developed in 4–7 years of T2DM	DR developed in >7 years of T2DM	p†	p‡
	(n = 152)	(n = 160)	(n = 724)		
Mild DR (n = 743)	113 (74.3)	136 (85.0)	494 (68.2)	0.019*	0.138
VTDR (n = 293)	39 (25.7)	24 (15.0)	230 (31.8)		

Data are presented as n (%).
DR, diabetic retinopathy; VTDR, vision-threatening diabetic retinopathy; T2DM, type 2 diabetes.
*Statistically significant; †Early vs intermediate; ‡Early vs late.

(64.1% vs. 46.5%, p = 0.042) than those who developed VTDR after 7 years of type 2 diabetes.

To explore the risk factors for developing VTDR in people with ≤3 years' type 2 diabetes, univariate and multivariate logistic regressions were performed to determine their independent correlates (Table 4). The univariate analysis indicated that higher levels of systolic blood pressure (OR = 1.02; 95% CI: 1.00–1.04; p = 0.029), higher glycated hemoglobin (OR = 1.21; 95% CI: 1.07–1.36; p = 0.002), and the presence of DN (OR = 2.74; 95% CI: 1.31–5.71; p = 0.007) were potential risk factors. Subsequent multivariate analysis demonstrated that higher glycated hemoglobin (OR = 1.14; 95% CI: 1.00–1.29; p = 0.043) and DN (OR = 2.31; 95% CI: 1.08–4.91; p = 0.030) were independent risk factors for the development of VTDR within the first three years of type 2 diabetes.

We also investigated the factors influencing the development of VTDR in the intermediate- and long-duration groups, the details of which are summarized in Tables S1, S2. Concurrent DPN was associated with developing VTDR in persons with 4–7 years of type 2 diabetes (OR = 2.52; 95% CI:1.01–6.29; p = 0.048) (Table S1). For the VTDR that occurred in the long-duration group, the independent risk factors included younger age at diabetes onset (OR = 0.95; 95% CI:0.92–0.98; p <0.001), female gender (OR = 1.42; 95% CI:1.02–1.96; p = 0.037), higher systolic blood pressure (OR = 1.01; 95% CI: 1.00–1.03; p = 0.014), and higher glycated hemoglobin (OR = 1.12; 95% CI: 1.03–1.22; p = 0.007), insulin use (OR = 1.53; 95% CI:1.09–2.16; p = 0.015), and the presence of DN (OR = 1.46; 95% CI:1.03–2.06; p = 0.034) (Table S2).

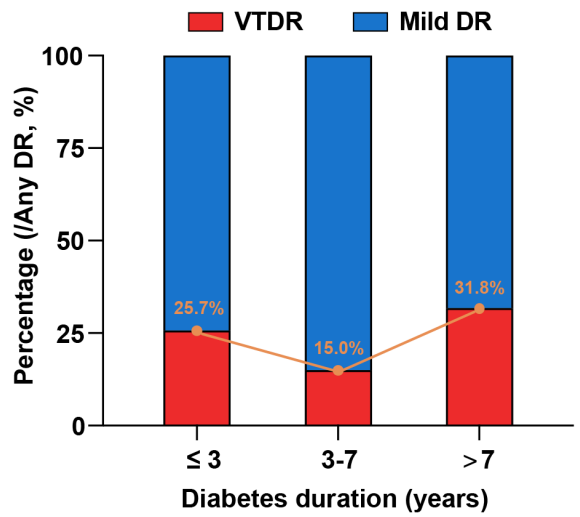

FIGURE 1
Distribution of different severities of DR stratified by diabetes duration. The proportions of the individuals who developed DR in the short-, intermediate, and long-duration groups were 25.7%, 15.0%, and 31.8%, respectively.

Discussion

In this study, we analyzed 2,961 individuals with type 2 diabetes to assess the incidence of DR and VTDR according to diabetes duration. Patients who developed retinopathy within the first three years of diabetes also had a relatively high risk of VTDR. Individuals who developed VTDR within the first three years of type 2 diabetes were more likely to have a family history of diabetes, higher glycated hemoglobin, male sex and were notably older at the onset of diabetes but younger when diagnosed with DR. Concurrent DN and higher glycated hemoglobin levels were predictors of VTDR development during the first three years after type 2 diabetes.

The risk of DR increases with diabetes duration. However, approximately one-fifth of the individuals have DR at the initial diagnosis of type 2 diabetes. Similarly, the incidence of VTDR is not directly proportional to diabetes duration, and varies depending on the severity of retinopathy. The findings of our study suggest that the first three years after diabetes diagnosis is the risk period for developing VTDR for persons with retinal changes. This is consistent with our clinical notion that ocular presentations vary greatly among individuals with newly diagnosed diabetes, most of whom do not have retinopathy; however, if people have diabetic retinal disorders, the initial conditions might have been severe.

DN, an established risk factor for DR development and progression, has been intensively investigated over the past few decades. An 8-year prospective cohort study conducted in 2,135 Chinese people with type 2 diabetes revealed that abnormal renal parameters both at baseline and during the follow-up period, including a high serum creatinine level, low estimated glomerular filtration rate, and high urinary albumin/creatinine ratio, were associated with the development of PDR (14). Elevated expression of vascular endothelial growth factor (VEGF) due to compromised glomerular filtration has been observed in both serum and renal tissues, which might contribute to the development of PDR (15, 16). The same applies to the association among DR, DN, and DPN. Bjerg et al. pointed out that patients with diabetes with any previous

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TABLE 3 Clinical variations of VTDR developed in different stages of T2DM.

Variables	VTDR developed in ≤3 years of T2DM (n = 39)	VTDR developed in 3–7 years of T2DM (n = 24)	VTDR developed in >7 years of T2DM (n = 230)	p†	p‡
Age at diabetes (y)	51.8 (48.0–55.0)	49.0 (44.3–58.3)	44.0 (38.0–50.3)	0.723	<0.001*
Age at DR (y)	52.0 (49.8–57.0)	55.0 (50.0–63.3)	60.0 (53.0–66.6)	0.173	<0.001*
Gender				0.836	0.042*
Male	25 (64.1)	16 (66.7)	107 (46.5)		
Female	14 (35.9)	8 (33.3)	123 (53.5)		
Family history of diabetes	26 (66.7)	9 (37.5)	144 (62.6)	0.024*	0.627
Body mass index (kg/m^2)	23.6 (21.9–26.8)	26.7 (23.5–28.0)	24.6 (22.5–27.0)	0.067	0.413
SBP (mmHg)	130 (120–140)	130 (125–149)	137 (122–150)	0.810	0.379
DBP (mmHg)	80 (70–90)	80.0 (78–80)	80 (75–90)	0.842	0.930
Glycated hemoglobin (%)	9.7 (8.0–11.7)	8.3 (7.1–10.2)	9.1 (7.8–10.4)	0.025*	0.164
Insulin use	22 (56.4)	14 (58.3)	165 (71.7)	0.911	0.054
Total cholesterol (mg/dL)	4.8 (4.4–5.5)	4.3 (3.9–5.3)	4.9 (4.2–5.8)	0.754	0.533
HDL cholesterol (mg/dL)	1.0 (0.9–1.3)	1.0 (0.9–1.1)	1.1 (0.9–1.3)	0.412	0.232
LDL cholesterol (mg/dL)	2.7 (2.3–3.6)	2.6 (2.3–3.4)	3.0 (2.4–3.6)	0.554	0.434
Triglycerides (mg/dL)	1.2 (0.8–2.9)	1.3 (1.1–1.8)	1.3 (0.9–2.0)	0.972	0.087
Serum creatinine	69.0 (57.0–78.3)	70.0 (58.5–84.0)	68.0 (53.5–84.0)	0.532	0.950
Uric acid	329 (246–397)	322 (275–410)	320 (260–364)	0.362	0.795
Urine microalbumin	105.8 (21.2–381.0)	44.3 (9.0–593.6)	54.3 (14.9–392.6)	0.721	0.866
Diabetic nephropathy	11 (28.2)	7 (29.2)	83 (36.1)	0.935	0.340
Diabetic peripheral neuropathy	7 (17.9)	7 (29.2)	67 (29.1)	0.441	0.148

Data are presented as median (interquartile range)/n(%).
DR, diabetic retinopathy; VTDR, vision-threatening diabetic retinopathy; T2DM, type 2 diabetes; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein.
*Statistically significant; †Early vs intermediate; ‡Early vs late.

microvascular complications had a higher risk of developing further microvascular complications than individuals without any complications (17).

Based on our observations, individuals who developed VTDR within the first three years of type 2 diabetes tended to be older at the onset of diabetes but younger at DR. The ages at diabetes diagnosis were 51.8 years, 49.0 years, and 44.0 years for individuals with VTDR in the short-, intermediate-, and long-duration groups, respectively. Type 2 diabetes is usually insidious at death, and its onset may occur 4–7 years before clinical diagnosis (6, 18). When diabetes is undiagnosed, risk factors for the microvascular disease may be neglected, such as hyperglycemia (19), dyslipidemia (19, 20), microalbuminuria (14), as well as metabolic memory (21), and all of these factors accelerate the development of DR. Consequently, it was not surprising to observe a reversed trend of the age at the diagnosis of DR, in which, the individuals with VTDR were youngest in the short-duration group (52.0 years), followed by 55.0 years in the intermediate-duration group and 60.0 years in the late-duration group. Clearly, the time interval between the diagnosis of type 2 diabetes and DR was shortest for individuals with VTDR in the short duration group. Prior data indicated that it took approximately 5 years from frank diabetes to detectable retinopathy (18). Therefore, the older age at diabetes diagnosis and younger age at DR diagnosis among individuals with VTDR in newly diagnosed type 2 diabetes can be partially explained by delays in seeking care for diabetes.

In this report, individuals with VTDR within the first three years of type 2 diabetes were more likely to be males. This finding is consistent with reports from India (22), England (10, 23, 24), and America (25, 26), which indicated that male sex is an independent

TABLE 4 Logistic regression for the predictors of developing VTDR within the first 3 years of T2DM.

Variables	Univariate		Multivariate	
	OR (95%CI)	p	OR (95%CI)	p
Age at diabetes (y)	1.00 (0.98–1.03)	0.810		
Age at diabetic retinopathy (y)	1.00 (0.98–1.02)	0.948		
Gender	0.97 (0.49–1.89)	0.918		
Male				
Female				
Family history of diabetes	1.81 (0.91–3.59)	0.089		
Body mass index (kg/m^2)	0.93 (0.85–1.02)	0.114		
SBP (mmHg)	1.02 (1.00–1.04)	0.029*	1.02 (0.99–1.04)	0.064
DBP (mmHg)	1.00 (0.97–1.03)	0.991		
Glycated hemoglobin (%)	1.21 (1.07–1.36)	0.002	1.14 (1.00–1.29)	0.043
Insulin use	1.32 (0.69–2.53)	0.410		
Total cholesterol (mg/dL)	1.04 (0.82–1.32)	0.720		
HDL cholesterol (mg/dL)	1.84 (0.57–5.94)	0.305		
LDL cholesterol (mg/dL)	1.00 (0.68–1.49)	0.991		
Triglycerides (mg/dL)	1.01 (0.88–1.15)	0.947		
Serum creatinine	1.01 (1.00–1.03)	0.089		
Uric acid	1.00 (0.99–1.00)	0.713		
Diabetic nephropathy	2.74 (1.31–5.71)	0.007*	2.31 (1.08–4.91)	0.030*
Diabetic peripheral neuropathy	1.97 (0.84–4.66)	0.121		

VTDR, vision-threatening diabetic retinopathy; T2DM, type 2 diabetes; OR, odds ratio; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein.
*Statistically significant.

risk factor for severe DR in type 2 diabetes, especially near the time of DM diagnosis. However, this relationship appears to weaken with increasing diabetes duration. In our study, females tended to have an increased risk of VTDR 7 years after diabetes. However, the reasons for this sex disparity remain unclear.

Patients who developed VTDR within the first three years were more likely to have a family history of diabetes. This finding suggests that heredity or genetic susceptibility might play a role in the etiology of VTDR in early onset retinopathy (27, 28). Familial patients may be more susceptible to hyperglycemia-induced damage.

This study provided an initial report that depicted an extraordinary accumulation of VTDR during the first three years of diabetes and explored its underlying causes. However, caution should be exercised when interpreting the findings owing to the limitations of this study. First, all participants were recruited from a single center, which might have led to selection bias, and our results may not be generalizable to the entire population of patients with type 2 diabetes. In addition, this study revealed associations, but not causations, owing to its observational design. Furthermore, lifestyle factors, including smoking status, alcohol consumption, sleeping status, and physical activity should also be considered when evaluating the development of DR. However, the strength of this study is the inclusion of a relatively large and homogeneous sample of comprehensive clinical data. The long follow-up time made our results more convincing.

In conclusion, our data provide some general guidance to clinicians that although the risk of having DR is not so high in persons with ≤3 years' duration of type 2 diabetes; however, if afflicted, the risk of VTDR should never be neglected, especially in patients with compromised glycated hemoglobin and/or DN. Consequently, more frequent retinal screening is warranted in individuals with newly diagnosed diabetes. In addition, individuals who are older at the time of diabetes diagnosis, males, those with a family history of diabetes, and those with compromised glycated hemoglobin may require closer monitoring.

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

The studies involving humans were approved by institutional review board of Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine Identifier: 2022KY024. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

JY: Conceptualization, Data curation, Methodology, Writing – original draft. BL: Validation, Formal analysis, Investigation, Writing – review & editing. YC: Conceptualization, Data curation, Investigation, Writing – review & editing. CG: Writing – original draft, Data curation, Methodology, Validation, Visualization. GD: Writing – original draft. QZ: Writing – original draft. DL: Writing – review & editing. SZ: Conceptualization, Formal analysis, Project administration, Supervision, Writing – review & editing. CZ: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. ZZ: Conceptualization, Project administration, Writing – review & editing.

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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.2023.1305378/full#supplementary-material

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Diabetes, periodontitis, and cardiovascular disease: towards equity in diabetes care

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Type 2 diabetes and its associated cardiovascular risk is an escalating epidemic that represents a significant public health burden due to increased morbidity and mortality, disproportionately affecting disadvantaged communities. Poor glycaemic control exacerbates this burden by increasing retinal, renal, and cardiac damage and raising healthcare costs. This predicament underscores the urgent need for research into cost-effective approaches to preventing diabetes complications. An important but often overlooked strategy to improve metabolic control in diabetic patients is the treatment of periodontitis. Our aim is to assess whether the inclusion of periodontitis treatment in diabetes management strategies can effectively improve metabolic control, and to advocate for its inclusion from an equity perspective. We conducted a comprehensive review of the literature from 2000 to 2023. We analyzed the pathophysiological links between periodontitis, diabetes, and atherosclerotic cardiovascular disease, all of which have inflammation as a central component. We also examined the inequalities in health care spending in this context. Our findings suggest that incorporating routine screening and treatment of periodontitis into national health programs, with coordinated efforts between physicians and dentists, is a cost-effective measure to improve metabolic control, reduce complications and improve the overall quality of life of people with diabetes.

KEYWORDS

type 2 diabetes mellitus, periodontitis, physicians and dentists, systemic diseases, inequality, inflammation, periodontal disease

Introduction

Type 2 diabetes (T2DM) is one of the fastest-growing global diseases of the 21st century, with a prevalence that has more than tripled in the last 20 years and affects 10.5% of the world's population aged 20–79 years (1). It is expected that these numbers will continue to grow, particularly in low-income countries, which have higher population growth compared to high-income countries. T2DM also generates a large impact on indirect health expenditure, i.e., workforce dropout, absenteeism and presenteeism (2), affecting the poorest countries, which account for the lowest percentage of global diabetes-related health expenditure but have the largest outflow from the national health budget attributed to T2DM (1, 2). This picture becomes even more striking when we consider that half of the people diagnosed with diabetes in the

United States achieve a glycosylated hemoglobin (HbA1c) < 7% (3), one of the markers with the highest predictive value for microvascular and macrovascular diabetes complications (4), with a 1% reduction in HbA1c being able to reduce the risk of any diabetes-related endpoint by 21% (5). More worryingly, only 1 in 4 or fewer adults with T2DM meet the American Diabetes Association (ADA) targets for blood pressure, cholesterol, and HbA1c to reduce their cardiovascular risk (6, 7). Failure to meet these targets results in a significant burden of disability and death (8), which has contributed to a tripling of global health expenditure on diabetes over the past 15 years (1), disproportionately affecting the poorest regions (1, 2). Much of this expenditure is attributable to atherosclerotic cardiovascular disease (ACVD), defined as coronary heart disease, cerebrovascular disease, or peripheral arterial disease of presumed atherosclerotic origin. ACVD is the leading cause of morbidity and mortality in people with diabetes, and the current paradigm of diabetes care requires that multiple cardiovascular risk factors be addressed simultaneously and aggressively (6).

This scenario implies a constant need to investigate new cost-effective methods to provide antidiabetic therapy and reduce T2DM cardiovascular risk and complications. An important but often neglected measure in the T2DM management is the treatment of periodontal disease (PD) (9). PD is a group of chronic inflammatory diseases that affect the tooth supporting structures and is classified into gingivitis, the mildest form of the disease, characterized by swollen, red and easily bleeding gums without loss of underlying alveolar bone, and periodontitis, an advanced state of the disease that involves both inflammatory changes and destruction of the periodontal ligament and supporting alveolar bone, which can lead to tooth loss if left untreated (10). Severe periodontitis has a worldwide prevalence of 5%–15% in the adult population (10) and, compared to other dental diseases, it has the highest correlation with chronic systemic diseases, especially T2DM. In diabetic patients, periodontitis is known to compromise glycemic control and perpetuate chronic inflammation, increasing the risk of microvascular and macrovascular complications (11).

In this article, we will review the pathophysiological and social links between PD and T2DM, focusing on the evidence for the benefits of periodontal treatment in controlling glycaemia, inflammatory markers, and cardiovascular risk. Our aim is to assess whether incorporating periodontal treatment into diabetes management can effectively improve metabolic control, with a significant impact on cardiovascular outcomes and the overall quality of life of patients with diabetes, with a particular focus on disadvantaged communities. We conducted a comprehensive review of the PubMed literature between 2000 and 2023 using relevant terms, PubMed functions, and citation tracking. Given the substantial growth in research on periodontitis and diabetes over the past 15 years, priority was given to recent literature, with particular attention to key studies on the systemic impact of periodontitis, standards of care, oral health management, and systematic reviews.

Inequality and neglect in oral health care: uncovering disparities and urging action

It is estimated that 90% of the world's population will suffer from some form of oral disease during their lifetime. In 2010, five oral diseases alone accounted for 18,814,000 disability-adjusted life years (DALYs), an increase of 21% since 1990 (10). This increase in DALYs is significantly higher for PD, which has risen by 57% in 20 years to become the leading oral disease in adults over the age of 35, along with other major non-communicable diseases (NCDs), such as diabetes (69.0%) (10). In addition, dental caries and PD are the leading causes of tooth loss and edentulism worldwide, resulting in difficulties with chewing, food intake, speech, self-esteem, quality of life and social interactions (10, 12). These conditions also hinder economic development. In Canada, more than 40 million hours are lost each year due to dental problems and treatment, representing a potential productivity loss of more than $1 billion (13).

Due to the high prevalence and the cumulative and recurrent nature of PD and other oral diseases, oral health care is one of the most expensive services provided by health systems; thus, with average annual expenditure in the European Union between 2008 and 2012 of 79 billion euros (€), exceeding the costs of stroke (€38.0 billion), cancer (€51.0 billion) and respiratory diseases (€55.0 billion) (10). However, the way in which governments address oral health varies widely between countries and among their own populations, with notable inequalities. A 2016 multinational comparison estimated the extent of social inequality in adult oral health in five countries, using education as an indicator of social position (12). Of the five Organization for Economic Cooperation and Development countries, the richest country, the United States, had the highest prevalence of edentulism at almost all levels of education, with high inequality, while in the poorest country, Chile, more than half of the population with the lowest level of education lacked functional dentition compared with 10% of the university population (12, 14).

Worldwide, several countries and health systems report a lack of oral health coverage and inequitable access, implying a significant impoverishment of communities due to out-of-pocket health expenditure (15, 16). Bernabé et al. (15) analyzed the impact of out-of-pocket dental health expenditure on catastrophic health expenditure (CHE) in households in low- and middle-income countries. They found that households that paid for dental care were more likely to experience CHE or impoverishment than those that did not pay for dental care. At the country level, of all the covariates analyzed, higher out-of-pocket health expenditure was the only factor associated with greater odds of both CHE and impoverishment (2).

Developed countries have developed strategies to address this problem (16–18), but in Latin America, only five governments have even assessed dental status in nationally representative samples of adults (19). The neglect of oral health, particularly in low- and middle-income countries, represents a major blind spot for public policies aimed at addressing NCD epidemics, as the relationship between dental conditions and other NCDs has been extensively studied, particularly PD, the dental condition with the strongest observed association with systemic diseases, particularly T2DM (20).

Impact of periodontitis on diabetes and cardiovascular risk

In 2012, the Joint EFP/AAP Workshop on Periodontitis and Systemic Disease concluded that there is consistent and strong epidemiological evidence that periodontitis increases the risk of ACVD (21), but the quality of the evidence at that time did not allow

causality to be inferred (22). In addition, diabetic patients with periodontitis have been shown to have a 6-fold higher risk of poor glycemic control and an increased risk of diabetic complications than patients with a healthy periodontium (11, 23). The mechanisms underlying these associations have been attributed to the relationship between local and systemic inflammation (24, 25) (Figure 1).

PD is initiated by the accumulation of pathogenic dental biofilm around the gingival margin (11). Diabetic patients have a 2–3 times higher risk of PD than non-diabetics, especially those with inadequate glycemic control (26), as chronic hyperglycemia and the accumulation of advanced glycation end products (AGEs) increase cytokine expression and oxidative stress in mononuclear and polymorphonuclear phagocytic cells (27), create a pro-inflammatory environment with high levels of serum concentrations of C-reactive protein (CRP), IL-1, IL-6, and TNF-α, and reduce both collagen synthesis and osteoblastic activity. These conditions facilitate the transformation of the subgingival microflora into an essentially high-risk gram-negative flora, especially with *Porphyromonas gingivalis* (*P. gingivalis*), generating dysbiosis and a chronic non-resolving and destructive inflammatory response on the connective tissue and alveolar bone, facilitating the entry of microbes into the bloodstream, and cause bacteremia (27, 28). *P. gingivalis* endotoxins (lipopolysaccharides – LPS) induce systemic responses. LPS facilitate the three critical steps in the pathogenesis of atherosclerosis: (1) increased levels of small and dense low-density lipoprotein (sd-LDL); (2) promotion of endothelial permeability by triggering the innate immune system response; and (3) stimulation of smooth muscle cells to produce a proteoglycan-enriched extracellular matrix in the intima that enhances lipoprotein binding and retention (29). It has also been shown that certain strains of *P. gingivalis* induce platelet activation and aggregation even in the absence of vascular injury, favoring thrombotic episodes (30, 31). In addition, the chronic pro-inflammatory response stimulated by LPS through the ulcerated bursal epithelium may reduce beta-cell function, increase insulin resistance and worsen dyslipidemia, creating a cycle of hyperglycemia and AGE protein binding, which reinforces the diabetic pathway of connective tissue

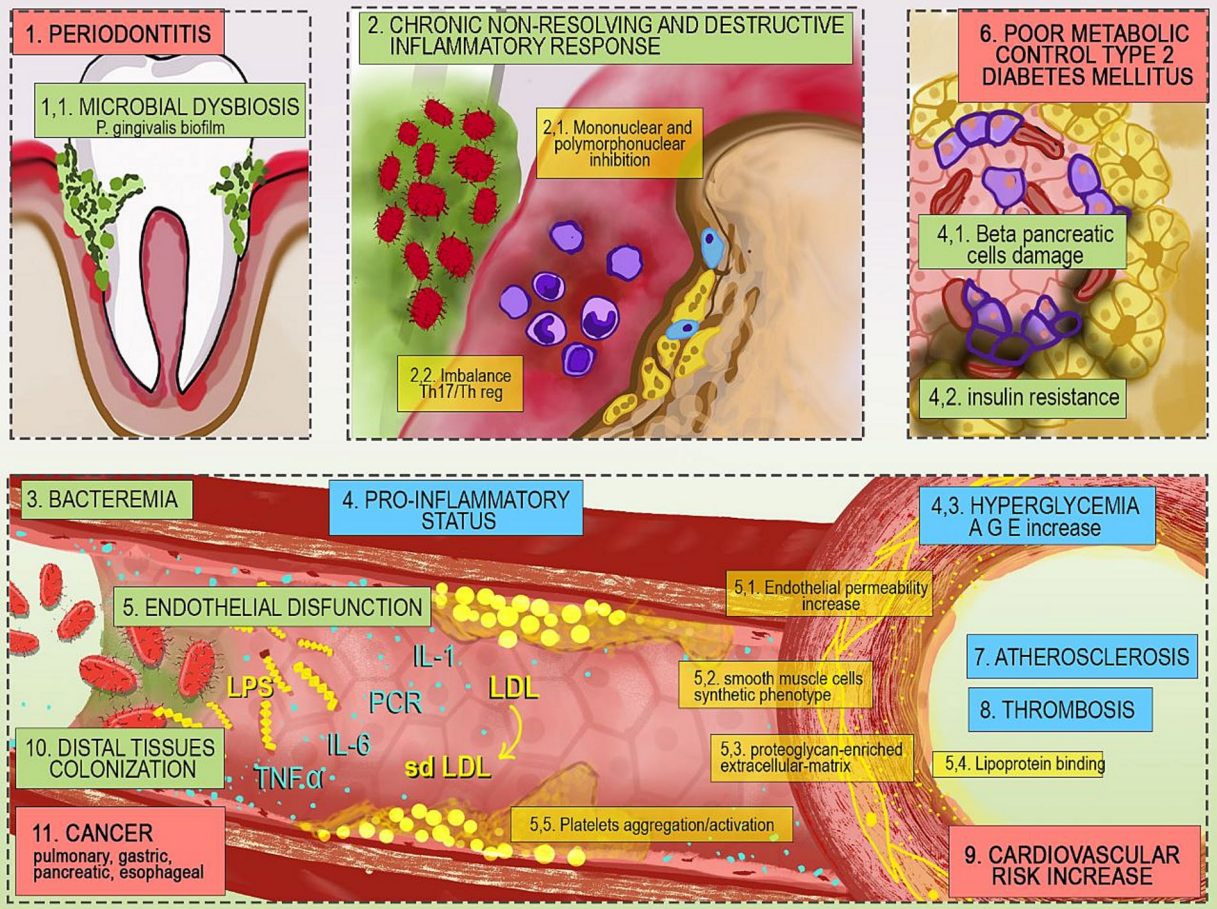

FIGURE 1
Mechanisms underlying the relationship between periodontitis and systemic diseases. 1. Microbial dysbiosis favors proliferation of *P. gingivalis*; 2. Bacterial infection destroys connective tissue and alveolar bone allowing the entry of various microbes into the bloodstream 3. The gram-negative pathogen *P. gingivalis* activates a pro-inflammatory status that 4. impairs pancreatic function and glycemic control; 5. The resulting endothelial dysfunction, combined with the action of bacterial endotoxins worsens 6. metabolic control and contributes to 7. Atherosclerosis, 8. Thrombotic phenomena and 9. Increased cardiovascular risk. 10. In addition, endothelial dysfunction allows colonization of distal tissues and 11. Increased risk of cancer of the lung, pancreas, esophagus, and stomach. Th17, T helper 17 lymphocyte; Threg, regulatory T helper lymphocyte; LPS, lipopolysaccharide; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; PCR, C-reactive protein; IL-1, interleukin 1; sdLDL, small and dense low-density lipoprotein; LDL, low-density lipoprotein; AGE, advanced glycation end products.

degradation, destruction, and proliferation, leading to more frequent and severe PD in people with diabetes, increased difficulty in controlling blood glucose and increased risk of ACVD (11, 27, 32).

Improving periodontitis management and metabolic control in individuals with diabetes

In addition to pharmacological treatment, the World Health Organization recommends a healthy diet, physical activity, and regular screening and treatment for complications to prevent adverse outcomes (33). In this context, any measure to improve glycemic control is welcomed.

The ADA concludes in its 2022 edition of the Standards of Medical Care in Diabetes, that the benefits of periodontal treatment in diabetic patients remain controversial (9) because, although the evidence clinically and statistically demonstrates a reduction in HbA1c in T2DM ranging from 0.27% to 0.48% at 3–4 months post-intervention with a reduction similar to that usually achieved by adding a second anti-diabetic agent to the pharmacological regimen (11, 34), and also comparable to the expected advantage from the insulin pen over the classical syringe method of administration in insulin users (35); the Cochrane review on this topic in 2015, rated this body of evidence as weak and insufficient to demonstrate that this effect could be sustained beyond 4 months (34). Since then, new evidence has been published. In 2018 a randomized clinical trial (RCT) by D'Aiuto et al. (36) showed that intensive and standardized periodontal treatment of moderate-to-severe periodontitis in adults with T2DM achieved a sustained 0.6% reduction in HbA1c after 12 months of follow-up. In 2021, new evidence (37) emerged on the efficacy of adding an adjunctive drug to non-surgical periodontal treatment with extended follow-up beyond 4 months. This RCT achieved a > 1% reduction in HbA1c at 3 and 6 months, with or without adjunctive antibiotic prophylaxis (37). The most recent Cochrane review in 2023 thus modified its conclusions, indicating that periodontal treatment with subgingival instruments improves glycemic control over 6 months in patients with both diabetes and periodontitis by a clinically meaningful proportion compared with no treatment or usual care, with moderate certainty of evidence (38), consistent with recent systematic reviews (39).

Periodontal treatment: a catalyst for inflammation control

In 2017, the CANTOS trial (40) showed that reducing vascular inflammation significantly reduced the rate of recurrent cardiovascular events even in the absence of concomitant lipid lowering. The main indicator of the reduction in inflammation was CRP, a known prognostic marker of vascular events associated with various metabolic conditions (29). In addition to the improvement in glycemic control, D'Aiuto et al. also confirm a reduction in inflammatory markers such as CRP and TNF-α after 6 and 12 months of follow-up with a lower 10-year risk of coronary heart disease, consistent with evidence of a direct association of periodontal therapy with lower rates of myocardial infarction and heart failure in T2DM (26). This improvement in the overall inflammatory status is also the proposed mechanism to explain the reduction in creatinine levels in patients with type 2 diabetes receiving intensive periodontal therapy (36, 41) and the lower incidence rate of end-stage renal disease in the general population following surgical treatment of periodontitis (42).

Critical considerations for public health policy: integrating periodontal treatment into diabetes management

The bidirectional relationship between PD and T2DM has long been recognized, to the extent that the recent EFP/AAP World Workshop proposed the inclusion of glycemic control in the diagnostic scheme for patients with periodontitis (43). While hyperglycemia increases the risk and severity of PD, periodontitis worsens metabolic control in T2DM (11, 26). Consistent with this evidence, a USA study published in 2009 found that using periodontal measures to assess T2DM risk significantly improved screening rates for prediabetes and diabetes (44), and early detection led to recommendations for cost-effective lifestyle changes that resulted in a significant proportion of patients with prediabetes normalizing their blood glucose levels (45). These results encouraged NICE in the UK to recommend the involvement of dentists and physicians in screening for T2DM (11).

On the other hand, PD is a highly relapsing chronic disease, in which inflammation promotes the growth of dysbiotic microbial communities of "inflammophilic" bacteria that perpetuate tissue destruction in a vicious cycle of mutually reinforcing dysbiosis and inflammation (46, 47). Therefore, a key element of therapeutic success is to restore a healthy and durable oral ecosystem with long-term stability to favor improvement in clinical parameters such as probing depth, bleeding on probing or suppuration (46), and inflammatory parameters, to control both dysbiosis and disease progression (47). Thus, regular examination and treatment of recurrent PD would likely contribute to metabolic control and stability in patients with T2DM. Experts have stated that asking about symptoms and performing a visual assessment of oral and periodontal changes is straightforward and should be part of the evaluation of diabetic patients to make the necessary referrals (9, 45).

The oral health community has led several initiatives as screening tools. In a pilot study in the US, chairside HbA1c testing was administered to 50 patients with periodontitis who reported one or more of the ADA's T2DM risk factors, detecting 32% of individuals with pre-diabetic HbA1c levels. Each HbA1c test costs US$9 (48). A more affordable option was demonstrated in Germany, where the Find-Risk questionnaire was modified to add the presence or absence of severe periodontitis and administered to general patients attending private dental services, with a positive predictive value of 35%. They were referred to a diabetologist for further diagnosis, where more than half of the patients had altered blood tests (49). Table 1 summarizes some recommendations for clinicians assessing patients with diabetes and dentists assessing adults with PD.

Inequalities in periodontal treatment and diabetes

There are several barriers to integrating PD prevention, control, and treatment into diabetes care programs. First, the current model

TABLE 1 Recommendations for physicians and dentists for screening and prevention of T2DM and PD.

Medical assessment of diabetes to screen PD		
Immediate referral to dentist	**Recommend professional oral examination**	**Prevention of PD**
Prior diagnosis of PD with no or poor follow-up of care.	Annual periodontal check-up for all persons with newly diagnosed diabetes mellitus, especially smokers.	Education: About the increased risk of PD, and the importance of watching for any signs or symptoms of gum disease.
Signs or symptoms of PD: • Bleeding gums during brushing or eating. • Loose teeth • Spacing or separation of the teeth • Bad oral odor and/or bad taste • Abscesses in the gums or gingival discharge.	Common oral problems in patients with diabetes: • Dry mouth • Burning mouth • Poor healing of oral wounds • Candida infections	Action: Advise that successful periodontal therapy can improve glycemic and metabolic control. Promote proper oral hygiene, i.e., twice-daily brushing for a minimum of 2 min, inter-dental cleaning, etc.
Dental assessment of patients with PD to screen T2DM		
Immediate referral to physician	**Recommend testing for prediabetes and T2DM**	**Prevention of T2DM**
Prior diagnosis of T2DM with little or no follow-up care. Ask about blood glucose and HbA1c levels.	Overweight or obese adults with one or more risk factors:	Education: About the increased risk of T2DM, especially in the presence of other risk factors.
In the presence of classical symptoms, such as: • Polydipsia • Polyuria • Polyphagia • Unexplained weight loss	• First-degree relative with diabetes • History of ACVD, use of anticoagulants or antiplatelet agents • Hypertension • Dyslipidemia or users of lipid-lowering drugs • Women with polycystic ovary syndrome • Physical inactivity • Conditions associated with insulin resistance, i.e., acanthosis nigricans, skin tags, etc.	Action: Referral to primary care, diabetes prevention program and/or nutrition programs available in your country.

of Westernized modern dentistry with a strong focus on treatment and high technology (50) greatly increases oral health expenditures, making it difficult for low- and middle-income countries to cover oral health services and increasing out-of-pocket expenditures for vulnerable populations, who are at greater risk of CHE and impoverishment (15). The prevalence of T2DM is directly related to lower socioeconomic status, less access to healthy food, lack of safety, and lower educational attainment in different countries (51). On the other hand, a low educational attainment has been shown to be a predictor of chronic PD, a relationship that is partly explained by the association between the educational attainment and T2DM (52), in addition to their common risk factors such as smoking, age, gender and overweight (20). A second barrier is the significant recruitment bias that exists in clinical studies of the effect of periodontal treatment on HbA1c, where the predominant participants are middle-aged white women with access to primary care providers, making it difficult to extrapolate results to those most at risk for PD and T2DM: older men, low socio-economic status, and racial/ethnic minorities (53).

In Latin America, Chile is one of the five countries that have assessed dental status in nationally representative samples of adults (19). In the early 2000s, this country embarked on a major health reform that included oral health, with the aim of improving access to and quality of health care and reducing health inequalities (54). Currently, inequalities in dental service utilization and untreated dental caries have been reduced. However, remaining teeth inequalities have increased, which can be explained by the application of low-complexity treatments with frequent dental extraction in low-income adults with free access to dental care (54). In addition, the Chilean health reform was designed to target the most prevalent diseases and at-risk populations. As a result, dental programs benefit certain age groups and pregnant women but ignore other populations with a high prevalence of oral disease, such as those with cardiovascular disease, diabetes or other NCDs (55).

Discussion

Both T2DM and PD are major causes of illness that can lead to disability and death (1, 2, 10). These conditions disproportionately affect low- and middle-income countries (12, 16). PD is two to three times more common in people with diabetes than in the general population (26). It is associated with a 6-fold higher risk of poor glycemic control and increased risk of complications compared with diabetic patients with a healthy periodontium (29). The effect of periodontal treatment on reducing HbA1c is comparable to the effect of adding a second drug to the antidiabetic pharmacological regimen (11). Despite historical controversy regarding the benefits of treating periodontitis in diabetes (9), recent evidence clearly confirms its benefits in terms of glycemic control, renal function, cardiovascular risk reduction, alleviation of chronic inflammation, and improvement in quality of life (36, 56); all with a clinically significant impact (38).

In addition, prevention of PD in T2DM may be a cost-effective strategy, involving regular check-ups in which clinicians inquire about symptoms and perform oral visual assessments (9, 11, 46). On the other hand, dentists can play an important role in the screening of T2DM through earlier medical evaluation in prediabetic stages (48, 49). Based on the evidence reviewed, it is also important to discuss the need to expand the services covered by T2DM insurance plans. This could make screening and improvement of glycemic status through PD treatment affordable in low- and middle-income countries.

Strengths of our study include a comprehensive review of the literature across multiple domains and the potentially high impact of our findings, given the widespread prevalence of both T2DM and PD. However, it is important to acknowledge its limitations. The certainty of our findings is limited by the exploratory nature of this review, which differs from the rigor of a systematic review. In future phases, our agenda includes evaluating the efficacy and safety of readily implementable interventions by both physicians and dentists to improve standards of care in the outpatient setting.

We must keep in mind the relapsing nature of PD and that durable metabolic control in T2DM may depend not only on the success of PD treatment, but also on regular follow-up to re-establish a healthy and durable oral ecosystem with long-term stability to favor improvement in clinical parameters and keep the inflammatory state in check. Finally, the prevention, treatment and control of PD in diabetic patients should be included in public health policies to improve metabolic control and quality of life and reduce diabetes complications not only in high-income patients but also in the most socially disadvantaged patients.

Author contributions

CS: Investigation, Validation, Writing – original draft. PO: Investigation, Validation, Writing – original draft. NF: Investigation, Validation, Visualization, Writing – original draft. BC: Investigation, Validation, Visualization, Writing – original draft. FA: Investigation, Validation, Visualization, Writing – original draft. FG: Conceptualization, Supervision, Validation, Writing – review & editing. IM: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing.

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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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Current knowledge of morbidities and direct costs related to diabetic foot disorders: a literature review

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Diabetes is a chronic disease associated with numerous complications including diabetic foot disorders, which are associated with significant morbidity and mortality as well as high costs. The costs associated with diabetic foot disorders comprise those linked to care (direct) and loss of productivity and poor quality of life (indirect). Due to the constant increase in diabetes prevalence, it is expected that diabetic foot disorder will require more resources, both in terms of caregivers and economically. We reviewed findings on management, morbidity, mortality, and costs related to diabetic foot disorder.

KEYWORDS

diabetic foot syndrome, infection, morbidity, cost, review

1 Introduction

Diabetes mellitus is a major public health concern with a rapidly increasing prevalence over the past several decades. Its worldwide prevalence is estimated to be approximately 10.5%, representing 536.6 million people, with a projected increase in 2045 to 12.2%, representing 783.2 million individuals. The greatest increase in prevalence is expected in areas currently undergoing an economic transition from low to middle-income levels (1). Several factors contribute to the current increase in the prevalence of type 2 diabetes, including a sedentary lifestyle, unhealthy diet, population aging, urban expansion, and economic growth (1). Diabetes mellitus is a leading cause of mortality, decreased life expectancy, and reduced quality of life worldwide. In the presence of diabetes, all-cause mortality is estimated to increase by two to three times (2, 3). Among the different anatomical complications of diabetes and associated metabolic syndromes, diabetic foot disorders are the most recurrent, and they represent an ever-increasing health care problem.

In this narrative review, we discuss the current knowledge on the impact of diabetes-related (foot) complications, including diabetic foot disorders, in terms of quality of life and direct costs related to its prevention and therapies. However, in this review targeting healthcare workers in resource-rich countries, we deliberately did not address the associated costs and burden in terms of economical, psychological, epidemiological, societal, lifestyle, or political aspects, for which specific literature is available (4, 5).

1.1 Diabetic foot management

A diabetic foot ulceration (DFU) multidisciplinary team approach is highly recommended and has been shown to be the most effective strategy for reducing the rates of amputation and mortality in diabetic foot disorders (6, 7). Ideally, this team should include a diabetologist, podiatrist, infectious disease specialist, orthopedic surgeon, vascular surgeon, physiotherapist orthotist, diabetes nurse, and an interventional radiologist (8, 9). The team's skills include wound care such as surgical and non-surgical wound debridement, adapted exudate control, vascular assessment, off-loading treatment, glycemia, lipid profile and blood pressure control, vascular and infection assessment, and, if required, revascularization procedures, antibiotic administration, and eventually amputation. The most important aspect of the DFU multidisciplinary team is that it comprises individuals with medical and surgical disciplines. Moreover, larger teams benefit from having a team leader and a team member structure, and clear referral pathways and care algorithms are important (10).

Wound debridement is performed to remove nonviable tissues that can interfere with wound healing by facilitating bacterial colonization and infection. Off-loading is a cornerstone of DFU management, as it allows redistribution of plantar pressure, promoting the healing process of DFU (11). The weight is then displaced to nearby areas that are not injured, thereby facilitating the healing process. Although offloading is fundamental, it can cause significant restrictions in daily life, mainly because of the resulting reduced physical movement.

In the presence of arterial insufficiency with hemodynamic impairment, a revascularization procedure that can be performed using an endovascular approach with angioplasty and stenting or surgical bypass grafting, or combination of these two if necessary, should be considered. Controlling plasma glucose levels can be beneficial, as hyperglycemia has been shown to be associated with delayed wound healing (12). The choice of anti-diabetic treatment must be individualized according to several parameters such as glycemic and weight goals, cardio-renal protection, side effects associated with treatments, mode of administration or even cost or accessibility (13). Stopping smoking is also beneficial for wound healing of DFU and must therefore always be considered in the management (14).

Antibiotic therapy is another important aspect that should be considered in DFU treatment (15). This is aimed at treating infection and not at healing the wound. It is crucial to investigate whether an infection is present according to the International Working Group on the Diabetic foot (IWGDF) guidelines. Empirical antibiotic treatment is often performed and should be based on a clinical suspicion of the causative bacteria, clinical severity, presence of previous microbiological culture results, presence of comorbidities such as chronic kidney failure, and antibiotic allergy history (16). If possible, a deep-wound surgical specimen should be obtained (7). Antibiotic treatment must be provided, with a narrow spectrum tailored to the microbiological results and the duration discussed by infectious disease specialists.

2 Diabetic complications

Diabetes mellitus is associated with various vascular complications that have traditionally been divided into two categories: macrovascular and microvascular pathologies. Macrovascular conditions include coronary heart disease, peripheral arterial disease (PAD), and stroke, while microvascular diseases include retinopathy, diabetic kidney disease (DKD), and peripheral neuropathy. These complications are very common, as approximately half of the individuals with diabetes have microvascular complications and more than a quarter have macrovascular complications (17). In diabetes, increased all-cause mortality rate is associated with cardiovascular, cerebrovascular, and chronic kidney diseases. According to the International Diabetes Federation, 6.7 million deaths can be attributed to diabetes in 2021 (18).

Other medical conditions commonly associated with diabetes include dementia, cancer, nonalcoholic fatty liver disease, and obstructive sleep apnea (19). These conditions now play a major role in the diabetes-related morbidity and mortality. For example, cancer is now considered the primary cause of death among individuals with diabetes in some countries, and the number of deaths attributed to dementia has significantly increased over the past several decades (20, 21). Owing to the many complications associated with diabetes, significant morbidity has been observed, resulting in an estimated 68 million disability-adjusted life years (22).

3 Diabetic foot disorders and amputations

Diabetic foot is a major complication of diabetes and includes a spectrum of injuries such as ulceration, infection, and destruction of tissue or bone. It is practically always the result of microvascular damage with neuropathy and/or macrovascular damage in the form of PAD (Figure 1). Diabetes is a serious and potentially devastating complication. The annual incidence of DFU is estimated to be less than 2.2%, and the risk of developing foot ulcers over the course of the life of a person with diabetes is greater than 30%. The rate of recurrence after wound healing reaches approximately 40% in the year after the episode (23). A meta-analysis of 67 studies mainly from Europe and Asia, including 801,985 individuals, showed a variation in its prevalence between 3% in Oceania and 13% in North

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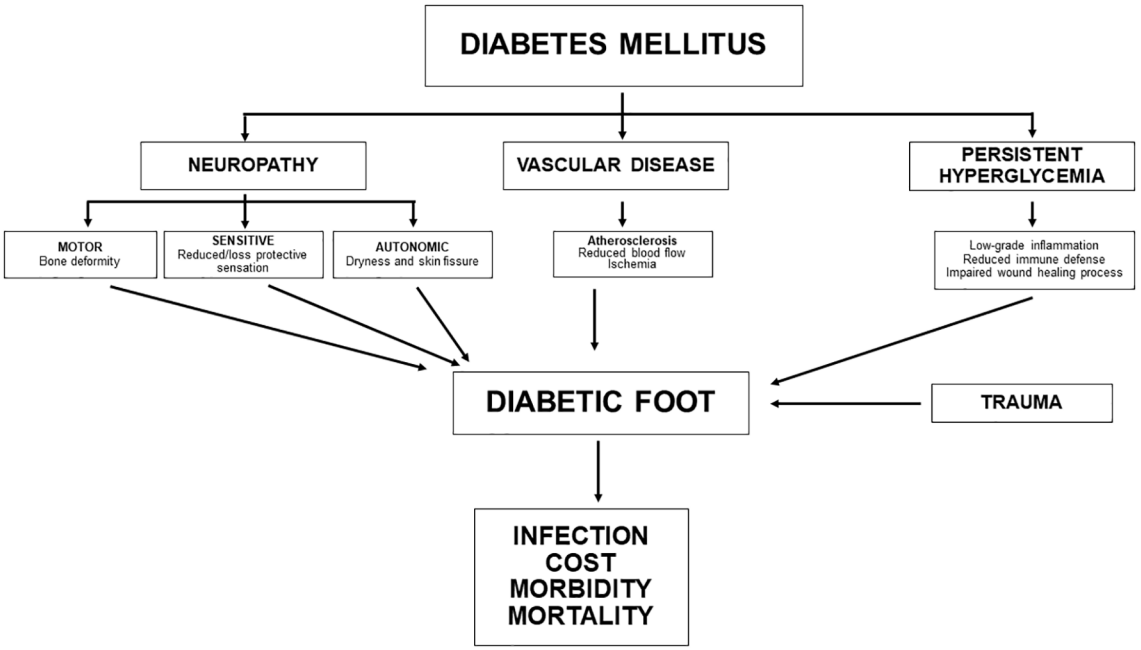

FIGURE 1
Pathophysiology of diabetic foot disorderss.

America, with a global average of 6.3% (24). Owing to the increasing prevalence of diabetes and prolonged life expectancy, the incidence of DFU is expected to increase in parallel.

For many vascular diseases, the most established risk factors for DFU are male sex, diabetes duration, HbA1c level, active smoking, high body mass index, PAD, and chronic kidney disease (24). Proinflammatory cytokine levels were also significantly higher in patients with diabetes with DFU than in those without DFU (25).

In the context of diabetic foot, adiponectin appears to promote wound healing as well as to protect against the development of atherosclerotic plaques and, therefore, cardiovascular (CV) diseases (26, 27). Its potential therapeutic role in diabetes, more specifically in diabetic foot, remains to be determined. Altogether, these findings highlight that diabetic foot is characterized by an exacerbated inflammatory state that promotes atherosclerosis; therefore, CV events contribute to the associated mortality in DFU. The increased CV risk in people with diabetes and DFU can be attributed, at least partially, to biochemical alterations, such as serum LDL > 130 mg/dL, hypertriglyceridemia, microalbuminuria, and proteinuria (28).

Peripheral arterial disease is an atherosclerotic narrowing of the peripheral arteries of the lower extremities and another contributor to DFU. The main risk factors of PAD are diabetes, hypertension, dyslipidemia, smoking, and age. Patients with diabetes with PAD are generally unaware of their condition, and signs and symptoms often only appear when the disease has already advanced, indicating that PAD remains largely underdiagnosed. When present, the signs and symptoms of PAD include pain in the lower limbs on exertion or at rest, non-healing wounds, ulcers, or gangrenes (29). The prevalence of PAD in patients with DFU is > 40% (30). The cornerstone of PAD management is revascularization of narrowed or occluded lower limb arteries to restore blood flow and induce wound healing. In PAD, the presence of diabetes is associated with more severe and distal arterial lesions, as well as a higher rate of amputation and mortality, compared with the absence of diabetes (31). In a series of 583 patients who underwent minor amputations due to diabetic foot osteomyelitis, 84% of those who required transtibial amputation over the follow-up period had concomitant PAD (32).

Diabetic foot ulceration generally develops after repetitive or minor (unrecognized) trauma to a part of the foot, with impaired wound healing due to PAD and/or peripheral neuropathy. Diabetic peripheral neuropathy (DPN) is the main cause of DFU. DPN can be sensitive, motor, or autonomic in nature. Sensitive neuropathy reduces the protective sensation of the feet, thereby reducing the detection of minor trauma, thermal injury, or overpressure in certain areas of the foot. Diabetic peripheral sensitive neuropathy is the primary component in more than half of all diabetic foot disorders (33). Motor neuropathy affects the biochemical aspects of foot ulceration, with progressive foot structural alteration leading to joint mobility impairment and anatomical deformation, causing detrimental and inappropriate pressure loads on the foot (34). Consequently, calluses appear on an area under high pressure, which promotes skin cracking, with perforation of the subcutaneous tissue contributing to DFU formation. Diabetic autonomic neuropathy elicits dryness, alterations in skin texture, edema, venous prominence, and nail loss, thereby leading to the development of DFU (35). Approximately half of all diabetic foot disorders are complicated by diabetic foot infection (DFI), and approximately 20% of moderate or severe infection episodes eventually require lower-extremity amputations at various levels

(23). One in five diabetic food disorders is associated with the presence of (chronic) osteomyelitis (36).

Diabetic foot infection is a leading cause of hospitalization among people with diabetes, representing 20% of hospital admissions in the United States of America (USA). DFI occurs mostly due to terminal ischemia that is not amenable to revascularization and slightly due to infection. However, infection can precipitate the need for the amputation of a chronically ischemic foot. Additionally, DFI is associated with a readmission rate of approximately 40% (37, 38). Diabetes-related lower extremity amputation is preceded in most cases by DFU, which can be associated with ischemia or infection and is the number one cause of non-traumatic lower limb amputations worldwide.

Amputation is often considered the last option for the management of non-salvageable limbs. The indications for this procedure are the presence of extensive necrotic tissues with possible rapid extension and several conditions for which the patient and clinician are of the opinion that amputation will yield better results in terms of the overall improvement in locomotor function and quality of life (39). The selection of the amputation procedure type depends on the extent of bone infection, degree of lower limb arterial insufficiency, severity of soft tissue injury, and patient's overall clinical state and functionality (40). The pursuit of antibiotics after amputation for an episode of DFI may not be needed (41). Globally, every 30 s, diabetes-related lower limb amputation is performed (42). It is estimated that approximately 90% and 67% of yearly amputations in the United Kingdom and USA, respectively, are related to diabetes (43). An episode of lower limb amputation should be considered a major risk factor for subsequent amputations as illustrated in a series of 102 individuals with transtibial amputation. In this cohort, at 2 years of follow-up after amputation, one-third of patients developed a diabetic foot disorder in the contralateral limb, and 10% underwent contralateral transtibial amputation (44). A series of 583 cases of diabetic foot osteomyelitis identified hind foot localization as an independent risk factor for limb loss (odds ratio, 5.4) (32).

4 Morbidity related to diabetic foot disorders

Diabetic foot disorders are associated with substantial morbidity and significantly reduced health-related quality of life (HRQoL) (42). They represent a considerable and increasingly prominent component of the global disability burden, with more than 2% of the global years lived with disability and almost two-thirds of all diabetes-related years lived with disability (45). A meta-analysis of 12 studies showed that people with DFU have significantly reduced HRQoL, especially in terms of their physical capacity and perception of general health (46).

Reduced HRQoL has been reported not only in patients with active ulcers and previous episodes of amputation but also in people whose ulcers have healed. A study of more than 300 participants with previous foot ulceration reported reduced HRQoL in most indicators, with the physical domain showing the greatest reduction in these patients compared with the control population (47).

In a study by the Eurodiale group, healing of DFU was reported to be associated with improvement in HRQoL. Another study that prospectively assessed the impact of hyperbaric oxygen therapy revealed a significantly higher mental quality of life, better social function, and reduced physical limitations in individuals who were healed compared with those who were not healed (48).

Functional recovery at the locomotor level in the postoperative period is crucial for a patient's quality of life and overall health. Indeed, after transtibial amputation, patients can be ambulated based on a perception of superior well-being as well as the fact that being ambulatory is associated with improvement in cardiovascular health, which can reduce morbidity and mortality (49). One disadvantage of the multidisciplinary management of DFU is that patients are required to attend multiple appointments with various healthcare workers, which may be considered burdensome. Altogether, diabetic foot disease is associated with low HRQoL, particularly with regard to the physical quality of life. However, currently, no gold standard tool exists to assess patient-reported outcomes in diabetic foot disorders, and healing of diabetic foot ulcers is ultimately associated with improvement in quality of life (50).

5 Mortality related to diabetic foot disorders

The 5-year overall mortality in individuals with DFU or diabetic Charcot arthropathy is approximately 30% and increases to more than 50% after a major amputation. Therefore, the survival rate after a major amputation is lower than the 5-year survival rate of patients with most local cancers (51). The risk factors associated with mortality in DFU are age, male sex, chronic kidney disease, and presence of PAD. Compared with that of patients of the same age and disease, the life expectancy of those with DFU is lower by five years (52, 53).

In addition, people with DFU or diabetic Charcot neuroarthropathy have an estimated reduction in life expectancy of 14 years (54). The severity of the level of amputation is correlated with the survival rate as shown in a study reporting a two-fold higher 2-year survival rate in patients with minor amputation (below the ankle) than in those with transtibial amputation (54).

The increased mortality risk in patients with DFU is primarily due to cardiovascular events. Patients with DFU have more CV risk factors, CV pathologies, and subclinical markers of CV diseases, compared with patients with diabetes without DFU (55). Patients with diabetic neuropathy display increased levels of inflammatory cytokines such as TNF-a, IL-1, and IL-6 (56).

Therefore, diabetic foot disease is associated with a high rate of mortality, although for patients, the fear of a major amputation remains higher than the risk of death (57). This highlights the fact that a significant proportion of mortality in diabetic foot complications is related to associated comorbidities, such as cardiovascular disease and DKD. However, DFU itself has been

shown to be an independent risk factor for premature mortality and lower limb amputation (58).

These observations have led to the suggestion of terminology modification in DFU by preferentially using the term "in remission" instead of "healed" to better illustrate the significant risk of recurrence for the patients and physicians and, therefore, the importance of regular and life-time follow-up after any episode of DFU (51).

6 General costs related to diabetes and diabetic foot disorders

Diabetes mellitus has incurred at least USD 966 billion in health expenditures worldwide, with a 316% increase over the past 15 year (18). Diabetic foot complications have major economic impacts on patients, families, and society. Information regarding expenditures related to diabetic foot is of particular importance, especially for public health policymakers, to encourage and develop prevention and therapeutic programs dedicated to this disorder. To properly assess the economic burden of diabetic foot diseases, it is crucial to consider direct and indirect costs such as primary care, podiatrist care, nursing care, special footwear, hospitalization, rehabilitation medicine, consequences of lower limb amputations, including loss of productivity, home care, family status and costs, and reduced quality of life (Table 1). Therefore, comparisons of the costs associated with diabetic foot between different areas of the world or countries remain complex, as the health economic machinery, including the reimbursement system, is not uniform. Furthermore, treatment approaches, reimbursement policies, and study designs remain heterogeneous among the published and available data worldwide. The severity of diabetic foot disorders is correlated with the associated costs. Indeed, expenditure related to DFU is higher if the severity of the ulcer is higher, and healthcare expenditures are five times higher in people with diabetes with DFU than in those without ulcer (59). Therefore, expenditures related to diabetic foot disease may vary greatly depending on the interventions used and the overall management approach. In Europe, the annual direct and indirect costs related to DFU are estimated at approximately USD 13 500 per person affected. In the UK, approximately 0.6% of the National Health Service budget is allocated to cover the management of DFU (60).

There are several studies that have evaluated the costs with the management of DFU. These costs vary greatly depending on the regions of the world and the periods during which the studies were carried out (Table 2).

In the United States of America, the direct expenditure attributed to diabetes was estimated at USD 237 billion in 2017, representing an increase of more than 25% from 2012, including 30% related to diabetic foot management. This cost is in the same range as that of cancer in 2015 (USD 80 billion) (51). In developing countries, the available evidence of expenditures related to diabetic foot remains limited, even though its burden is most likely to be higher. In Brazil, one study estimated the direct medical costs of inpatient and outpatient care for diabetic foot disorders. The annual cost represented 0.3% of all public health expenditures and mainly comprised outpatient care rather than inpatient care (87% vs. 13%, respectively) (71). It remains very difficult to correctly estimate the unrelated costs of transport, which vary according to different factors such as area, medical density and the literature currently available is limited. Transport is a real barrier for patients, especially those living in rural areas but also in urban areas, Due in particular to traffic jams, parking problems, travel fatigue and access limitations when using wheelchairs (73). Telemedicine could represent a step forward in this aspect and make it possible to reduce transport-related contracts. A recent randomized study showed that in patients with DFU, the addition of monitoring by telemedicine with a specialized nurse makes it possible to reduce costs and hospital stays compared to conventional monitoring and also probably indirectly costs and transport constraints (74). DFU is associated with a high emotional load, favoring anxiety and

TABLE 1 Direct and indirect cost associated with diabetic foot disorders.

Direct cost
Primary care
Global diabetes management including glucose control
Medical and nursing ambulatory care
Podiatrist associated care
Wound dressing
Debridement
Antibiotics
Microbiological analysis
Offloading devices
orthopedic diabetic shoes
Radiological imaging
Revascularization procedure
Orthopedic surgery
Amputation
Hospitalization
Physiotherapy
Orthopedic prostheses
Indirect cost
Loss of productivity
Under employment/unemployment
Absenteeism
Reduce family asset
Early retirement
Transportation
Psychotherapy
Home care

TABLE 2 Studies reporting the cost of diabetic foot.

Authors, year	Country	Number of individuals	Type of care	Cost
Alshammary, 2020 (61)	Saudi Arabia	99	Episode of DFU	1783 USD
Apelqivst, 1994 (62)	Sweden	197	All ulcer types; total direct costs	8659 USD
Ashry, 1998 (63)	USA	5062	Lower-extremity amputations, hospital charges only	27930 USD
Danmusa UM, 2016 (64)	Nigeria	1573	Episode of DFU	1104 USD
Girod, 2003 (65)	France	239	Monthly healthcare expenditure for DFU	1265 USD
Greenidge, 2021 (66)	Barbados	50	Episode of DFU	7272 USD
Jodheea-Jutton, 2022 (67)	India	7487	Episode of DFU	1960 USD
Kerr, 2014 (60)	The UK	72 459	Per episode of DFU	7539 USD
Lo, 2021 (68)	Singapore	1729	Mean cost per patient-year	3368 USD
Lu, 2020 (69)	China	3654	Episode of DFU	¥21827
Oksuz, 2016 (70)	Turkey	5000	Out-patient and in-patients costs of DFU care	14 288 USD
Prompers, 2007 (59)	Nine European countries	821	DFUs, total annual total direct and indirect cost	10,091 Euros
Toscano, 2018 (71)	Brazil	12994	Inpatient cost of DFU care	306 USD
Van Acker, 2000 (72)	Belgium	167	Direct and indirect expenditure per episode of DFU	10 572 USD

USD, United States dollar; ¥, Yen.

depression (75). The presence of depression will not only necessitate costly psychological support, but will also have an impact on quality of life by reducing it. The psychological cost associated with DFU could be considered an indirect cost of care, but is not yet clearly evaluated and estimated in the literature, and would therefore require future analysis. Reduced mobility and performance in the workplace will lead to frustration and emotional distress, making it even more difficult to provide comprehensive care (76). The loss of productivity and the risk of unemployment and/or early retirement may have also significant impact on the family asset, but remain to be properly quantified (76).

Therefore, preventive approaches to reduce DFU and lower limb amputation are considered a fundamental way to decrease the high costs associated. Available and regular diabetic foot care is a major way to decrease amputation rates in individuals with diabetes. A multi-disciplinary team approach remains crucial, as it has been shown to decrease amputation rates by approximately 85% and reduce the risks associated with DFU, leading to a higher quality of life (77, 78). The costs related to DFU are expected to increase alongside the prevalence of diabetes.

Future detection and implementation of cost-saving and cost-effective measures for the management of DFU are required to decrease its healthcare burden. Therefore, future research with economic comparisons of several strategies is required in the field of DFU.

7 Conclusions

In conclusion, diabetic foot disorders are associated with high rates of morbidity and mortality and have a major impact on health-related expenditures. As the prevalence of diabetes continues to increase, concerted efforts are needed in terms of prevention and treatment to reduce the burden associated with its complications, such as diabetic foot disorders. There is still ample scope for improving the current organization of available preventive and therapeutic care for diabetic foot disorders in various areas of the world. Therefore, new global strategies are urgently needed to counteract the deleterious effect of diabetes.

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The interplay between gingival crevicular fluid microbiome and metabolomic profile in intensively treated people with type 1 diabetes - a combined metagenomic/metabolomic approach cross-sectional study

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Aims: This study aimed to assess the gingival crevicular fluid (GCF) microbiome and metabolome of adults with type 1 diabetes (T1D) treated with continuous subcutaneous insulin infusion (CSII).

Methods: In this cross-sectional study, the GCF of adults with T1D treated with CSII and non-diabetic controls were sampled, and metagenomic/metabolomic analyses were performed.

Results: In total, 65 participants with T1D and 45 healthy controls with a mean age of 27.05 ± 5.95 years were investigated. There were 22 cases of mild gingivitis (G) in the T1D group. There were no differences considering the Shannon and Chao indices and β-diversity between people with T1D and G, with T1D without G, and healthy controls. Differential taxa were identified, which were mainly enriched in people with T1D and G. Acetic acid concentration was higher in people with T1D, regardless of the presence of G, than in healthy controls. Propionic acid was higher in people with T1D and G than in healthy controls. Isobutyric and isovaleric acid levels were higher in individuals with T1D and G than in the other two subgroups. The concentration of valeric acid was lower and that of caproic acid was higher in people with T1D (regardless of gingival status) than in healthy controls.

Conclusions: The identification of early changes in periodontal tissues by targeting the microbiome and metabolome could potentially enable effective prevention and initial treatment of periodontal disease in people with T1D.

KEYWORDS

type 1 diabetes, continuous subcutaneous insulin infusion, gingival crevicular fluid, microbiome, metabolome, gingivitis

Highlights

- To our knowledge, this is the first study to investigate the GCF microbiome in people with T1D. The study population was treated with modern technologies, such as CSII, demonstrating good glycemic control.
- Intensively treated people with T1D with satisfactory glycemic control and non-diabetic individuals generally showed good oral and periodontal health.
- With relatively well-controlled diabetes, slight differences in glycemic control did not significantly affect the oral microbiome, which was comparable to that observed in people without diabetes.
- Despite good metabolic control of diabetes, people with T1D in our study had a higher prevalence of mild gingivitis than healthy controls. This subpopulation exhibited shifts in the GCF microbiome and metabolome, resembling those in periodontitis.

1 Introduction

The oral cavity, also known as the mouth or buccal cavity, is the first section of the digestive system (1–3). It consists of several distinct microbial niches, such as tooth surfaces, gingiva, gingival sulci, and mucosal surfaces of the tongue, cheeks, lips, and palate (4). Species residing in the oral cavity are regarded as part of the oral microbiome (5), which is one of the most important microbial complexes in humans (6). It has been reported to include over 1000 species of bacteria, with a few lesser-known taxa emerging from the most recent studies, archaea, which are less abundant and diverse than bacteria, approximately 100 species of fungi, and a rich virome (7). Although the oral microbiome is defined as all microorganisms residing in the human oral cavity and its extensions (reaching the distal part of the esophagus), most studies have focused on samples obtained from the oral cavity itself (4). They can have both positive and detrimental effects on general health and the local state of the oral cavity (5, 6). The link between the oral microbiome and organisms is bidirectional. Diseases affect microbial composition and function, and microorganisms modify their susceptibility to disease states, course, and prognosis. One such condition is diabetes (8–10).

Type 1 diabetes (T1D) is a chronic autoimmune disease in which pancreatic beta cells responsible for insulin production are destroyed. People with T1D account for 5–10% of the population with diabetes (11). Continuous subcutaneous insulin infusion (CSII) using an insulin pump is one of the most notable advancements in diabetes technology. Insulin pump therapy has become the preferred treatment for T1D, as it mimics the physiological secretion of insulin better than multiple daily injections. People with T1D treated with CSII therapy compared to traditional multiple daily insulin injections achieve improved glycemic control (12).

Diabetes not only affects the oral microbiome but also increases the risk of multiple local oral abnormalities in the oral cavity, affecting the quality of life of people with diabetes (13). People with diabetes are highly susceptible to dental caries, tooth loss, and periodontal disease (PD) (14, 15). The mechanisms responsible include quantitative and qualitative salivary changes, formation of advanced glycosylation end products, and their deposition in tissues, leading to vascular dysfunction due to hyperglycemia and accompanying atherosclerosis (13, 16).

Alterations in the oral microbiome of people with diabetes have been extensively investigated; however, most studies have focused on type 2 diabetes. The oral microbiome of individuals with T1D has rarely been the subject of extensive research. Interestingly, the results often contrast and cannot be generalized. Microbiome shifts have been reported to affect the immune function and metabolic control in this population (17, 18). Moreover, reports tend to focus on those with poor oral health, caries, or PD (19, 20).

Subgingival plaque accumulation is associated with the supragingival environment (21). Caries and PDs, which are common oral biofilm-related diseases, are caused by resident microorganisms in the oral cavity (22). The *red complex* is a specific group of bacteria considered to play a major role in the development of adult PD. These bacteria include *Porphyromonas gingivalis, Treponema denticola*, and *Tannerella forsythia* (23). People with T1D and good metabolic control of diabetes without a history of oral pathologies, are still underreached, showing alterations in the oral microbiome, such as a greater abundance of *Streptococcus* spp., *Actinomyces* spp., and *Rothia* spp., than healthy controls (17). Additionally, some studies have

implemented traditional bacterial identification methods (24, 25). Therefore, the oral microbiome signature in T1D has not yet been established.

Novel approaches to the holistic investigation of phenomena include metagenomics, transcriptomics, proteomics, and metabolomics to identify the differences between healthy and diseased participants and their underlying mechanisms (26–28). Metabolomics can target various environments, such as stools, saliva, and gingival crevicular fluid (GCF), metabolites and metabolomics which can be associated with several diseases, including diabetes and PD (29–32). The roles of various metabolites in the human body, such as in the gut, respiratory tract, genitourinary tract, and oral cavity, are complicated and not well understood. In some cases, different concentrations of the same metabolite can have opposite effects depending on its location. Moreover, metabolites delivered in nutrients can influence microbiome composition, but these substances are also produced by bacteria, implicating complex bidirectional relationships (33).

Usually, saliva has been investigated, with only a few studies assessing the GCF microbiome or its metabolome. The GCF, which is derived from periodontal tissues, plays an important role in preserving the junctional epithelium and other periodontal structures (34, 35). It may play a dual role – either maintaining periodontal health and assuring the antimicrobial defense of the periodontium, as it contains immune cells, antibodies, and cytokines, or, when altered by acute or chronic immune processes, is responsible for the emergence of PD (36, 37). GCF contains multiple proteolytic and hydrolytic enzymes, bone-related biomarkers, cell death, and tissue breakdown products. Oral bacteria and products of their metabolism can also be identified in the GCF and add to the complexity of this oral niche (35). Thus, GCF analysis has the potential to become a predictive, preventive, and personalized medical approach for the diagnosis of PDs. Although GCF is inherently associated with PD, its metabolomics have rarely been investigated (29).

The present study aimed to assess the oral GCF microbiome and metabolome status in the group of adult people with T1D, homogenous with respect to the mode of diabetes management (CSII) and glycemic control.

2 Methods

2.1 Study design and participants

This was a cross-sectional study consecutively that recruited 110 adult participants. Sixty-five people with T1D were treated with continuous subcutaneous insulin infusion (CSII) in the Outpatient Clinic of the Department of Metabolic Diseases and Diabetology of the University Hospital in Krakow, an academic referral center for diabetes in southeastern Poland. Patients were matched with 45 non-diabetic controls.

Between October 1 and December 31, 2022, patients attending the clinic who met the inclusion criteria were offered the opportunity to participate in the study. After obtaining written consent, the sampling date was set and participants were instructed on how to prepare for the study procedures. The inclusion criteria were age 18–35 years, T1D diagnosed at least 1 year before recruitment, treatment with CSII for at least 6 months, and informed consent to participate. The exclusion criteria were pregnancy or breastfeeding and comorbidities, such as metabolic syndrome, cardiovascular disease, cancer, severe liver failure, or kidney failure. Diagnosis of T1D was confirmed based on the Diabetes Poland criteria. Data on age, sex, comorbidities, diabetes duration (on the day of sampling), glycated hemoglobin (HbA1c), and T1D treatment were extracted from the medical records. HbA1c levels were measured using high-performance liquid chromatography. Individuals with diabetes were matched with non-diabetic controls. The ratio of controls per case was set at 0.7:1.

Information on daily oral hygiene routines, frequency of dental appointments, and history of dental procedures were recorded. Examination of the oral cavity was performed by a trained dentist in a specially prepared room equipped with a dental chair and shadowless lamp to ensure maximal privacy for the participants. During periodontal examination, a WHO 621 periodontal probe was used to assess the Gingival Index (38), Gingival Sulcus Bleeding Index (39), and Plaque Index (39). PerioCP probe-15 was utilized to assess the Clinical Attachment Level (CAL) and Pocket Probing Depth. The oral health status was assessed using the Oral Hygiene Index (40), Community Periodontal Index, and Treatment Needs (41).

Microbiological samples were collected from the oral cavity by refraining from brushing the teeth with any kind of toothpaste or rinsing the oral cavity with any kind of mouthwash for 12 h prior to the visit. Additionally, information on the previous use of selected types of oral health products (i.e., toothpaste containing triclosan, mouthwash containing chlorhexidine, or any oral topical agent) was recorded. On the day of the examination, the participants refrained from brushing their teeth and drinking, eating, or smoking for 1 h before the microbiological samples were collected. To prevent salivary contamination of GCF, pieces of sterile gauze were used to remove excess saliva from the mucosal and dental surfaces. PerioPaper Strips were used to collect GCF samples. The strips were placed in the gingival pocket for 30–45 s until the surface was soaked. After the collection, strips were placed in 1 mL of Liquid Amies in a plastic screw cap tube (COPAN ESwab™).

2.2 DNA extraction and 16S rRNA sequencing

Genomic DNA was extracted and purified from PerioPaper Strips using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) with modifications to the bacterial protocols. DNA purity was measured on a NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and quantified using fluorimetry with the Qubit dsDNA High Sensitivity Assay (Thermo Fisher Scientific, Carlsbad, CA, USA). Bacterial 16S rRNA libraries were prepared using an Ion 16S™ Metagenomics Kit and an Ion Plus Fragment Library Kit as previously described (42). Next, constructed libraries were sequenced on an Ion Torrent Personal Genome Machine (PGM) platform (Thermo Fisher Scientific,

Waltham, MA, USA) using Ion PGM™ Hi-Q™ View Kit (Thermo Fisher Scientific, Waltham, MA, USA).

2.3 GCF microbiome metabolite quantification

The concentrations of short-chain fatty acids (SCFAs) and trimethylamine derivatives were determined using liquid chromatography coupled with mass spectrometry (Waters Acquity Ultra Performance Liquid Chromatograph, Waters TQ-S triple-quadrupole mass spectrometer, Waters). Waters MassLynx software was used for instrument control and data acquisition. Waters TargetLynx was used to process the data. To evaluate metabolites' concentrations, one strip with gingival crevicular fluid (PerioPaper Strip) was incubated with 50 µL PBS for 30 min to extract all analytes. SCFAs and lactic acid analysis were based on derivatization using 3-nitrophenylhydrazine and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide-pyridine solution. LC-MS/MS analysis was performed in the negative electrospray ionization multiple-reaction monitoring mode. The SCFAs were separated using a Waters BEH C18 column (1.7 µm, 2.1 mm x 50 mm) and a Waters BEH C18 guard column (1.7 µm, 2.1 mm x 5 mm). A 1 mL of formic acid in 1 L of water was used as mobile phase A, and 1 mL of formic acid in acetonitrile was used as mobile phase B. The flow rate of the mobile phase was set at 0.6 mL/min. To determine trimethylamine (TMA), choline, carnitine, betaine and glycerophosphorylcholine, only 20 µL of a sample was used. TMA was performed using a butyl bromoacetate solution as the derivative reagent. The LC-MS/MS method has been previously described (43).

2.3 Statistical analysis

The null hypothesis used in the study was that there are significant differences in the alpha and beta diversity of the oral GCF microbiome between people with T1D treated with CSII and healthy non-diabetic controls.

Metagenomic analyses included comparisons between healthy controls and people with T1D and gingivitis (G) vs. those with T1D without G. Individuals with T1D were additionally divided into quartiles based on HbA1c and the first and fourth quartiles were compared. Metabolomic analyses included a comparison between healthy controls and people with T1D and G vs. those T1D without G. Additionally, we analyzed the correlation between metabolite concentrations and HbA1c%. In the metagenomic-metabolomic analysis, correlations between the identified bacterial taxa and selected metabolite concentrations were compared.

The PS Imago Pro ver. 8.0 and Statistical ver. (13) were used for all the statistical analyses. When data were missing, a complete case selection approach was used. Normality of the continuous variable distribution was assessed using the Shapiro–Wilk test. Differences between groups were analyzed using Student's t-test or nonparametric tests (Mann–Whitney U test, Kruskal–Wallis ANOVA), when appropriate. Continuous variables were presented as arithmetic means ($\bar{x}$) ± standard deviations (SD) or as the median with interquartile range (IQR) when the data were not normally distributed. The distribution of categorical variables was described as counts and percentages. Statistical testing was performed to compare categorical variables using an independent sample chi-square test or Fisher's exact test, when appropriate. Statistical significance was set at $p < 0.05$. The Bonferroni method was used to correct multiple comparisons. Power calculations using the RNASeqPower package estimated a power of 90% for a coverage depth of 10x, sample size of 45 (each group), coefficient of variation of 0.5, and effect size (fold-change) of 1.5.

The unmapped BAM files were converted into FASTQ files using Picard SamToFastq (44). Additional steps of the analysis were performed using the Mothur version 1.47 software (45). FASTQ files were converted to FASTA format. For the analyses, only sequences 200–300 bp in length with an average base quality of 20 in a sliding window of 50 bases and a maximum homopolymer length of 10 were used. Chimeric sequences were identified using the VSEARCH chimera detection algorithm with default parameters (46)and the internal sequence collection as the reference database. Chimeric sequences were removed and the remaining 16S rRNA sequences were classified using the Wang method and the SILVA bacterial 16S rRNA database (47) for reference (release 138) with an 80% bootstrap cut-off.

Differential taxon abundances were assessed using a mixed-effects model implemented in LinDA (48). The nonparametric Shannon diversity index and Chao1 richness index were determined using Mothur, with differences in the values of the indices assessed using the Mann–Whitney U-test. Bray–Curtis indices and principal coordinate analysis (PCoA) were performed using the vegan package (49). FDR-adjusted (50) P-values ≤ 0.05 were considered statistically significant. For SCFAs and amino acids, correlations with bacteria were determined using the Spearman's coefficient.

2.4 Ethics and reporting guidelines

This study involving humans was approved by the Jagiellonian University Bioethics Committee (Komisja Bioetyczna Uniwersytetu Jagiellońskiego). The study was conducted in accordance with local legislation and institutional requirements. All the participants provided written informed consent to participate in this study.

This study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines. This checklist has been added to the Supplementary File.

3 Results

A total of 110 participants were included in the study: 65 people with type 1 diabetes and 45 matched healthy controls. The mean age of the sampled population was 27.05 ± 5.95 years. Sixty percent of participants were male. The mean duration of diabetes was 15.5 ± 8.4 years. All people with T1D were treated with continuous subcutaneous insulin infusion. The mean Hba1c% was 6.97 ±

0.95% (53 ± 2.2 mmol/mmol). No baseline differences were observed between the groups (Supplementary Table 1).

None of the participants had dental implants or prostheses. There were no cases of periodontitis in the study population, and 22 cases of mild G were observed in the T1D group. The selected dental indices and the prevalence of edentulism are summarized in Supplementary Table 1.

3.1 GCF microbiome analysis

Taxonomic profiling revealed that *Firmicutes* was the most abundant phylum (mean: 34%), followed by *Proteobacteria* (mean: 27%), *Bacteroidota* (mean: 16%), *Actinobacteriota* (mean: 12%), and *Fusobacteriota* (mean: 7%) (Figure 1). At the genus level *Pasteurellaceae*_unclassified, *Haemophilus*, *Actinomyces*, *Veillonella*, and *Fusobacterium* were the most abundant genera (Figure 1).

To identify the potential differences in the structure of the GCF microbiome, we first evaluated the α- and β-diversity in subgroups of T1D cases and controls divided according to clinical status. The α-diversity was analyzed using the Shannon index, a marker of bacterial richness and evenness, and the Chao index, a marker of richness. The β-diversity was analyzed using PCoA of Bray-Curtis distances.

GCF community composition did not differ between T1D cases and controls considering insignificant differences in the Shannon and Chao indices (Figure 2A, B), whereas there were borderline significant differences in the β-diversity (p =0.058, Figure 2C). In the first comparison between people with T1D without G and healthy controls, after adjusting for multiple comparisons, no differentiating taxa were identified. Next, we compared the GCF microbiomes of people with T1D and G to those with T1D and no gingival pathology. We found as many as 31 differential taxa at the adjusted p-value significance level when comparing people with T1D and G to those without G. All but one taxon, *Cutibacterium* spp. (p adjusted 0.04; FC -0.88), were overrepresented in participants with G (Supplementary Table 2). Third, people with T1D and G were compared with healthy controls. There were 38 differentially expressed taxa at the adjusted p-value significance level. All but one taxon, *Hemophilus* spp. (adjusted 0.026; FC 1-.79), were overrepresented in T1D participants with G (Supplementary Table 3).

A side-by-side comparison of the differential taxa between people with T1D and G and those with T1D without G vs. those with T1D and G and healthy controls is presented in Table 1. In total, 20 taxa were different in both comparisons: 11 were specific only for comparisons between people with T1D and G vs. those with T1D without G, and 17 were present only in comparisons between people with T1D and G and healthy controls. Most taxa were enriched in people with T1D with G, apart from *Cutibacterium* ssp., which was more abundant than that in people with T1D without G vs. those with T1D and G, and *Hemophilus* spp. which was enriched in healthy controls vs. in people with T1D and G (Table 1).

FIGURE 1
Krona charts of the genera with a mean abundance greater than 1% of the total found in the gingival crevicular fluid samples.

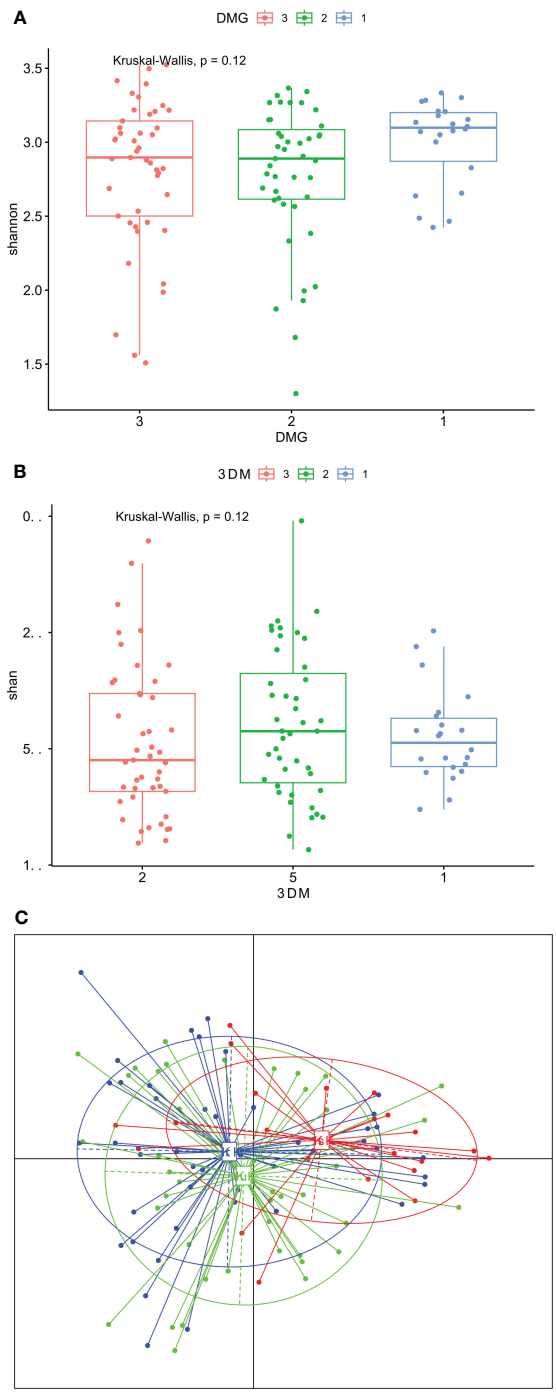

FIGURE 2
(A–C). Gingival crevicular fluid microbiome composition of people with type 1 diabetes and healthy controls. **(A)** – Shannon diversity; **(B)** – Chao diversity; **(C)** - PCoA (beta diversity); group 1 – people with type 1 diabetes and gingivitis (G), group 2 – people with type 1 diabetes without gingivitis, group 3 – healthy controls.

Additionally, within the T1D group, those with HbA1c% in the first and fourth quartiles were compared, regardless of their gingival status. We further explored the association between the community structure of the GCF microbiome and HbA1c levels in people with T1D. There were no significant differences in the Shannon and Chao indexes and β-diversity (Supplementary Figure 1A–C). Two genera, Family_XIII_UCG-001 (padj 0.08; FC 2.53) and *Prevotellaceae*_YAB2003_group (p-adjusted 0.08; FC 2.25), tended to be overrepresented in the fourth quartile compared to the those in the first quartile of HbA1c after adjustment for multiple comparisons (Supplementary Table 4).

3.2 Metabolome

When comparing three groups – healthy controls without G, people with T1D without G, and people with T1D and G, after adjusting for multiple comparisons, there were some significant differences in the concentrations of the selected metabolites (Table 2). Acetic acid concentration was higher in people with T1D than in healthy controls, regardless of the presence of G. Propionic acid was significantly higher in people with T1D and G than in healthy controls. Isobutyric and isovaleric acid levels were higher in people with T1D and G than those in the other two subgroups. In contrast, valeric acid was lower and caproic acid was higher in individuals with diabetes (regardless of gingival status) than those in healthy controls (Figures 3A-E). There were some borderline insignificant results for the selected SCFAs and TMA (Figures 3F-H). In the correlation analysis between HbA1c and SCFA concentrations in people with T1D, there was only one significant result for VA, but the correlation was weak (Spearman's correlation coefficient -0.272, p=0.031; Supplementary Table 5).

3.3 Integrated metagenomic–metabolomic analysis

In the metagenomic–metabolomic analysis, we analyzed the correlations between the identified bacterial taxa and selected metabolite concentrations. The results are shown in Figures 4A, B.

There were 85 and 46 correlations with absolute value above 0.25 for amino acids and SCFAs, respectively. All correlations were statistically significant at a nominal p-value <0.05 (Supplementary Table 6). Among the amino acids, TMA had the strongest positive correlation with 20 correlations with coefficients above 0.4 (Supplementary Table 6). There were also two strong negative correlations (coefficient below -0.4). The correlations with SCFAs were of low strength, with only one absolute value above 0.4. Most correlations between SCFA concentrations and bacterial genera were weak or moderate. *Bergeyella* spp. were negatively correlated with BA, IBA, and MeB. *Kingella* spp. were positively correlated with VA. *Micrococcae* were negatively correlated with AA, PA, and MeB. *Rothia* spp. were negatively correlated with AA and PA. *Streptococcae* were negatively correlated with AA, PA, MeB, IBA, ICA, and IVA. *Veilonella* spp. were positively correlated with PA, and *Treponema* spp. with IVA. Trimethylamine was the only metabolite that showed moderate correlation with the selected bacterial genera. The strongest positive correlations were observed with *Treponema* spp., *Tanerella* spp., *Filifactor* spp., *Tanerella* spp., and *Porphyrimonas* spp. (Table S6). The greatest negative

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TABLE 1 Side-by-side comparison of differential taxa between people with T1D and gingivitis (G) and those with T1D without G vs. those with T1D and G vs. healthy controls.

Taxon	Differential species T1D with G vs. T1D without G	Differential species T1D with G vs. healthy controls
***Peptoanaerobacter* (100)**	X	
***Anaeroglobus* (100)**		X
***Bacteria*_unclassified (100)**	X	X
***Bacteroidales*_unclassified (100)**		X
***Bacteroides* (100)**		X
***Bacteroidia*_unclassified (100)**		X
***Campylobacter* (100)**	X	X
***Campylobacterales*_unclassified (100)**	X	X
***Clostridia*_unclassified (100)**	X	
***Clostridia*_vadinBB60_group_ge (100)**	X	X
***Cutibacterium* (100)**	X †	
***Defluviitaleaceae*_UCG-011 (100)**	X	
***Desulfobacterota*_unclassified (100)**	X	
***Desulfobulbus* (100)**	X	X
Family_XIII_UCG-001 (100)	X	
***Filifactor* (100)**	X	
***Firmicutes*_unclassified (100)**		X
***Fretibacterium* (100)**	X	
***Fusobacteriaceae*_unclassified (100)**	X	X
***Fusobacteriales*_unclassified (100)**	X	X
***Fusobacterium* (100)**	X	X
***Haemophilus* (100)**		X †
***Lachnospirales*_unclassified (100)**	X	X
***Lactobacillus* (100)**		X
***Muribaculaceae*_ge (100)**		X
***Mycoplasma* (100)**	X	
***Negativicutes*_unclassified (100)**		X
***Oscillospiraceae*_unclassified (100)**		X
***Peptoanaerobacter* (100)**		X
***Peptostreptococcaceae*_unclassified (100)**	X	X
***Peptostreptococcales-Tissierellales*_fa_unclassified (100)**	X	X
***Phocaeicola* (100)**	X	

(Continued)

TABLE 1 Continued

Taxon	Differential species T1D with G vs. T1D without G	Differential species T1D with G vs. healthy controls
***Prevotella* (100)**		X
***Prevotellaceae*_ge (100)**	X	
***Prevotellaceae*_NK3B31_group (100)**	X	X
***Prevotellaceae*_UCG-001 (100)**	X	X
***Prevotellaceae*_unclassified (100)**		X
***Proteobacteria*_unclassified (100)**		X
***Rikenellaceae*_RC9_gut_group (100)**	X	X
***Rikenellaceae*_unclassified (100)**	X	X
***Slackia* (100)**		X
***Spirochaetaceae*_unclassified (100)**	X	X
***Spirochaetota*_unclassified (100)**	X	X
***Synergistaceae*_unclassified (100)**	X	X
***Tannerella* (100)**	X	X
***Tannerellaceae*_unclassified (100)**		X
***Treponema* (100)**	X	X
***Veillonellaceae*_unclassified (100)**		X

The abundance of a taxon was higher in people with type 1 diabetes than in the compared group unless otherwise marked †.
T1D, type 1 diabetes; G, gingivitis.
X, A taxon was present in a subgroup.

correlations were observed for *Streptococcus* spp., *Hemophilus* spp., *Rothia* spp., *Basfia* spp., and *Flavobacterium* spp.

4 Discussion

4.1 Microbiome

To date, most oral microbiome studies on people with T1D have included children or individuals with a wide range of metabolic controls. Furthermore, saliva is usually analyzed. To the best of our knowledge, this is the first study to investigate the GCF microbiome in people with T1D. In our study, we aimed to fill the knowledge gap in people with longstanding T1D treated with modern technologies, such as CSII, demonstrating good glycemic control.

In this study, we showed that intensively treated people with T1D with satisfactory glycemic control and non-diabetic individuals did not differ considering the Shannon and Chao indices, but β-diversity tended towards significant differences, especially pronounced for people with concomitant T1D and G. There were no differences within the T1D subgroup when comparing participants in the first and fourth quartile of HbA1c, that is, those with the best and worst metabolic control of diabetes.

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TABLE 2 Results of metabolomic analyses.

Metabolite	T1D with G	T1D without G	Healthy controls	Adjusted p value
Lactic acid [mmol/l]	93.9 (62.6-180.4)	123.3 (39.8-177.6)	79.8 (55.8-148.3)	NS
Acetic acid [mmol/l]	206.6 (134.8-451.3)*	181.8 (122.0-264.1)#	125.9 (88.4-179.9)*#	<0.001
Propionic acid [mmol/l]	13.5 (7.0-32.4)*	10.9 (6.0-14.4)	7.2 (7.5-11.3)*	0.006
Isobutyric acid [mmol/l]	1.3 (0.6-4.4)*#	0.6 (0.4-1.5)*	0.5 (0.3-1.1)#	<0.001
Butyric acid [mmol/l]	2.9 (1.3-7.5)	1.6 (1.0-2.8)	1.6 (0.8-3.2)	0.09
2-metylobutyric acid [mmol/l]	0.6 (0.2-1.7)	0.3 (0.2-0.7)	0.3 (0.2-0.5)	0.068
Isovaleric acid [mmol/l]	0.9 (0.3-2.6)*#	0.3 (0.1-0.8)#	0.3 (0.1-0.5)*	0.03
Valeric acid [mmol/l]	15.0 (9.8-16.9)*	13.8 (11.2-18.3)#	21.3 (19.1-24.7)*#	<0.001
Isocaproic acid [mmol/l]	0.4 (0.1-1.0)	0.2 (0.1-0.6)	0.2 (0.1-0.4)	NS
Caproic acid [mmol/l]	2.4 (0.7-4.4)*	2.9 (0.8-4.2)#	0.8 (0.6-0.9)*#	<0.001
Trimethylamine [umol/l]	19.3 (7.8-38.6)	10.5 (5.1-38.6)	8.7 (19.3 (7.8-38.6)	0.059
Betaine [umol/l]	171.5 (56.3-482.2)	109.5 (73.0-304.3)	119.4 (37.2-209.0)	NS
Glycerophosphorylcholine [umol/l]	74.4 (41.0-108.5)	96.3 (55.9-156.8)	76.4 (47.5-142.7)	NS
Choline [umol/l]	351.1 (163.5-553.4)	323.8 (202.3-509.9)	432.3 (215.1-631.5)	NS
Carnitine [umol/l]	71.1 (34.7-111.8)	49.0 (29.6-73.9)2	47.7 (35.2-73.2)	NS

Data are presented median (interquartile range).
T1D, type 1 diabetes; G, gingivitis; NS, not significant.
*# significant difference in post-hoc analysis at p>0.05.

This is an important conclusion of the study. With relatively well-controlled diabetes, slight differences in glycemic control did not significantly affect the oral microbiome, which was comparable to that observed in people without diabetes. Despite good metabolic control of diabetes, people with T1D had a higher prevalence of mild G than healthy controls. People with T1D and G show apparent shifts in the GCF microbiome and metabolome, which have been associated with periodontitis.

In one study on the oral microbiome in children with metabolically stable T1D, differences between T1D participants and healthy children included reduced Shannon diversity in the T1D group. However, no differences in bacterial diversity have been reported in a cohort similar to that in the present study (51). When comparing participants after stratification by HbA1c level, the differences in microbiome composition were barely notable in our cohort, which is in line with other studies (17, 51). In contrast, another study investigated a cohort of people with severely uncontrolled T1D. Those with high HbA1c levels were characterized by significantly decreased Chao and Shannon indices and an increased Simpson index of the oral microbiome (52). A comparison between individuals with T1D without periodontal pathology and healthy controls did not reveal any significant differences in the GCF microbiome, with no apparent differentiating taxa between these two groups. This may suggest that in T1D population intensive treatment and good glycemic control along with the lack of oral pathology can increase the probability of preserving the "healthy" GCF microbiome in this population.

Previous studies comparing the oral microbiome in people with T1D and non-diabetic populations have reported some distinctions. They showed a significantly high abundance of *Streptococcus* spp., *Actinomyces* spp., and *Rothia* spp (17).. In another study, commensal *Streptococcus* spp., *Granulicatella* spp., *Rothia* spp., and *Rhodococcus* spp. were decreased in diabetic children as well as *Veillonella* spp. and *Prevotella* spp. However, these T1D children presented with severe glycemic dysregulation (52). Importantly, in studies comparing the oral microbiome between people with T1D with non-diabetics, a thorough assessment of oral health status has not been performed widely, in contrast to this study. However, the results of the selected reports are similar to ours (51). Within the T1D subgroup (regardless of gingival pathology), the abundance of *Anaerovoracaceae* and *Prevotellaceae* was higher in those with poor metabolic control. The higher prevalence of *Prevotella* in individuals with T1D and worse diabetes control may be associated with a higher risk of PD in this subgroup. A poor-quality, high-sugar diet in T1D has been reported to be associated with a high abundance of *Prevotella copri* (53). This was linked to an altered periodontal status (54, 55). *Anaerovoracaceae* is another novel taxon that has been reported in oral microbiome studies. These genes were found to be enriched in individuals with diabetic retinopathy (56).

Additional analyses comparing individuals with T1D and G and those with only T1D or non-diabetic individuals with a healthy periodontium revealed some interesting differences. G seemed to be responsible for the majority of these discrepancies, as multiple taxa differentiated participants with G from those without such pathology, including some associated with various oral pathologies, such as *Fusobacterium* spp (57)., *Negativicutes* (58), *Prevotella* spp (54, 55)., *Tanerella* spp., and *Treponema* spp (59).. Previously, periodontally healthy diabetic participants had lower species richness than healthy controls, but also had higher loads of

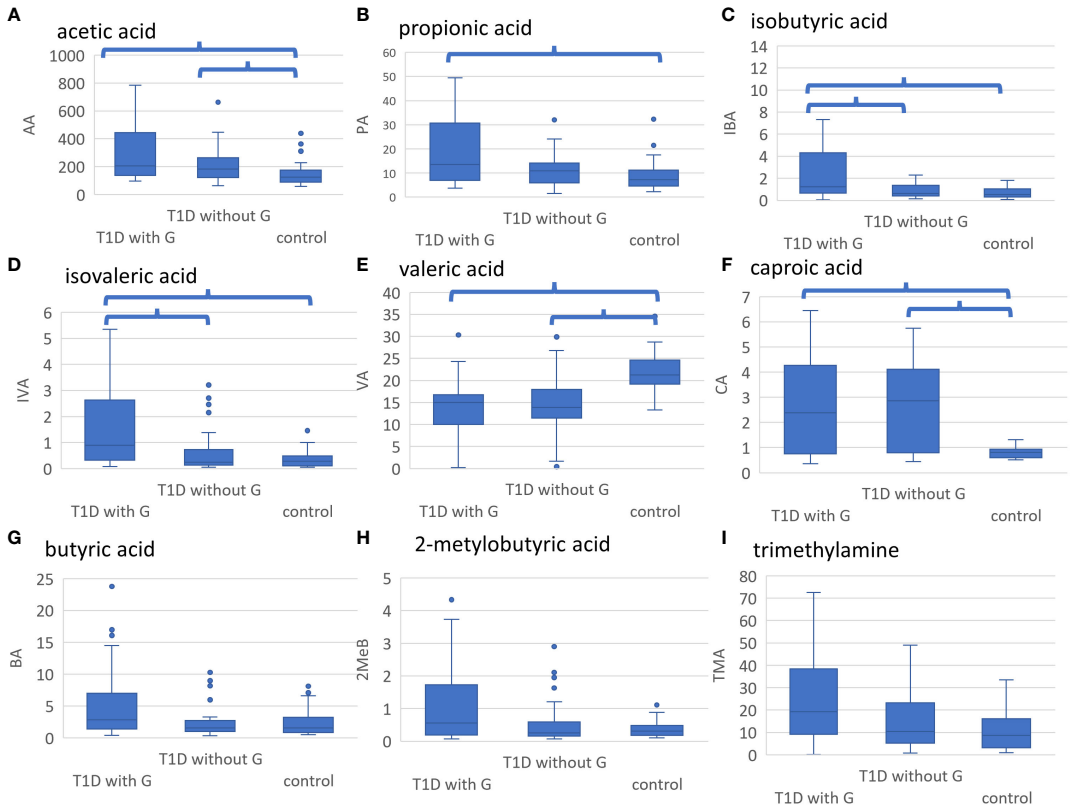

FIGURE 3
(A-I). Results of metabolomic analyses. Healthy controls without gingivitis (G), people with T1D without G and those with T1D and G were compared. Significant differences after adjustment with p value <0.05 are marked. **(A)** AA was significantly higher in people with T1D and G and those with T1D without G vs. healthy controls, **(B)** PA was significantly higher in people with T1D and G vs. healthy controls, **(C)** IBA was significantly higher in people with T1D and G vs. those with T1D without G and healthy controls, **(D)** IVA was significantly higher in people with T1D and G vs. those with T1D without G and healthy controls, **(E)** VA was significantly higher in healthy controls vs. all people with T1D, **(F)** CA was significantly lower in healthy controls vs. all people with T1D, **(G)** BA tended to be higher in people with T1D and G vs. those with T1D without D and healthy controls (not statistically significant), **(H)** 2MeB tended to be higher in people T1D and G vs. those with T1D without G and healthy controls (not statistically significant), **(I)** TMA tended to be higher in people with T1D and G vs. those with T1D without G and healthy controls (not statistically significant). LA, lactic acid; AA, acetic acid; PA, propionic acid; IBA, isobutyric acid; BA, butyric acid; 2MeB, 2-metylobutyric acid; IVA, isovaleric acid; VA, valeric acid; ICA, isocaproic acid; CA, caproic acid; TMA, trimethylamine; GPA, glycerophosphorylcholine; A-H, concentration in mmol/l; I, concentration in umol/l.

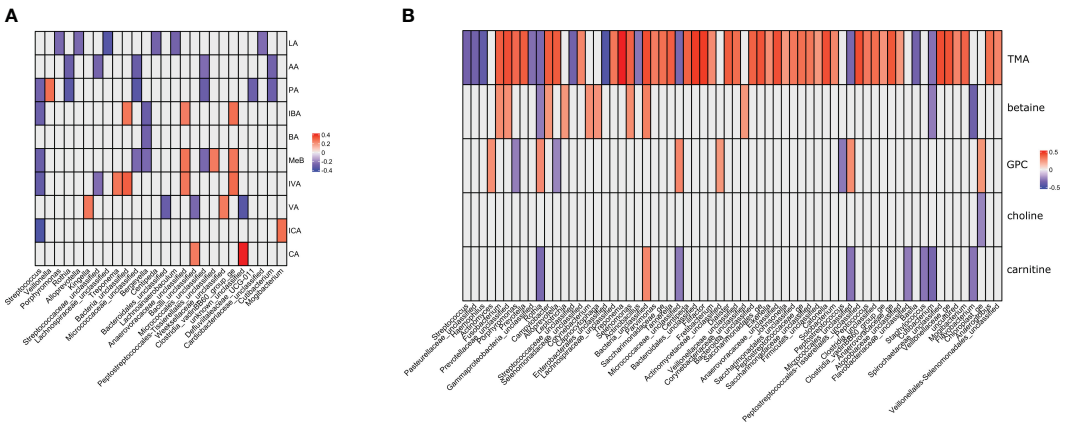

FIGURE 4
(A, B). Metagenomic–metabolomic analyses between the identified bacterial taxa and selected metabolites concentrations. Correlation coefficients significant at p>0.05 are presented and color-coded. **(A)** lactic acid and short chain fatty acids. **(B)** trimethylamine and its metabolites. LA, lactic acid; AA, acetic acid; PA, propionic acid; IBA, isobutyric acid; BA, butyric acid; 2MeB, 2-metylobutyric acid; IVA, isovaleric acid; VA, valeric acid; ICA, isocaproic acid; CA, caproic acid; TMA, trimethylamine; GPA, glycerophosphorylcholine.

red complex species responsible for the development of periodontal pathology, potentially putting them at risk of developing periodontitis (59, 60). There is no consensus on whether oral microbiome diversity is higher (61) or lower (60) in people with diabetes and PD. Specifically, the subgingival tissue microbiome showed a relatively high abundance of *Leptotrichiaceae, Neisseriaceae, Lactobacillus, Corynebacterium*, *Pseudomonas, Saccharibacteria, Aggregatibacter, Neisseria, Gemella, Eikenella, Selenomonas, Actinomyces, Capnocytophaga, Fusobacterium, Veillonella, Streptococcus*, and *Actinomyces*. For *Filifactor, Prevotella*, and *Parvimonas*, low abundances were observed (59). To date, studies have mainly included individuals with periodontitis. However, in our study, the spectrum of PD was limited to mild cases of G. This and the good metabolic control of diabetes may have been one of the reasons for the discrepancies between our data and those of previous research.

A more detailed analysis of the differences in differentiating taxa between people with T1D and G and T1D without it vs. those with T1D and G and non-diabetic controls revealed some interesting observations. Comparisons within people with T1D omit the impact of diabetes on any potential differentiating taxa, showing, in our opinion, the core differences between those with and without G. Nevertheless, quantitatively, there were more differentiating taxa in the analysis of nondiabetics than in those with T1D. Qualitatively, some taxa that differentiated people with T1D and G vs. non-diabetics but their abundances were similar within people with T1D included *Bacteroides* spp., *Firmicutes* spp., and *Lactobacillus* spp. This suggests that individuals with T1D, even those without clinically visible signs of periodontal pathology, show specific shifts in the GCF microbiome, putting them at risk of developing PD.

4.2 Metabolome

SCFAs are formed from saturated aliphatic organic acids containing one–six carbon atoms (62). SCFAs are produced by gut microbes during fiber fermentation (63). Considering the gut microbiome, SCFAs have also been investigated as potential additives in the regular diet to positively influence insulin sensitivity, obesity, diabetes control, and immune modulation to counter autoimmune diseases (63, 64). SCFAs, as products of bacterial metabolism, can also be found in periodontal pockets; however, there are only singular studies on this subject (65). Bacteria known to produce SCFAs include *Porphyromonas gingivalis, Treponema denticola, Aggregatibacter actinomycetemcomitans, Prevotella intermedia*, and *Fusobacterium nucleatum* (66). GCF SCFAs produced locally in gingival pockets seem to play a role opposite to that in the gut and are considered responsible for local pathologies, such as PD. The complexity is added by data suggesting that specific nutrients that modify the level of SCFAs production in the gut can improve periodontal status (67, 68). Finally, not only are SCFAs produced by bacteria, but dietary SCFAs can also influence the microbiome composition, implicating complex bidirectional associations (33).

In our study, the concentrations of selected SCFAs, including acetic acid and caproic acid, were higher in people with T1D than in those without diabetes, whereas isobutyric and isovaleric acids were higher in people with T1D and G than in the remaining participants. However, the concentration of valeric acid was lower in people with diabetes than in healthy controls. These findings are in agreement with the results of the integrated metagenomic–metabolic analysis. For example, *Treponema* spp. were positively correlated with isovaleric acid concentrations, which were higher in participants with T1D and G. Interestingly, we did not identify differences in the abundance of *Streptococcus* spp. between T1D participants and healthy controls but identified negative correlations between *Streptococcus* spp. and acetic acid, isobutyric acid, isocaproic acid, and isovaleric acid concentrations. The levels of these metabolites were high in the T1D subgroup. This, in concurrence with the abovementioned findings in differential taxa between the studied groups, may suggest the first stage towards the development of an abnormal oral microbiome and, further, the emergence of PD.

Along with the early alterations in the microbiome discussed above, disturbances in metabolite concentrations may be treated as a marker of oral cavity acidification, an auxiliary predisposing factor for caries and PD (69). This is consistent with previous observations showing an abundance of acid-producing bacteria in individuals with diabetes. As this process progresses, it can lead to destabilization of the balance between *Streptococcus* spp. and the emergence of clinical oral pathology (70).

Acetate, derived primarily from microorganisms on the skin in the oral cavity and the gastrointestinal, urogenital, and respiratory tracts, has immunomodulatory effects (71). They are also involved in tissue development, nutrient absorption, and metabolism (72–74). Acetate plays a role in maintaining the intestinal barrier (75, 76). The metabolic processes affected by acetate include the accumulation of body fat, liver lipids, and cholesterol synthesis (77). Acetate in the gut is predominantly produced by *Prevotella* and *Bifidobacterium* spp (78).. Acetate is also used by *Firmicutes* to produce butyrate (79). The serum concentrations of acetate and propionate in individuals with T1D have been reported to be lower than those in individuals without diabetes (17). Importantly, the roles of serum and gut acetate seem to contradict that of oral acetate. Although serum acetate may protect against the emergence of anti-islet cell autoantibodies (80), local oral acetate administration may also promote periodontal pathology.

In type 2 diabetes, propionate acts locally on tissues, improving insulin sensitivity, suppressing cholesterol synthesis, and lowering the risk of cardiovascular disease (81). In a mouse model, gut integrity is ensured by the healthy commensalism of lactate- and butyrate-producing bacteria, with non-butyrate-producing bacteria preventing optimal mucin synthesis in individuals with type 1 diabetes (82). Butyrate has also been intensely investigated as a supplement to improve immune function, strength, and physical function and alleviate symptoms of gastrointestinal tract diseases (83).

We did not observe any changes in GCF butyrate concentrations between people with T1D and healthy controls. One possible explanation for this may be that the gingival pocket was the site of sampling in our study, as most studies have assessed its levels in the gut or serum. Moreover, none of the participants had PD. Nevertheless, we hypothesized that the observed alterations in

SCFAs concentrations may reflect a predisposition towards the development of an early preclinical stage of PD.

A few studies that investigated oral propionate and butyrate have reported that their levels are increased in PDs, regardless of the diagnosis of diabetes (84). In participants with PD, the GCF concentrations of acetic, propionic, and butyric acids were positively correlated with *P. gingivalis*, *T. denticola, F. alocis*, *T. socraskii, F. nucleatum, and T. forsythia* (85, 86).

In vitro studies have shown that high concentrations of butyrate can induce apoptosis in gingival fibroblasts, leading to periodontal tissue damage (87, 88). High concentrations of lactic acid and a wide range of SCFAs, including acetic, propionic, butyric, and isovaleric acids, in GCF have been observed in patients with aggressive periodontitis. Interestingly, the probing depth and attachment loss were positively correlated with the concentrations of the selected SCFAs (85). As butyrate can have both detrimental and beneficial effects, this is likely attributed to the tissues where it is produced, i.e., where it acts locally, and its concentration (89).

The functional and physiological effects of isobutyric and 2-methylbutanoic acids are poorly understood. Valeric acid in the gut is produced by the microbial metabolism of lactic acid and propionic acids (90). A study of women with gestational diabetes (GDM) showed that the levels of isobutyric, isovaleric, valeric, and caproic acids were high in women with GDM (91). The authors linked high levels of inflammation to hyperglycemia and a dysbiotic gut microbiome in this population (91). Women with GDM have also been reported to have a high abundance of *Prevotella* spp., a potential caproic acid-producer, which leads to increased caproic acid production (92). Our findings on valeric, isovaleric, and caproic acids in the GCF of people with T1D are novel and partially in contrast with these reports, requiring further investigation and analysis in conjunction with other potential factors, such as diet or body composition.

Trimethylamine and its metabolites were not significantly different between the subgroups in our study, with TMA being the only metabolite nearing significance, with numerically higher concentrations in people with T1D and G. Trimethylamine levels are elevated in patients with PD (93). In our study, trimethylamine concentrations were positively correlated with typical red complex bacteria (*Tanerella* spp., *Treponema* spp., and *Fusobacterium* spp.) that are responsible for the development of PDs.

4.3 Limitations

The inclusion of only relatively young people with T1D with good glycemic control may be regarded as both a limitation and a strength. This is a homogeneous subgroup representative of a large portion of the T1D population. The percentage of well-controlled people with T1D within the entire T1D population will probably increase, at least in developed countries, with more common usage of advanced technologies, such as continuous glucose measurements and semi-automated or hybrid insulin pumps. However, our findings cannot be extrapolated to patients with poor metabolic control. Notably, our group was relatively young and free of advanced complications of diabetes. Thus, the results of our analysis cannot be extrapolated to older populations or individuals with advanced micro-and macrovascular complications of diabetes, such as renal failure and advanced cardiovascular diseases. We did not observe any cases of PD in our population, which may have affected our microbiome and metabolome findings. In this study, we focused on limited confounders related to microbiome and metabolome results. As more data regarding diet and body structure remain to be analyzed, the acquired picture may not be fully explained. Finally, measurements of fecal and serum SCFA levels may poorly reflect biologically active SCFA levels. Only up to 10% are found in these types of samples, as active SCFAs are constantly produced, utilized by microbial cross-feeding, or interact with host cells (17).

5 Conclusions

The GCF microbiome in intensively treated people with T1D with satisfactory glycemic control and healthy gingival tissues was similar to that in non-diabetic controls. People with T1D and G show clear shifts in the GCF microbiome and metabolome. In this cohort of people with T1D, HbA1c% did not have a significant impact on SCFA concentrations in the correlation analysis. By contrast, the GCF microbiome appeared to have a significant relationship with SCFAs. This suggests that despite good metabolic control of diabetes, people with T1D are susceptible to the development of PDs. This was demonstrated by early changes in the structure of the GCF microbiome and altered concentrations of selected metabolites in this environment.

To summarize, the identification of early changes in periodontal tissues by targeting microbiome and metabolome changes could potentially enable effective prevention and initial treatment of PD in people with T1D.

Data availability statement

The data presented in the study are deposited in the NCBI BioProject repository, accession number ID: 1064953.

Ethics statement

This study involving humans was approved by the Jagiellonian University Bioethics Committee (Komisja Bioetyczna Uniwersytetu Jagiellońskiego). The study was conducted in accordance with local legislation and institutional requirements. All the participants provided written informed consent to participate in this study.

Author contributions

IG-M: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. MKa: Data

curation, Formal analysis, Investigation, Validation, Visualization, Writing – original draft, Writing – review & editing. MD: Data curation, Formal analysis, Software, Validation, Visualization, Writing – review & editing. ES: Data curation, Formal analysis, Software, Validation, Visualization, Writing – review & editing. NŻ-L: Data curation, Formal analysis, Software, Validation, Visualization, Writing – review & editing. MKu: Data curation, Formal analysis, Software, Validation, Visualization, Writing – review & editing. TK: Methodology, Resources, Supervision, Writing – review & editing.

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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.2023.1332406/full#supplementary-material

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Associations between type 2 diabetes mellitus and chronic liver diseases: evidence from a Mendelian randomization study in Europeans and East Asians

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Objective: Multiple observational studies have demonstrated an association between type 2 diabetes mellitus (T2DM) and chronic liver diseases (CLDs). However, the causality of T2DM on CLDs remained unknown in various ethnic groups.

Methods: We obtained instrumental variables for T2DM and conducted a two-sample mendelian randomization (MR) study to examine the causal effect on nonalcoholic fatty liver disease (NAFLD), hepatocellular carcinoma (HCC), viral hepatitis, hepatitis B virus (HBV) infection, and hepatitis C virus (HCV) infection risk in Europeans and East Asians. The primary analysis utilized the inverse variance weighting (IVW) technique to evaluate the causal relationship between T2DM and CLDs. In addition, we conducted a series of rigorous analyses to bolster the reliability of our MR results.

Results: In Europeans, we found that genetic liability to T2DM has been linked with increased risk of NAFLD (IVW : OR =1.3654, 95% confidence interval [CI], 1.2250-1.5219, p=1.85e-8), viral hepatitis (IVW : OR =1.1173, 95%CI, 1.0271-1.2154, p=0.0098), and a suggestive positive association between T2DM and HCC (IVW : OR=1.2671, 95%CI, 1.0471-1.5333, p=0.0150), HBV (IVW : OR=1.1908, 95% CI, 1.0368-1.3677, p=0.0134). No causal association between T2DM and HCV was discovered. Among East Asians, however, there was a significant inverse association between T2DM and the proxies of NAFLD (ALT: IVW OR=0.9752, 95% CI 0.9597-0.9909, p=0.0021; AST: IVW OR=0.9673, 95%CI, 0.9528-0.9821, p=1.67e-5), and HCV (IVW: OR=0.9289, 95%CI, 0.8852-0.9747, p=0.0027).

Notably, no causal association was found between T2DM and HCC, viral hepatitis, or HBV.

Conclusion: Our MR analysis revealed varying causal associations between T2DM and CLDs in East Asians and Europeans. Further research is required to investigate the potential mechanisms in various ethnic groups, which could yield new insights into early screening and prevention strategies for CLDs in T2DM patients.

KEYWORDS

Mendelian randomization, type 2 diabetes mellitus, nonalcoholic fatty liver disease, hepatocellular carcinoma, viral hepatitis, hepatitis B virus infection, hepatitis C virus infection

1 Introduction

Diabetes mellitus, an enormous public health concern, imposes staggering health and financial burdens worldwide (1, 2). There are two primary types of diabetes: Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM) (3). Approximately 10% of adults worldwide suffer from T2DM, making it the most prevalent form of diabetes (4).T2DM, characterized by hyperglycemia resulting from disturbed glycemic homeostasis (5), is precipitated by defective insulin secretory responses and action. The liver, which is essential to glucose homeostasis by supplying endogenous sugar, is the first organ to receive insulin following its release from pancreatic islets (6). Moreover, hepatic glucose metabolic disorders could contribute to fast hyperglycemia in diabetic patients (7, 8).

Considering the important role of the liver in the pathogenesis and treatment of diabetes, various liver diseases have been associated with T2DM (9, 10). Previous studies have demonstrated a connection between T2DM and chronic liver diseases (CLDs), such as Non-alcoholic fatty liver disease (NAFLD), hepatocellular carcinoma (HCC) and viral hepatitis (11–13). A large meta-analysis from 156 studies estimated that the global prevalence of NAFLD among patients with T2DM was 65.04% (14). It was reported that coexistence of NAFLD and T2DM is common in everyday outpatient practice (15). There is a bidirectional relationship between NAFLD and T2DM that is confirmed by epidemiological data, clinical picture, diagnosis and pathomechanisms (16). However, the complex causal link between NAFLD and T2DM is still controversial, and it is important to bring the attention of clinicians and researchers to the relationship between these two metabolic diseases in order to prevent adverse clinical outcomes (16). Viral hepatitisis an acute or chronic inflammation of the liver parenchyma caused by viruses. There are 5 common types of viral hepatitis: A, B, C, D, and E, and most deaths from viral hepatitis are due to hepatitis B and hepatitis C (17). It is worth noting that hepatitis B Virus (HBV) and hepatitis C Virus (HCV) infections were reportedly higher among T2DM patients (18). However, other studies reported that the risk of HCV infection was rather low in patients with T2DM (19, 20). The discrepancies may be attributable to the sample sizes, target populations, control sources, potential biases from residual confounding, and reverse causation of the respective studies. These highlight how crucial it is to manage T2DM in CLDs patients and to make a firm determination of the causal relationships between T2DM and CLDs.

Mendelian Randomization (MR), a developing epidemiological technique, examines the effect of genotypic variation (exposure) on a phenotype (outcome) from a genetic standpoint. Using germline genetic variance as instrumental variables (IVs), MR analysis assesses causation between exposure and outcomes, thereby avoiding biases caused by confounding variables or reverse causation (21). Previous MR research assessed the relationship between NAFLD and T2DM in Europeans but skipped East Asians (22). To comprehensively evaluate the role of T2DM in CLDs across both demographics, we conducted a comprehensive two-sample MR study.

2 Materials and methods

2.1 Study design

A two-sample MR study was designed to investigate the causative role of T2DM on the risk of five CLDs (NAFLD, HCC, viral hepatitis, HBV and HCV). Three critical assumptions for MR analysis must be satisfied (see Figure 1A) (1): IVs should be associated with exposure factors; (2) IVs should not be associated with confounding factors; and (3) IVs should only affect the outcome through the exposure (23). The study relied on publicly available genome-wide association studies (GWAS) data. We estimated the relationships between T2DM and CLDs in Europeans and East Asians and Figure 1B depicts the study's overall workflow.

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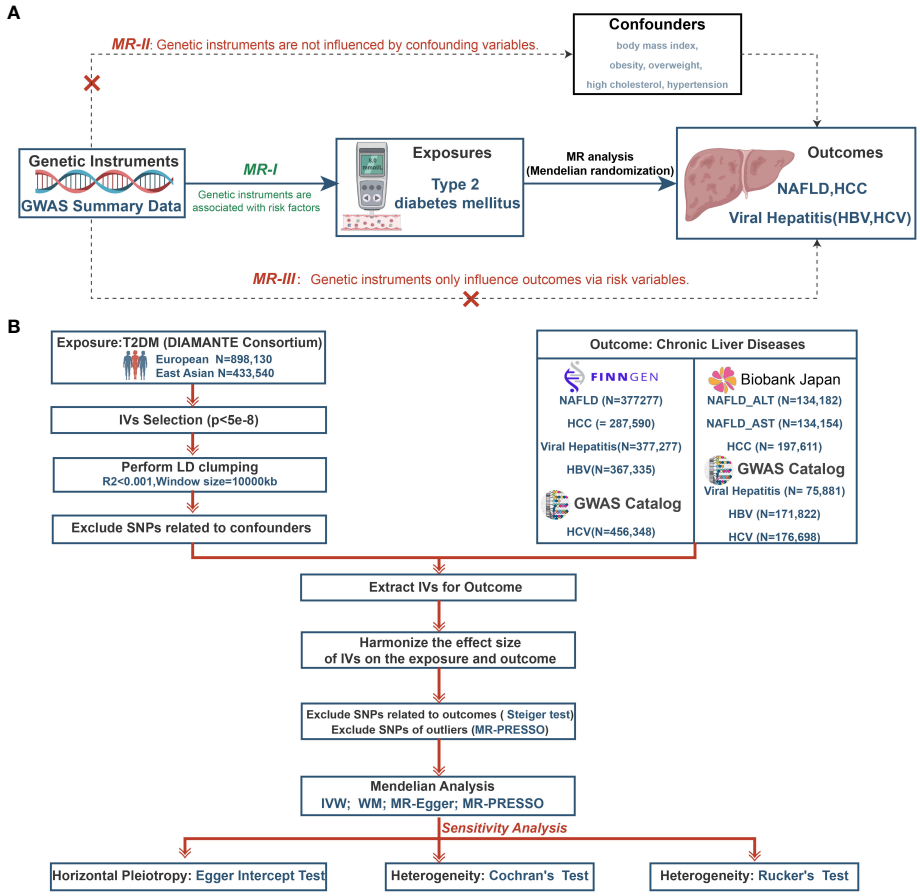

FIGURE 1
The study design of MR analysis **(A)** and the overall workflow **(B)**. MR, mendelian randomization; GWAS, genome-wide association study; NAFLD, non-alcoholic fatty liver disease; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; T2DM, type 2 diabetes mellitus; DIAMANTE, Diabetes Meta-analysis of Trans-ethnic Association Studies; IVs, instrumental variables; LD, linkage disequilibrium; SNPs, single-nucleotide polymorphisms; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; MR-PRESSO, Mendelian randomization pleiotropy residual sum and outlier; IVW, inverse-variance weighted; WM, weighted median.

2.2 Data sources

2.2.1 Genetic association datasets for T2DM

The GWAS summary statistics for T2DM were obtained from the Diabetes Meta-analysis of Trans-ethnic Association Studies (DIAMANTE) Consortium, including 898,130 European individuals (n=74,124 case patients and 824,006 control participants) and 433,540 East Asian individuals(n=77,418 case patients and 356,122 control participants) (24).

2.2.2 Genetic association datasets for CLDs

Summary statistics from the GWAS of European populations for four outcomes were procured from the FinnGen consortium, which is collecting genetic data based on FinnGen registries. For this study, we utilized data from their R9 release (https://r9.finngen.fi/). This release includes data regarding NAFLD (2,275 cases and 375,002 controls), HCC (453 cases and 287,137 controls), viral hepatitis (2,143 cases and 375,134 controls), and HBV (885 cases and 366,450 controls). The HCV summary statistics (219 cases and 456,129 controls) were obtained from Jiang's research (25) and were also available in the GWAS catalog (https://www.ebi.ac.uk/gwas/).

In the absence of valid GWAS for NAFLD in East Asians, we used Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels from the Biobank Japan GWAS as surrogates for NAFLD, following a precedent established in a previous MR study (26). We eventually obtained summary statistics for East Asian for NAFLD (ALT: n=134,182, AST: n=134,154), and HCC (1,866 cases and 195,745 controls) from the IEU OPEN GWAS PROJECT (https://gwas.mrcieu.ac.uk/). The remaining summary statistics for viral hepatitis (117 cases and 75,764 controls), HBV (2,234 cases and 169,588 controls), and HCV (7,110 cases and 169,588 controls) were obtained from studies conducted by Walters RG and Sakaue S (27, 28). Table 1 provides a comprehensive summary of the data sources used for each exposure and outcome.

TABLE 1 Profiles of exposure and outcomes in GWAS datasets.

Trait	GWAS ID (Data sources)	Data Type	Case	Control	Ethnicity	Consortium
T2DM	PMID: 35551307	Exposure	74,124	824,006	European	DIAMANTE
T2DM	PMID: 35551307	Exposure	77,418	356,122	East Asian	DIAMANTE
NAFLD	finngen_R9_NAFLD	Outcome	2,275	375,002	European	FinnGen biobank
HCC	finngen_R9_C3_HEPATOCELLU_CARC_EXALLC	Outcome	453	287,137	European	FinnGen biobank
Viral Hepatitis	finngen_R9_AB1_VIRAL_HEPATITIS	Outcome	2,143	375,134	European	FinnGen biobank
HBV	finngen_R9_K11_CHRONHEP	Outcome	885	366,450	European	FinnGen biobank
HCV	GCST90041714(PMID: 34737426)	Outcome	219	456,129	European	GWAS catalog
ALT	bbj-a-6(PMID: 29403010)	Outcome	134,182		East Asian	Biobank Japan
AST	bbj-a-8(PMID: 29403010)	Outcome	134,154		East Asian	Biobank Japan
HCC	bbj-a-158(PMID: 32514122)	Outcome	1,866	195,745	East Asian	Biobank Japan
Viral Hepatitis	GCST90246018(PMID: 37601966)	Outcome	117	75,764	East Asian	GWAS catalog
HBV	GCST90018584(PMID: 34594039)	Outcome	2,234	169,588	East Asian	GWAS catalog
HCV	GCST90018585(PMID: 34594039)	Outcome	7,110	169,588	East Asian	GWAS catalog

GWAS, genome-wide association study; ID, identity; T2DM, type 2 diabetes mellitus; DIAMANTE, Diabetes Meta-analysis of Trans-ethnic Association Studies; NAFLD, non-alcoholic fatty liver disease; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase.

2.3 Genetic instrument selection and evaluation

The single-nucleotide polymorphisms (SNPs) associated with T2DM were selected based on the following criteria (1): The phenotypes should be significantly related with IVs ($P < 5 \times 10^{-8}$). (2) The related linkage disequilibrium (LD) of r^2<0.001 and clumping with a 10000kb window were considered. (3) IVs should have at least five variants as biallelic SNPs (29).

Variance (R2) and the F-statistic were utilized to evaluate the robustness of IVs in order to avoid tool bias. F-statistic was computed for each SNP using the following formula: $F = (beta/se)^2$ (30). To reduce bias caused by weak IVs, an F-statistic greater than 10 was considered significant for the IV-exposure association (31). Statistical power was calculated for each outcome using the online tool (https://shiny.cnsgenomics.com/mRnd/) (32). A sufficient strength of over 80% was advised. We also searched all eligible SNPs with PhenoScanner V2(http://www.phenoscanner.medschl.cam.ac.uk/) to exclude SNPs associated with potential confounders including body mass index, obesity, overweight, high cholesterol, hypertension, and alcohol consumption (33–35). In addition, we harmonized the summary statistics and removed SNPs with palindromic sequences.

2.4 Statistical analysis

As the primary method, the random-effect inverse-variance weighted (IVW) approach was utilized. Three additional MR methods, the weighted median (WM), MR-Egger, and Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO), were also employed in this study. The IVW method is an extension of the Wald ratio estimator based on meta-analytic principles that can generate relatively precise estimates that are not influenced by horizontal pleiotropy (36). MR-Egger and WM could provide more stable estimates and correct potential pleiotropy in a broader set of scenarios but are less efficient (37, 38). The MR-PRESSO was implemented to identify and remove pleiotropic outliers so that the causal impact estimate is obtained (39). Considering the multiple comparisons, the Bonferroni method was performed to rectify overall type I errors, and p<0.01 (0.05/5) was considered statistically significant association, while p<0.05 was considered suggestive association (22, 40).

Multiple sensitivity analyses were implemented to guarantee the uniformity and reliability of the MR results. Cochran's and Rucker's Q test were used to detect heterogeneity, where P<0.05 indicated the presence of heterogeneity (41). Using the MR-Egger intercept test, we then evaluated the directional horizontal pleiotropy; p>0.05 indicated that there was no directional horizontal pleiotropy (42). Finally, leave one-out analysis was performed to evaluate whether the MR estimate was driven or biased by a single SNP (43). To explore the possible reverse causality, we performed the Steiger filtering test to evaluate the direction of causality for each IV on exposure and outcome. When their directions are "FALSE", related SNPs were removed and the MR analysis would be reassessed (44). A two-sided p-value < 0.05 was considered statistically significant. All statistical analyses were performed in R software 4.3.1 (R Foundation for Statistical Computing) using R packages "Two Sample MR".

3 Results

3.1 Selection of IVs

A total of 44 SNPs associated with potential confounders of NAFLD (body mass index, obesity, overweight, high cholesterol, and hypertension) were removed, and 131 SNPs were selected for

analysis in Europeans. In East Asians, 17 and 17 SNPs associated with possible confounders of the ALT and AST were eliminated. Through the application of MR-PRESSO, outlier SNPs (rs79747549) linked with ALT were dropped. In the end, 65 and 66 SNPs corresponding to ALT and AST were chosen from East Asians as candidates (Supplementary Table S1). Regarding the SNPs associated with HCC, three SNPs, namely rs429358, rs28663084, and rs1260326, were excluded from the analysis based on the results of MR-PRESSO and the Steiger test, as well as their association with potential confounding variables. Ultimately, a set of 169 SNPs were identified as IVs in the European population. In the study conducted on the HCC patients of East Asians, two SNPs, namely rs9948462 and rs1260326, were eliminated from the analysis depending on the MR-PRESSO and Steiger test, as well as their association with potential confounding variables. Eventually, a total of 83 SNPs were chosen for further investigation, as outlined in Supplementary Table S2. For viral hepatitis, a total of 173 and 85 SNPs were selected in Europeans and East Asians, respectively. Specifically, Europeans selected 173 SNPs for HBV and 178 SNPs for HCV. In comparison, East Asians selected 86 SNPs for HBV and 86 SNPs for HCV (Supplementary Table S3). Supplementary Table S4 shows the detailed eliminated SNPs in the selection of IVs. It is worth noting that all selected IVs exhibited F-statistics greater than 10.

3.2 Causal effect of T2DM on NAFLD

In Europeans, the risk of NAFLD was found to be higher among patients with T2DM (IVW: OR=1.3654, 95% confidence interval [CI] 1.2250-1.5219, p=1.85e-8; WM: OR=1.2013,95%CI, 1.0295-1.4019, p=0.0199; MR-Egger: OR=1.2540, 95%CI, 0.9891-1.5899, p=0.0638, MR-PRESSO: OR=1.3654, 95%CI, 1.2250-1.5219, p=1.08e-7, Figure 2 and Supplementary Figure S1). Despite the presence of heterogeneity (Cochran's Q test: p=0.0035 and Rucker's Q test: p=0.0033, Supplementary Table S5), no significant outliers were identified, and the similar result from MR-PRESSO and leave-one-out analysis (Supplementary Figure S1) supports the consistency of the conclusion. Also, no directional horizontal pleiotropy was observed in the MR analysis (MR-Egger intercept=-0.0063, p=0.4306, Supplementary Table S5). Furthermore, the MR Steiger test yielded no indication of reverse causality.

In East Asians, however, the risk of elevated ALT (IVW: OR=0.9752, 95%CI, 0.9597-0.9909, p=0.0021; WM: OR=0.9734, 95%CI, 0.9528–0.9944, p=0.0133; MR-Egger: OR=0.9597, 95% CI, 0.9155-1.0060, p=0.0919; MR-PRESSO: OR=0.9752, 95%CI, 0.9597-0.9909, p=0.0031, Figure 3 and Supplementary Figure S2) and AST (IVW: OR=0.9673, 95%CI, 0.9528-0.9821, p=1.67e-5; WM: OR=0.9604, 95%CI, 0.9401-0.9811, p=0.0002; MR-Egger: OR=0.9401, 95%CI, 0.8994-0.9826, p=0.0080; MR-PRESSO: OR=0.9673, 95%CI, 0.9528-0.9821, p=5.74e-5; Figure 3 and Supplementary Figure S3) was found to decrease in patients with T2DM. In the sensitivity analysis, there was no evidence of horizontal directional pleiotropy (ALT: MR-Egger intercept=0.0013, p=0.4807; AST: MR-Egger intercept=0.0024, p=0.1828, Supplementary Table S5). Despite the ALT index exhibited heterogeneity (ALT: Cochran's Q test: p=0.0423 and Rucker's Q test: p=0.0392; AST: Cochran's Q test: p=0.1105 and Rucker's Q test: p=0.1270, Supplementary Table S5), the leave-one-out analysis (Supplementary Figure S3) validate the reliability of the conclusion in Asian inhabitants.

3.3 Causal effect of T2DM on HCC

For HCC, a possible causal relationship was observed between T2DM and HCC in European populations (IVW: OR=1.2671, 95%

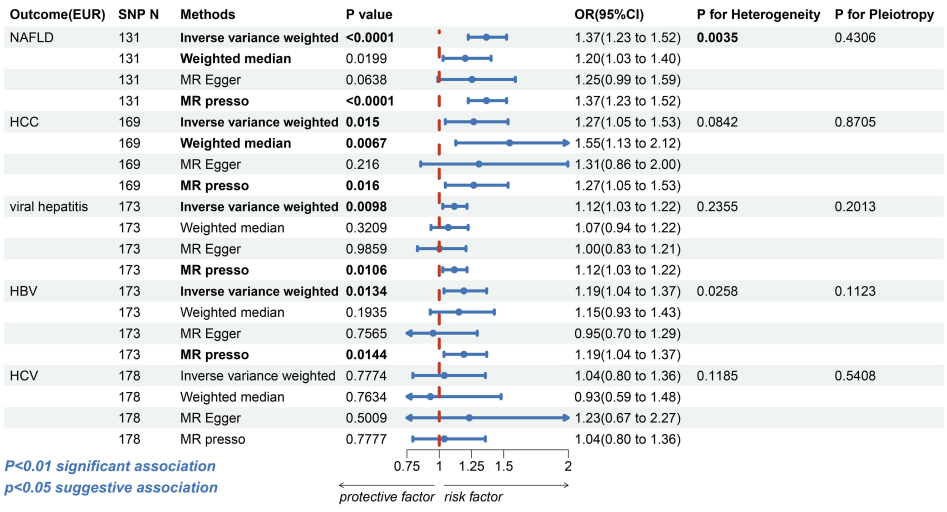

FIGURE 2
The forest plot depicting the genetic susceptibility to T2DM in Europeans with CLDs. T2DM, type 2 diabetes mellitus; CLDs, chronic liver diseases; EUR, Europeans; NAFLD, non-alcoholic fatty liver disease; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; SNP, single-nucleotide polymorphisms; IVW, inverse-variance weighted; WM, weighted median; MR-PRESSO, Mendelian randomization pleiotropy residual sum and outlier; OR, odds ratio; CI, confidence interval. Statistical significance was set at p< 0.05.

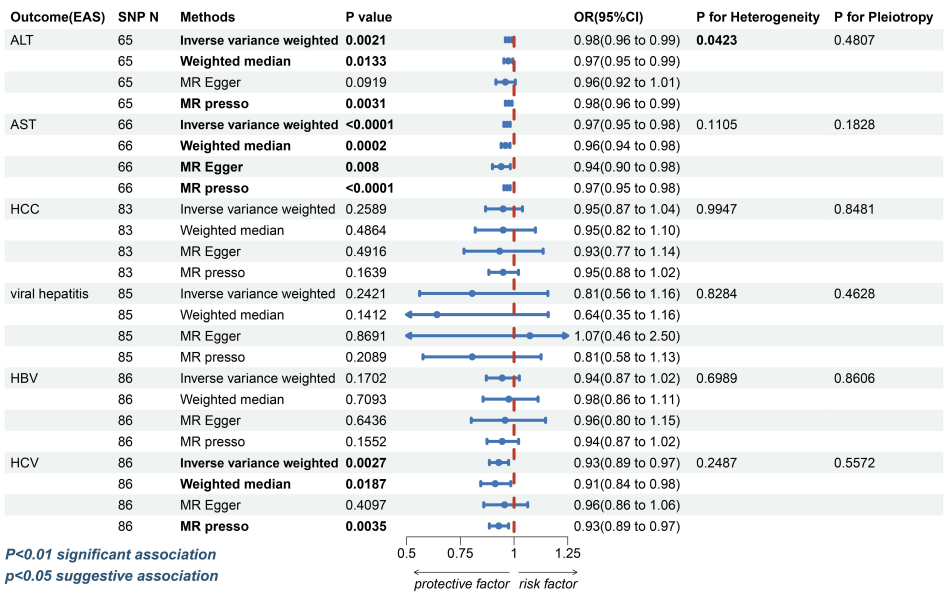

FIGURE 3
The forest plot depicting the genetic susceptibility to T2DM in East Asians with CLDs. T2DM, type 2 diabetes mellitus; CLDs, chronic liver diseases; EAS, East Asians; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; SNP, single-nucleotide polymorphisms; IVW, inverse-variance weighted; WM, weighted median; MR-PRESSO, Mendelian randomization pleiotropy residual sum and outlier; OR, odds ratio; CI, confidence interval. Statistical significance was set at p< 0.05.

CI, 1.0471-1.5333, p=0.0150; WM: OR=1.5455, 95%CI 1.1280-2.1175, p=0.0067; MR-Egger: OR=1.3076, 95%CI 0.8563-1.9965, p=0.2160; MR-PRESSO: OR=1.2671, 95%CI 1.0471-1.5333, p=0.0160, Figure 2 and Supplementary Figure S4). However, the association between T2DM and HCC (IVW: OR=0.9493, 95%CI, 0.8673-1.0391, p=0.2589; WM: OR=0.9491, 95% CI 0.8194-1.0094, p=0.4864; MR-Egger: OR=0.9332, 95%CI 0.7671-1.1354, p=0.4916; MR-PRESSO: OR=0.9493, 95%CI, 0.8828-1.0208, p=0.1639, Figure 3 and Supplementary Figure S5) was insignificant among East Asian individuals. Neither heterogeneity nor horizontal pleiotropy was seen across any humanity (Supplementary Table S5).

3.4 Causal effect of T2DM on viral hepatitis

Next, we discovered that patients with T2DM in European populations had an increased risk of contracting viral hepatitis (IVW: OR=1.1173, 95%CI, 1.0271-1.2154, p=0.0098; WM: OR=1.0703, 95%CI, 0.9360-1.2239, p=0.3209; MR-Egger: OR=1.0017, 95%CI, 0.8310-1.2075, p=0.9859; MR-PRESSO: OR=1.1173, 95% CI, 1.0271-1.2154, p=0.0106, Figure 2 and Supplementary Figure S6). More specifically, T2DM was possibly linked with HBV (IVW: OR=1.1908, 95%CI, 1.0368-1.3677, p=0.0134; WM: OR=1.1525, 95%CI, 0.9305-1.4274, p=0.1935; MR-Egger: OR=0.9526, 95%CI, 0.7009-1.2946, p=0.7565; MR-PRESSO: OR=1.1908, 95%CI, 1.0368-1.3677, p=0.0144, Figure 2 and Supplementary Figure S7). The association between T2DM and HCV was insignificant (IVW: OR=1.0393, 95%CI, 0.7956-1.3575, p=0.7774; WM: OR=0.9314, 95%CI, 0.5866-1.4791, p=0.7634; MR-Egger: OR=1.2330, 95%CI, 0.6708-2.2664, p=0.5009; MR-PRESSO: OR=1.0393, 95%CI, 0.7956-1.3575, p=0.7777, Figure 2 and Supplementary Figure S8). There was no evidence of horizontal directional pleiotropy in any of these MR analyses (Supplementary Table S5). Notwithstanding, the analysis with HBV revealed heterogeneity (Cochran's Q test: p=0.0258, Rucker's Q test: p=0.0319); however, the leave-one-out analysis validate the conclusion's validity (Supplementary Figure S7).

In East Asians, the causal relationship between T2DM and viral hepatitis, HBV, was considered invalid by four different methods (Figure 3 and Supplementary Figures S9, 10). Nevertheless, it was discovered that patients with T2DM had a lower risk of contracting HCV (IVW: OR=0.9289, 95%CI, 0.8852- 0.9747, p=0.0027; WM: OR=0.9123, 95%CI, 0.8450-0.9849, p=0.0187; MR-Egger: OR=0.9559, 95%CI, 0.8591-1.0635, p=0.4097; MR-PRESSO: OR=0.9289, 95%CI, 0.8852-0.9747, p=0.0035, Figure 3 and Supplementary Figure S11). Horizontal pleiotropy and heterogeneity were not observed in any of the three diseases (Supplementary Table S5).

4 Discussion

To the extent of our knowledge, this is the first study leveraging MR to comprehensively investigate the association of genetic predictors of T2DM with CLDs across various ethnic backgrounds. Our analysis indicates that T2DM was probably linked with NAFLD and viral hepatitis, and possibly associated with HCC, and HBV in Europeans. In East Asians, our findings suggest a significant inverse correlation between T2DM and ALT, AST, and HCV. However, we did not find any causal association between T2DM and HCC, viral hepatitis, and HBV. Figure 4.

Epidemiological data has revealed that the age-adjusted relative risk of NAFLD is approximately 5.36 times greater in individuals with T2DM as opposed to the general non-diabetic population (45). Prior MR studies also presented evidence supporting correlations between genetic predisposition to T2DM and an escalated risk of NAFLD, particularly within European populations (46). These findings align with our MR analysis, albeit our study was conducted on a larger sample size. Numerous potential mechanisms may underpin the associations such as insulin resistance (IR), altered lipid metabolism, and inflammation (47). T2DM is classified as a chronic low-grade inflammatory disease characterized by the elevated levels of interleukin-6 and tumor necrosis factor (TNF)-α (48, 49). By stimulating cellular kinase and inhibiting kappa B kinase, these inflammatory cytokines can induce IR (50). IR may deliver substrates and precursors (e.g., free fatty acids, glucose, and glycerlol) for *de novo* lipogenesis and mitochondrial β-oxidation, processes that induce hepatic steatosis and increase the susceptibility to NAFLD (47, 51, 52). Moreover, higher blood glucose levels can activate carbohydrate response element-binding protein (ChREBP) signaling pathway, which stimulates the expression of several glycolytic genes, and ChREBP overexpression induced stearoyl-CoA desaturase 1 expression, increasing liver fat content (50, 53, 54).

NAFLD patients usually have abnormal liver function (55). Previous studies have shown that ALT, AST and AST/ALT ratio can predict NAFLD, and AST/ALT are closely related to IR and T2DM (55, 56). High circulating ALT and AST are widely used proxies of NAFLD, although they are not specific markers of NAFLD (57). Interestingly, our MR studies showed there was an inverse association between T2DM and NAFLD Proxies (ALT, AST) in East Asians. A retrospective investigation conducted in coastal Eastern India revealed that NAFLD patients of Indian descent exhibited a lower body mass index (BMI) and a lower prevalence of diabetes when compared to those in Western populations (58). Hence, we formulated the hypothesis that, with the exception of genetic predisposition and environmental exposures, the lifestyle and body weight in the West may account for a portion of these variations among populations and ethnicities but it remains to be validated.

Regarding the relation between T2DM with HCC, a recent meta-analysis reported that T2DM is associated with a significantly higher risk of HCC (12). Another larger cohort study also showed that the incidence of HCC was three times higher than the general population compared to patients with T2DM (59). Several factors could explain this observation, firstly, long-term T2DM can cause oxidative stress and telomere shortening, which induces DNA damage, apoptosis and chromosomal instability in hepatocytes, increasing the risk of HCC (60, 61). Secondly, IR is highly suspected of being a cancerogenic condition, which may attribute to hyperinsulinemia and increased bioavailable insulin-like growth factor I (IGF-I) (59, 62). In Europeans, our MR study identified a causal effect of T2DM on HCC that was only suggestive; in East Asians, no causal effect was observed. The potential explanation for this association specific to ethnicity is still unknown. The observed discrepancies in outcomes across ethnic groups may potentially be attributed to variations in residual confounding and selection bias, factors that are unavoidable (26, 63). Additionally, environmental, genetic, dietary, and lifestyle factors may account for the inverse result. Thus, additional research is required to examine the ethnic variations in disease risk profiles in order to develop more effective treatment approaches.

Additionally, numerous studies have investigated the correlation between T2DM and viral hepatitis, such as HBV and HCV. HBV infection is the most prevalent chronic viral infection on a global scale, affecting roughly 30% of the world's population in their lives; over 350 million individuals are chronic carriers of the virus (64). Also, it is estimated that around 71.1 million people are chronically infected with HCV, and that 1.75 million new cases of HCV infection were identified in 2015 (65). Previous study shows that patients with T2DM are more likely to be infected with HBV and HCV (18). This could be attributed to a multitude of possible factors. Firstly, HCV replication may be favored by hyperinsulinemia and/or the increased serum levels of free fatty acids observed in patients with T2DM (13). Then, T2DM has been associated with an immunocompromised condition, resulting in disruptions of immune function that potentially heighten the

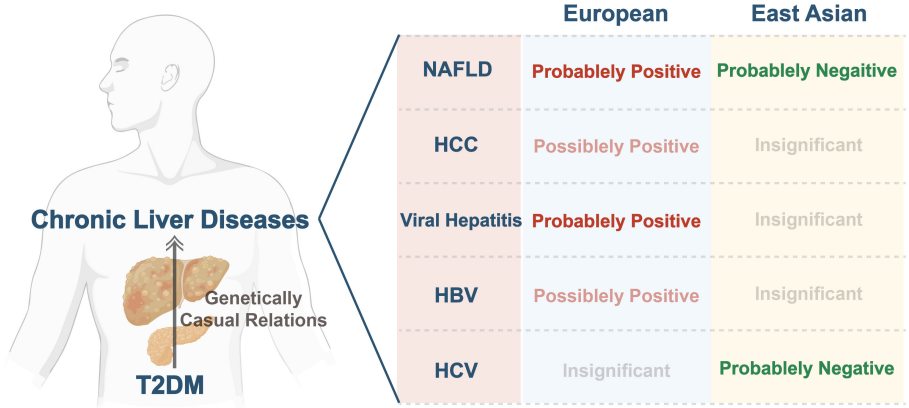

FIGURE 4
Summary of genetically causal relations between T2DM and CLDs across various ethnic backgrounds. T2DM, type 2 diabetes mellitus; CLDs, chronic liver diseases; NAFLD, non-alcoholic fatty liver disease; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus.

vulnerability to infection with HBV and HCV (66, 67). Also, it is worth noting that the process may be significantly influenced by interleukin-6, tumor necrosis factor-alpha, and various other immune-mediated pathways (9, 66, 68). Within the European population, this MR study revealed a suggestive association between T2DM and HBV and a positive correlation between T2DM and viral hepatitis. On the other hand, we discovered an inverse relationship between T2DM and HCV and absent relation between T2DM and viral hepatitis, HBV in East Asians. Considering the divergent outcomes observed in Eurasian populations, we posit that the relationship between T2DM and CLDs may indeed exhibit ethnic variations. Certainly, we consider the need for further investigation into potential molecular mechanisms and pathways involved in these ethnic disparities. Future studies, particularly those conducted in diverse ethnic populations, should delve deeper into understanding the intricate interplay between T2DM and CLDs. Our MR study also has certain shortcomings. First, comparatively small numbers of cases, such as NAFLD and viral hepatitis in East Asians, may result in low precision and potentially false negative results, which could influence the different MR results in different ancestral origins. Nevertheless, we have implemented the most extensive database of these diseases available, and additional research encompassing larger case sizes and ethnic backgrounds is necessary to definitively ascertain this correlation. Next, because there were zero SNPs as IVs when we set the threshold at 5e-8 in the GWAS of CLDs, we were unable to perform reverse MR analysis to estimate associations between CLDs and T2DM. Furthermore, the implementation of summarized GWAS precludes the ability to perform subgroup analyses according to gender and age. To mitigate such biases, future studies may employ within-family genome-wide association studies (GWASs) (69).

5 Conclusion

In summary, this research employed MR analysis to deduce a causal relationship between T2DM and CLDs by utilizing GWAS data from various ethnic populations. Our results showed the positive association between T2DM and NAFLD, viral hepatitis, HCC and HBV infection but not with HCV infection in Europeans. In contrast, in East Asians, T2DM was negatively associated with NAFLD and HCV but was not associated with HCC, viral hepatitis or HBV. The result revealed varying causal associations between T2DM and CLDs in East Asians and Europeans. Further clinical trials should be conducted in individuals of various ancestral origins to investigate the interaction between T2DM and CLDs in order to generate novel concepts for early screening and prevention of CLDs in patients with T2DM, as suggested by 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 authors.

Author contributions

YZ: Conceptualization, Formal Analysis, Writing – original draft, Writing – review & editing. DL: Data curation, Methodology, Software, Writing – original draft. HS: Data curation, Methodology, Writing – original draft. WLu: Data curation, Software, Writing – original draft. JQ: Formal Analysis, Writing – original draft. SW: Project administration, Resources, Writing – review & editing. YG: Methodology, Writing – original draft. RL: Project administration, Writing – review & editing. FH: Resources, Writing – review & editing. JL: Data curation, Formal Analysis, Writing – review & editing. WLi: Conceptualization, Project administration, Validation, Writing – review & editing. FW: Conceptualization, Project administration, Supervision, Validation, Writing – review & editing.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1338465/full#supplementary-material

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METS-IR and all-cause mortality in Korean over 60 years old: Korean genome and epidemiology study-health examinees (KoGES-HEXA) cohorts

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Background: The metabolic score for insulin resistance index (METS-IR) is a novel non insulin-based marker that indicates the risk for metabolic syndrome and type 2 diabetes mellitus (T2DM). However, METS-IR has not been investigated in relation to all–cause mortality. We investigated the longitudinal effect of METS-IR on all–cause mortality in a significantly large cohort of Korean adults over 60 years old.

Methods: Data were assessed from 30,164 Korean participants over 60 years of age from the Korean Genome and Epidemiology Study-Health Examinees (KoGES-HEXA) cohort data, linked with the death certificate database of the National Statistical Office. The participants were grouped into three according to METS-IR tertiles. We used multivariate Cox proportional-hazard regression models to prospectively assess hazard ratios (HRs) for all-cause mortality with 95% confidence intervals (CIs) over an 11-year postbaseline period.

Results: During the mean 11.7 years of follow-up, 2,821 individuals expired. The HRs of mortality for METS-IR tertiles were 1.16 (95% CI, 1.01–1.34) in T3 after adjustment for metabolic parameters, but the T2 did not show statistical significance towards increases for incident mortality respectively. In subgroup analysis depending on the cause of mortality, higher METS-IR was associated with cancer mortality (HR, 1.23, 95% CI, 1.01–1.51) but not with cardiovascular mortality (HR, 1.14, 95% CI, 0.83–1.57) after adjustment for the same confounding variables.

Conclusion: The METS-IR may be a useful predictive marker for all-cause mortality and cancer mortality, but not for cardiovascular mortality in subjects over 60 years of age. This implies that early detection and intervention strategies for metabolic syndrome could potentially benefit this identified group.

KEYWORDS

METS-IR, mortality, aging, cardiovascular disease, cancer, insulin resistance

Introduction

Aging is the process characterized by the continual accumulation of changes that result in sequential transformations as one advances in age (1). It stands as the most significant and unalterable factor contributing to the risk of diseases and mortality (2). In recent decades, while advancements in public health have led to reduced mortality rates and increased life expectancy (3), an undeniable challenge has emerged. The notable increase in life expectancy has given rise to a growing population of individuals afflicted by chronic diseases associated with aging (4). Particularly, in today's reality, where an unhealthy lifestyle accelerates susceptibility to diseases, this trend is gaining momentum (5). As a result, our focus is shifting towards the importance of healthy life expectancy (6). In a world marked by rapid demographic changes and a progressively aging population, the importance of managing the health of the older population has gained unprecedented significance.

Insulin resistance (IR) is defined as a state in which insulin exhibits reduced responsiveness in target tissues, despite its sufficient secretion (7). The unfavorable metabolic changes and disrupted glucose metabolism induced by IR can ultimately lead to the generation of oxidative stress and trigger inflammatory responses that result in cellular damage (8). Consequently, IR is associated with chronic conditions such as metabolic syndrome, hypertension and T2DM, and it extends to the development of serious health issues, including cardiovascular diseases (CVD), cancer, and, in some instances, mortality (9, 10). Globally, the leading causes of mortality are primarily dominated by CVD and cancer (11). In addition, the prevalence of IR is increasing worldwide, driven by factors such as excessive nutrition and sedentary modern lifestyles (12). Within the context of global aging and these prevailing trends, early detection of IR in the elderly is valuable as it enables the evaluation of the risk for major diseases like CVD and cancer, along with their associated mortality, and facilitates preventive management.

The hyperinsulinemic-euglycemic clamp is considered the gold standard for assessing IR (13). However, this method is often impractical for routine IR assessment, leading to the development of several surrogate IR markers to meet this demand (14, 15). Among these markers, the recently introduced novel non-insulin-based IR marker, METS-IR, has been proposed and validated against the hyperinsulinemic-euglycemic clamp, confirming its efficacy (16). METS-IR is a convenient tool for routine health monitoring, utilizing easily assessable metrics such as fasting plasma glucose (FPG), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and body mass index (BMI). This simplicity makes it highly accessible and sustainable for primary care in the older population. Additionally, previous studies targeting patients without diabetes revealed that METS-IR outperformed metabolic syndrome in predicting ischemic heart disease, a key age-related ailment (17, 18). In comparison to the metabolic syndrome, acknowledged as a risk factor for major age-associated diseases with insulin resistance as its primary pathophysiology (19), METS-IR demonstrated its utility and excellence as an alternative marker for insulin resistance.

While METS-IR is widely used as an indicator of IR and has been linked to conditions such as hypertension, T2DM, and CVD in previous studies (17, 20–22), there is currently no research, to our knowledge, that has explored the connection between METS-IR and both all-cause and cause-specific mortality in individuals aged 60 and above. Therefore, we prospectively investigated the relationships between METS-IR and all-cause, as well as cause-specific mortality, among individuals aged over 60 years within the Korean population.

Abbreviations: METS-IR, the Metabolic score of Insulin Resistance; T2DM, Type 2 Diabetes mellitus; KoGES-HEXA, Korean Genome and Epidemiology Study-Health Examinees; HR, Hazard ratio; CI, Confidence interval; WC, Waist circumference; LDL, Low-density lipoprotein; IR, Insulin resistance; CVD, Cardiovascular disease; FPG, Fasting plasma glucose; TG, Triglyceride; HDL-C, High-density lipoprotein cholesterol; BMI, Body mass index; IRB, Institutional review board; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; GGT, γ-glutamyltransferase; ICD, International Classification of Diseases; ANOVA, Analysis of variance; IGFBP, Insulin-like growth factor binding protein.

Materials and methods

Study design and participants

The Health Examinees Cohort (HEXA) is a large, government-funded prospective cohort study to identify genetic and

environmental factors for common complex diseases in. The cohort of participants consisted of community dwellers and participants, men and women, aged ≥ 40 years at baseline who were recruited from the National Health Examinee Registry. These participants were recruited during the baseline survey, conducted from 2004 to 2013, at 38 health examination centers and hospitals in the eight regions of South Korea. The participants were then asked to return periodically to complete the follow-up surveys by mail and telephone. Details of the study have been published elsewhere (23). For the analysis of this study, anonymized data of 173,195 participants aged ≥ 40 years were linked with the death certificate database of the National Statistical Office. The data set of those consists of anthropometric and clinical measurements, lifestyle (i.e., diet, smoking, alcohol drinking, and physical activity), and the Food Frequency Questionnaire. In the current study, we included a total of 30,164 participants whose medical history and mortality records were available.

This study investigated the risk factors for metabolic syndrome (METS-IR) in the population over 60 years of age. Figure 1 shows a flow chart describing the study. Out of a total of 173,195 participants, we excluded 130,379 who were under 60. Additionally, 2,846 participants with missing covariates were excluded, along with another 9,806 due to follow-up loss. Participant follow-up employed both active and passive methods. Active methods involved sending information leaflets by mail and making phone calls, while passive methods identified cases through Korean health-related databases (24). Main reasons for follow-up refusal included changes in contact information, being too busy to attend, and not responding to phone calls (23). Imputation analysis was conducted to address the issue of excessive exclusions due to follow-up loss, confirming no significant bias resulting from missing data (Supplementary Table S5). Ultimately, after these exclusions, 30,164 participants remained in the study. This study was conducted under the Institutional Review Board (IRB) approval of Yongin Severance Hospital (IRB number: 9-2023-0018).

Data collection

Every participant granted informed consent for baseline data and biospecimen collection, and underwent both an interview and physical examination. Ethical approval was secured from the institutional review boards of the National Research Institute of Health and collaborators of the KoGES groups (23). Each participant completed a comprehensive questionnaire that captured information about his or her lifestyle and medical history. Smoking status was divided into never-smoker, ex-smoker, and current smoker. Regular alcohol drinker was defined as consuming more than 140 grams per week, based on the frequency of alcohol consumption reported by the subjects. Body weight and height were measured with an accuracy of 0.1 kg and 0.1 cm, respectively. Participants were instructed to wear light indoor clothing and not to wear shoes during measurement. BMI was calculated by dividing weight divided by height squared (kg/m^2). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were assessed by means of a standard mercury sphygmomanometer (Baumanometer, W.A. Baum Co Inc., Copiague, NY, USA) while participants were in a seated and rested for 10 minutes Mean arterial pressure was calculated from the measured SBP and DBP values. Hypertension was defined as an SBP ≥140 mmHg, a DBP ≥90 mmHg, or current use of hypertension medication. Blood samples were collected from the subjects through an antecubital vein after a 12-hour overnight fast. Concentrations of FPG, total cholesterol, TG, HDL-C, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) were measured enzymatically using a Chemistry Analyzer (Hitachi 7600, Tokyo, Japan up to August 2002 and ADVIA 1650, Siemens, Tarrytown, NY from September 2002). In the HEXA cohort, efforts are made to achieve standardized results through the centralization of sample preparation and management. Each year, on-site inspections, internal and external quality control, inter-laboratory comparisons, measurement traceability evaluations, and

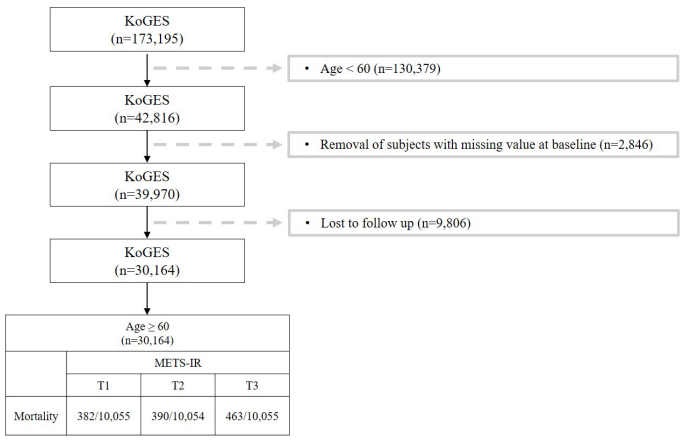

FIGURE 1
Flowchart for the selection of study participants.

trend analysis assessments are consistently conducted for the diagnostic testing institutions. These ongoing checks aim to enhance the reliability of test results (25).

Assessment of METS-IR

The METS-IR index was computed using the following formulas (16): ln (2 × FPG [mg/dL] + TG [mg/dL]) × BMI (kg/m^2)/ln (HDL-C [mg/dL]).

Study outcomes

Mortality status was determined by linking data to the unique personal identification key code system since the HEXA cohort is connected to national data sources that contain mortality records, from the Korea National Statistical Office. Participants were continuously followed from the baseline survey data to the the time of the mortality event, the study end date, or the date of last contact. Participant mortality was monitored from January 2001 to December 2019, with the cause of mortality classified based on the International Classification of Diseases (ICD) codes as listed in the National Mortality Index. All-cause mortality represents all deaths with specified and unknown causes, cancer mortality includes deaths under ICD-10 codes C00-C97, and CVD mortality includes deasths under ICD-10 codes I00-I99.

Statistical analysis

We categorized the participants into three groups according to the base METS-IR level. The cut-off level of METS-IR is 33.2 and 38.0 in subjects older than 60 years (17, 20, 26–30). All the data presented in this study include means with standard deviations or frequency with percentages. The baseline characteristics of the study population were compared according to METS-IR tertiles using Pearson's chi-squared test for categorical variables and an analysis of variance (ANOVA) for continuous variables. The primary variables under examination included individual demographic details, anthropometric and biochemical parameters, and lifestyle factors. Kaplan–Meier curves were used to evaluate the cumulative incidence of all-cause mortality, cancer-related and CVD-related mortality. The log-rank test was used to determine whether the distributions of cumulative incidence function for mortality differed among the groups. In multivariable analysis, with the lowest tertiles of all-cause mortality value as the reference group, hazard ratios (HRs) and 95% confidence intervals (CIs) for incident mortality were calculated using the Cox proportional hazards model after adjustment for potential confounding variables. The models included participant characteristics related to mortality in the Korean population as potential confounders (31). For all analyses, R software (version 4.0.2; R Foundation for Statistical Computing, Vienna, Austria) was used. All statistical tests conducted were two-sided, with P values of <0.05 were considered to be statistically significant.

Results

Baseline characteristics

During the mean 11.7 years of follow-up period, 2,821 all-cause mortality, 1,235 cancer mortality, 514 CVD mortality, and 1,072 mortality from other reasons occurred among the 30,164 participants. Table 1 displays the baseline characteristics of the study population according to the METS-IR tertiles. Those in the third METS-IR tertiles had higher levels or proportions of BMI, waist circumference (WC), SBP, TG, FPG, liver enzymes, and lower levels of HDL-C. The third tertile of the METS-IR in population also had the highest proportion of current smoker, hypertension, and T2DM.

Hazard ratios for all-cause mortality

Table 2 summarizes the association between baseline METS-IR and all-cause mortality within the study population. In the unadjusted model, there was no association with higher all-cause mortality for the highest METS-IR (all-cause mortality: METS-IR [16.7, 33.2] vs METS-IR [38.0, 77], HR, 1.05; 95% CI, 0.96–1.15). However, In the fully adjusted model, contorolling for age, sex, WC, SBP, DBP, creatinine, low-density lipoprotein (LDL) cholesterol, smoking status, alcohol intake, exercise, liver enzymes, hypertension and T2DM, it was found that the highest METS-IR was significantly associated with all-cause (all-cause mortality: METS-IR [16.7, 33.2] vs METS-IR [38.0, 77], HR, 1.16; 95% CI, 1.01–1.34). Additionally, as depicted in Figure 2, the Kaplan-Meier survival curve, when stratified by METS-IR tertiles, illustrated an elevation in the cumulative incidence of all-cause mortality corresponding to an increase in METS-IR (log-rank test, P < 0.001).

Subgroup analysis for incident mortality

In the subgroup analysis depending on the cause of death, higher METS-IR was associated with cancer mortality but this association was not observed in CVD mortality (Table 3).

(cancer mortality: METS-IR [16.7, 33.2] vs METS-IR [38.0, 77], HR, 1.23; 95% CI, 1.01–1.51; CVD mortality: METS-IR [16.7, 33.2] vs METS-IR [38.0, 77], HR, 1.14; 95% CI, 0.83–1.57).

Discussion

In a community-based population of Korean adults aged over 60 years, our research has substantiated a significant positive association between METS-IR and all-cause mortality, even after adjusting for confounding factors. Additionally, when conducting a cause-specific analysis, METS-IR demonstrated a notable positive correlation with cancer-related mortality, while no statistically significant results were observed concerning CVD-related mortality.

Defining the older adults is a challenging task, considering the various biological, demographic, and sociological perspectives.

TABLE 1 Baseline characteristics of the study population according to the METS-IR tertiles in individuals older than 60 years.

Characteristic	Total	Group 1	Group 2	Group 3	*p* value
	(n=30,164)	T1 [16.7, 33.2] (n = 10,055)	T2 (33.2, 38.0] (n = 10,054)	T3 (38.0, 77] (n = 10,055)	
Sex (men)	12,837 (42.6)	3,948 (39.3)	4,225 (42)	4,664 (46.4)	<0.001
Age (years)	64.3 ± 3.3	64.2 ± 3.3	64.3 ± 3.3	64.4 ± 3.3	0.014
Body mass index (kg/m^2)	24.3 ± 2.8	21.7 ± 1.8	24.3 ± 1.5	26.9 ± 2.3	<0.001
Waist circumference (cm)	83.4 ± 8.1	77.3 ± 6.5	83.6 ± 5.8	89.4 ± 6.8	<0.001
Systolic blood pressure	127.1 ± 15.1	124.1 ± 15.1	127.4 ± 14.8	129.7 ± 14.9	<0.001
Diastolic blood pressure (mmHg)	77.2 ± 9.4	75.6 ± 9.3	77.3 ± 9.3	78.6 ± 9.3	<0.001
LDL-cholesterol (mg/dl)	119.0 ± 31.1	116.0 ± 29.6	122.7 ± 31.6	118.4 ± 32.7	<0.001
HDL-cholesterol (mg/dl)	51.8 ± 12.4	60.6 ± 12.3	50.9 ± 9.5	43.9 ± 8.9	<0.001
Triglyceride (mg/dl)	126.6 ± 64.9	91.1 ± 40.2	123.0 ± 45.5	165.9 ± 72.2	<0.001
Fasting plasma glucose (mg/dl)	98.7 ± 23.1	93.2 ± 16.2	98.3 ± 22.0	104.7 ± 28.1	<0.001
ALT	22.6 ± 17.7	19.9 ± 19.1	22.1 ± 14.4	25.8 ± 18.7	<0.001
AST	24.8 ± 14.4	24.6 ± 18.5	24.3 ± 11.3	25.6 ± 12.2	<0.001
Creatinine	0.9 ± 0.3	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.3	<0.001
Smoking status, n (%)					<0.001
Never smoker	21,005 (69.6)	7,333 (72.9)	7,052 (70.1)	6,620 (65.8)	
Former smoker	6,364 (21.1)	1,821 (18.1)	2,165 (21.5)	2,378 (23.6)	
Current smoker	2,795 (9.3)	901 (9)	837 (8.3)	1,057 (10.5)	
Alcohol intake, n (%)					<0.001
Never drinker	17,556 (58.2)	5,920 (58.9)	5,822 (57.9)	5,814 (57.8)	
Former drinker	1,658 (5.5)	483 (4.8)	541 (5.4)	634 (6.3)	
Current drinker	10,950 (36.3)	3,652 (36.3)	3,691 (36.7)	3,607 (35.9)	
Regular exercise (Yes)	1,6282 (54)	5,501 (54.7)	5,668 (56.4)	5,113 (50.9)	<0.001
Hypertension, n (%)	10,967 (36.4)	2,572 (25.6)	3,645 (36.3)	4,750 (47.2)	<0.001
Diabetes, n (%)	3,928 (13)	775 (7.7)	1,246 (12.4)	1,907 (19.0)	<0.001

P-values were calculated using 1-way ANOVA or Pearson's chi-square test.

However, it is a common practice to define the older population as individuals aged 60 or 65 and older for statistical and administrative purposes (32, 33). A previous study, aimed at investigating the relationship between changes in plasma proteomics across the lifespan and the occurrence of diseases, discovered a noteworthy trend. It revealed that among individuals aged 60 and older, there was a substantial enrichment of proteins associated with CVD. This finding strongly suggests an increased likelihood of CVD incidence in this age group (34). In this context, it is essential to establish correlation metrics for major diseases like CVD and associated mortality in the population 60 years and older.

Insulin plays a crucial role in maintaining glucose homeostasis primarily through promoting glucose uptake and exerts diverse effects on systemic target cells like muscle, adipose tissue and liver (9). When IR occurs, compensatory hyperinsulinemia develops to maintain normal glucose levels (35). IR can contribute to the development of atherosclerosis and the advancement of arterial plaque (36). Disturbed insulin signaling within the cells lining the innermost layer of blood vessels, which play a role in atherosclerosis, including endothelial cells, vascular smooth muscle cells, and macrophages, is thought to be a contributing factor. In other words, the original role of insulin in the endothelial cells, which is to counteract atherosclerosis, becomes impaired under conditions of IR, potentially accelerating the progression of atherosclerosis (37). Indeed, Prior investigations have demonstrated a correlation between IR and an elevated likelihood of CVD (17, 38, 39). The association between IR and cancer has also sparked widespread interest, leading to extensive research. It has been suggested that IR may contribute to an increased risk of cancer development (40–42). Tumorigenesis can be caused by the hallmark of IR, hyperinsulinemia, and the role of the insulin/insulin-like growth factor system associated with it (43).

TABLE 2 Hazard ratios and 95% confidence intervals for All-cause mortality according to METS-IR tertiles in subjects older than 60 years.

		Group 1	Group 2	Group 3	*p* for trend
		T1 [16.7, 33.2] (n = 10,055)	T2 [33.2, 38.0] (n = 10,054)	T3 [38.0, 77] (n = 10,055)	
New cases of death, n		923	905	993	
Mean follow-up, years		11.59	11.75	11.73	
Pearson-years of follow-up		116,497	118,172	117,943	
Incidence rate/1000 person -years		7.92	7.66	8.42	
Model 1	HR (95% CI)	1.00 (reference)	0.95 (0.87-1.05)	1.05 (0.96-1.15)	0.240
	p value	–	0.325	0.250	
Model 2	HR (95% CI)	1.00 (reference)	1.11 (1.00-1.23)	1.40 (1.22-1.60)	<0.001
	p value	–	0.054	<0.001	
Model 3	HR (95% CI)	1.00 (reference)	1.10 (0.99-1.22)	1.33 (1.16-1.53)	<0.001
	p value	–	0.083	<0.001	
Model 4	HR (95% CI)	1.00 (reference)	1.05 (0.95-1.17)	1.16 (1.01-1.34)	0.030
	p value	–	0.354	0.030	

Model 1: Unadjusted.
Model 2: adjusted for age, sex and WC.
Model 3: adjusted for age, sex, WC, SBP, DBP, ALT, AST, creatinine and LDL.
Model 4: adjusted for age, sex, WC, SBP, DBP, ALT, AST, creatinine, LDL, smoke, drink, exercise, HTN and DM.

Given these points, it can be inferred that higher IR may lead to increased mortality related to cancer and CVD. Some studies have yielded diverse results regarding the correlation between IR and mortality, encompassing both all-cause and cause-specific outcomes (27, 44–53). When interpreting our results within the context of previous research, both concordant and discordant findings emerge. In the analysis of the association between IR and cause-specific mortality, no study demonstrated a significant association with both cancer-related and CVD-related mortality. Some studies established an association between IR and all-cause mortality (44, 45), along with either CVD (27, 46–49) or cancer-related mortality (50, 51), while others failed to find associations with mortality (52, 53). This implies that understanding the shared pathophysiology, such as insulin resistance, between CVD and cancer, while simultaneously comprehending the distinct characteristics of each disease, is crucial for formulating tailored strategies for each. CVD and cancer are complex, multifactorial conditions, not attributable to a single cause (54). In some aspects, even the outcomes of shared pathways may differ between the occurrence and management of CVD and cancer. Angiogenesis is recognized as a driving force in cancer, characterized by pathological neovascularization. Therapeutic strategies aim to inhibit angiogenesis. Conversely, in ischemic diseases, angiogenesis is considered a therapeutic potential. In this context, insulin-like growth factor binding proteins (IGFBPs), a group of seven proteins essential for the transportation of insulin-like growth factor, play a significant role in angiogenesis. Each member within the IGFBP family exhibits varying effects on angiogenesis. IGFBP-1 and IGFBP-2 demonstrate pro-angiogenic effects, while IGFBP-4 and IGFBP-5 primarily exhibit anti-angiogenic properties. IGFBP-3 and IGFBP-7, depending on the cellular environment, possess dual characteristics, showing both pro- and anti-angiogenic effects (55).

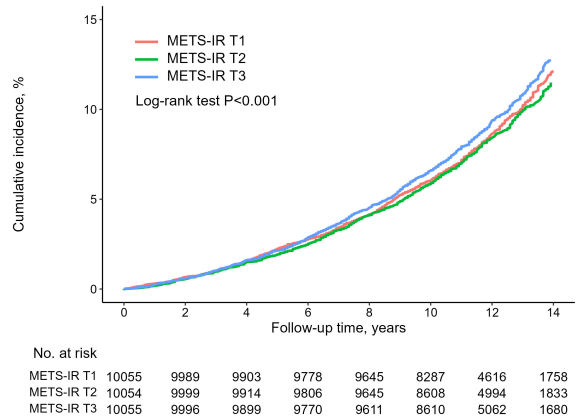

FIGURE 2
Kaplan-Meier curve for all-cause mortality.

Considering the reasons for inconsistent findings among previous studies, including our research results, several factors come to mind. Firstly, each study targeted a diverse study population with variations in age, sex, race, and other characteristics, and there were differences in sample sizes. Secondly, the variation in IR assessment methods across studies also contributes to these disparities. We utilized METS-IR as our IR marker, which is a variable with multiple components. Previous studies have used traditional IR marker like homeostatic model sssessment for insulin resistance (HOMA-IR) (45, 48–51), as well as non-insulin-based IR markers like the triglyceride glucose index (52). The former may face challenges in large-scale studies due to procedural and cost issues, while the latter lacks the inclusion of nutritional factors.

TABLE 3 Multivariate Cox proportional-hazards regression models for cancer mortality and CVD mortality according to METS-IR tertiles in subjects older than 60 years.

		Cancer mortality				CVD mortality			
		Group 1	Group 2	Group 3	*p* for trend	Group 1	Group 2	Group 3	*p* for trend
		T1 [16.7, 33.2] (n = 10,055)	T2 [33.2, 38.0] (n = 10,054)	T3 [38.0, 77] (n = 10,055)		T1 [16.7, 33.2] (n = 10,055)	T2 [33.2, 38.0] (n = 10,054)	T3 [38.0, 77] (n = 10,055)	
New cases of death, n		382	390	463		158	159	197	
Mean follow-up, years		11.59	11.75	11.73		11.59	11.75	11.73	
Pearson-years of follow-up		116,497	118,171	117,942		116,497	118,172	117,943	
Incidence rate/ 1000 person -years		3.28	3.30	3.93		1.36	1.35	1.67	
Model 1	HR (95% CI)	1.00 (reference)	1.00 (0.87-1.15)	1.19 (1.04-1.36)	0.011	1.00 (reference)	0.98 (0.78-1.22)	1.22 (0.99-1.50)	0.057
	p value	–	0.979	0.012		–	0.844	0.063	
Model 2	HR (95% CI)	1.00 (reference)	1.09 (0.93-1.28)	1.39 (1.13-1.71)	0.001	1.00 (reference)	1.04 (0.81-1.34)	1.39 (1.01-1.90)	0.037
	p value	–	0.288	0.002		–	0.743	0.041	
Model 3	HR (95% CI)	1.00 (reference)	1.10 (0.94-1.29)	1.38 (1.12-1.69)	0.002	1.00 (reference)	1.03 (0.80-1.32)	1.30 (0.95-1.78)	0.097
	p value	–	0.244	0.002		–	0.844	0.103	
Model 4	HR (95% CI)	1.00 (reference)	1.06 (0.91-1.25)	1.23 (1.01-1.51)	0.048	1.00 (reference)	0.99 (0.77-1.27)	1.14 (0.83-1.57)	0.418
	p value	–	0.45	0.045		–	0.921	0.429	

Model 1: Unadjusted.
Model 2: adjusted for age, sex and WC.
Model 3: adjusted for age, sex, WC, SBP, DBP, ALT, AST, creatinine and LDL.
Model 4: adjusted for age, sex, WC, SBP, DBP, ALT, AST, creatinine, LDL, smoke, drink, exercise, HTN and DM.

METS-IR was able to overcome these limitations and provide more support in predicting cardiovascular metabolic diseases by integrating BMI (17). Moreover, In a previous study utilizing factor analysis, results similar to our study's conclusions were derived. They formed a cluster of IR-related biomarkers, including higher BMI, FPG, TG, uric acid, and GGT activity and lower HDL-C levels. This cluster predicted cancer mortality, while HOMA-IR and fasting insulin failed to do so (56). The use of a multivariable marker, including BMI, to represent IR is similar between this study and our research. This suggests that multivariable markers, such as METS-IR, can be effective in predicting cancer-related mortality.

We conducted research beyond the initially studied population of 60 years and older to examine whether there are differences in the association between METS-IR and mortality based on age, specifically for those aged 65 and older, and those aged 40 to less than 60 (Supplementary Tables S1–S4). The findings indicate that METS-IR did not demonstrate significant associations with all-cause mortality and cause-specific mortality in both the 40 to less than 60 age group and the 65 and older age group. Reflecting on the reasons for this, in the 65 and older age group, the limited sample size appears to be a contributing factor. Additionally, for the 40 to 59 age group, the relatively low mortality rate and the distinctive characteristics of this age group, which focuses on a comparatively younger demographic, may have hindered the attainment of statistically significant results due to an insufficient follow-up duration to observe mortality occurrences.

On the other hand, there are still many aspects to address behind the potential use of METS-IR as an indicator for predicting all-cause mortality, particularly cancer-related mortality, in individuals aged 60 and above. In our study, we did not differentiate cancer types, and information regarding incidence rates was unavailable.

Additional research on cancer types holds the potential to provide information on specific cancers that METS-IR may reflect, especially considering METS-IR's inclusion of BMI as a marker. This suggests the possibility of obtaining insights into obesity-related cancers. Furthermore, additional studies that include the timing of occurrence can shed light on the possibility of being diagnosed with rapidly progressing cancers in terms of cancer-related mortality. Additionally, these studies may reveal the importance of internal factors, such as IR, in leading to mortality, even in the absence of differences in cancer types. Furthermore, prior research has suggested that genetic variations in insulin receptor genes can elevate the risk of obesity-related cancers (57). Considering this, by confirming cancers predominantly predicted by METS-IR and conducting additional studies to identify genetic variations associated with these cancers, we can establish the foundation for personalized medicine. This involves utilizing METS-IR to assess and manage individuals with such genetic variations.

Our study has both strengths and limitations. One key strength lies in its large, population-based cohort design, with a relatively higher number of mortality cases compared to other studies. Conversely, limitations include the fact that we focused solely on Korean adults aged 40 and older, limiting the generalizability of our findings to other countries, all age groups, and ethnic groups. Additionally, the information on the incidence rates of CVD and cancer is insufficient, and the study outcomes are restricted to mortality rates. Evaluation based on the types of cancer was not conducted. Finally, It is also possible that there are other residual confounding factors that were not adequately controlled for.

Conclusions

METS-IR demonstrated a positive correlation with both all-cause and cancer-related mortality, making it a reliable predictor of mortality in individuals over 60 years old. These results highlight a previously unrecognized subgroup of elderly individuals at a significantly heightened risk of cancer-specific mortality. Early detection and intervention strategies could potentially benefit this identified group.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://nih.go.kr/ko/main/contents.do?menuNo=300563.

Ethics statement

The studies involving humans were approved by Yongin Severance Hospital (IRB number: 9-2023-0018). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

HR: Conceptualization, Writing – original draft, Writing – review & editing. DJ: Conceptualization, Writing – original draft, Writing – review & editing. SH: Methodology, Writing – original draft, Writing – review & editing. BP: Methodology, Writing – original draft. YL: Funding acquisition, Supervision, Writing – review & editing.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1346158/full#supplementary-material

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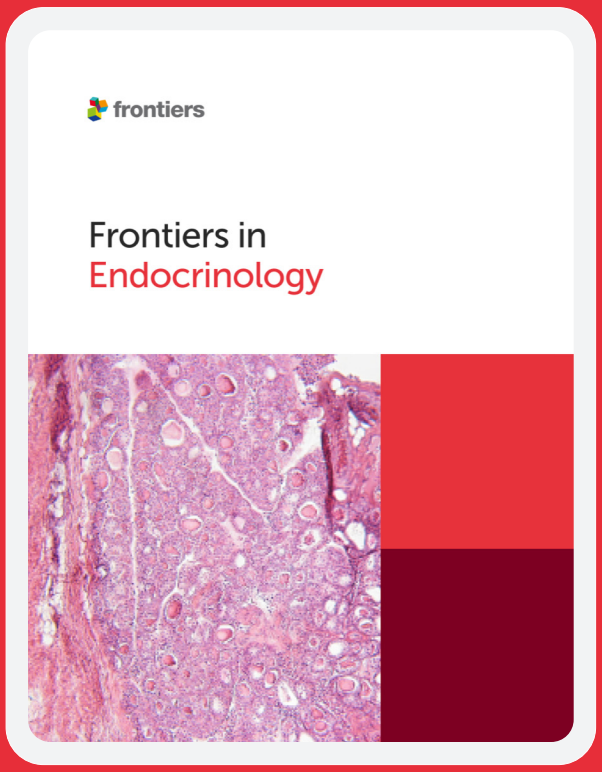

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