NUTRACEUTICALS FOR THE RECOVERY OF COVID-19 PATIENTS

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NUTRACEUTICALS FOR THE RECOVERY OF COVID-19 PATIENTS

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Editorial: Nutraceuticals for the recovery of COVID-19 patients

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COVID-19, dietary patterns, nutraceuticals, vegetarian diet, vitamin D

Editorial on the Research Topic
Nutraceuticals for the recovery of COVID-19 patients

The novel coronavirus disease (COVID-19) unleashed sudden and unprecedented mortality on global populations and fosters a lingering health burden. In our call for papers on the theme ***Nutraceuticals for the recovery of COVID-19 patients***, we purposively invited topics on the immunomodulatory effects of nutrients and bioactive compounds falling into the narrow definition of nutraceuticals and functional foods (1–3) as well as dietary supplements and designed diets (4), given the knowledge gaps in adjunctive therapy management for the post-infection stages of COVID-19. Falling within this research theme are 10 papers covering dietary protein (Shariatpanahi et al.), melatonin (Su et al.), curcumol (Yang et al.), herbal tea (Hsieh et al.), dietary supplements (Hashemi et al.), and dietary patterns (Ebrahimzadeh et al.; Hou et al.; Vajargah et al.) as well as vitamin D (Bogliolo et al.; Chiang et al.) (Figure 1).

Malnutrition is prevalent in COVID-19-infected patients, particularly in those with a greater severity of the disease and who are critically ill (1). A major complication associated with the initiation of feeding in malnourished patients is refeeding syndrome (2). In a prospective cohort study, Shariatpanahi et al. assessed patients for their risk of developing refeeding syndrome and those who did develop it. They found the incidence of refeeding syndrome was relatively high in the majority of critically ill COVID-19 patients, but increased protein intake was associated with reduced occurrence of refeeding syndrome.

The protective role of vitamin D in COVID-19 sufferers (3) is commonly researched for its immunomodulatory and anti-inflammatory action at the level of endothelial function (4–6), and is highly recommended as an adjuvant therapy for COVID-19 (7). In this special issue, a prospective observational multicenter study by Bogliolo et al. showed that very low 25(OH) vitamin D levels were highly prevalent in patients with severe COVID-19, but low 25(OH) vitamin D levels were not associated with high mortality outcomes in moderate to severe cases of COVID-19. This finding is contrary to a meta-analysis of seven systematic reviews (7) that showed that vitamin D supplementation reduces the risk of mortality, need for intensive care, and mechanical

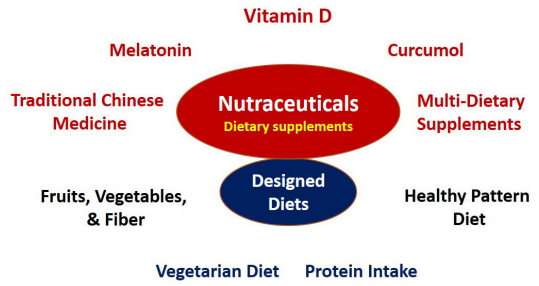

FIGURE 1
Nutraceutical supplements and various dietary patterns for the recovery of COVID-19 patients.

ventilation requirements in COVID-19 patients. Another direction in vitamin D adjuvant therapy is in kidney disease, given the concern that COVID-19 patients who are asymptomatic or have mild symptoms show dynamic changes in renal function (8), whilst patients with chronic kidney disease (CKD) frequently have vitamin D deficiency and increased susceptibility to infection. In their review article, Chiang et al. highlight the double burden of increased risk for vitamin D deficiency in CKD patients due to the coexistence of immune activation and immune deficiency, and proposed mechanisms by which vitamin D administration could modulate the immune system and alleviate the pathological consequences of COVID-19. A further benefit of vitamin D supplementation would be to reduce the severity of acute kidney injury in COVID-19 patients *via* reducing soluble urokinase-type plasminogen activator receptor levels.

Factors such as age, sex, and comorbidities are key determinants of illness severity and progression of COVID-19. The review article by Su et al. centers attention on the decline in melatonin levels exacerbated by aging, with a strong implication of compromised mitochondrial redox activities which could explain the higher death rate of COVID-19 in older age groups. Declining melatonin levels are closely related to mitochondrial dysfunction, and its reversal with melatonin supplementation could limit virus-related diseases. Hence, melatonin in elderly people may be warranted in the treatment of COVID-19.

The special edition introduces curcumol as a common traditional Chinese medicine (TCM), isolated from Rhizoma Curcumae with well-documented anti-viral activity (9). By using network pharmacology and systematic bioinformatics analysis, Yang et al. identified seven core targets of curcumol therapy for lung adenocarcinoma (LUAD) patients infected with COVID-19. These targets influence cell-signaling associated with the Warburg effect, which supports SARS-CoV-2 replication and inflammatory response. Comparative transcriptomic analysis specified the effects of curcumol through control of cell cycle, DNA damage response, and cell apoptosis. The combination of TCM and standard management in treating patients with COVID-19 in Taiwan was examined using Jing Si Herbal Tea (JSHT). A prospective cohort study by Hsieh et al. that recruited patients with mild to moderate COVID-19 suggests JSHT combined with standard management may prevent critical status and mortality. Effective improvements in measured outcomes such as reverse transcription–polymerase chain reaction cycle threshold value, C-reactive protein level, and Brixia score occurred in male and older patients (≥60 years), suggesting that three main pathophysiological pathways, anti-infective, anti-inflammation, and anti-thrombosis, were potentially targeted (10).

The ability of purposive dietary patterns to protect against respiratory viral infections and reduce associated inflammation and oxidative stress is also examined in this special edition. A retrospective evaluation of COVID-19 patients by Hou et al. found COVID-19 symptom severity was significantly and inversely associated with adherence to a self-reported vegetarian diet compared to those consuming a non-vegetarian diet, with the latter group having a higher risk in contracting critically severe COVID-19. A cross-sectional study of COVID-19 hospitalized patients by Vajargah et al. showed higher consumption of fruits, vegetables, and fiber was inversely linked with COVID-19 severity, clinical symptoms, hospitalization, and convalescence duration, and concentrations of inflammatory markers. Fruits and vegetables are rich in fiber and a good source of anti-inflammatory and immune-boosting vitamins, minerals, and antioxidants (11). In contrast, the pre-COVID-19 status of habitual food intake could be an environmental factor affecting inflammation status in the body (12) and potentiate outcome response to COVID-19 infection (13). Ebrahimzadeh et al. retrospectively evaluated 250 recovered COVID-19 cases to explore diet pattern effects using a self-reported web-based food questionnaire. They found cases reporting a higher adherence to a healthy diet pattern were associated with lower inflammatory markers levels and lower risk of COVID-19 severity, hospitalization, and convalescence duration.

Was consumption of immune-boosting supplementation critical to offering protection during the COVID-19 pandemic? Hashemi et al. in a cross-sectional study involving 300 adult men and women with COVID-19, probed recent and long-term supplement intakes using a questionnaire. Short-term use (∼ 2 months) saw improvements in blood urea nitrogen and higher serum 25(OH)D levels whilst long-term use achieved significantly lower invasive oxygen support, lactate dehydrogenase (LDH), fewer days of fever, and higher serum 25(OH)D levels.

The papers included under the theme of *Nutraceuticals for the recovery of COVID-19 patients* are highly relevant to the

emergence of long COVID symptoms as a health burden and the need for encouraging more research in this area.

Author contributions

KC-L wrote the introduction and the conclusion. TK wrote the central part with comments on the cited papers and references. Both authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Efficacy and Safety of Complementary Therapy With Jing Si Herbal Tea in Patients With Mild-To-Moderate COVID-19: A Prospective Cohort Study

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Background: Since late 2019, there has been a global COVID-19 pandemic. To preserve medical capacity and decrease adverse health effects, preventing the progression of COVID-19 to severe status is essential. Jing-Si Herbal Tea (JSHT), a novel traditional Chinese medicine formula was developed to treat COVID-19. This study examined the clinical efficacy and safety of JSHT in patients with mild-to-moderate COVID-19.

Methods: In this prospective cohort study, we enrolled 260 patients with mild-to-moderate COVID-19. The enrolled patients were divided into the JSHT ($n = 117$) and control ($n = 143$) groups. Both groups received standard management. The JSHT group was treated with JSHT as a complementary therapy.

Results: Compared with standard management alone, JSHT combined with standard management more effectively improved the reverse transcription–polymerase chain reaction cycle threshold value, C-reactive protein level, and Brixia score in the adult patients with mild-to-moderate COVID-19, especially in the male and older patients (those aged ≥60 years). The results revealed that the patients treated with JSHT combined with standard management had 51, 70, and 100% lower risks of intubation, Medisave Care Unit admission, and mortality compared with those receiving standard management only.

Conclusions: JSHT combined with standard management more effectively reduced the SARS-CoV-2 viral load and systemic inflammation and alleviated lung infiltrates in the patients with mild-to-moderate COVID-19, especially in the male and older patients (those aged ≥60 years). JSHT combined with standard management may prevent critical status and mortality in patients with mild-to-moderate COVID-19. JSHT is a promising complementary therapy for patients with mild-to-moderate COVID-19.

Keywords: mild-to-moderate COVID-19, Jing Si Herbal Tea, complementary and alternative medicine, traditional Chinese medicine, phytomedicine, herbal medicine

INTRODUCTION

In late 2019, multiple cases of novel viral pneumonia were reported in Wuhan City, China. Since then, the SARS-CoV-2 virus has spread rapidly, leading to the global COVID-19 pandemic (1), which has considerably affected patient health and health-care systems (2). SARS-CoV-2 is primarily transmitted through direct or indirect respiratory tract exposure (3). After binding to the angiotensin-converting enzyme 2 (ACE2) receptor on the respiratory epithelium, SARS-CoV-2 begins replicating, migrates down to the airway, and enters alveolar epithelial cells in the lungs, thus resulting in asymptomatic infection progressing to severe respiratory failure (3). Most unvaccinated patients develop asymptomatic, mild, or moderate COVID-19 infection. However, in some patients, epithelial cells in the respiratory tract stimulate immune cells, resulting in a cytokine storm, which is considered the leading cause of the severe clinical manifestation and mortality in patients with COVID-19 (4). To preserve medical capacity and reduce adverse health effects on individuals, preventing the progression of asymptomatic, mild, or moderate COVID-19 infection into severe COVID-19 is essential.

In January 2022, five SARS-CoV-2 variants have been designated as variants of concern (VOC) by the World Health Organization: Alpha, Beta, Gamma, Delta, and Omicron (5). The variants have been spread worldwide, and the dominant VOC have changed over time (5). Various possible mutation pathways of SARS-CoV-2 have been proposed, including ligand-receptor interactions, genomic alterations, lysosomal activity, recombination, conditional mutations, complementation, genetic robustness, and benign relationship (6). Global pandemic outbreaks and genome sequence profiles revealed that SARS-CoV-2 has a high mutation potential and high infectivity, enabling the virus to evolve resistance to newly developed therapeutic strategies rapidly (6). As the pandemic progressed, the mutation of the SARS-CoV-2 genome led to different clinical and socio-economical impacts, which observed dynamic risks of transmissibility, vaccine breakthrough infection, hospitalization, and critical condition (5).

Many COVID-19 vaccines have been proven to prevent hospitalization and mortality in adults (7). To date (January 19, 2022), 60.1% of the world population has received at least one dose of a COVID-19 vaccine. However, only 9.6% of people in low-income countries have received at least one dose (8). The BNT162b2 vaccine in 12-to-15-year-old recipients has been reported favorable safety and highly effective against COVID-19 (9). However, due to the limited information from short-term trials and high post-inoculation severe adverse effects and deaths, there are still concerns that lag the vaccination rate on children/adolescents (10).

In 2020, compared with most industrialized countries, Taiwan was not considerably affected by the COVID-19 pandemic, which can be attributed to rapid national border control, nonpharmaceutical interventions, and cooperation between the government and people (11, 12). However, an outbreak of the Alpha variant of SARS-CoV-2 occurred in mid-May 2021, at which point a relatively low proportion of individuals had received the vaccine (0.55 doses administered per 100 people) (13). Except for standard management, potential complementary therapies for preventing severe COVID-19 should be investigated.

Emerging evidence indicates that the combination of traditional Chinese medicine (TCM) and standard management may play a vital role in treating patients with COVID-19 (14–17). The findings of *in vitro* pharmacological assays demonstrated the efficacy of Taiwan Chingguan Yihau (NRICM101) in inhibiting the spike protein/ACE2 interaction, 3CL protease activity, viral plaque formation, and cytokine production (e.g., production of interleukin [IL]-6 and tumor necrosis factor [TNF]-α) (14). Because of its antiviral and anti-inflammatory effects, NRICM101 may be used to inhibit SARS-CoV-2 invasion and proliferation (14); however, the changes that can be achieved in clinical parameters and outcomes remain unclear. During the pandemic, the Chinese government recommended 6 TCM formulas, referred to as "3-drugs-3-formulas," for treating SARS-CoV-2 infection (18). Five of the formulas were based on the core Maxing Shigan decoction, which possesses anti-inflammatory and antiviral properties (18). Studies have reported that TCM as an adjuvant therapy combined with conventional treatment may be effective and safe for treating mild-to-moderate COVID-19 (15–17). However, because most of the results have been reported as odds ratios, including the improvement rate of symptoms or lung images, quantification of the clinical effects is difficult.

Jing Si Herbal Tea (JSHT), a novel TCM formula, was developed to treat COVID-19. The ingredients of JSHT have been reported to exert anti-SARS-CoV-2, anti-inflammatory, and antithrombotic effects, thus targeting the main pathophysiological pathways in COVID-19 (14, 19–23). We hypothesized that JSHT effectively shortens the viral shedding duration and alleviates respiratory and systemic inflammation. This study analyzed the clinical efficacy and safety of JSHT in patients with mild-to-moderate COVID-19.

MATERIALS AND METHODS

Study Design and Participants

In this prospective cohort study, we enrolled patients with mild-to-moderate COVID-19 upon admission (immediately after the confirmation of COVID-19 infection) from one medical center in New Taipei City between May 1 and August 31, 2021. The study protocol and informed consent form were approved by the Research Ethics Committee of Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation (No. 10-X-045). All participants provided written informed consent.

The inclusion criteria were as follows: (1) laboratory-confirmed positive COVID-19 based on reverse transcription–polymerase chain reaction (RT-PCR) testing, (2) age $\geq$ 18 years, and (3) mild-to-moderate COVID-19 (24). The exclusion criteria were as follows: (1) critical status requiring mechanical ventilation; (2) having severe systemic disease (i.e., malignancy or autoimmune, liver, or renal diseases); (3) pregnancy or lactation in women; (4) participation in other clinical trials within 3 months; (5) history of allergy to the investigational medications; and (6) other conditions judged by the investigators.

The enrolled patients were divided into the JSHT and control groups. Patients in both groups were treated with standard management—supportive treatment, oxygen therapy, symptomatic therapies, and COVID-19-specific medications—in accordance with the Interim Guidelines for Clinical Management of SARS-CoV-2 Infection (11th edition, 2021) (25). Patients in the JSHT group were treated with JSHT as a complementary therapy.

The patients' baseline demographic and clinical characteristics were collected before the research program upon admission (on day 1 [D1]). The following variables were recorded: sex, age, body mass index (BMI), smoking status, hemogram value, serum biochemistry profile, and Charlson comorbidity index. The key clinical parameters: peripheral oxygen saturation (SpO2), fraction of inspired oxygen (FiO2), neutrophil-to-lymphocyte ratio (NLR), RT-PCR cycle threshold (CT) value, C-reactive protein (CRP) level, and Brixia score (26), and serum cytokine levels (IL-6, IL-8, and IL-10) levels were also examined and recorded. In addition, the key clinical parameters, liver and renal function parameters, and cytokine levels were evaluated and recorded after the research program (on day 8 [D8]). The use of concomitant medications (antibiotics, dexamethasone, remdesivir, and tocilizumab), clinical outcomes (including intubation, Medisave Care Unit [MICU] admission, discharge, and mortality), and adverse events during and after the research program were also recorded.

Jing Si Herbal Tea

JSHT is a novel TCM herbal formula developed by the Buddhist Tzu Chi Medical Foundation, Taiwan. The ingredients of JSHT include Houttuyniae Herba (Hanyu Pinyin: Yu Xing Cao; scientific name: *Houttuynia cordata* Thunb.; HC; percentage by weight: 14.18%), Perillae Folium (Zi Su Ye; *Perilla frutescens*; PF; 7.09%), Glycyrrhizae Radix et Rhizoma (Gan Cao; *Glycyrrhiza glabra*; GG; 7.09%), Artemisiae Argyi Folium (Ai Ye; *Artemisia argyi*; AA; 21.28%), *Anisomeles indica* (L.) Kuntze (Yu Zhen Cao; AI; 21.28%), Platycodonis Radix (Jie Geng; *Platycodon grandiflorus*; PG; 14.18%), Ophiopogonis Radix (Mai Men Dong; *Ophiopogon japonicus*; OJ; 14.18%), and Chrysanthemi Flos (Ju Hua; *Chrysanthemum morifolium*; CM; 0.71%). Aqueous extraction of the herbs was filtered and concentrated to obtain a JSHT potion. The JSHT used in the current study was a standardized manufactured product with vacuum packaging that contained a single-dose (225 mL) potion. The JSHT group were administered a single-dose potion 3 times daily for 7 days.

Evaluation of IL-6, IL-8, and IL-10 Concentrations by Using Enzyme-Linked Immunosorbent Assay

We evaluated the concentrations of IL-6, IL-8, and IL-10 in the serum of patients with COVID-19 by using the following enzyme-linked immunosorbent assay (ELISA) kits: IL-6 (88-7066, Thermo Fisher Scientific Inc., Waltham, MA, USA), IL-8 (88-8086, Thermo Fisher Scientific Inc.), and IL-10 (88-7106, Thermo Fisher Scientific Inc.). After performing ELISA in accordance with the manufacturer's instructions, absorbance was read at 450 nm on a Tecan infinite M200 PRO reader (Tecan, Maennedorf, Switzerland).

Statistical Analysis

All statistical analyses were conducted using GraphPad Prism 9 for macOS (Version 9.2.0, GraphPad Software, San Diego, CA, USA, www.graphpad.com). The baseline demographic and clinical characteristics are presented as the patient number (%) and mean ± standard deviation (SD). Outcome measurements are presented as the mean ± SD. For intragroup and intergroup comparisons, categorical variables were examined using Fisher's exact test and the chi-square test, whereas continuous variables were examined using the independent *t*-test, Wilcoxon signed-rank test, and Mann–Whitney U test. Analysis of covariance (ANCOVA) for statistical control was performed using software R 4.1.1 for Windows (27) in comparisons of changes in clinical parameters with baseline intragroup difference. A 2-tailed *P* value of <0.05 was considered statistically significant.

RESULTS

Cohort Characteristics

Figure 1 presents the study flow diagram. A total of 354 patients with mild-to-moderate COVID-19 infection were admitted to Taipei Tzu Chi Hospital between May 1 and August 31, 2021. After applying the exclusion criteria, 94 patients were excluded (68 patients who were intubated within 1 week or admitted to the MICU immediately after hospitalization, 12 patients who had impending respiratory failure and a do-not-resuscitate order, 5 patients who were enrolled in other trials, 3 patients who had drug allergies, 4 patients who were pregnant, 1 patient who had human immunodeficiency virus infection, and 1 patient who had tuberculosis infection). Overall, 260 patients were included in this study and divided into the JSHT group (117 patients) and the control group (143 patients).

Table 1 lists the baseline demographic and clinical characteristics of the patients. No significant differences in sex, age, BMI, smoking status, hemogram value, serum biochemistry profile, or Charlson comorbidity index were observed between the JSHT and control groups. The mean age and BMI of the 117 participants in the JSHT group were 55.42 ± 15.38 (range, 20–95) years and 24.59 ± 15.38 (range, 17.48–32.89) kg/m^2, respectively. The mean age and BMI of the 143 participants in the control group were 53.96 ± 16.97 (range, 19–97) years and 25.58 ± 4.66 (range, 16.11–44.58) kg/m^2, respectively.

Regarding the key clinical parameters, no significant differences in SpO2, FiO2, NLR, or CRP were observed between the JSHT and control groups. However, the mean RT-PCR CT value was significantly lower (P =0.006) in the JSHT group (21.21 ± 6.10; range 9–34) than in the control group (23.38 ± 6.62; range 11–38). The mean Brixia score was significantly higher (P = 0.003) in the JSHT group (2.03 ± 2.22; range 0–10) than in the control group (1.27 ± 1.61; range 0–7). The JSHT group had higher viral load and more severe lung infiltrates at admission than did the control group.

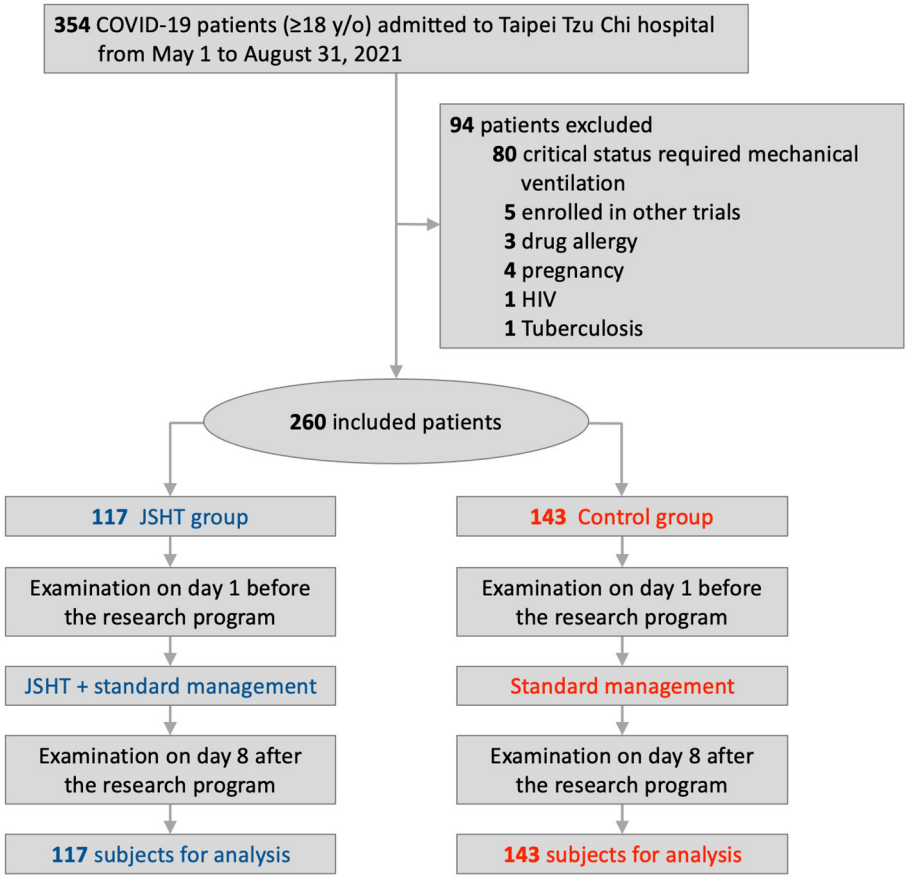

FIGURE 1 | Study flow diagram.

Management During the Study

The patients in both the groups received standard management. The use of concomitant medications (antibiotics, dexamethasone, remdesivir, and tocilizumab) and oxygen therapy with high-flow nasal cannula (HFNC) was recorded. No significant differences in the use of concomitant medications or oxygen therapy with HFNC were noted between the JSHT and control groups (**Supplementary Table S1**).

Clinical Effects of Complementary Therapy With JSHT

The clinical effects of complementary therapy with JSHT on the patients with mild-to-moderate COVID-19 infection are presented in **Figure 2** and **Supplementary Table S2**. The results demonstrated that standard management resulted in significantly improved SpO2, NLR, CT value, CRP level, and Brixia score after the study (on D8 compared with D1) in the control group. JSHT combined with standard management resulted in significantly improved SpO2, CT value, CRP level, and Brixia score after the study (on D8 compared with D1). The increase of CT value after the study was significantly higher ($P = 0.001$) in the JSHT group (8.14 ± 4.90) than in the control group (5.20 ± 6.99; **Figure 2D**). The decrease of CRP after the study was significantly greater ($P = 0.044$) in the JSHT group (-3.48 ± 5.15 mg/dL) than in the control group (-2.17 ± 4.96 mg/dL; **Figure 2E**). The decrease of Brixia score after the study was significantly greater ($P < 0.0001$) in the JSHT group (-0.50 ± 1.99) than in the control group (0.55 ± 2.14; **Figure 2F**).

Concerning the intragroup differences of baseline CT value and Brixia score between the JSHT and control groups, we conducted ANCOVA using the baseline values as covariates to further evaluate the results. The ANCOVA results revealed that after the study, the CT value and Brixia score were more significantly improved in the JSHT group ($P < 0.001$ and $P < 0.0001$, respectively). JSHT combined with standard management more effectively reduced the SARS-CoV-2 viral load and systemic inflammation and alleviated lung infiltrates in the patients with mild-to-moderate COVID-19 infection.

Subgroup Analysis of the Clinical Effects of Complementary Therapy With JSHT

We conducted subgroup analysis to investigate the clinical effects of complementary therapy with JSHT under different risk factors, including age (≥60 or <60 years), (28) BMI (≥30 or <30), (29) and sex (male or female) (30) (**Figure 3**). The results demonstrated that JSHT combined with standard

TABLE 1 | Baseline demographics and clinical characteristics upon admission.

Variable	JSHT group	Control group	*p* value
Number of patients, n	117	143	
Male, *n* (%)	56 (47.9)	70 (49.0)	0.960
Female, *n* (%)	61 (25.1)	73 (51.0)	
Age, year, mean ± SD	55.42 ± 15.38	53.96 ± 16.97	0.472
BMI, kg/m^2, mean ± SD	24.59 ± 3.16	25.58 ± 4.66	0.056
Smoking status, n (%)			0.254
Never-smoker	87 (74.4)	117 (81.8)	
Current smoker	17 (14.5)	12 (8.4)	
Ex-smoker	13 (11.1)	14 (9.8)	
Hemogram value, mean ± SD			
Leukocyte count, 10^9/L	5,257.09 ± 1,758.39	5,496.63 ± 2,230.94	0.345
Neutrophil count, %	68.59 ± 11.28	70.37 ± 11.31	0.208
Lymphocyte count, %	22.86 ± 10.22	20.85 ± 9.62	0.105
Hemoglobin, g/dL	13.49 ± 1.86	13.62 ± 2.13	0.597
Platelet count, 10^3 μL	198.09 ± 64.06	194.66 ± 77.65	0.702
Serum biochemistry profile, mean ± SD			
AST, U/L	33.15 ± 20.82	37.84 ± 28.10	0.165
ALT, U/L	33.78 ± 33.07	32.15 ± 31.08	0.690
Total bilirubin, mg/dL	0.58 ± 0.21	0.62 ± 0.41	0.395
BUN, mg/dL	15.52 ± 22.06	14.90 ± 10.62	0.772
Creatinine, mg/dL	0.83 ± 0.41	1.14 ± 2.08	0.088
D-dimer	755.36 ± 880.67	1,005.28 ± 1,330.51	0.132
Ferritin	570.56 ± 695.06	682.13 ± 1,296.09	0.514
Charlson comorbidity index, mean ± SD	1.75 ± 1.73	1.75 ± 1.93	0.987
Key clinical paremeters, mean ± SD			
SpO_2, %	95.86 ± 1.86	95.96 ± 2.43	0.323
FiO_2, %	23.28 ± 11.03	22.72 ± 9.63	0.659
Neutrophil-to-lymphocyte ratio	4.52 ± 5.73	4.93 ± 4.63	0.152
RT-PCR CT value	21.21 ± 6.10	23.38 ± 6.62	0.006*
CRP, mg/dL	4.33 ± 5.00	3.89 ± 4.33	0.450
Brixia score	2.03 ± 2.22	1.27 ± 1.61	0.003*

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BUN, blood urea nitrogen; CRP, c-reactive protein; FiO_2, fraction of inspired oxygen; JSHT, Jing Si Herbal Tea; RT-PCR CT value, reverse transcription polymerase chain reaction cycle threshold value; SpO_2, peripheral oxygen saturation. *Indicates statistical significance ($p < 0.05$).*

management reduced the SARS-CoV-2 viral load in all subgroups. Furthermore, JSHT combined with standard management significantly and more effectively reduced systemic inflammation in the patients aged ≥60 years ($P = 0.0004$) and male patients ($P = 0.0092$). In addition, JSHT combined with standard management significantly and more effectively alleviated lung infiltrates in the male patients ($P = 0.0154$). In summary, JSHT was observed to be suitable for all the groups of adult patients, especially male and older patients (those aged ≥60 years).

Effects of Complementary Therapy With JSHT on Cytokine Levels

The baseline IL-6 levels were 8.69 ± 17.79 pg/mL and 11.45 ± 21.78 pg/mL in the JSHT and control groups, respectively ($P = 0.549$), whereas the corresponding baseline IL-8 levels were 9.73 ± 9.43 pg/mL and 11.83 ± 10.36 pg/mL ($P = 0.368$). The baseline IL-10 levels were 10.10 ± 9.07 pg/mL and 9.33 ± 7.70 pg/mL in the JSHT and control groups, respectively ($P = 0.842$). No significant differences in IL-6, IL-8, or IL-10 level were noted between the JSHT and control groups. The IL-6, IL-8, and IL-10 levels were significantly lower after the study in both groups. However, the changes in these levels did not significantly differ between the groups (**Supplementary Table S3**).

Effects of Complementary Therapy With JSHT on Clinical Outcomes

Details regarding the effects of complementary therapy with JSHT on clinical outcomes are presented in **Table 2**. The incidence of intubation after the study was 1.7% in the JSHT group and 3.5% in the control group. The relative risk was 0.49 (95% CI: 0.11–2.12, $P = 0.6102$). The incidence of MICU admission after the study was 3.4% in the JSHT group and 4.9% in the control group. The relative risk was 0.70 (95% CI: 0.22–2.18, $P = 0.7805$). The incidence of mortality after the study was 0.0% in the JSHT group and 2.8% in the control group.

TABLE 2 | Clinical outcomes after the study.

Variable	JSHT group	Control group	Relative risk JSHT/Control	*p* value	95% CI
Critical status, *n* (%)					
Intubation	2 (1.7)	5 (3.5)	0.49	0.6102	0.11 to 2.12
MICU admission	4 (3.4)	7 (4.9)	0.70	0.7805	0.22 to 2.18
Clinical outcome, *n* (%)					
Discharge	117 (100)	139 (97.2)	1.03	0.1879	0.99 to 1.04
Mortality	0 (0)	4 (2.8)	0.00	0.1879	0.00 to 1.16

JSHT, Jing Si Herbal Tea; MICU, Medisave Intensive Care Unit.

The relative risk was 0.00 (95% CI: 0.00–1.16, $P = 0.1879$). The results suggested that the patients with mild-to-moderate COVID-19 infection treated with JSHT combined with standard management had 51%, 30%, and 100% lower risks of intubation, MICU admission, and mortality, respectively, compared with the patients treated with standard management only. Although no significant differences were discovered between the groups, JSHT combined with standard treatment tended to prevent critical status and mortality in the patients with mild-to-moderate COVID-19 infection. To validate the results, double-blinded, prospective, randomized controlled trials using a larger population are warranted in the future.

Safety of Complementary Therapy With JSHT

We examined adverse effects, liver function, and renal function to evaluate the safety of complementary therapy with JSHT. Only 4 patients developed diarrhea after receiving JSHT, and their symptoms disappeared spontaneously within 3 days. No other adverse effects were observed. The liver and renal function values (aspartate aminotransferase, alanine aminotransferase, total bilirubin, blood urea nitrogen, and creatinine) were within normal ranges in both the JSHT and control groups before and after the study. The results indicate that complementary therapy with JSHT is safe for patients with mild-to-moderate COVID-19 infection (**Supplementary Table S4**).

DISCUSSION

This is the first study to investigate the efficacy and safety of JSHT combined with standard management in patients with mild-to-moderate COVID-19. The results demonstrated that JSHT combined with standard management more effectively reduced the SARS-CoV-2 viral load and systemic inflammation and alleviated lung infiltrates in adult patients with mild-to-moderate COVID-19, especially in male and older patients (those aged ≥60 years). In addition, the patients treated with JSHT combined with standard management had 51, 30, and 100% lower risks of intubation, MICU admission, and mortality compared with the patients treated with standard management only. This finding indicates the potential of complementary JSHT treatment in preventing critical status and mortality.

The hyperinflammatory response induced by SARS-CoV-2 is a major cause of disease severity and death in COVID-19 (31). Increased IL-6 level is associated with COVID-19 severity and poor prognosis (32). Zhang et al. reported that an IL-6 concentration of >37.65 pg/mL was predictive of in-hospital death (33). Our findings revealed low IL-6 levels in both the JSHT and control groups before and after the study, indicating that the included patients with COVID-19 had relatively low disease severity and low mortality risk. IL-8 is a proinflammatory cytokine that may recruit neutrophils to the infected areas and is associated with tissue damage (34). Li et al. demonstrated that IL-8 is a sensitive biomarker in patients with mild or severe COVID-19, whereas IL-6 is a biomarker of severe COVID-19 (34). Ma et al. reported that patients with COVID-19 with a high IL-8 level (≥10.65 pg/mL) had a significantly longer illness duration than did those with a low IL-8 level (<10.65 pg/mL) (35). We observed low serum IL-8 levels in both the JSHT and control groups after the study. IL-10 has potent anti-inflammatory and immunosuppressive effects (31). An increase in the IL-10 level can be interpreted as an attempt to suppress hyperinflammation (36). However, a marked early increase in the level of the proinflammatory cytokine IL-10 may be associated with COVID-19 severity (37). Han et al. reported that IL-6 and IL-10 are disease severity predictors of COVID-19 (38). Our results revealed low serum IL-10 levels in both the JSHT and control groups after the study. Although CRP levels were more significantly improved in the JSHT group compared with the control group, no significant differences in changes in IL-6, IL-8, or IL-10 levels were observed between the groups. The multiple active ingredients of JSHT may exert an anti-inflammatory effect through multiple signaling pathways and thus contribute to reducing systemic inflammation.

JSHT potentially targeted 3 main pathophysiological pathways: anti-infective, anti-inflammation, and anti-thrombosis. HC, GG and PF exert an anti-infective effect against SARS-CoV-2 (14, 19, 39–41). The main protease (M^{pro}) of SARS-CoV-2 is a crucial enzyme of coronaviruses and plays a pivotal role in mediating viral replication and transcription (42). HC blocks binding between ACE2 and the spike protein of SARS-CoV-2 (14). Regarding the active ingredients of HC, 6-hydroxyondansetron has higher binding affinity toward 2 SARS-CoV-2 receptor proteins, namely M^{pro} and papain-like protease (PL^{pro}). In addition, quercitrin has been identified

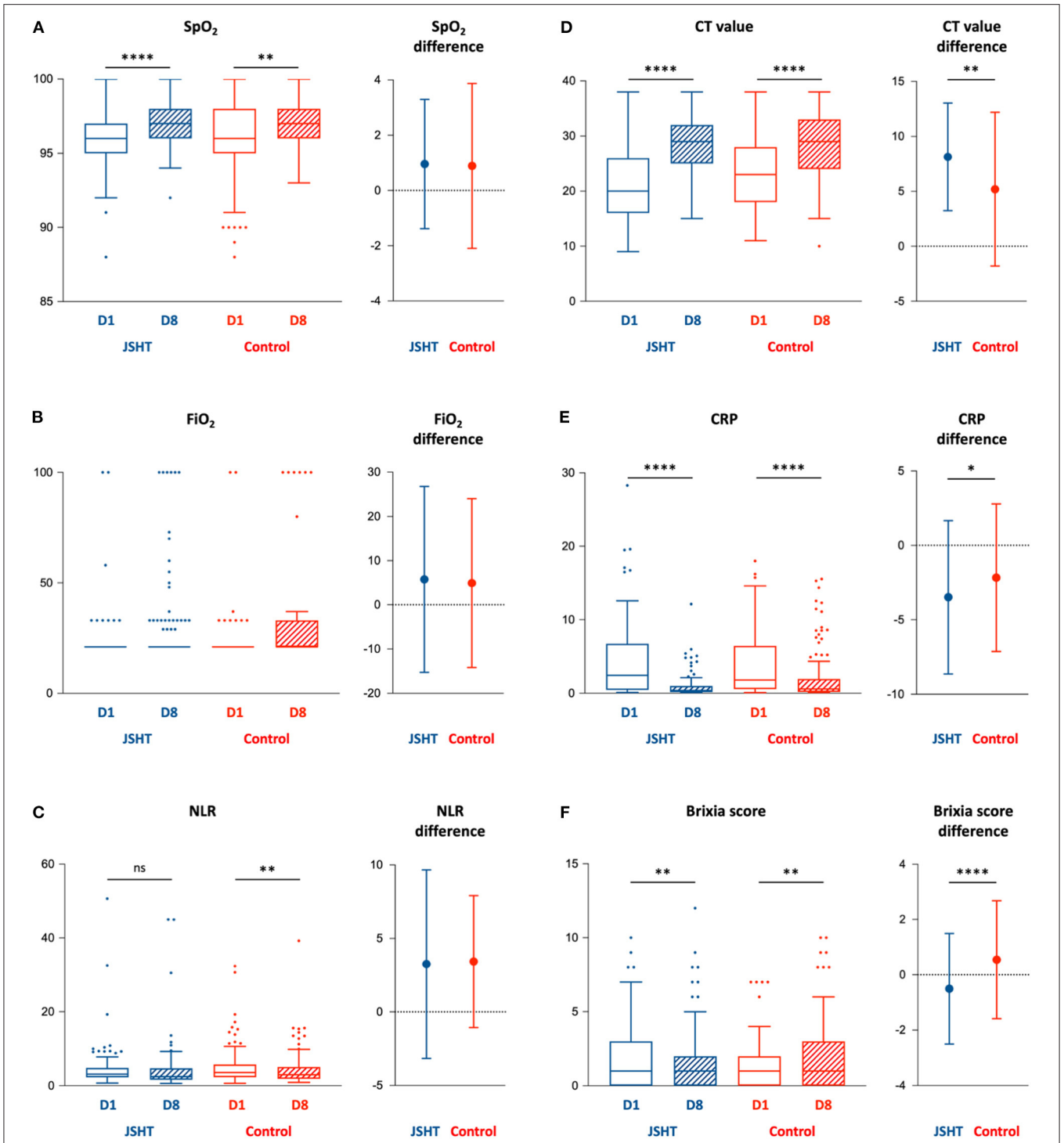

FIGURE 2 | Key parameters before (D1) and after (D8) the study. **(A)** SpO_2; **(B)** FiO_2; **(C)** NLR; **(D)** CT value; **(E)** CRP; **(F)** Brixia score. BMI, body mass index; CRP, c-reactive protein; CT value, Reverse Transcription Polymerase Chain Reaction cycle threshold value; FiO2, fraction of inspired oxygen; JSHT, Jing Si Herbal Tea; NLR, Neutrophil-to-lymphocyte ratio; SpO_2, peripheral oxygen saturation. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$.

as another promising inhibitor because it exhibits the highest binding affinity toward the ADP ribose phosphatase of SARS-CoV-2 (19, 39). GG exerts an anti-infective effect (19). The main active ingredients of GG, glycyrrhizin, targeted the ACE2 receptor with structural affinity and prevented SARS-CoV-2 entry in silicon and docking studies (40, 41). Moreover, PF exerts

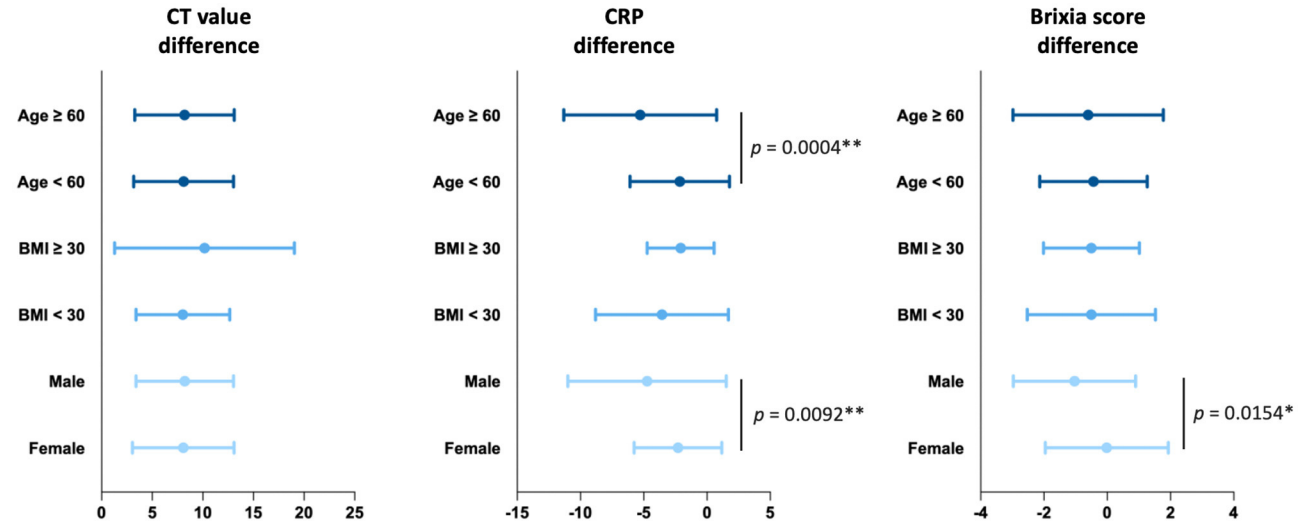

FIGURE 3 | Subgroup analysis of the effects of JSHT on differences in the CT value, CRP level, and Brixia score. CRP, c-reactive protein; CT value, Reverse Transcription Polymerase Chain Reaction cycle threshold value. *$p < 0.05$, **$p < 0.01$.

an anti-infective effect and significantly reduced the pulmonary viral load (*in vivo* anti-SARS-CoV-2 assay) in a female golden Syrian hamster model (19).

HC, AA, AI, and PG exerts anti-inflammatory effects against pulmonary inflammation. The phytoconstituents of HC, including afzelin, hyperoside and, quercitrin, could reduce inflammation (43). HC extracts and the bioactive molecules in HC possess both anti-inflammatory and anti-oxidative properties (43). AA and its active compound, dehydromatricarin A, markedly reduced pulmonary inflammation by suppressing inducible nitric oxide synthase (iNOS) expression and nuclear factor kappa B (NF-κB) phosphorylation, thus reducing the levels of TNF-α and IL-6 (20). AI inhibited the inflammatory mediator nitric oxide and TNF-α and IL-12 production in lipopolysaccharide/IFN-γ-activated macrophages (21). PG was discovered to exert apophlegmatic, antitussive, anti-inflammatory, and antioxidative effects (22).

Thrombotic events that occur in COVID-19 are strongly associated with increased disease severity and poor clinical outcomes (44). Distinctive microvascular abnormalities in COVID-19 include endothelial inflammation, intercellular junction disruption, and microthrombus formation (44). OJ exerted antithrombotic effects by inhibiting venous thrombosis mainly through protecting endothelial cells and reducing leukocyte–endothelial cell adhesion (23).

Based on the *in vivo* and *in vitro* experiments, NRICM101 inhibits SARS-CoV-2 invasion and proliferation through its antiviral and anti-inflammatory properties (14). In clinical practice, NRICM101 was used as an optional alternative therapy in treating COVID-19 in Taiwan and was observed to be effective. However, statistically analyzed real-world data have not been reported. According to previous meta-analysis studies, the combination of traditional Chinese herbal medicine with conventional therapy was effective and safe in the treatment of mild to moderate COVID-19, which improved lung CT parameters, CRP, and clinical symptoms (fever, cough, and fatigue) (15–17). The formulas showed similar therapeutical effects as JSHT (15–17). According to the preliminary data, the worldwide dominant Omicron variant shows increased transmissibility compared to Delta and may evade vaccine-induced immunity (45). As more patients may quarantine at home, JSHT, suitable for mild-to-moderate COVID-19, is also suggested for home care management.

Limitation of the Study

This study has some limitations that should be addressed. First, this is not a double-blinded, randomized controlled trial. Second, the dosage and duration of JSHT treatment were established empirically. Studies with more groups and different dosages and durations are warranted. Third, the molecular mechanisms through which JSHT combats COVID-19 remain unclear. Double-blinded, prospective, randomized controlled trials with a larger population and basic studies investigating the underlying mechanisms should be conducted to comprehensively investigate the effects of JSHT.

CONCLUSIONS

JSHT combined with standard management more effectively reduced the SARS-CoV-2 viral load and systemic inflammation and alleviated lung infiltrates in patients with mild-to-moderate COVID-19, especially in male and older patients (those aged ≥60 years). JSHT combined with standard management may prevent critical status and mortality in patients with mild-to-moderate COVID-19. Concerning the efficacy and safety of JSHT, we suggest JSHT as a promising complementary therapy for patients with mild-to-moderate COVID-19.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee of Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CYS, Y-CC, and Y-KW: study conception and design. C-HL, K-WT, and Y-KW: data collection. P-CH, I-ST, and Y-KW: statistical analysis. P-CH, C-HL, K-WT, and Y-KW: interpretation of results. P-CH and Y-KW: drafting manuscript. CYS and Y-CC: supervision. Y-KW: project administration. All authors reviewed the results and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.832321/full#supplementary-material

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Refeeding Syndrome and Its Related Factors in Critically Ill Coronavirus Disease 2019 Patients: A Prospective Cohort Study

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Background and Aim: Malnutrition and its complications is usually neglected in critically ill COVID-19 patients. We conducted the present study to investigate the prevalence of refeeding syndrome and its related factors in this group of patients.

Methods: In this prospective cohort study, 327 patients were assessed for being at risk and developing refeeding syndrome. The criteria was ASPEN consensus recommendations for refeeding syndrome released in 2020. Malnutrition was assessed based on global leadership initiative on malnutrition (GLIM) criteria. The relation between actual protein, calorie intake, and refeeding syndrome was also evaluated *via* cox regression model. The data concerning calorie and protein intake were gathered for 5 days after initiating feeding. The daily protein and calorie intake were divided by kilogram body weight in order to calculate the actual protein (g/kg/day) and energy (kcal/kg/day) intake.

Results: Among the subjects, 268 (82%) were at risk of refeeding syndrome and 116 (36%) got involved in this syndrome. Malnutrition, according to the GLIM criteria, was found in 193 (59%) of the subjects. In the at-risk population, the risk of refeeding syndrome was reduced by 90% with the rise in protein intake (CI; 0.021–0.436, $P = 0.002$), increased by 1.04 times with the increase in age (CI; 1.032–1.067, $P < 0.001$), and by 1.19 times with the rise in the days from illness onset to admission (CI; 1.081–1.312, $P < 0.001$) in adjusted cox model analysis.

Conclusion: The incidence of refeeding syndrome is relatively high, which threatens the majority of critically ill COVID-19 patients. Increased protein intake was found to reduce the occurrence of refeeding syndrome.

Keywords: malnutrition, GLIM, muscle loss, protein intake, calorie intake

Abbreviations: ASPEN, American Society of parenteral and enteral nutrition; APACHE, Acute Physiology and Chronic Health Evaluation; BMI, Body Mass Index; COVID-19, Coronavirus disease 2019; GLIM, global leadership initiative on malnutrition; ICU, Intensive care unit; to Nitrogen Ratio; RT-PCR, Real-time fluorescence polymerase chain reaction; RS, Refeeding syndrome.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) infection, primarily identified in December 2019, strongly affects the patient's nutritional status. COVID-19-infected patients are considered to be at a high risk of malnutrition (1). This risk particularly increases in severe and critical cases. Several factors could be behind a malnourished state; inflammation and hypercatabolism, anorexia, depression, anxiety, Hypoguesia, Hyposmia, immobilization, digestive discomforts (nausea, diarrhea, and pain), and comorbidity all lead to a malnourished state with loss of lean body mass and negative nitrogen balance (2, 3).

One of the complications associated with the initiation of feeding in malnourished patients is refeeding syndrome. Refeeding syndrome, a life-threatening condition, is a metabolic disorder characterized by electrolyte disturbances and shifting fluid following reintroducing oral, enteral, and parenteral nutrition in malnourished patients (4). It is well known that malnutrition is prevalent in COVID-19 infected patients, especially in severe and critically ill ones (5). Unfortunately, a big proportion of hospital staff are not informed about the importance of malnutrition and the precautions needed to initiate feeding. In COVID-19 cases, in particular, nutrition therapy is neglected on a number of occasions due to the special condition of this disease. Therefore, it is necessary to be careful about the risk of developing refeeding syndrome. Since there is no universally accepted definition for refeeding syndrome, the data in this regard are controversial (4). In a study conducted on critically ill patients, using the hypophosphatemia as the diagnostic criteria for refeeding syndrome, its incidence was reported to be 34% (6). Additionally, in a prospective cohort study on patients hospitalized both in ward and intensive care unit (ICU), this rate was 2% based on the following diagnostic criteria: severely low electrolytes (potassium, magnesium, and phosphorus), fluid overload, and disturbance of organ function (7). Recently, the American Society for Parenteral and Enteral Nutrition (ASPEN) consensus recommendations for refeeding syndrome have been released (4). Herein, we used these recommendations to evaluate the incidence of refeeding syndrome in critically ill COVID-19 patients and investigate its association with calorie and protein intake.

MATERIALS AND METHODS

Study Design and Participants

The current prospective cohort study was conducted from January 2021 to August 2021. The study was approved by the Ilam University of Medical Sciences Ethics Committee (IR.MEDILAM.REC.1399.270) and informed consent was obtained from patients. Critically ill adult patients with the age of 18 years and older, who were sequentially hospitalized in ICU with positive real-time fluorescence polymerase chain reaction (RT-PCR) for COVID-19, were included in the study. The critically ill subjects were defined as those with respiratory failure, shock, or multiorgan dysfunction, who should have been treated in ICU (8). The exclusion criteria were the absence of data concerning weight, height, and nutritional intake, in addition to any causes for electrolyte abnormalities, such as parathyroidectomy or use of phosphate binders. In the patients with a readmission during the study period, the data from the first admission were considered for analysis (**Figure 1**).

Data Collection

During the first 48 h of admission, prior to edema formation, anthropometric measurements were done to assess malnutrition and the risk of refeeding syndrome. The ASPEN consensus recommendation criteria were used to determine whether the patients were at risk of refeeding syndrome (4). The collected data were: (1) low BMI, (2) percentage of weight loss, (3) muscle loss, (4) subcutaneous fat loss, (5) low nutritional intake prior to hospitalization, (6) low serum levels of electrolytes (phosphorus, potassium, and magnesium), and (7) higher-risk comorbidities. ASPEN criteria added the physical exam findings, including loss of subcutaneous fat and muscle mass, to the previous National Institute for Health and Care Excellence (NICE) criteria.

Feeding was started during the first 48 h of admission to the ICU. After feeding, the patients were followed for the occurrence of refeeding syndrome. Refeeding syndrome is defined by ASPEN Consensus Recommendations as: (1) a decrease in any 1, 2, or 3 of serum phosphorus, potassium, and/or magnesium levels and/or organ dysfunction as a result of a decrease in any of these and/or due to thiamine deficiency, and (2) occurring within 5 days of reinitiating or substantially increasing energy provision.

Hypokalemia, hypophosphatemia, and hypomagnesemia were defined as the serum levels less than 3.5 mg/dL (3.5 mmol/L), 2.5 mg/dL (0.8 mmol/L), and 1.46 mg/dL (0.6 mmol/L), respectively, based on hospital reference for adults. Moreover, the patients were observed on a daily basis for any symptoms of hypokalemia, hypomagnesemia, hypophosphatemia, and thiamine deficiency.

Malnutrition was assessed by Global Leadership Initiative on Malnutrition (GLIM) criteria. The data about calorie and protein intake were obtained from the medical records during hospitalization. Furthermore, calories from propofol or any macronutrient infusion were recorded for each patient. As refeeding syndrome occurs within 5 days of reinitiating or substantially increasing energy provision, the mean calorie and protein intake were calculated for 5 days after starting feeding in each patient for statistical analysis. If refeeding syndrome occurred in any patients earlier than 5 days, the average calorie intake was limited to the days before getting the syndrome. To calculate the actual protein intake (g/kg/day), daily protein intake was divided by kilogram body weight. In addition, the daily calorie intake was divided by kilogram body weight to obtain the actual energy (kcal/kg/day) intake.

To calculate non-protein calorie to nitrogen ratio (NPC: N), the mean calorie of protein intake was subtracted from the mean total energy intake, and then divided by the mean nitrogen intake. Nitrogen intake was estimated by dividing the actual protein intake to 6.25.

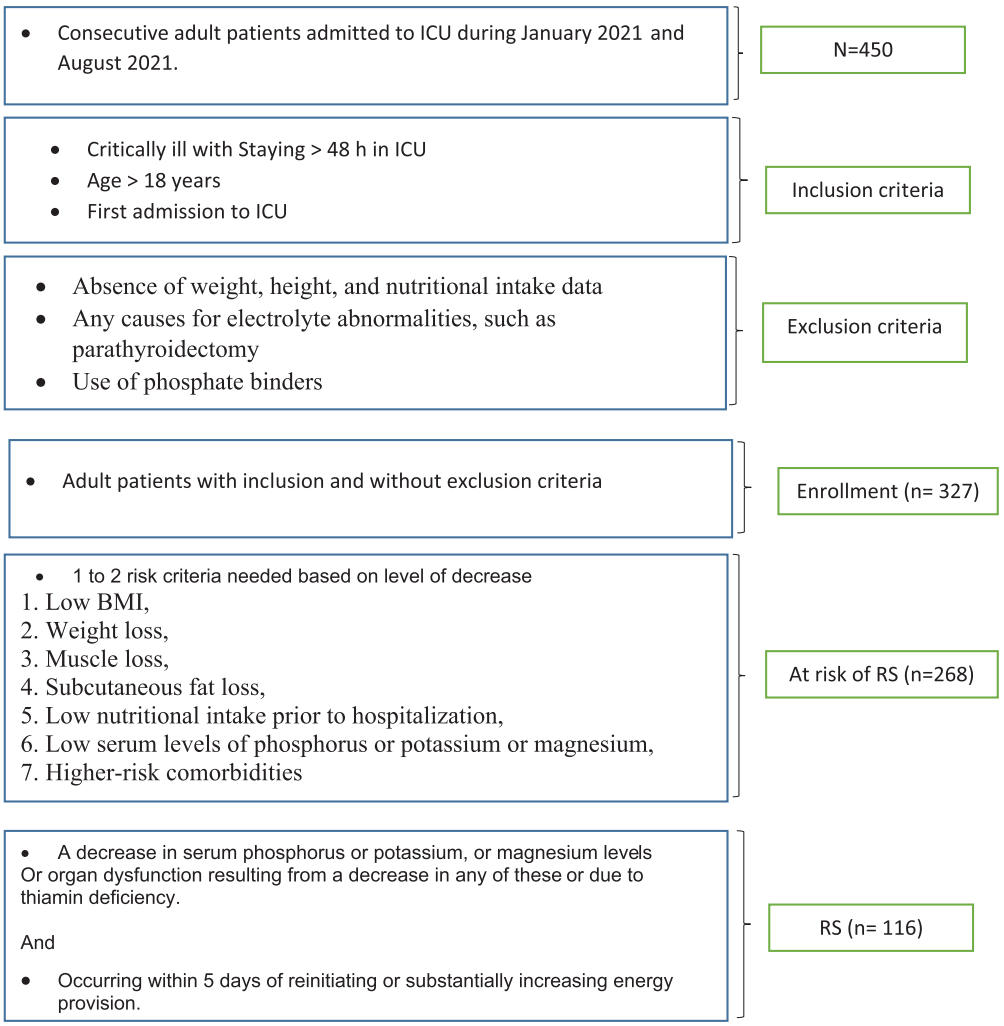

FIGURE 1 | Flowchart of patients.

Outcome Measures

The primary outcome measure was the incidence of refeeding syndrome. The secondary outcome measure was the relation between calorie and protein intake with the occurrence of refeeding syndrome.

Statistical Analysis

The data were analyzed through the use of SPSS software version 22.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, United States). *P*-values equal to or less than 0.05 were considered as the level of significance. Kolmogorov-smirnov (K-S) test was conducted to examine the distribution normality of the variables. All the variables were normally distributed, so the continuous variables were regarded as means ± standard deviation (SD). The categorical data were presented as proportions (number and percentage).

In the patients at risk of refeeding syndrome, the Cox regression analysis was utilized to estimate the hazard ratio (HR) of refeeding syndrome with the actual calorie and protein intake and NPC: N with and without adjustments for confounder variables with $P < 0.05$, including, age, comorbidity, and the number of days from illness onset to admission.

RESULTS

Characteristics of the Population

A total of 327 patients were included in the study. Among these patients, 268 (82%) were at risk of refeeding syndrome. Overall, 116 subjects (36%) developed refeeding syndrome and 158 (47%) died. Moreover, 193 (59%) of the critically ill COVID-19 patients were malnourished based on GLIM criteria.

Characteristics of the Patients at Risk of Refeeding Syndrome

Table 1 shows the demographic and clinical characteristics of the patients who were at risk of refeeding syndrome. Among the criteria in the ASPEN concerning the risk of refeeding syndrome,

the highest prevalence of the syndrome belonged to reduced food intake prior to hospitalization, followed by weight loss, low BMI, fat/muscle loss, and electrolyte disturbances.

193 (73%) at-risk patients were malnourished. 116 (43%) at-risk patients were involved in refeeding syndrome.

No differences were observed between the two groups concerning sex and APACHE II score. The mean age of the subjects in the group with refeeding syndrome was significantly higher than that in the group without refeeding syndrome. Furthermore, the rate of comorbidity and the average days from illness onset to admission were higher in those with refeeding syndrome.

Among the cases at risk of the syndrome, 126 (47%) died in the hospital; this rate was twice as high as that in the patients with refeeding syndrome.

Table 2 shows the feeding characteristics of patients. The mean actual protein intake (g/kg/day) was significantly lower in those involved in refeeding syndrome in comparison with the patients without refeeding syndrome. There were no differences in the mean actual energy intake (Kcal/kg/day) and NPC: N between the two groups.

To determine the relationship between food intake and refeeding syndrome, cox regression analysis was performed. The results revealed that with the increase in the actual protein intake (g/kg/day), hazard ratio of refeeding syndrome decreased by about 96% (**Table 3**). Similar to age, the days from illness onset to admission and comorbidity were significantly associated with refeeding syndrome in crude model (HR = 1.160, 1.062, 2.298, respectively). As shown in **Table 3**, adjusted cox regression analysis was employed for controlling these covariates. The results showed that hazard ratio of refeeding syndrome decreased by 90% with the increase in protein intake. Furthermore, hazard ratio of refeeding syndrome rose by about 1.05 times with the increase in age and by 1.19 times with the increase in the days from onset of illness before admission.

There was no association between the mean actual energy intakes (Kcal/kg/day), NPC: N, and the occurrence of refeeding syndrome in cox regression analysis.

DISCUSSION

In the present study, which was conducted on 327 critically ill COVID-19 patients, 82% were at risk of refeeding syndrome and 36% were involved in refeeding syndrome. Refeeding syndrome was developed only in the patients who were at risk of refeeding syndrome. In other words, 43% of the at-risk subjects were involved in refeeding syndrome. On the other hand, the highest rate of mortality was observed in the patients with refeeding syndrome, in a way that among 158 dead cases, 85 had refeeding syndrome, 41 were at risk of refeeding syndrome, and 32 were in none of these two groups. Another interesting finding was that with the increase in protein intake, the risk of refeeding syndrome was reduced by 90% in the at-risk population. This association was not seen between the syndrome and energy and NPC: N.

TABLE 1 | Demographic and clinical characteristics of at risk patients, stratified by Refeeding syndrome.

Variable	At risk for RS, total (*n* = 268)	Non- RS (*n* = 152)	RS (*n* = 116)	*P*-value
Age, Year	61.04 ± 12.36	56.80 ± 11.51	66.59 ± 11.22	<0.001
Male	139 (52)	84 (55)	55 (47)	0.20
APACHE II	16.05 ± 2.90	15.98 ± 2.69	16.11 ± 3.06	0.71
Weight (Kg)	71,76 ± 12.90	72.35 ± 13.73	71.00 ± 11.72	0.39
Comorbidity	113 (42)	53 (35)	60 (52)	0.006
Malnutrition (GLIM)	193 (72)	96 (63)	97 (84)	<0.001
Reduced food intake prior hospitalization	225 (84)	109 (72)	116 (100)	<0.001
Anorexia/weakness	213 (80)	97 (64)	116 (100)	<0.001
Weight loss	169 (63)	72 (47)	97 (84)	<0.001
Low BMI	35 (13)	8 (5)	27 (23)	<0.001
Muscle mass loss	20 (8)	8 (5)	12 (10)	0.11
Subcutaneous fat loss	68 (25)	28 (18)	40 (34)	0.002
Baseline potassium	4.10 ± 0.59	4.13 ± 0.62	4.08 ± 0.54	0.47
Baseline phosphate	3.05 ± 0.62	3.09 ± 0.65	3.01 ± 0.60	0.29
Baseline magnesium	1.80 ± 0.34	1.83 ± 0.37	1.77 ± 0.31	0.15
Medication				
Antiviral	268 (100)	152 (100)	116 (100)	1
Antibiotic	154 (57)	74 (49)	80 (69)	0.001
Glucocorticoid	268 (100)	152 (100)	116 (100)	1
Days from illness onset to admission	7.89 ± 1.72	7.57 ± 1.54	8.24 ± 1.86	0.002
Admission from				0.005
Emergency	140 (52)	68 (45)	72 (62)	
Ward	128 (48)	84 (55)	44 (38)	
Mortality	126 (47)	41 (27)	85 (73)	<0.001

RS, Refeeding syndrome; APACHE, Acute Physiology and Chronic Health Evaluation; GLIM, Global Leadership Initiative on Malnutrition. Data are reported as X^2 test (n,%) or T-test (mean, SD).

TABLE 2 | Feeding characteristics of at risk patients, stratified by Refeeding syndrome.

Variable	At risk for RS, total (*n* = 268)	Non- RS (*n* = 152)	RS (*n* = 116)	*P*-value
Feeding start time (day)	1.48 ± 0.5	1.41 ± 0.49	1.57 ± 0.49	0.08
Type of feeding				
Enteral, oral	180 (67)	105 (69)	75 (65)	0.44
Parenteral (propofol, macronutrients)	88 (33)	47 (31)	41 (35)	
Mean actual protein intake (g/kg/day)	0.56 ± 0.11	0.59 ± 011	0.52 ± 0.11	<0.001
Mean actual energy intake, (Kcal/kg/day)	13.64 ± 2.87	13.42 ± 3.04	13.93 ± 2.62	0.15
NPC:N	125.62 ± 43.39	122.26 ± 44.92	130.02 ± 41.07	0.14

RS, Refeeding syndrome; NPC: N; Non-protein Calorie to Nitrogen Ratio. Data are reported as X^2 test (n,%) or T-test (mean, SD).

TABLE 3 | Cox regression model for refeeding syndrome.

	HR	CI	*P*-value	Adjusted HR	CI	*P*-value
Mean actual protein intake (g/kg/day)	0.042	0.011–0.155	<0.001	0.095	0.021–0.436	0.002
Days before admission	1.160	1.050–1.281	0.003	1.191	1.081–1.312	<0.001
Age	1.062	1.046–1.080	<0.001	1.049	1.032–1.067	<0.001
Comorbidity	2.298	1.591–3.320	<0.001	1.156	0.788–1.695	0.45

Increased age and days from illness onset to admission had also a minor association with refeeding syndrome.

To the best of our knowledge, there has been no reports of the prevalence of refeeding syndrome in critically ill COVID-19 patients to date. However, ASPEN recommends that in critically ill COVID-19 patients, trophic nutrition be started and slowly increased to a full dose of 15 to 20 kcal/kg in the first week to prevent refeeding syndrome (9). Furthermore, there is no universal definition for refeeding syndrome. The current study applied recent ASPEN consensus recommendation criteria to detect the critically ill COVID-19 patients who were at risk of refeeding syndrome or suffering from it. The reported prevalence of refeeding syndrome in other diseases is variable and underestimated at ICU admission as reported in literature review (10). In a study conducted on 178 patients, who acutely admitted to the department of internal medicine, applying the NICE criteria and taking hypophosphataemia as the main indicator for the presence of this syndrome, 14% developed refeeding syndrome and 54% were considered to be at risk of the syndrome (11). In a retrospective study in a neurocritical care unit, 328 neurocritically ill patients who received total enteral nutrition were assessed for refeeding syndrome. The occurrence of refeeding syndrome was 17.1% based on low phosphate level (12). Valizade et al. reported that the incidence of refeeding syndrome was 21.4% in a general critical care unit with no definition for refeeding syndrome diagnosis (13). In our study, the reason behind the higher incidence of both at-risk and involved cases in refeeding syndrome was the use of a more comprehensive tool for the assessment.

In the present work, 72% of the at-risk patients had malnutrition based on GLIM criteria. In fact, the criteria defined by ASPEN consensus recommendation for the risk of refeeding syndrome are similar to those for assessment of malnutrition by GLIM criteria (14). The only difference is the item of prefeeding serum electrolyte disturbances, which is specific to the at risk for refeeding syndrome criteria. This finding showed the importance of conducting a nutritional assessment in critically ill COVID-19 patients *via* a comprehensive tool, such as GLIM, to find those at risk of refeeding syndrome.

This manuscript also demonstrated the tight relationship between the amount of protein intake and the prevention of refeeding syndrome. ASPEN consensus recommendations for avoidance and treatment of refeeding syndrome in at-risk adults have not recommended an appropriate amount of protein intake at this time (4). Additionally, regarding calorie intake, it has recommended initiating with 10–20 kcal/kg for the first 24 h with gradually increasing it. In our research, the mean calorie intake was 13.58 ± 2.83 in the at-risk patients and there was no significant difference between the two groups. This amount of calorie is in agreement with ASPEN recommendations, which is the reason behind non-association between calorie intake and refeeding syndrome in our study. We also found that older patients and those who spent more days from illness onset to admission were more involved in refeeding syndrome.

The mean NPN: N ratio was 125.6 in the total population at risk of refeeding syndrome. This value was higher in the patients with refeeding syndrome although the difference was not significant. It was demonstrated that in the critically ill patients, an NPC: N ratio of 100:1 or less may be optimal (15). Most enteral formulas have a high NPC: N ratio; therefore, considering protein supplementation beside the formula intake may be of value in these patients.

Our investigation had certain strengths and limitations. The incidence of refeeding syndrome in critically ill COVID-19 patients and its association with calorie and protein intake were studied herein for the first time. Unfortunately, it was a single-center study and due to the lack of previous research on this topic, the sample size was not calculated. Moreover, the data concerning the height and weight of certain patients were self-reported by the patients or caregivers.

CONCLUSION

The current study indicated that most critically ill COVID-19 patients are at risk of refeeding syndrome. The incidence of refeeding syndrome remained relatively high despite the decreased calorie intake. Increased protein intake was found to reduce the incidence of this syndrome.

As most enteral formulas have a high NPC: N ratio, addition of modular protein supplements for critically ill COVID-19 patients should be considered. Evaluation of malnutrition at admission is valuable to detect patients at risk of refeeding syndrome. Furthermore, with the increase in age and the days from onset of illness to admission, the risk of refeeding syndrome augments.

DATA AVAILABILITY STATEMENT

The datasets used and analyzed during this study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by IR.MEDILAM.REC.1399.270. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SS, ZV, MV, and ES performed the material preparation and data collection and analysis. ZV wrote first draft of the manuscript. All authors commented on previous versions of the manuscript, read and approved the final manuscript, and contributed to the study conception and design.

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Network Pharmacology and Comparative Transcriptome Reveals Biotargets and Mechanisms of Curcumol Treating Lung Adenocarcinoma Patients With COVID-19

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The coronavirus disease 2019 (COVID-19) pandemic has led to 4,255,892 deaths worldwide. Although COVID-19 vaccines are available, mutant forms of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have reduced the effectiveness of vaccines. Patients with cancer are more vulnerable to COVID-19 than patients without cancer. Identification of new drugs to treat COVID-19 could reduce mortality rate, and traditional Chinese Medicine(TCM) has shown potential in COVID-19 treatment. In this study, we focused on lung adenocarcinoma (LUAD) patients with COVID-19. We aimed to investigate the use of curcumol, a TCM, to treat LUAD patients with COVID-19, using network pharmacology and systematic bioinformatics analysis. The results showed that LUAD and patients with COVID-19 share a cluster of common deregulated targets. The network pharmacology analysis identified seven core targets (namely, AURKA, CDK1, CCNB1, CCNB2, CCNE1, CCNE2, and TTK) of curcumol in patients with COVID-19 and LUAD. Clinicopathological analysis of these targets demonstrated that the expression of these targets is associated with poor patient survival rates. The bioinformatics analysis further highlighted the involvement of this target cluster in DNA damage response, chromosome stability, and pathogenesis of LUAD. More importantly, these targets influence cell-signaling associated with the Warburg effect, which supports SARS-CoV-2 replication and inflammatory response. Comparative transcriptomic analysis on *in vitro* LUAD cell further validated the effect of curcumol for treating LUAD through the control of cell cycle and DNA damage response. This study supports the earlier findings that curcumol is a potential treatment for patients with LUAD and COVID-19.

Keywords: lung adenocarcinoma, COVID-19, curcumol, network pharmacology, transcriptome

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KEY POINTS

- COVID-19-/LUAD-associated and curcumol targets were identified.
- Prognostic value of curcumol against LUAD and COVID-19 was characterized.
- We identified seven core pharmacological targets of curcumol, namely, AURKA, CDK1, CCNB1, CCNB2, CCNE1, CCNE2, and TTK, in treating LUAD and COVID-19.
- Comparative transcriptomic analysis specified the effects of curcumol for treating LUAD through control of cell cycle, DNA damage response, and cell apoptosis.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a novel disease characterized by high infectivity and rapid spread. Widespread community transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to a global pandemic. As of 4 March 2022, 440,807,756 confirmed cases of COVID-19 and 5,978,096 deaths had been reported worldwide, according to the World Health Organization (WHO) (https://worldhealthorg.shinyapps.io/covid/). A considerable proportion of patients with COVID-19 related critical illness have comorbidities, which are associated with increased mortality.

The prospective cohort studies have demonstrated that patients with COVID-19 with underlying malignancies have a higher mortality rate than those without cancer (1, 2). The recent studies have shown that the levels of angiotensin-I-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) in patients with lung adenocarcinoma (LUAD) are significantly increased, and the increased levels of these enzymes are associated with susceptibility of patients with LUAD to SARS-CoV-2 (3), because ACE2 serves as an important binding site for SARS-CoV-2, leading to facilitate viral entry into target host cells (4). Lung adenocarcinoma is the most common subtype of lung cancer. The development of LUAD is stepwise, beginning with atypical adenomatous hyperplasia (AAH), and progressing to adenocarcinoma *in situ* (AIS) and then to minimally invasive adenocarcinoma (MIA) (5, 6). Most of the patients are diagnosed with advanced disease and have poor prognosis. Gene mutations (such as EGFR, KRAS, and BRAF mutations) and tumor inflammatory microenvironment are strongly associated with LUAD pathogenesis (7, 8).

A meta-analysis of patients with lung cancer and COVID-19, which included 13 studies, showed that the pooled mortality of patients with lung cancer and COVID-19 (up to 25–42%) was significantly higher than the mortality of patients with other cancers (9, 10). This may be due to different pathophysiological factors, such as pulmonary compromise and smoking history, in patients with lung cancer, compared with other cancers (11). Therefore, there is an urgent need to identify drugs to treat patients with lung cancer and COVID-19. In addition to helping these patients, identification of such drugs will also relieve the pressure on respiratory healthcare services.

The current research findings have shown that traditional Chinese medicine (TCM), such as *Lianhua Qingwen Keli*, Honeysuckle Flower Cold-Relieving Granules, and *Xuebijing* injection can be effective in preventing COVID-19 and relieving clinical symptoms of COVID-19. These TCM were officially recommended by the National Medical Products Administration, as adjunctive therapy, in the treatment of COVID-19 (12). TCM can potentially be used for the treatment of comorbidities associated with COVID-19, and has received worldwide attention. Curcumol, a sesquiterpenoid isolated from Curcumae rhizoma, has been shown to have various therapeutic effects, including anticancer, antioxidant, antimicrobial, and anti-inflammatory effects (13). An *in vitro* study by Li et al. showed that curcumol suppresses proliferation of the LUAD cells, A549 and H460, by arresting the cell cycle, altering the expression of apoptosis signaling pathways and inducing tumor cell apoptosis (14). In chronic asthmatic mice, curcumol was found to reduce pulmonary inflammation and airway remodeling by decreasing cytokine levels (15). In addition, curcumol was reported to inhibit LUAD growth and metastasis and overcome tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) resistance in lung cancer (16). The results of the aforementioned studies show that curcumol may be a potential treatment for patients with LUAD and COVID-19.

In the current study, we used network pharmacology, comparative transcriptome, and systematic bioinformatics analysis to investigate the use of curcumol for COVID-19 and LUAD treatment. We aimed to identify possible therapeutic targets and to unfold the molecular mechanisms underlying the therapeutic effects of curcumol in COVID-19 and LUAD, using clinicopathological analysis, gene ontology, KEGG enrichment analysis, ingenuity pathway analysis (IPA) and molecular docking.

MATERIALS AND METHODS

Identification of Common Deregulated Targets Between COVID-19 and LUAD

For the identification of COVID-19-associated targets, the keywords "coronavirus COVID-19," "coronavirus Disease 2019," "severe acute respiratory syndrome coronavirus 2," and "COVID-19" were subjected to different databases, including the Genecards database (17), Online Mendelian Inheritance in Man (OMIM) database (https://omim.org/), Therapeutic Target Database (TTD) (18), Comparative Toxicogenomics Database (CTD) (19), and National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/), genes with the relevance score >1 were obtained from the databases. To identify LUAD-associated targets, the transcriptome data of patients with LUAD were obtained from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/) on 26 July 2021. Using the *limma* package in R on Bioconductor software, genes with FDR < 0.05, and $|\text{logfold change}| > 2$ were considered as differentially expressed genes (DEGs) (20).

Abbreviations: LUAD, lung adenocarcinoma; COVID-19, coronavirus disease 2019; TCGA, The Cancer Genome Atlas; GO, Gene Ontology; BP, Biological process; KEGG, Kyoto Encyclopedia of Genes and Genomes; IPA, Ingenuity Pathway Analysis.

Identification of Pharmacological Targets of Curcumol in LUAD and COVID-19 Treatment

The pharmacological targets of curcumol were determined using various online tools and databases, including Swiss Target Prediction database and Bioinformatics Analysis Tool for Molecular mechANism of TCM (BATMAN-TCM) (21). The target genes were subjected to UniProt for human database correction. The common deregulated genes between COVID-19 and LUAD, that were previously identified, were intersected and compared with the targets of curcumol. Interactions between common targets were analyzed using the STRING database (version 11.0) (22) and Cytoscape software (version 3.6.1) (23). The Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 was used for the gene ontology (GO) function enrichment analysis and KEGG enrichment analysis, to understand the functional roles of targets and signaling pathways controlled by the genes.

Binding of Curcumol to Predicted Targets

Molecular docking analysis was used to investigate the possible binding between curcumol and its predicted targets. The protein structures of the core targets were searched for from the Protein Data Bank (PDB) database (24). Identified protein structures were then docked with curcumol using the AutoDock Vina program and docking analysis was conducted (25).

The Roles of Curcumol Target Genes in LUAD Pathogenesis

To determine the pathological roles of the core targets in LUAD, Cox proportional hazards models were applied in univariate analysis of survival as a function of clinical variables and gene expression.

Cell Culture

The human lung adenocarcinomic cell lines of A549 were incubated with high glucose dulbecco's modified eagle medium (DMEM) medium (ThermoFisher, Cat. No. 11965118), supplemented with 0.5% penicillin–streptomycin (ThermoFisher, Cat. No. 15140122) and 5% fetal bovine serum (ThermoFisher, Cat. No. 10082147) under 5% CO_2 at 37°C.

Cell Proliferation Assays

The cells were seeded in a 96-well plate at a cell density of 2×10^4 cells per well, with eight replicate wells. The cells were treated with different concentrations of curcumol (0.1–100 μM) for 48 h. After the incubation, the cell viability was measured by the CCK-8 assay (Data Inventory Biotechnology) as described previously (26). The colorimetric product formed was measured at an absorbance of 450 nm and 600 nm, $\Delta OD = OD_{450nm} - OD_{600nm}$.

The RNA Sequencing

After the treatment of the cell with 100 μM curcumol for 48 h, the total RNA of the cell was extracted using Trizol reagent (Thermofisher) following the manufacturer's instruction. The RNA quality and quantity were assessed by using Bioanalyzer 2,100 and RNA 6,000 Nano LabChip Kit (Agilent), high-quality RNA samples with RNA integrity number (RIN) number higher than 7.0 were used to construct sequencing library. The average insert size for the final complementary DNA (Cdna) library was about 300 bp. Then 2 × 150 bp paired-end sequencing (PE150) was performed on an Illumina Novaseq™. The high-quality clean reads were mapped to the Human genome reference (Homo sapiens Ensembl v96) using HISAT2 software (version: hisat2-2.0.4) (27). StringTie and ballgown were used to determine the gene expression level (28). The genes with a $1.5 <$ fold change (treatment/control) < 0.75 and –log10 (q-value) > 1.3 were considered as DEGs. The DEGs were subjected to the DAVID v6.8 analysis (29) and IPA (https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis) to delineate the molecular mechanism underlying the effect of curcumol for treating LUAD.

RESULTS

Identification of Pharmacological Targets of Curcumol in COVID-19 and LUAD Treatment

Using the relevant databases, we identified a total of 8,339 targets associated with COVID-19 **(Figure 1A)**. Using the TCGA database, we found 5,538 differential expressed genes associated with LUAD **(Figure 1A)**. When we compared the COVID-19- and LUAD-associated targets, we found 882 shared targets **(Figure 1A)**, among which 216 were downregulated and 666 were upregulated, in patients with LUAD **(Figure 1B)**. To understand the pharmacology of curcumol, a network pharmacology analysis was conducted. We identified 151 curcumol-associated targets using the mentioned databases **(Figure 1A)** and, after comparison of the curcumol-associated targets with the COVID-19/ LUAD-associated targets, we found 28 targets shared by curcumol, COVID-19, and LUAD **(Figure 1A)**. The molecular network analysis using Cytoscape highlighted seven core targets of curcumol, namely, AURKA, CDK1, CCNB1, CCNB2, CCNE1, CCNE2, and TTK in COVID-19 and LUAD (**Figure 1C** and **Table 1**).

Binding of Curcumol to Targets CDK1, TTK, and AURKA

Three protein structures, CDK1, TTK, and AURKA, out of the seven core targets were available on the PDB database (AURKA, ID:2J50; CDK1, ID:5HQ0, and TTK, ID:6N6O). These protein structures were subjected to docking analysis with curcumol using the AutoDock Vina program. The results were displayed using PyMOL (version 2.3), which showed that curcumol formed a hydrogen bond with LYS-162 (3.2 Å) of AURKA (PDB ID: 2J50) **(Figure 2A)**, and the binding affinity of curcumol for AURKA was −6.4 kcal/mol. A similar bindings were observed between curcumol and the amino acid residue LEU-83 (2.2 Å) of CDK1 (PDB ID: 5HQ0) **(Figure 2B)** and between curcumol and the amino acid residue LYS-529 (3.0 Å) of TTK (PDB ID: ID:6N6O) **(Figure 2C)**. The binding affinities of curcumol for CDK1 and TTK were −3.2 kcal/mol and −4.7 kcal/mol, respectively. Our data suggested the direct binding of curcumol to its targets.

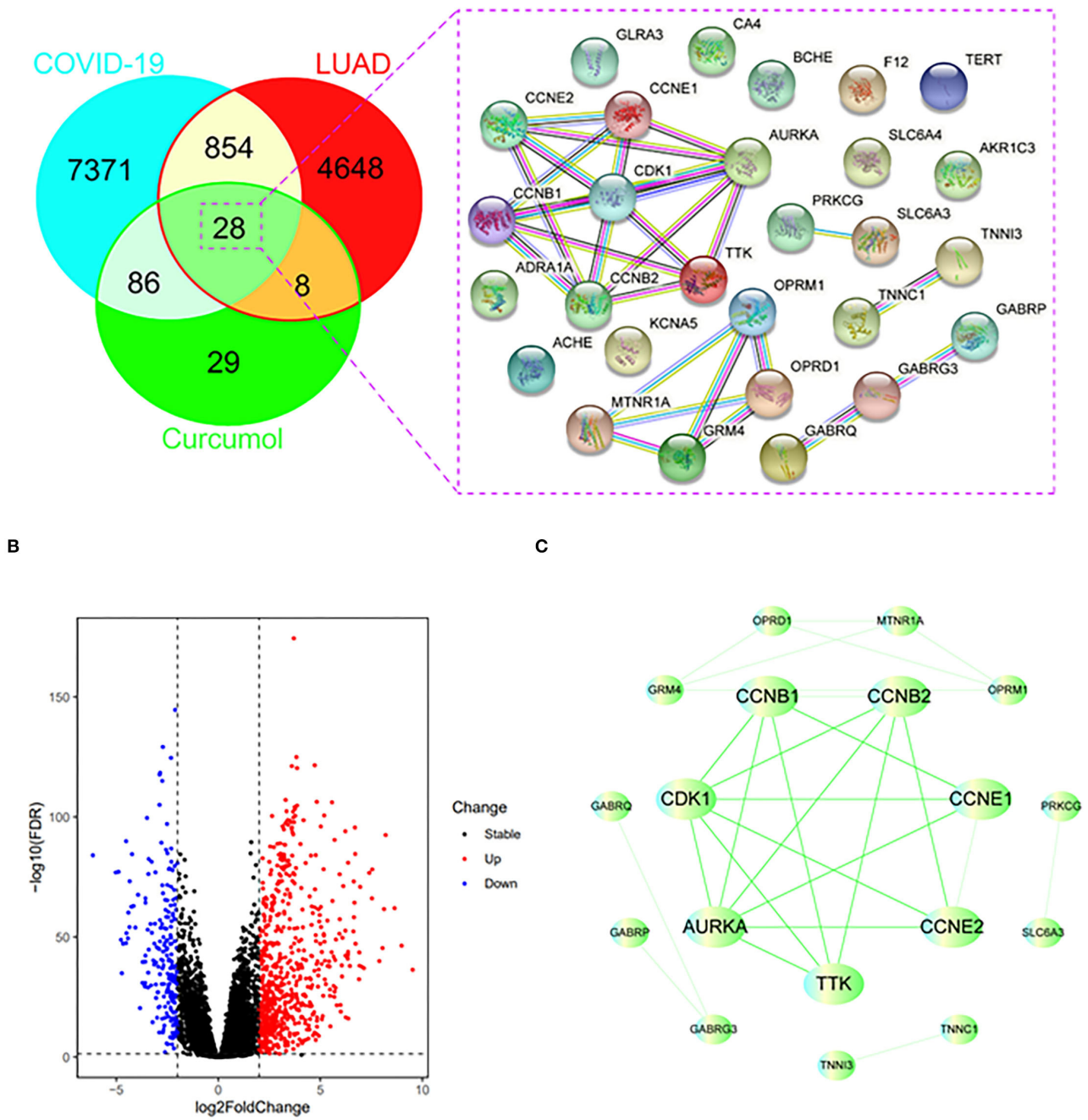

FIGURE 1 | Identification of curcumol-targeted COVID-19- LUAD-associated targets. **(A)** Venn diagram showing the intersecting targets of curcumol /COVID-19/LUAD. **(B)** Volcano plot showing the expression level of differential expressed COVID-19- LUAD-associated genes in patients with LUAD. The genes with $|\log_2$ (fold change)$| > 1$ and $-\log10(FDR) > 1.3$ were considered as differential expressed genes. **(C)** Protein–protein interaction analysis of curcumol/COVID-19/LUAD-intersecting genes using STRING tool.

The Roles of Curcumol Target Genes in LUAD Pathogenesis

The results of the hazards models showed that the expression of curcumol targets in COVID-19 and LUAD was significantly associated with the relative risk of survival [AURKA ($p = 0.001$, hazard ratio, 1.086–1.399); CDK1 ($p < 0.001$, hazard ratio, 1.099–1.401); CCNB1 ($p < 0.001$, hazard ratio, 1.144–1.499); CCNB2 ($p < 0.001$, hazard ratio, 1.089–1.388); CCNE1 ($p = 0.012$, hazard

ratio, 1.032–1.283); CCNE2 ($p = 0.026$, hazard ratio, 1.019–1.341); TTK ($p = 0.004$, hazard ratio, 1.053–1.307)] in patients with LUAD **(Figure 3A)**. The results of survival analysis using the Kaplan–Meier estimator also showed that patients with LUAD with greater expression of the genes, AURKA, CDK1, CCNB1, CCNB2, CCNE2, and TTK, had poorer overall survival rates **(Figure 3B)**. In addition, the correlation analysis highlighted that increased expression of AURKA was associated with advanced stages of LUAD and the increased number of lymph nodes containing tumor **(Figure 3C)**.

Curcumol Target Genes Mediated DNA Damage Response and Cell Cycle Control

To further understand the biological roles of curcumol in the treatment of COVID-19 and LUAD, the identified core targets were subjected to GO enrichment analysis and KEGG pathway enrichment analysis. The results of GO analysis showed that curcumol targets (AURKA, CDK1, and CCNB1) could mediate biological processes related to DNA damage response by controlling DNA integrity and the DNA damage checkpoint (**Figure 4A** and **Supplementary Table 1**), or by regulating cyclin-dependent protein kinase activity, which could lead to cell cycle arrest at different cell cycle checkpoints, such as cell cycle G1/S phase transition and cell cycle G2/M phase transition (**Figure 4B** and **Supplementary Table 1**). These responses are controlled by the seven curcumol targets (**Figure 4B** and **Supplementary Table 1**). The other possible outcome of mediation of biological processes related to the DNA damage response by these targets is the alteration of cellular assembly processes, such as organelle fission, mitotic nuclear envelope disassembly, chromosome segregation, and mitotic spindle organization (**Figure 4C** and **Supplementary Table 1**). In addition, curcumol targets were found to regulate many important processes involved in LUAD carcinogenesis, such as histone phosphorylation, oxidative phosphorylation, and cellular respiration (**Figure 4D** and **Supplementary Table 1**).

The GO cellular components and molecular functions analyses showed that the curcumol targets play roles in many enzymatic complexes related to cell cycle control, such as in cyclin-dependent protein kinase holoenzyme complex and in serine/threonine protein kinase complex (**Figure 5A** and **Supplementary Table 2**). More importantly, the targets are involved in chromosome organization, specifically, in organization of centromeric region of chromosomes, kinetochores, mitotic spindle pores, and telomeric regions (**Figure 5A** and **Supplementary Table 2**). The results of the molecular function analysis further highlighted the effects of curcumol on cyclin-dependent protein serine/threonine kinase regulator activity, histone kinase activity, and cyclin binding (**Figure 5A** and **Supplementary Table 2**). KEGG pathway analysis was used to investigate the pharmacological effect of curcumol on the regulation of cell-signaling pathways. The results showed that curcumol could target many cell-signaling pathways related to cancer development (**Figure 5B** and **Supplementary Table 3**), including the p53 signaling pathway, FoxO signaling pathway, and PI3K–Akt signaling pathway (**Figure 5B** and **Supplementary Table 3**). The curcumol targets were also found to play a role in immune response to viral infections, like T-cell leukemia virus 1 infection, papillomavirus infection, and immunodeficiency virus 1 infection (**Figure 5B** and **Supplementary Table 3**).

TABLE 1 | All seven core genes of curcumol against COVID-19 and LUAD.

Protein Name	Symbol	Uniprot ID
Aurora kinase A	AURKA	O14965
Cyclin-dependent kinase 1	CDK1	P06493
G2/mitotic-specific cyclin-B1	CCNB1	P14635
G2/mitotic-specific cyclin-B2	CCNB2	O95067
G1/S-specific cyclin-E1	CCNE1	P24864
G1/S-specific cyclin-E2	CCNE2	O96020
Dual specificity protein kinase TTK	TTK	P33981

Curcumol Inhibited the Cell Proliferation of LUAD Through the Control of Cell Cycle and DNA Damage

To investigate the effect of curcumol on lung cancer, an *in vitro* LUAD model A549 was used. Our result showed that the treatment of curcumol caused a significant dose-dependent inhibition of cell proliferation in LUAD as compared to the control group **(Figure 6A)**. Then comparative transcriptomic analysis was conducted to delineate the corresponding molecular mechanism. By comparing the gene expression profile of control and curcunol treatment group, we found 348 DEGs, including 206 upregulated genes and 142 downregulated genes (**Figure 6B** and **Supplementary Table 4**). The DEGs were used for the DAVID and IPA analysis to understand the biological alteration and gene network mediated by curcumol treatment in LUAD. The result of GO enrichment showed that the curcumol treatment controlled biological processes related to cell cycle and gene transcription of LUAD (**Figure 6C** and **Supplementary Table 5**). More importantly, curcumol could trigger DNA damage response, leading to cell death and cell apoptosis in LUAD (**Figure 6C** and **Supplementary Table 5**). These results further supported the above findings from network pharmacology. Finally, gene networking of IPA highlighted the involvement of transcription and translation factors, enzymes, kinases, phosphatases, and receptors in curcumol-mediated cell cycle and DNA response (**Figure 6D** and **Table 2**).

DISCUSSION

Using the network pharmacology analysis, we identified seven core targets of curcumol against COVID-19 and LUAD. These seven targets, namely, CCNB1, CCNB2, CCNE1, CCNE2, AURKA, CDK1, and TTK, have been reported to play critical roles in the carcinogenesis and development of LUAD. These targets mediate signaling pathways, such as PI3K/AKT and p53, that are associated with the Warburg effect, which supports SARS-CoV-2 replication and inflammatory response (30, 31). Four cyclin family members (CCNB1, CCNB2, CCNE1, and CCNE2) were identified as core targets in our analysis. Also, cyclin B1 (CCNB1) is associated with poor prognosis in LUAD (32). It is a downstream effector

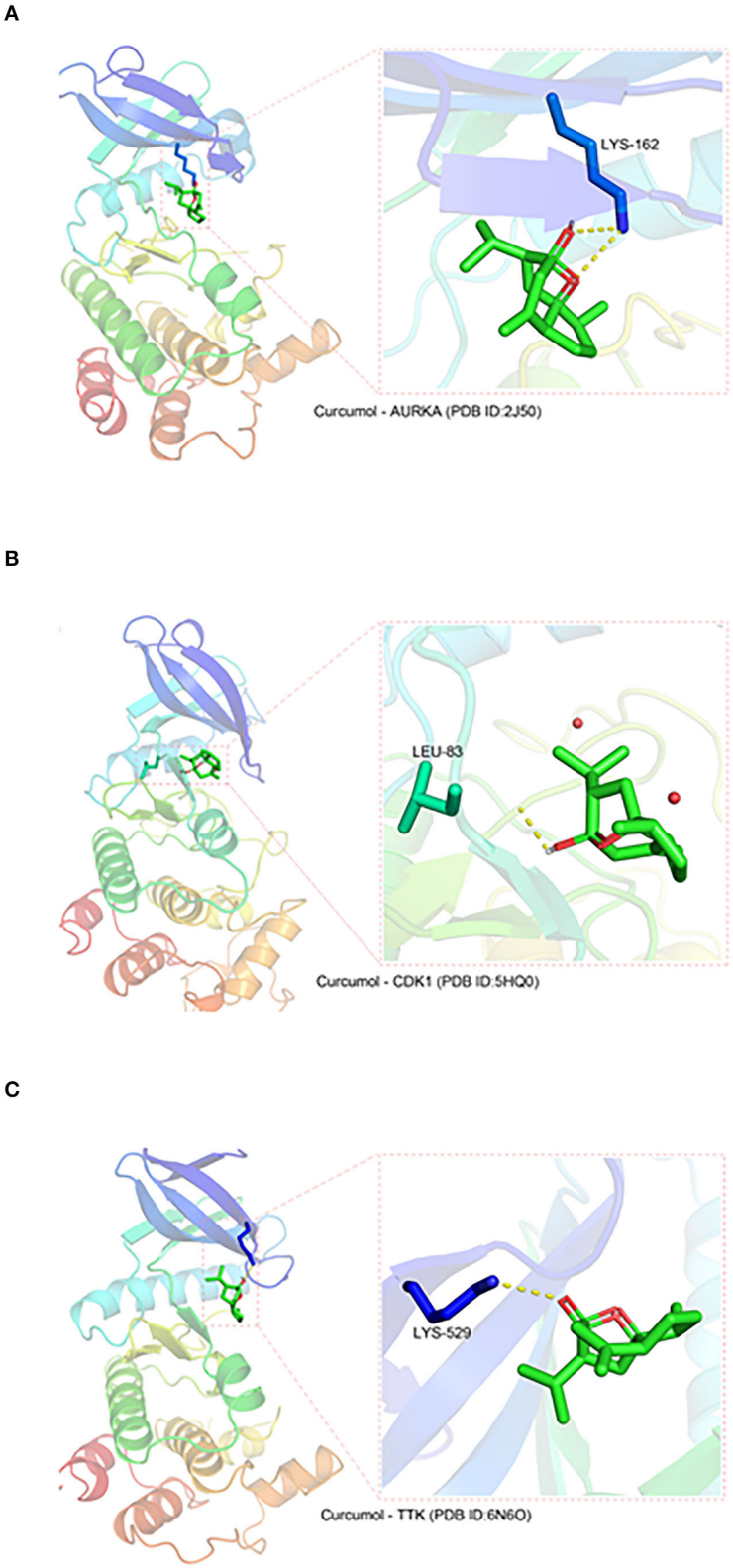

FIGURE 2 | The direct binding of curcumol to CDK1, TTK, and AURKA. The protein structures of CDK1, TTK, and AURKA, obtained from the PDB database. Using the programs Autodock and Visual Molecular Dynamics for visualization, hydrogen bonds can be seen between curcumol and **(A)** the amino acid residue LYS-162 (3.2 Å) of AURKA (PDB ID: 2J50), **(B)** the amino acid residue LEU-83 (2.2 Å) of CDK1 (PDB ID: 5HQ0), and **(C)** the amino acid residue LYS-529 (3.0 Å) of TTK (PDB ID: ID:6N6O).

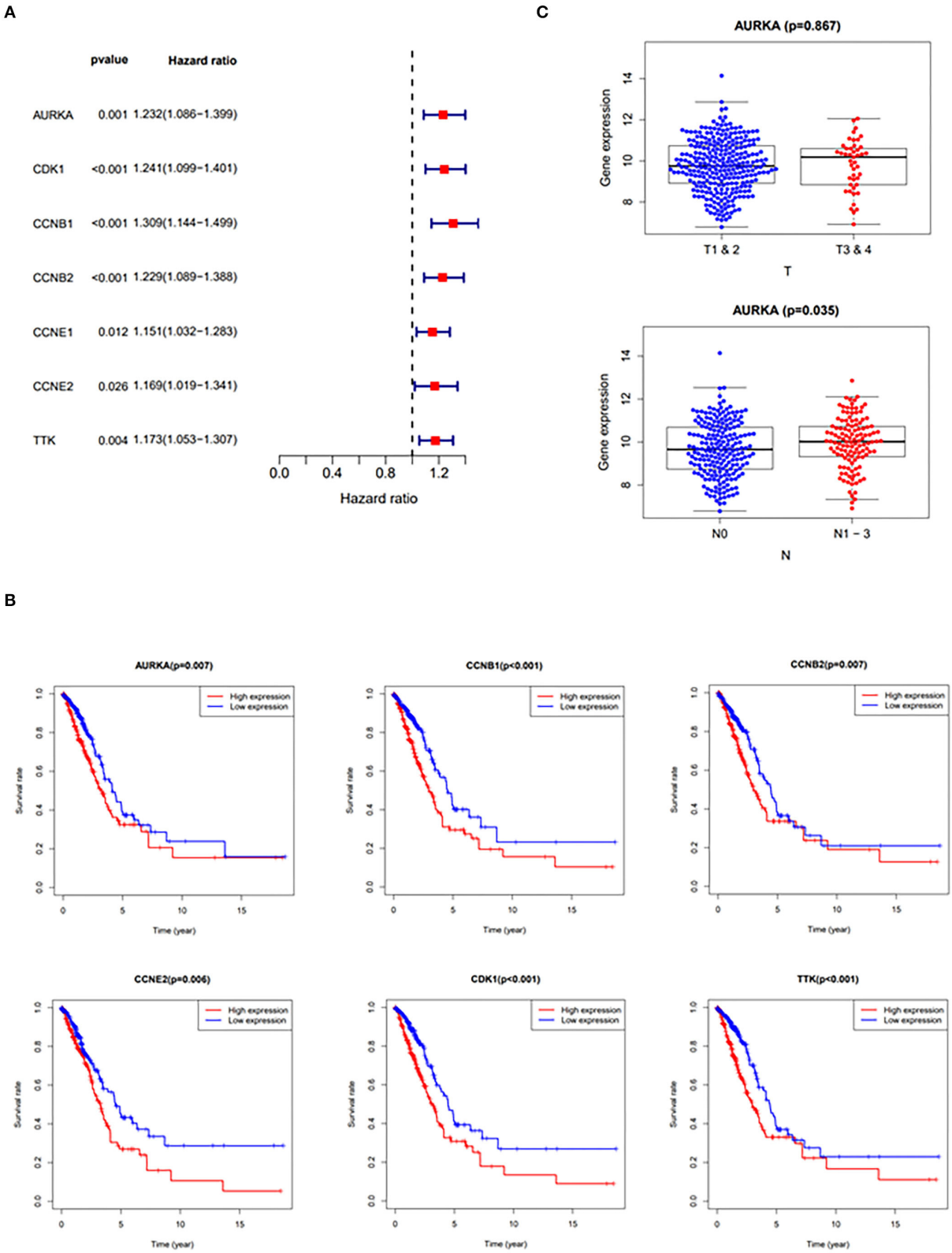

FIGURE 3 | Clinicopathologic analysis of curcumol-targeted COVID-19/LUAD-associated targets. **(A)** The Univariate Cox proportional hazards models showing that the expression of curcumol target genes in COVID-19 and LUAD was significantly associated with relative risk of survival in the patients with LUAD. **(B)** The survival analysis using Kaplan–Meier estimator showing that the patients with LUAD with higher expression of AURKA, CDK1, CCNB1, CCNB2, CCNE2, and TTK had poorer overall survival rates. **(C)** The higher expressions of AURKA in patients with LUAD are associated with advanced stages of LUAD. T, Staging of tumor; N, Number of lymph nodes containing tumor.

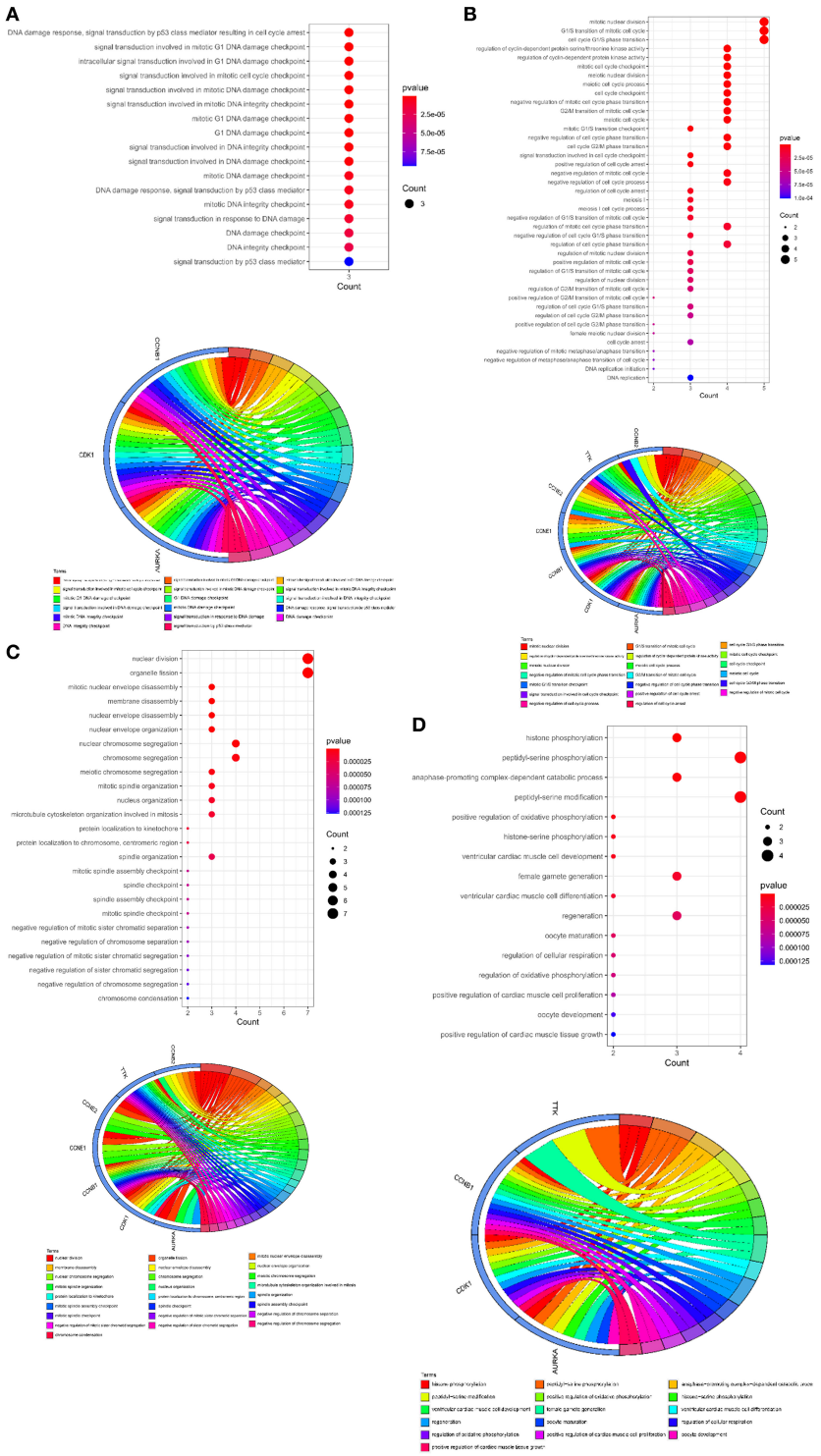

FIGURE 4 | Functional characterization of curcumol-targeted COVID-19/ LUAD-associated targets. **(A)** The GO enrichment analysis highlighted the biological processes related to DNA damage response that are controlled by curcumol-targeted COVID-19/LUAD-associated targets. The circos plot showing the involvement of AUKRA, CDK1, and CCNB1 in the enriched biological processes. **(B)** The GO enrichment analysis highlighted the biological processes related to cell cycle control regulated by curcumol-targeted COVID-19/LUAD-associated targets. Circos plot showing the involvement of AUKRA, CDK1, CCNB1, CCNB2, CCNE1, CCNE2, and TTK in the enriched biological processes. **(C)** The GO enrichment analysis highlighted the biological processes related to cellular assembly processes controlled by curcumol targeted COVID-19/LUAD-associated targets. Circos plot showing the involvement of AUKRA, CDK1, CCNB1, CCNB2, CCNE1, CCNE2, and TTK in the enriched biological processes. **(D)** The GO enrichment analysis highlighted the biological processes related to carcinogenesis of LUAD. Circos plot showing the involvement of AUKRA, CDK1, CCNB1, and TTK in the enriched biological processes. The size of the dot represents the number of targets. The color intensity of the dot represents the significance of the processes.

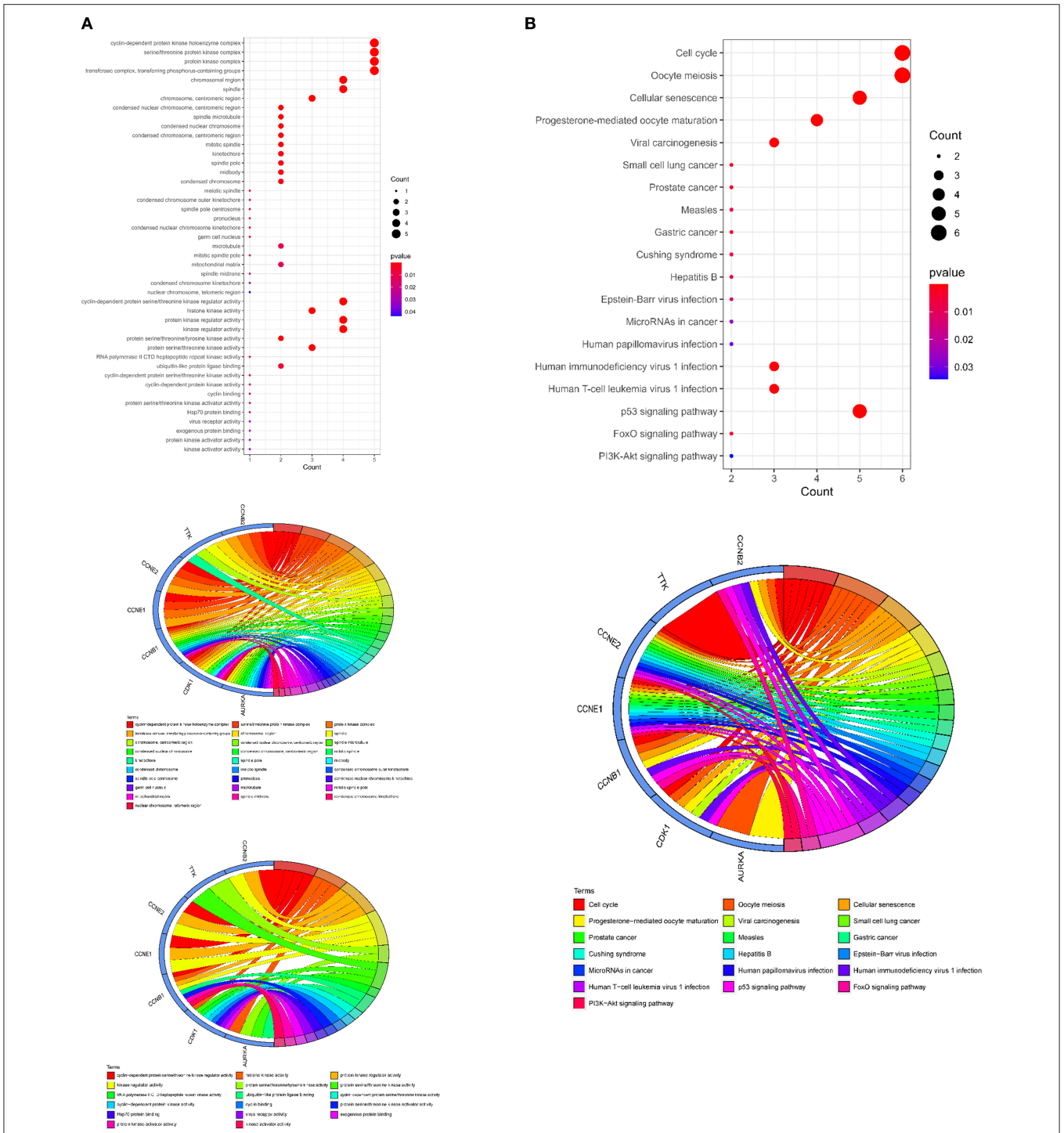

FIGURE 5 | Molecular functions and signaling pathways controlled by curcumol-targeted COVID-19/LUAD-associated targets. **(A)** The GO enrichment analysis highlighted the involved enzymatic complexes, chromosome compartments, and molecular functions in curcumol-targeted COVID-19/LUAD-associated targets. The size of the dot represents the number of targets. The color intensity of the dot represents the significance of the terms. The Circos plot shows the involvement of AUKRA, CDK1, CCNB1, CCNB2, CCNE1, CCNE2, and TTK in the enriched terms. **(B)** The KEGG analysis highlighted the cell-signaling pathways related to cancer development, mediated by curcumol, against COVID-19 and LUAD. The size of dot represents the number of targets. The color intensity of the dot represents the significance of the pathways. The Circos plot shows the involvement of AUKRA, CDK1, CCNB1, CCNB2, CCNE1, CCNE2, and TTK in the enriched signaling.

of monoacylglycerol lipase (MGLL), a key enzyme in lipid metabolism which plays an oncogenic role in LUAD progression and metastasis (33). The cyclin family member CCNB1 also contributes to lung inflammation and oxidative stress (34, 35); CCNB2 is an independent predictor of the prognosis of patients with LUAD (36). The functional characterization

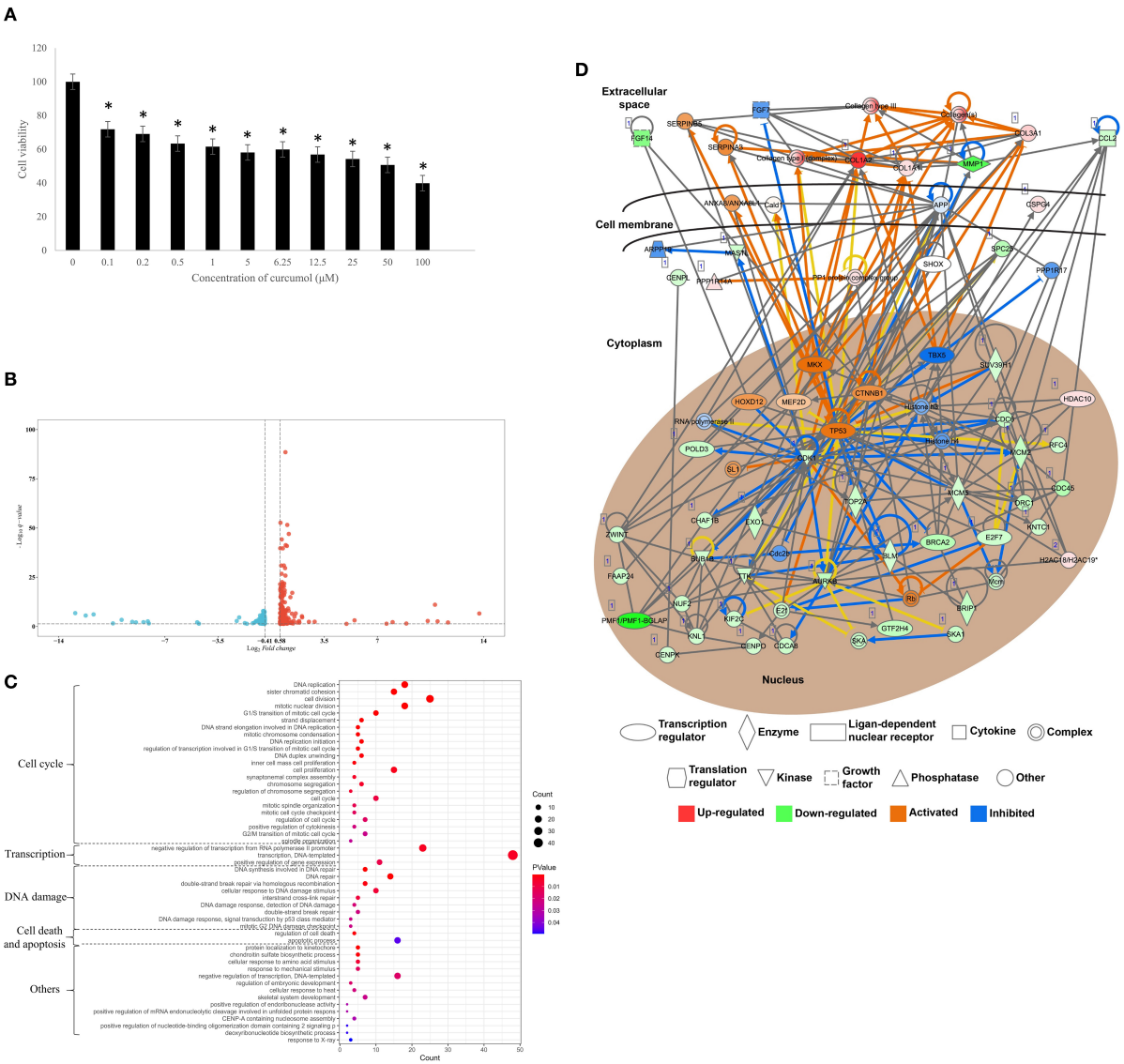

FIGURE 6 | Curcumol-targeted genes involved in cell cycle and DNA damage of LUAD cell model A549. **(A)** The curcumol treatment (100 μM) inhibited cell proliferation of LUAD cell in a dose-dependent manner; $N = 8$ and three experiments. **(B)** The comparative transcriptomic analysis showed the differential gene expression in LUAD cell caused by curcumol treatment (100 μM). Genes with a 1.5 < fold change (treatment/control) < 0.75 and –log10 (q-value) > 1.3 were considered as DEGs. Blue dots represented downregulated genes and red dots represented upregulated genes. **(C)** The GO enrichment analysis highlighted the importance of curcumol-dysregulated genes in cell cycle, DNA damage, and cell apoptosis of LUAD cell. The size of bubble represented the number of DEGs involved in the processes, the color of bubble represented the significance of the processes. **(D)** Gene networking of IPA highlighted the involvement of DEGs in cell cycle and DNA damage response. Red color represented the upregulated genes, green color represented downregulated genes. Orange represented the predicted activated molecules and blue color represented the predicted inhibited molecules in the signaling. * represented $p < 0.05$, as compared to control group.

further highlighted the involvement of CCNB2 in inducing cell cycle arrest and apoptosis in LUAD cells (37). Moreover, the high levels of CCNB2 activate inflammation-induced motility in LUAD (38). Additionally, a study by Ma et al. revealed that knockdown of CCNB2 suppressed proliferation of the LUAD cell line (39). In addition to cyclin B members, our results show that cyclin E members, such as CCNE1 and CCNE2, are also targeted by curcumol; CCNE1 plays a role in progression, cell proliferation, and cell cycle arrest of lung cancer cells (40–42).

Using molecular docking, we found that curcumol binds directly to a group of kinases, including CDK1, TTK, and Aurora kinase A (AURKA). AURKA, a cell cycle kinase, is associated with many cancer types (43). An *in vitro* study of human LUAD cell lines demonstrated that AURKA plays an important role in the proliferation of LUAD cells, through the regulation of multiple

TABLE 2 | Highlighted IPA canonical pathways.

Ingenuity canonical pathways	–log (*p*-value)	Gene symbol
Kinetochore metaphase signaling pathway	1.61E+01	AURKB,BUB1B,CDCA8,CDK1,CENPK,CENPL,CENPO,H2AC18/H2AC19,KIF2C,KNL1, KNTC1,MASTL,NUF2,PMF1/PMF1-BGLAP,PPP1R14A,SKA1,SPC25, STAG3,TTK,ZWINT
Cell cycle control of chromosomal replication	4.89E+00	CDC45,CDC6,CDK1,MCM2,MCM5,ORC1,TOP2A
Role of BRCA1 in DNA damage response	3.04E+00	BLM,BRCA2,BRIP1,E2F7,FAAP24,RFC4
Tumor microenvironment pathway	3.00E+00	CCL2,COL1A1,COL1A2,COL3A1,CSPG4,FGF14,MMP1, MMP17,MMP28
NER (Nucleotide Excision Repair, Enhanced Pathway)	1.82E+00	CHAF1B,GTF2H4,POLD3,RFC4,TOP2A
Mismatch repair in eukaryotes	1.68E+00	EXO1,RFC4
Cyclins and cell cycle regulation	1.51E+00	CDK1,E2F7,HDAC10,SUV39H1
Role of CHK proteins in cell cycle checkpoint control	1.34E+00	CDK1,E2F7,RFC4

downstream effectors, such as RAF-1, CCND2, CCND3, CDK4, PAK4, and EGFR (44). In addition to its function as a cell cycle kinase, AURKA is considered as an inhibitor which prevents the chromatin assembly of functional replisomes, leading to sensitization of cancer cells to combination therapy (45). Further functional characterization studies of AURKA report that AURKA suppression enhances the radiosensitivity of lung cancer and its response to EGFR inhibitors (46, 47). In addition, the downregulating AURKA inhibits docetaxel chemoresistance in LUAD (48), suggesting that AURKA is a promising target for LUAD therapy. Many studies have also demonstrated the autoimmune and inflammatory roles of AURKA *via* regulation of M1 macrophage polarization (49, 50).

Our results showed that the curcumol targets the other cell cycle gatekeeper kinases, such as cyclin-dependent kinase 1 (CDK1); CDK1 is a potential prognostic biomarker of/and target for lung cancer (51); CDK1 activity is critical for JAK/STAT3 signaling activation, and the inhibition of CDK1 can suppress lung cancer (52). In addition, an *in vitro* study of LUAD cells showed that reduced CDK1 activity led to cell cycle arrest and promotion of apoptosis in LUAD (53, 54). CDK1 controls many effectors involved in cell cycle regulation, such as FOXM1, TRAP1, and GCN1 (55–57). Furthermore, CDK1 plays a role in the DNA damage response. For example, CDK1 ensures optimal Fun30 phosphorylation and checkpoint activation at DNA double-strand breaks and plays an important role in the DNA damage response by preventing the formation of lagging chromosomes (58, 59). Also, CDK1 ensures accurate chromosomal segregation *via* the activity of acetyltransferase TIP60 and chromatin remodeller RSF1 (60, 61). Cumulative studies have reported that DNA damage is associated with higher mortality in patients with COVID-19 (62). Kinase threonine tyrosine kinase (TTK) is a critical component of the spindle assembly checkpoint (63). Also, TTK is a biomarker for prognosis of Non-small cell lung cancer (64), and the upregulation of TTK increases the cancer progression in lung cancer (65). In addition, the TTK antagonism has marked antineoplastic effects against LUAD (66). The targeting of CDK1 and TTK by curcomol provides an opportunity to treat patients with LUAD and COVID-19. The results were further validated by using comparative transcriptomic analysis on LUAD cell. The treatment of curcumol could inhibited cell proliferation of LUAD through its control on cell cycle and DNA damage response.

CONCLUSIONS

In conclusion, we identified the pharmacological targets and the therapeutic mechanisms of curcumol in the treatment of COVID-19 and LUAD, including immune response, DNA damage response, and cell cycle arrest, and regulation of cell-signaling pathways such as the p53 signaling pathway, FoxO signaling pathway, and PI3K-Akt signaling pathway. The results were further supported by the comparative transcriptomic analysis on *in vitro* LUAD cell, suggesting that curcumol has potential for treating patients with LUAD and COVID-19. However, further Pre-clinical study is needed to warrant the findings of the present study before the clinical use.

DATA AVAILABILITY STATEMENT

Sequencing data of transcriptome sequencing that support the findings of this study have been deposited in the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject) with the BioProject accession code PRJNA793079.

AUTHOR CONTRIBUTIONS

RL, KL, and XC contributed to the conception, design of the manuscript, drafted this manuscript, and revised this manuscript. LY, HX, XLi, YL, HZ, XLin, and TC contributed to the acquisition, analysis, and interpretation of data in this manuscript. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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BRIEF RESEARCH REPORT
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COVID-19 Illness Severity in the Elderly in Relation to Vegetarian and Non-vegetarian Diets: A Single-Center Experience

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The first wave of the coronavirus disease 2019 (COVID-19) outbreak in Taiwan occurred in May 2021. The risk for and severity of this disease vary and are highly dependent on personal habits and comorbidities. Moreover, the gut microbiome, which may be affected by diet, is highly susceptible with regard to the risk and severity of infectious diseases such as COVID-19. The relationship between dietary habits, nutritional status, and the effects of these factors on the immune system in the context of a global pandemic is an extremely important topic of immediate concern. Hence, the aim of this study was to explore the effect of vegetarian and non-vegetarian diets on COVID-19 severity during the pandemic. We conducted a retrospective evaluation of 509 patients who had been diagnosed with COVID-19 at a single medical center between May 2021 and August 2021. Patients were divided into three groups according to disease severity. For patients aged ≥65 years, COVID-19 symptom severity was statistically significantly and inversely associated with the adherence to a vegetarian diet ($p = 0.013$). Moreover, subgroup analysis results showed that older COVID-19 patients and those with a non-vegetarian diet had a higher risk of contracting critically severe COVID-19 [adjusted odds ratio (OR) $= 5.434$, $p = 0.005$]. Further research is needed to determine the effects of dietary habits on COVID-19 risk and severity during the global pandemic.

Keywords: COVID-19, diet, Taiwan, illness severity, nutritional status

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INTRODUCTION

In Taiwan, the first confirmed case of coronavirus disease 2019 (COVID-19) occurred on January 21 2020, and the first wave of the COVID-19 pandemic outbreak occurred in May 2021. Community COVID-19 transmission was confirmed as many sources of infection could not be traced. Many locally confirmed cases were identified as uncomplicated or mild COVID-19 according to SARS-CoV-2 RT-PCR (i.e., severe acute respiratory syndrome coronavirus 2 reverse transcription polymerase chain reaction assay) results and clinical symptomology. These confirmed cases were admitted to centralized quarantine centers in accordance with governmental mandates.

Hotels in Northern Taipei were remodeled during the pandemic in order to serve as quarantine centers, and were supported by medical centers. More specifically, these makeshift quarantine centers provided medical care for confirmed cases of asymptomatic to mild COVID-19. If the disease progression of the confirmed cases worsened, these patients were transferred to their respective medical centers for appropriate treatment. Accordingly, there were different levels of COVID-19 severity in this study population (i.e., patients recruited from quarantine centers that were supported by a single medical center), which is hence suitable for research on disease risk factors in the hospital setting.

Dietary influence on the gut microbiome is an extremely important topic overall, as well as in light of susceptibility with regard to the varying risk and severity of infectious diseases, according to nutritional patterns, such as the possibility of healthy plant-based food intake modulating COVID-19 risk (1).

Merino et al. administered web-based surveys targeting healthcare workers with substantial exposure to COVID-19 patients, and analyzed the influence of self-reported dietary patterns on COVID-19 outcomes and severity (2, 3). These researchers found that plant-based diets were associated with a low risk of moderate to severe COVID-19.

The relationship between dietary habits, nutritional status, and the effects of these factors on the immune system in the context of a pandemic is an extremely important topic of immediate concern (3–6). It seems that a healthy lifestyle may reduce the severity of COVID-19 symptomology, and these preliminary findings prompt further investigation (7). Evidence with regard to specific dietary patterns that may support the optimal alleviation of COVID-19 risk and symptomology have not been widely researched. In this study, we aimed to evaluate the association between self-reported dietary patterns and the severity of COVID-19 in a hospital setting.

MATERIALS AND METHODS

Subject Selection

To evaluate the relationship between vegetarian diets and the severity of COVID-19 symptoms, we retrospectively collected data from medical records of patients housed at quarantine centers. Dietary data were queried in questionnaires asking about dietary patterns 1 year before the date of subjects' COVID-19 diagnosis. Data were collected from the corresponding support hospital in Northern Taiwan.

In this cross-sectional study, 509 patients between 20 and 95 years of age, who had been diagnosed with COVID-19 (Roche Cobas® SARS-CoV-2 PCR Reagent; Roche, Basel, Switzerland), were recruited between May 2021 and August 2021. During the pandemic, all 509 COVID-19 patients had not received COVID-19 vaccinations.

Patients were divided into three groups according to disease severity, as shown in **Table 1**. Patients with incomplete data on the demographic questionnaire and symptom surveys were excluded from the current study.

This study was approved by the Institutional Review Board (IRB) of the Buddhist Taipei Tzu Chi General Hospital (10-X-141) and was conducted in accordance with the principles of the Declaration of Helsinki and its later amendments. The study protocol was reviewed, and an informed consent waiver was received from the IRB. Patient privacy rights, including with regard to any individual person's data in any form (including medical information, images, and videos), were strictly observed.

Diagnosis and Management

COVID-19 disease severity was defined as either uncomplicated, mild, moderate, severe, or critical illness according to National Institute of Health (NIH) guidelines (8). Uncomplicated COVID-19 involves symptoms such as an upper respiratory

TABLE 1 | Various coronavirus disease 2019 (COVID-19) classification levels.

Category	Laboratory tests	Medical care	Symptoms
Uncomplicated to mild COVID-19	Positive SARS-CoV-2 PCR result or a positive antigen test result	Receiving outpatient or inpatient care, but not HFNC oxygen or mechanical ventilation	Patients with mild symptoms and patients with an uncomplicated upper respiratory tract viral infection may present with fever, cough, sore throat, nasal congestion, malaise, headache, or muscle pain
Moderate to severe COVID-19	Laboratory-confirmed SARS-CoV-2 infection	Standard of care: need for urgent hospital treatment	Patients with severe pneumonia and clinical signs of pneumonia (fever, cough, dyspnea, fast breathing) and one or more of the following: • Respiratory rate > 30 breaths/min • Severe respiratory distress • SpO_2 < 94% on room air • PaO2/FiO2 < 300 • Infiltration > 50%
Critical COVID-19	Presumed or confirmed SARS-CoV-2 infection	Standard of care: ICU admission for respiratory or cardiovascular organ support; need for intubation, rescue strategies, or oxygenation (i.e., achieving a change in the PaO_2/FiO_2 ratio)	Presence of acute respiratory distress syndrome, respiratory failure requiring ventilation, sepsis, or septic shock

COVID-19, coronavirus disease 2019; HFNC, high-flow nasal cannula; ICU, intensive care unit; PaO_2/FiO_2, ratio of the partial pressure of oxygen in the arterial blood to the fraction of inspired oxygen; SARS-CoV-2 PCR, severe acute respiratory syndrome coronavirus 2 reverse transcription polymerase chain reaction.

tract viral infection and the presence or absence of fever, cough, sore throat, nasal congestion, malaise, headache, and/or muscle pain with no other complications. Mild COVID-19 is defined according to symptoms such as cough with expectoration, fever, general body pain, and weakness. Moderate COVID-19 is associated with a combination of symptoms that are more severe but are not life threatening. Severe COVID-19 describes patients with clinical signs of pneumonia (fever, cough, dyspnea, fast breathing) and one or more of the following symptoms: a respiratory rate of >30 breaths/min, severe respiratory distress, ratio of the partial pressure of oxygen in the arterial blood to the fraction of inspired oxygen (PaO_2/FiO_2) of <300, an oxygen saturation (SpO_2) of <94% on room air, or infiltration of >50%.

In this study and according to the NIH guidelines, patients with critical COVID-19 were confirmed to have a SARS-CoV-2 infection and were admitted to the intensive care unit (ICU) for respiratory and/or cardiovascular organ support, including intubation, rescue strategies, and/or oxygenation (i.e., eliciting changes in the PaO_2/FiO_2 ratio). The clinical symptoms characterizing critically severe COVID-19 include the presence of acute respiratory distress syndrome, respiratory failure requiring ventilation, sepsis, and/or septic shock.

The study questionnaire was given to subjects while they were inpatients. The questionnaire included four parts: patient characteristics, type of diet, comorbidities and complications, and pneumonia severity. With regard to patient characteristics, we collected information on sex, age, weight, treatment locations, and diet type (i.e., vegetarian or non-vegetarian). Moreover, we collected data on meat, vegetarian, and other specific diet types retrospectively (i.e., 1 year before the date of COVID-19 diagnosis).

Comorbidities and complications were measured using the Charlson Comorbidity Index (CCI) (9). This index assigns a score for specific diseases, with a higher score indicating a more severe condition and, consequently, a worse prognosis. Underlying comorbidities, such as disabling neurologic conditions, chronic obstructive or restrictive lung disease, coronary atherosclerotic disease, congestive heart failure, liver cirrhosis, end-stage renal disease, diabetes, metastatic cancer, and acquired immunodeficiency syndrome, were recorded.

The severity of pneumonia was measured using the CURB-65 scale (confusion, uremia, respiratory rate, BP, age ≥ 65 years), which is based on confusion (i.e., being newly disoriented with regard to questions relevant to person, place, or time), blood urea nitrogen (BUN) levels of >20 mg/dl, a respiratory rate of ≥30 breaths/min, blood pressure parameters (systolic blood pressure <90 mmHg or diastolic blood pressure ≤60 mmHg), and an age ≥65 years (10). Patients received a score of 1 if any of the symptoms described within the above five items were noted; the highest obtainable score was 5. A score of 0 or 1 indicated that the patient should be treated as an outpatient, a score of 2 indicated a short stay in the hospital or careful observation as an outpatient, and a score of 3–5 indicated that the patient should be hospitalized and that ICU admission should be considered.

Statistical Analysis

Descriptive statistics were used to present the demographic characteristics of the enrolled study participants. Categorical variables are expressed as numbers and percentages, and continuous variables are expressed as means ± standard deviations.

Categorical variables were examined using the chi-square test and Fisher's exact test, and continuous variables were examined using one-way analysis of variance (ANOVA).

Logistic regression was performed to investigate the factors associated with COVID-19 severity after adjusting for other potentially confounding factors. The data were analyzed using SPSS statistical software (version 24.0, IBM Corp, Armonk, NY, USA). A p-value of <0.05 was considered the threshold for statistical significance.

RESULTS

A total of 906 COVID-19 infected patients comprised the eligible study population (these patients were infected during the year 2021). The study population was enrolled retrospectively during the pandemic to investigate the association between adherence to a vegetarian diet and COVID-19 severity. The hotel of the quarantine center was closed in August 2021. Thus, no more uncomplicated or mild COVID-19 patients were enrolled after August 2021. No patient became critical after the COVID-19 vaccination policy was implemented in our country, so we stopped enrolling patients in August 2021. Therefore, 319 patients who were not admitted to the hospital during the study period (May 2021 to August 2021) were excluded from the current study. A total of 587 patients were examined for eligibility.

The questionnaire was not completed in detail by 78 of these eligible patients during admission, including eight critical, 23 moderate to severe, and 47 uncomplicated COVID-19 cases. Finally, 509 patients were included in this study; these cases had completed the entirety of follow-up (**Figure 1**).

Table 2 shows subject characteristics as well as the results of statistical analyses comparing patients grouped by severity. Subjects with critical COVID-19 were more likely to be older in age and have a higher body mass index (BMI). A non-vegetarian diet was most prevalent in the critical COVID-19 group, though this finding was not statistically significant.

In **Table 3**, we focus on older patients aged ≥65 years. Due to different age groups having very different COVID-19 severities (based on previously published statistics), the older population may be a more indicative group with regard to highlighting the benefits of a vegetarian diet.

Overall, there were fewer vegetarians in the critical COVID-19 group, indicating a possible protective effect in vegetarians. Moreover, the severity of COVID-19 symptoms showed a borderline significant association ($p = 0.062$) in patients with higher adherence to a vegetarian diet (**Table 2**). For patients aged ≥65 years, COVID-19 symptom severity was statistically significantly associated ($p = 0.013$) with adherence to a vegetarian diet (**Table 3**). The crude odds ratio (OR)

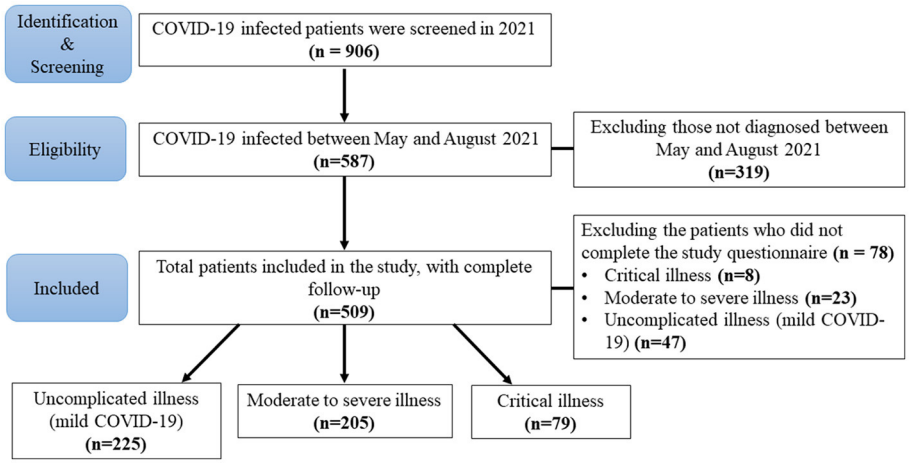

FIGURE 1 | Flow diagram of the study enrollment process. A total of 906 COVID-19 (coronavirus disease 2019) infected patients comprised the eligible study population as of 2021. In order to study vegetarian diets in connection with COVID-19 severity, 319 patients were excluded due to not being evaluated between May 2021 and August 2021. Hence, a total of 587 patients were examined for eligibility. The questionnaire was not completed in detail by 78 patients during admission, which included eight critical, 23 moderate to severe, and 47 uncomplicated (mild) COVID-19 patients. A total of 509 patients with complete follow-up data were included in this study.

TABLE 2 | Characteristics of COVID-19 patients by severity status.

		Category			
	Total (*N* = 509)	**Uncomplicated illness to mild COVID-19 (*n* = 225)**	**Moderate to severe COVID-19 (*n* = 205)**	**Critical COVID-19 (*n* = 79)**	***p*-value**[†]
Sample size, *N*					
Sex					0.316
Women	269 (52.8)	119 (52.9)	114 (55.6)	36 (45.6)	
Men	240 (47.2)	106 (47.1)	91 (44.4)	43 (54.4)	
Age, years	52.17 ± 16.57	44.26 ± 14.20	55.66 ± 15.42	65.63 ± 13.85	0.000*
Weight (kg)	66.57 ± 13.79	65.97 ± 14.39	66.27 ± 11.89	69.60 ± 16.38	0.169
Body mass index (kg/m^2)	24.73 ± 4.26	24.16 ± 4.28	24.90 ± 3.52	26.31 ± 5.62	0.002*
CCI	1.28 ± 1.35	0.80 ± 1.23	1.48 ± 1.30	2.15 ± 1.28	0.000*
CCI = 0	213 (41.8)	137 (60.9)	64 (31.2)	12 (15.2)	0.000*
1 ≤ CCI ≤ 2	189 (31.7)	61 (27.1)	96 (46.8)	32 (40.5)	
CCI ≥ 3	107 (26.5)	27 (12.0)	45 (22.0)	35 (44.3)	
Self-reported diets					0.374
Non-vegetarian	487 (95.7)	214 (95.1)	195 (95.1)	78(98.7)	
Vegetarian	22 (4.3)	11 (4.9)	10 (4.9)	1 (1.3)	
Diet type					0.062
Non-vegetarian	487 (95.7)	214 (95.1)	195 (95.1)	78 (98.7)	
Vegan	6 (1.2)	1 (0.4)	5 (2.4)	0 (0.0)	
Ovo-lacto vegetarian	13 (2.6)	10 (4.4)	3 (1.5)	0 (0.0)	
Lacto-vegetarian	1 (0.2)	0 (0.0)	1 (0.5)	0 (0.0)	
Ovo-vegetarian	2 (0.4)	0 (0.0)	1 (0.5)	1 (1.3)	
Pescatarian	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

[†] *p-value comparing critical or moderate to severe cases with uncomplicated or mild severity cases. CCI, Charlson Comorbidity Index; COVID-19, coronavirus disease 2019. *p < 0.05.*

was 19.78 ($p = 0.009$) when evaluating disease severity level (critical vs. mild disease) in connection with vegetarian or non-vegetarian diet. Subject ages were highest in those with critical COVID-19 ($p = 0.014$).

TABLE 3 | Characteristics of older COVID-19 patients aged ≥65 years by severity status.

	Total ($N = 136$)	Category			p-value[†]
		Uncomplicated illness to mild COVID-19 ($n = 23$)	Moderate to severe COVID-19 ($n = 66$)	Critical COVID-19 ($n = 47$)	
Sample size, N					
Sex					0.316
Women	61 (44.9)	9 (39.1)	34 (51.5)	18 (38.3)	
Men	75 (55.1)	14 (60.9)	32 (48.5)	29 (61.7)	
Age, years	72.46 ± 6.71	69.91 ± 4.51	71.86 ± 6.31	74.55 ± 7.62	0.014*
Weight (kg)	65.40 ± 11.21	65.96 ± 14.97	64.30 ± 8.74	66.74 ± 12.06	0.572
Body mass index (kg/m^2)	24.98 ± 3.65	24.85 ± 4.45	24.82 ± 2.84	25.34 ± 4.28	0.787
CCI	2.94 ± 0.82	3.09 ± 1.12	2.85 ± 0.77	3.00 ± 0.72	0.410
CCI=0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
1≤CCI≤2	43 (31.6)	7 (30.4)	24 (36.4)	12 (25.5)	0.471
CCI ≥3	93 (68.4)	16 (69.6)	42 (63.6)	35 (74.5)	
Self-reported diets					0.013*
Non-vegetarian	127 (93.4)	19 (82.6)	61 (92.4)	47 (100.0)	
Vegetarian	9 (6.6)	4 (17.4)	5 (7.6)	0 (0.0)	
Particular diet type					0.077
Non-vegetarian	127 (93.4)	19 (82.6)	61 (92.4)	47 (100.0)	
Vegan	3 (2.2)	1 (4.3)	2 (3.0)	0 (0.0)	
Ovo-lacto vegetarian	4 (2.9)	3 (13.0)	1 (1.5)	0 (0.0)	
Lacto-vegetarian	1 (0.7)	0 (0.0)	1 (1.5)	0 (0.0)	
Ovo-vegetarian	1 (0.7)	0 (0.0)	1 (1.5)	0 (0.0)	
Pescatarian	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

[†] *p value comparing critical or moderate to severe cases with uncomplicated or mild cases. COVID-19, coronavirus disease 2019. *p < 0.05; crude odds ratio = 19.78; p = 0.009 when evaluating disease severity level in connection with diet type.*

In further subgroup analysis, the vegetarian group had no differences in BMI (kg/m^2) when compared to the non-vegetarian group (vegetarian vs. non-vegetarian group: 24.01 ± 3.40 vs. 24.77 ± 4.30, $p = 0.414$). There were no differences between vegetarian and non-vegetarian groups with respect to the other variables, including gender, smoking status, age, weight, CCI, and CURB-65.

We also conducted various subgroup analyses, the results of which are described below. For example, the study population was divided into four subgroups according to age and diet type. We further divided the subjects into low-low risk (age < 65 years, vegetarian diet), low-high risk (age < 65 years, non-vegetarian diet), high-low risk (age ≥ 65 years, vegetarian diet), and high-high risk (age ≥ 65 years, non-vegetarian diet) subgroups (**Supplementary Table 1**). In the multivariate analysis, which adjusted for all variables that had a p of <0.05 in the univariate analysis (**Supplementary Table 1**), COVID-19 severity was statistically significantly associated with BMI [adjusted OR = 1.064, 95% confidence interval (CI): 1.017–1.116, $p = 0.008$] and categorization into the "high-high risk" subgroup (adjusted OR = 5.434, 95% CI: 1.624–18.826, $p = 0.005$).

DISCUSSION

In our study, the severity of COVID-19 symptoms showed borderline significance in patients with higher adherence to a vegetarian diet. Although these findings trend in the direction of a meaningful association between adherence to a vegetarian diet and COVID-19 symptom severity, these findings could also simply be due to chance. Furthermore, when we stratified the study population by groups defined according to age and vegetarian diet status (**Supplementary Table 1**), COVID-19 severity was found to be statistically significantly associated with older age and a non-vegetarian diet.

Age is well known to be associated with immune system strength and overall immune functioning, and may, thus, reduce individual's capacity to deal with infections effectively and may also affect the likelihood of developing chronic inflammation (11). An age-matched study may help elucidate these findings during future research efforts.

In addition, we evaluated COVID-19 severity in connection with CCI scores, with higher scores associated with greater COVID-19 severity. These results were similar to those of a previous meta-analysis (12), in which each point increase in CCI scores increased mortality risk by 16%. Moreover, a higher mean CCI score was also statistically significantly associated with mortality and disease severity in this prior study. Recently, a well-established observational study likewise explored the association between the CCI and severe COVID-19 outcomes (13). In the current study, we obtained the same results as in previous publications, namely that CCI scores are associated with COVID-19 severity (13).

We note that a recent study suggested strengthening the immune system through dietary habits and specific nutrients (14). However, it is important to acknowledge that dietary habits are changing on many levels worldwide, including as multiple regulations and lockdowns take effect (15, 16).

Prior studies have reported that plant-based diets are nutrient-dense, and include high concentrations of polyphenols, carotenoids, fiber, vitamins A, C, and E, folate, iron, potassium, and magnesium (17, 18). Plant-based diets have known benefits in terms of preventing hypertension and cardiovascular disease (19, 20). Vegetarian plant-based diets also strengthen the immune system, reduce inflammation and oxidative stress (14), and may help prevent chronic kidney disease and preserve kidney function (21). Hence, we propose the hypothesis that vegetarian diets might prevent heart and kidney diseases as well as strengthen the immune system and lower comorbidities. The presence of fewer comorbidities may influence the severity of COVID-19 indirectly.

An epidemiologic prospective cohort study by Merino et al. (1) suggests that plant-based foods were associated with lower severity of COVID-19, especially in areas of higher socioeconomic deprivation. In our study, the hospital setting enrolled a more severe and critical COVID-19 population and when there was no COVID-19 vaccination during pandemics, our findings agree with the results of their study. However, we did not explore other supplements of Vitamin B12, D3, or blood tests in our study population because it was a retrospective study. Therefore, we recommend that future studies include vaccination status and vitamin supplements intake data to clarify the components of a vegetarian diet that may reduce COVID-19 severity.

This study has several limitations. First, too few vegetarians were recruited in this study, thus limiting our study power. Moreover, our study population did not include any patients with asymptomatic COVID-19 and our study population may thus be limited in terms of representativeness, generalizability, and external validity. Further, large study populations and multi-center studies in quarantine hotels and responding hospitals are needed to further clarify the possible protective effect on vegetarians of old age.

Second, the study questionnaire was not sufficiently detailed. For example, we only asked about the adherence to a vegetarian diet over the course of 1 year. A more detailed and long-term investigation of vegetarian as compared with non-vegetarian diets is necessary in order to clarify the relationship between diet and COVID-19 severity in the future.

CONCLUSION

For older COVID-19 patients, we found that a non-vegetarian diet was associated with a higher risk of critical COVID-19 severity. Additional research is necessary in order to determine the effects of dietary habits on COVID-19 risk and severity during the global pandemic.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

For this retrospective study, informed consent was waived by the IRB, and the privacy rights of patients, which cover any individual's data in any form (including individual details, images, or videos), were upheld. This study was approved by the Institutional Review Board of Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation (approval number 10-X-141) and conducted according to the amended Declaration of Helsinki. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Y-CH and Y-CC conceived the study, designed the trial, obtained the research funding, analyzed and interpreted the data, and contributed to manuscript preparation. Y-CH and W-LS supervised the conduct of the trial, as well as data collection, and revised the manuscript for critical content. W-LS provided statistical advice with regard to the study design, and analyzed the data. Y-CH drafted the manuscript, and all authors contributed substantially to its revision. All authors take responsibility for the article as a whole. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.837458/full#supplementary-material

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ORIGINAL RESEARCH
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Association of Recent and Long-Term Supplement Intakes With Laboratory Indices in Patients With COVID-19 in Tehran, Iran, During 2020

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Background: Although previous studies observed the relationship between individual dietary supplements and enhancing body resistance against viruses, few studies have been conducted regarding the role of different supplements in treatment of COVID-19. This article aims to determine the association of recent and long-term supplement consumption on the biochemical indices and impatient duration among patients with COVID-19.

Methods: In this cross-sectional study on 300 adult men and women with COVID-19, recent and long-term supplement intakes were investigated by using a questionnaire. In addition, lifestyle was also assessed in aspects of fruits and vegetable consumption, physical activity, sleeping duration, fluid intake, and smoking status. Furthermore, the laboratory and paraclinical parameters were obtained from medical records. The relationship between supplement intake with the length of hospitalization and clinical laboratory tests was investigated by one-way analysis of variance (ANOVA).

Results: Those patients with supplement intake in the last 2 months had a significantly lower amount of blood urea nitrogen (BUN) (31.31 ± 13.87 vs. 37.57 ± 19.77 mg/dL, *P*: 0.002) and higher serum 25(OH)D (28.13 ± 14.09 vs. 23.81 ± 13.55 ng/mL, *P*: 0.03). Subjects with long-term supplement intake had a significantly lower invasive oxygen support (0.00 vs 5.10 %, *P*: 0.05), lactate dehydrogenase (LDH) (498.11 ± 221.43 vs. 576.21 ± 239.84 U/L, P: 0.02), fewer days of fever (0.49 ± 3.54 vs. 2.64 ± 9.21, *P*: 0.02), and higher serum 25(OH)D (31.03 ± 13.20 vs. 22.29± 13.42 ng/mL, $P < 0.001$). The length of hospital stay was practically the same between groups who received and did not receive supplementation during the 2 months prior to hospitalization (6.36 ± 3.32 vs. 6.71 ± 4.33 days, *P*: 0.004). Similarly, people who took supplements during the past year had practically similar hospitalization lengths (6.29 ± 4.13 vs. 6.74 ± 3.55 days, *P*: 0.004).

Conclusion: In conclusion, although practically the length of hospital stay was the same in both groups of supplement consumers and others, immune-boosting supplements were associated with improved several laboratory indices. However, due to the cross-sectional nature of our study, further longitudinal studies seem to be essential.

Keywords: supplement, COVID-19, BUN, ICU, general ward, creatinine, CRP

INTRODUCTION

The new variation of coronavirus, SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), which triggers COVID-19, is undoubtedly the foremost dangerous coronavirus with the greatest morbidity and death rate. Human society experienced the most important acute health trouble due to the coronavirus pandemic in recent year (1). The crude mortality ratio is 1.63% based on Worldometer by January 21, 2022 (2), which can vary in different countries depending on age and the presence of comorbidities (1).

As of January 21, 2022, 9,807,128,664 doses of the COVID-19 vaccine have been administered worldwide, according to covidvax; however, maintaining social distancing and carrying face masks are recommended in public spaces to avoid the transmission of COVID-19 (3). Furthermore, lifestyle factors, including dietary intake, exercise, smoking, alcohol intake, screen time, and sleep, may also additionally have a crucial influence on COVID-19 vulnerability (4). Hence, many researchers highlighted the importance of taking suitable dietary interventions and nutritional supplements to boost our immune systems. The relationship between taking protein, omega-3, vitamin A, B complex, C, D, E, minerals like zinc, selenium, iron, copper, and magnesium supplements and improving the immune system has been investigated (5–16). Previous studies have also investigated the effects of glycophosphopeptical AM3 on respiratory diseases (17, 18). Furthermore, a review study has discussed the potential prophylactic and therapeutic benefits of glycophosphopeptical AM3 on COVID-19 (19). Vitamin D, lactoferrin, and selenium supplementation have possibly desirable effects on COVID-19 prevention (11, 20–25). It is recently reported that D3 intake for a few weeks at 10 000 IU/d followed by 5,000 IU/d to reach the minimum of 150 nmol/L for 25(OH)D serum is a prophylactic measure to COVID-19 for high-risk people (8). The researchers recently reported vitamin D, zinc, omega-3, vitamin C, selenium, magnesium, lactoferrin, L glutamine, gut microbiota, nigella sativa, and propolis supplementation could be considered as possible COVID-19 treatments (15, 22, 25–33). Early dietary supplementation in suspicious COVID-19 cases can contribute to better treatment results, and good nutritional status is essential prior to the other medical measures (33).

The necessity of immune-boosting supplementation is one of the most common ambiguities during the COVID-19 pandemic. Furthermore, in patients with COVID-19, possible differences in length of hospital stay and biochemical parameters in people who took the supplements with others are yet to be determined. In general, the benefits of taking immune-boosting supplements during the COVID-19 pandemic are not completely conclusive. Previous studies have observed relationships between appropriate nutritional status and enhancing body resistance against viruses (33–35). However, the information about supplementation as a preventive and treatment measure for COVID-19 remains controversial. Although there are reports on patients with COVID-19 nutritional supplement effects, there is no study on multi-dietary supplement's role in the prevention and treatment of COVID-19 simultaneously. This article aims to determine the association of recent and long-term supplement consumption before disease incidence on the biochemical indices and impatient duration among infected patients to COVID-19.

MATERIALS AND METHODS

Study Design and Population

The main aim of this cross-sectional study was to investigate the association of recent and long-term supplement intakes with laboratory indices and length of hospital stay in hospitalized patients with COVID-19. Participants were recruited from patients with COVID-19 hospitalized in Ziaeian hospital affiliated with Tehran University of Medical Sciences. The data collection process was performed from March 24, 2020, to November 20, 2020. This study was performed on 300 adults aged 18 years and older admitted to Ziaeian Hospital who were randomly selected. Subjects were confirmed patients with COVID-19 diagnosed by the general practitioner (GP) regarding laboratory results, lungs CT scan, and clinical symptoms simultaneously. Consumption of dietary supplements in the last 2 months and the past year was classified into recent and long-term supplement intakes, respectively. Supplement intake modulating the immune system function, such as A, C, D, E, and B vitamins, zinc, selenium, iron, omega-3, and protein, as well as probiotics, was studied. Written informed consent was obtained from all participants. Inclusion criteria were as follows: no pregnancy, no lactation, and no serious condition, such as dementia, history of a large stroke, and active cancer within 5 years. Patients with non-communicable diseases, such as diabetes, hypertension, hyperlipidemia, hypothyroid, kidney disease, and fatty liver, were included. This study was approved by the ethical committee of Tehran University of Medical Sciences with the ethics code IR.TUMS.VCR.REC.1399.036 and grant number 99-1-212-47266.

Assessment of Anthropometric and Obesity Characteristics

Anthropometric parameters, such as weight, height, and body mass index of patients, were measured at admission. Weight

was measured with light clothing and using a digital scale with an accuracy of 100 grams, and height was also obtained using a stadiometer with an accuracy of 0.1 cm after removing the shoes. To reduce possible errors, all these measurements were performed by a healthcare staff. Using a demographic questionnaire, patient sudden and severe weight changes in the last month were assessed. Moreover, to evaluate weight changes during hospitalization, subjects' weight was measured at the time of discharge. Finally, body mass index was computed by dividing weight (kg) to the square of height (m^2).

Assessment of Usual Dietary and Supplement Intake

Daily energy intake, and macro- and micronutrients of the subjects were obtained using 24-h recall based on the number of hospitalization days during hospital stay. Using USDA modified food composition table for Iranian foods, the daily macro- and micronutrient intake of each patient was calculated (36). Patients were evaluated regarding following a special diet before the onset of the disease, servings of fruit consumption per day during the past year, number of daily main meals received, type and amount of bread, meat, dairy, and oil consumed, and the frequency of fast-food consumption. Moreover, the amount of main meals and snacks consumed during hospitalization in comparison with the amount consumed before the onset of the disease was obtained using questionnaire. In addition, fluid intake habits regarding the frequency of carbonated beverages consumption and daily water intake were assessed based on questionnaire data. Finally, to assess the nutrition status of each participant, an overall nutrition score was applied. We obtained the overall nutrition score for each participant by summing the scores of the type of meat consumed, the type of bread consumed, the amount of dairy consumed, the amount consumed of fruits and vegetables, and the consumption of sweetened carbonated beverages. The points allocated were as follows: consumption of red meat 0, chicken 1, fish 2, consumption of <1 glass of dairy 0, 1 glass of dairy 1, 2 glass of dairy 2, 3 glass of dairy 3, >3 glass of dairy 4, consumption of carbonated beverages 0 and non-consumption 1, consumption of white bread 0 and whole wheat bread 1, consumption of 1 unit of fruits 1, 2 units of fruits 2, 3 units of fruits 3 and consumption of <1 unit of vegetables 0, 1 unit of vegetables 1, 2 units of vegetables 2, 3 units of vegetables 3, and >3 units of vegetables 4 points were assigned. To evaluate the recent intake of supplements, we considered taking supplements during 2 months before hospitalization. We also obtained supplement intake during 1 year before admission to assess long-term supplementation. Long-term (over 1 year) and more recently (over the last 2 months) vitamin, mineral, probiotic, omega-3s, and protein supplement intake have been evaluated, and subjects have been asked to list their possible consumed supplements and state the received dose. Using supplement intake questionnaire, received supplements involved in the immune system function, such as A, C, D, E, and B vitamins, zinc, selenium, iron, omega-3, and protein, as well as probiotics, were obtained.

Assessment of Comorbidities and Medications

Patient current comorbidities, such as diabetes, hypothyroid, asthma, arthroses, Alzheimer's disease, rheumatoid arthritis, chronic obstructive pulmonary disease, end-stage renal disease, Parkinson's, kidney failure, hepatitis B, meningitis, human immunodeficiency virus, cerebral vascular accident, chronic kidney disease, mental disease, obesity, and immune system deficiency, were obtained using medical records. In addition, based on medical record data, cardiovascular disease risk factors, including hypertension, hyperlipidemia, ischemic heart disease (IHD), deep vein thrombosis (DVT), congenital heart defects (CHD), coronary artery disease (CAD), and congestive heart failure (CHF), were assessed. Likewise, using questionnaire, a history of heart valve replacement, dialysis, coronary artery bypass graft surgery (CABG), liver surgery, chemotherapy, and various types of transplants were obtained. Furthermore, received medications during the hospitalization period with emphasis on immune system suppressors, such as corticosteroids and their dosage, were extracted from the questionnaire. Patients were also evaluated in terms of antiviral drugs azithromycin, heparin, oseltamivir, Kaletra, ribavirin, favipiravir, remdesivir, atazanavir, and ivermectin consumption based on medical record data. In addition to current diseases and drugs, a history of diseases and drugs was also obtained regarding medical records. The history of lipid-lowering, anticoagulant, psychiatry, statin, angiotensin-converting enzyme inhibitors (ACEIs), and angiotensin II receptor blocker (ARB) drugs were assessed using the questionnaire. The possible underlying disease, as well as the severity of the disease, was stated in the questionnaire based on the paraclinical findings in the medical records.

Assessment of Clinical and Paraclinical Findings

Laboratory clinical tests were performed at the time of hospital admission using conventional methods, such as routine blood tests, cardiometabolic factors, renal function, liver enzymes, coagulation factors, and inflammation profile. The serum levels of important laboratory and paraclinical parameters, including blood urea nitrogen (BUN), creatinine, albumin, ferritin, 25(OH)vitamin D, c-reactive protein (CRP), d-dimer, and hemoglobin, were extracted using a questionnaire. In addition, according to medical records, other paraclinical findings in terms of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALKPH) hepatic enzymes, electrolytes, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), fasting blood sugar (FBS), HCO3, pH, partial pressure of oxygen (PO2), partial pressure of carbon dioxide (PCO2), and troponin serum levels were obtained. To measure CRP, troponin, ferritin, sodium, potassium, albumin, AST, ALT, LDH, creatinine, and urea, Abbott's Alinity Systems (Abbott, Chicago, IL, USA) were applied on plasma samples added with lithium heparin (Becton Dickinson, Franklin Lakes, NJ, USA). FBS levels were determined using the glucose oxidase-phenol 4-aminoantipyrine peroxidase (GOD-PAP) colorimetric method. Routine blood factors, such as white blood cell (WBC),

red blood cell (RBC) and platelet count, neutrophils, lymphocytes and HCT percentage, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and erythrocyte sedimentation rate (ESR), were obtained from anticoagulated blood samples with ethylenediaminetetraacetic acid tripotassium (K3 EDTA; Becton Dickinson, Franklin Lakes, NJ, USA) using Sysmex XN hematology analyzer and reagents (Sysmex Corporation, Kobe, Japan). Partial thromboplastin time (PTT), partial prothrombin time (PT), international normalized ratio (INR), and D-dimer were measured by benchtop analyzer STA compact Max 3 (Stago, Asnières-sur-Seine, France) and reagents on serum evacuated to 3.2% sodium citrate coagulation tubes (Becton Dickinson, Franklin Lakes, NJ, USA). The patient's symptoms, such as general pain, headache, cough, sore throat, joint pain, respiratory problems, anorexia, runny nose, lethargy, diarrhea, and vomiting, were documented in a questionnaire based on the evidence in the patient medical records.

Assessment of Other Variables

Related data on sociodemographic variables, including age, sex, education, occupation, residency, family status, and marital status, were extracted from the demographic questionnaire. Female participants were assessed in aspects of the physiologic condition, such as pregnancy, lactation status, number of deliveries, and menopausal status, additionally. Lifestyle-related variables, including smoking status, current and past history of alcohol consumption and drug abuse, daily physical activity, facing stressful events in the last 6 months, and sleep duration changes, were obtained using a questionnaire. Finally, using questionnaire, the method of diagnosis of COVID-19 disease, the extent of contact with COVID-19 patients before infection, inpatient or non-hospital treatment, place of hospitalization, duration of hospitalization in addition to general condition at the time of discharge from the hospital were assessed.

Statistical Analysis

Participants were categorized regarding recent and long-term supplement consumption. We applied one-way ANOVA and chi-square tests for continuous and categorical variables, respectively, to compare sociodemographic characteristics across different supplement intake categories. The relationship between more recent and long-term supplement intakes with the length of hospitalization was assessed using one-way ANOVA. Similarly, the association of recent and long-term supplementation with clinical laboratory tests was investigated by one-way ANOVA. By applying the chi-square test, recent and long-term supplement consumption relationships with the symptoms of disease (fever, cough, headache, runny nose, and sore throat) were examined. Moreover, the prevalence of various comorbidities and medications across different categories of supplement intake was assessed using the chi-square test. Potential confounders which were likely to affect the outcomes of our study were adjusted in three phases using ANCOVA. One of the factors that may affect the laboratory indices and duration of hospitalization is medication. We examined the interaction of drugs with supplements in such a way that in a separate model, we performed statistical adjustments. If the effect of the interaction of drugs with supplements was significant, then the effect would disappear. In model 1, variables of gender, age, history and the current onset of diseases, and history and current medications were adjusted. The length of hospitalization in different categories of supplement intake following gender, age, history and current onset of diseases, and history and current medications adjustment is presented in model 1 (**Figure 1**). Furthermore, in model 2, the *P*-values were controlled by body mass index, hemoglobin, and nutrition overall score, additionally, which were included as covariates (**Figure 1**). Finally, in model 3, multiple variable adjustments were performed and the existence of respiratory support, fever, blood oxygen level, partial pressure of carbon dioxide, albumin, 25(OH)D, and c-reactive protein serum levels were included as covariates, additionally.

RESULTS

Out of 300 subjects in the present study, 164 and 138 patients had recent and long-term supplement intake, respectively. 54.7% of the participants were males and 45.3% were females with a mean age of 51.95 $\pm$ 15.34 years. **Table 1** and **Supplementary Table 1** represent the sociodemographic characteristics of patients with COVID-19 who were included in the present study. People who have been supplemented in the last 2 months were younger, slightly higher educated, obese, and more stressed than those who did not take the supplement recently. Similarly, people who received supplements in the past year were people with younger age, more percentage of women, slightly more traveling experience, more sleep-alteration, and more stressful events than those who did not receive the supplement. There was no significant difference in marital status, the number of family members, job classification and comorbidities except for obesity in the two groups of recent supplementation and non-recent supplementation. No significant difference was observed in marital status, number of family members, job classification and comorbidities except for immune deficiency and asthma across different long-term supplementation groups. Anthropometric and obesity characteristics of patients with COVID-19 across recent and long-term supplementation categories are presented in **Table 2**. In people who have recently taken supplements, more weight changes have been observed, approaching the marginal significance ($P = 0.07$).Similarly those subjects with recent supplement intake showed significantly more prevalence of obesity ($P = 0.04$). Supplementation in recent years has been associated with less weight change ($P = 0.002$). History of different diseases and diagnosis methods among patients with COVID-19 are presented in **Supplementary Table 2**. Dietary intake during hospitalization among patients with COVID-19 in different supplement categories intake has been provided in **Supplementary Table 3**. There was a significant difference in the amount of breakfast and dinner consumption of those who took the supplement recently and those who did not take the supplement recently (P =0.004, 0.04). The use of different drugs did not differ much between the two groups. No significant difference was found in terms of dietary intake

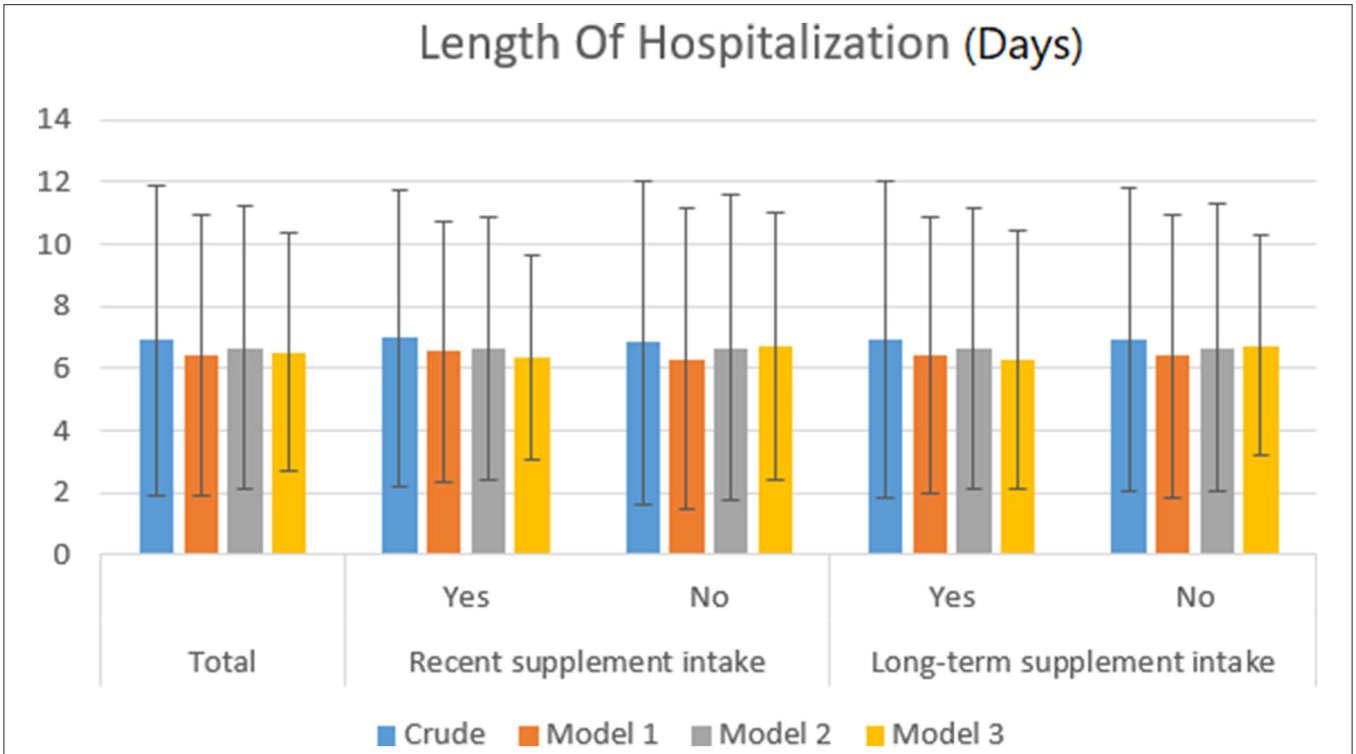

FIGURE 1 | Length of hospitalization in different supplement intake categories.

and medication in those who took or did not take supplements in the past year. **Supplementary Table 4** illustrates the usual dietary intake among patients with COVID-19 in different supplement categories intake. There was a significant difference in the frequency of fast food consumption, consumption of lipid-lowering drugs and statins in those subjects with recent supplements compared to those who did not take supplements recently. Beverage and anticoagulants consumption was significantly different in those who did and did not receive supplementation in the past year ($P = 0.05$, 0.01). Biochemical indices in different supplement intake categories are provided in **Supplementary Table 5**. Alkaline phosphatase (146.64 ± 67.21 vs. 176.96 ± 143.10 IU/L, *P*: 0.03) and MCV (84.70 ± 6.56 vs, 86.39 ± 5.19 fl, *P*: 0.02) were significantly lower in the group that had taken the supplement in the last 2 months. Subjects with long-term supplement intake had significantly lower LDH (498.11 ± 221.43 vs. 576.21 ± 239.84 U/L, *P*: 0.02), hematocrit (39.46 ± 6.03 vs. 40.81 ± 5.23 %, *P*: 0.04) MCV (84.47 ± 6.58 vs. 86.35 ± 5.35 fl, *P*: 0.008), and significantly higher lymphocytes (25.15 ± 11.29 vs. 21.93 ± 10.90 %, *P*: 0.01). Important parameters in different supplement categories are illustrated in **Table 3**. Recent supplementation was associated with lower BUN (31.31 ± 13.87 vs. 37.57 ± 19.77 mg/dL, *P*: 0.002) and higher 25(OH)D serum levels (28.13 ± 14.09 vs. 23.81 ± 13.55 ng/mL, *P*: 0.03). 25(OH)D serum level was significantly higher in those who had taken supplements in the past year (31.03 ± 13.20 vs. 22.29 ± 13.42; $P < 0.001$). Clinical and para-clinical parameters have been provided in **Table 4**. Compared to those who have not taken supplements recently, no parameter was significantly different in the supplemented group. After performing multiple variable adjustments, those with recent (0.70 ± 4.41 vs. 3.07 ± 9.96 days, $P < 0.001$) and long-term supplementation intake had fewer days of fever (0.74 ± 4.70 vs. 2.80 ± 9.46 days, $P < 0.001$). Furthermore, recent (91.83 ± 10.03 vs. 85.89 ± 21.06 %, $P < 0.001$) and long-term supplement consumption was associated with higher blood oxygen pressure after statistical adjustments (91.50 ± 11.20 vs. 86.77 ± 19.83 %, $P < 0.001$).

The length of hospitalization after gender, age, history and the current onset of diseases and history and current medications adjustment are presented in model 1 (**Table 5** and **Figure 1**). The *P*-values were controlled by body mass index, hemoglobin and nutrition overall score, which were included as covariates additionally in model 2 (**Table 5** and **Figure 1**). In model 3, *P*-values were controlled by the existence of respiratory support, fever and blood oxygen level, partial pressure of carbon dioxide, albumin, 25(OH)D, c-reactive protein serum levels, in addition to the above variables which were included as covariates. Although, those who recently supplemented had slightly fewer hospital stays, the length of hospitalization was practically the same (6.36 ± 3.32 vs. 6.71± 4.33 days, *P*: 0.004). Similarly, People who took supplements in the past year also had similar hospitalization lengths (6.29 ± 4.13 vs. 6.74± 3.55 days, *P*: 0.004) (**Table 5** and **Figure 1**).

DISCUSSION

In this cross-sectional study, we did not find a significant inverse relationship between supplementation in the last 2 months and

TABLE 1 | Sociodemographic characteristics of patients with COVID-19 according to recent, long-term, and during hospitalization intakes.

	Total	Recent supplement intake		Long-term supplement intake	
		No	Yes	No	Yes
Sample size, *n*	300 (100%)	138 (46%)	162 (54%)	162 (54%)	138 (46%)
Age (years)	51.95± 15.34	54.72± 15.28*	49.59±15.05	53.57 ± 15.37*	50.04 ± 15.14
Gender					
Men	164 (54.7%)	87 (63.1%)*	77 (47.5%)	101 (62.3%)*	63 (45.6%)
Women	136 (45.3%)	51 (36.9%)*	85 (52.5%)	61 (37.7%)*	75 (54.4%)
Number of family members	3.36± 1.29	3.46 ±1.47	3.29± 1.11	3.46 ± 1.39	3.25 ± 1.15
Marital status					
Single	143 (49.8%)	63 (47.4%)	80 (51.9%)	80 (52.2%)	63 (47%)
Married	143 (49.8%)	69 (51.9%)	74 (48.1%)	72 (47%)	71 (53%)
Divorced	1 (0.3%)	1 (0.8%)	0 (0%)	1 (0.6%)	0 (0%)
Patient caregiver					
Him/Herself	26 (8.7)	10 (7.2)	16 (9.9)	15 (9.3%)	11 (8%)
Spouse	50 (16.7%)	26 (20.3%)	24 (16.4%)	23 (14.2%)	27 (19.6%)
Children	31 (10.3%)	15 (11.7%)	16 (10.9%)	15 (9.3%)	16 (11.6%)
Spouse & children	192 (64%)	87 (67.9%)	105 (71.9%)	108 (66.7%)	84 (60.9%)
Nurse	1 (0.3%)	0 (0%)	1 (0.6%)	1 (0.6%)	0 (0%)
Smoking status					
Never smoker	260 (86.7%)	117 (84.7%)	143 (89.3%)	140 (86.4%)	120 (88.2%)
Former smoker	30 (10%)	15 (10.8%)	15 (9.3%)	16 (9.9%)	14 (10.3%)
Current smoker	8 (2.7%)	6 (4.3%)	2 (1.2%)	6 (3.7%)	2 (1.5%)
Smoking duration (years)	1.15 ± 5.40	1.29 ± 5.63	1.03 ± 5.21	1.04 ± 5.22	1.27 ± 5.62
Smoking person in family					
Yes	40 (13.3%)	17 (12.4%)	23 (14.2%)	20 (12.4%)	20 (14.4%)
No	259 (86.3%)	120 (87.5%)	139 (85.8%)	141 (87.6%)	118 (85.6%)
Alcohol history	11 (3.7%)	8 (5.8%)	3 (1.8%)	8 (4.9%)	3 (2.1%)
Drug abuse history	4 (1.3%)	3 (2.1%)	1 (0.6%)	3 (1.8%)	1 (0.7%)
Life style habit					
Sleep change					
Yes	152 (50.7%)	69 (50.7%)	83 (51.2%)	69 (42.9%)*	83 (60.6%)
No	146 (48.7%)	67 (49.3%)	79 (48.8%)	92 (57.1%)	54 (39.4%)
Sleep duration before illness	7.57± 2.07	7.49± 1.93	7.63 ± 2.18	7.52 ± 2.27	7.62 ± 1.82
Sleep duration after illness	6.86±2.26	6.69 ±2.46	7 ±2.07	6.75 ± 2.22	6.98 ± 2.31
Taking sleeping pills before illness	8 (2.7%)	5 (3.6%)	3 (1.8%)	3 (1.8%)	5 (3.6%)
Facing a stressful event in the last 6 months					
Yes	125 (4.7%)	48 (35%) *	77 (47.5%)	52 (32.2%)*	73 (52.8%)
No	174 (58%)	89 (65%) *	85 (52.4%)	109 (67.8%)*	65 (47.2%)
Physiologic condition					
Breast feeding					
Yes	3 (1%)	2 (1.4%)	1 (0.6%)	1 (0.6%)	2 (1.4%)
No	297 (99%)	136 (98.6%)	161 (99.4%)	161 (99.4%)	136 (98.6%)
Menopausal condition					
Menopause	63 (21%)	26 (18.8%)	37 (22.8%)	33 (20.3%)	30 (21.7%)
Non-menopausal	237 (79%)	112 (81.2%)	125 (77.2%)	129 (79.7%)	108 (78.7%)
Responder to the questionnaire					
Patient	1 (0.3%)	0 (0%) *	1 (0.6%)	1 (0.6%)*	0 (0%)
Other person	299 (99.7%)	138 (100%) *	161 (99.4%)	161 (99.4%)*	138 (100%)

B.Sc., A Bachelor of Science; M.Sc., A Master of Science; PH.D, Doctor of Philosophy. Qualitative variables are reported as frequency (percent). Quantitative variables are reported as mean ± SD.

*P-value is reported based on one-way ANOVA test.*P-value is <0.05 and considered as statistically significant.*

TABLE 2 | Anthropometric and obesity characteristics of patients with COVID-19.

	Total	Recent supplement intake		Long-term supplement intake	
		No	Yes	No	Yes
Height (cm)	168 ± 9.33	168.68 ± 9.56	167.43 ± 9.12	168.68 ± 9.37	167.22 ± 9.26
Weight (kg)					
Weight before illness (kg)	81.84 ± 16.34	81.88 ± 17.42	81.80 ± 15.41	82.24 ± 17.23	81.37 ± 15.27
Weight after illness (kg)	77.75 ± 15.88	77.23 ± 16.58	78.19 ± 15.30	77.94 ± 16.60	77.52 ± 15.05
Sudden weight change in the last 6 months					
Yes	47 (15.7%)	21 (15.4%)	26 (16%)	22 (13.7%)	25 (18.1%)
No	251 (84.3%)	115 (84.6%)	136 (84%)	138 (86.3%)	113 (81.9%)
Sudden weight change in the past year					
Yes	110 (36.7%)	58 (42.3%)	52 (32%)	72 (44.7%) *	38 (27.5%)
No	189 (63%)	79 (57.7%)	110 (68%)	89 (55.3%)	100 (72.5%)
Obesity					
Yes	15 (5%)	3 (2.1%) *	12 (7.4%)	7 (4.3%)	8 (5.7%)
No	285 (95%)	135 (97.9%)	150 (92.6%)	155 (95.7%)	130 (94.3%)
BMI (kg/m^2)	27.48 ± 5.10	27.07 ± 5.25	27.83 ± 4.95	27.34 ± 5.44	27.65 ± 4.68

BMI, body mass index. Qualitative variables are reported as frequency (percent). Quantitative variables are reported as mean ± SD.
*P-value is reported based on one-way ANOVA test. *P-value is <0.05 and considered as statistically significant.*

the length of hospital stay among Iranian patients with COVID-19. Similarly, people who received supplements in the past year had similar hospitalization duration. Recent supplementation was significantly associated with lower BUN and higher serum 25(OH)D. Furthermore, 25(OH)D serum levels were significantly higher in those who had long-term supplementation during the past year. Moreover, recent supplementation was slightly associated with lower blood creatinine levels. To the best of our knowledge, this is the first study to completely examine the association of recent and long-term consumption of various supplements with different laboratory indicators and the length of hospital stay in patients with COVID-19.

The results of our study showed that people who took supplements modulating the immune system during the year before admission needed significantly less invasive support. This result was consistent with a cohort study that showed that the combination of vitamin D, magnesium, and B12 intake was associated with a reduced need for respiratory support and (or) hospitalization in the ICU (37). The possible mechanism of vitamin D is that it has an effect on NF-κB and reduces pro-inflammatory cytokines and reduces the risk of cytokine storm (38). Magnesium has a positive effect by acting as a cofactor in the synthesis and activation of vitamin D (39). Vitamin B12 also has a positive effect on the gut microbiota and promotes the innate and adaptive immunity of COVID-19 patients (40). Moreover, our study showed that adjuvant supplementation was significantly associated with lower BUN during the 2 months prior to admission. Our results were consistent with the findings of a clinical trial study that showed the effect of omega-3s on BUN reduction in the short term. The mechanism of action of omega-3 is probably by preventing the binding of the SARS-CoV-2 virus to the human ACE2 receptor and activating the host proteases TMPRSS2 and cathepsin L (41). Also, the results of our study were in line with the clinical trial study performed on healthy individuals, which showed a decrease in the ratio of BUN to creatinine after vitamin D supplementation (42). The possible mechanism of this favorable effect of vitamin D supplementation is an alteration in the composition of the microbiota gut, especially increasing Bacteroidetes to Firmicutes ratio (42). Our study findings showed a significant decrease in blood creatinine. This finding was consistent with the systematic review and meta-analysis findings on the use of probiotics and synbiotics that indicated a decrease in blood creatinine levels (43). We observed a significant reduction in CRP, which confirmed the findings of a clinical trial study that showed a significant reduction in inflammatory outcomes following supplementation with vitamins A, B, C, D, and E (44). Our study findings showed a significant decrease in blood LDH levels in supplemented groups. Previous evidence suggests that vitamin D supplementation significantly reduces LDH and CRP serum levels (45). Previous observational studies have mainly focused on the association of specific supplement intake rather than various supplements in COVID-19. A population-based study investigated the association of calcitriol supplementation with mortality in CKD patients and reported a significant association between supplementation and reduction in risk of COVID-19 infection and mortality rate (46). This study has been performed on CKD patients, and it is difficult to extend the results of this study to the general population. Also, some variables may not be considered in the matching process, which is an inherent limitation of this type of study. Furthermore, the study examined only calcitriol, but our study investigated all supplements involved in the immune system (46). A cross-sectional study examined the association of vitamin D supplementation and its outcomes in 19 patients and approached the significance of higher mortality rates (47). However, two of the three groups of participants in the study were either patients with Parkinson's or Parkinson's caregivers, which made

TABLE 3 | Important parameters in patients with COVID-19 in different supplement intake categories.

	Total	Recent supplement intake		Long-term supplement intake	
		No	Yes	No	Yes
BUN (mg/dL)	34.18± 17.09	37.57 ± 19.77*	31.31 ± 13.87	35.28 ±17.02	32.89 ± 17.15
Adjusted	34.44 ± 17.66	38.72 ± 20.35*	30.58 ± 13.88	35.68 ± 16.94*	33.06 ± 18.46
Cr (mg/dL)	1.14 ± 0.65	1.22 ± 0.89	1.07 ± 0.32	1.14 ± 0.62	1.14 ± 0.69
Adjusted	1.17 ± 0.72	1.25 ± 0.96*	1.11 ± 0.39	1.11 ± 0.42*	1.24 ± 0.95
Alb (g/dL)	4.17 ± 0.4	4.12 ± 0.42	4.21 ± 0.38	4.16 ± 0.39	4.18 ± 0.40
Adjusted	4.18 ± 0.38	4.14 ± 0.39*	4.22 ± 0.37	4.17 ± 0.37*	4.19 ± 0.40
Ferritin (ng/ml)	307.8 ± 270.34	299.20 ± 250.52	314.62 ± 285.97	320.16 ± 267.26	293.09 ± 274.64
Adjusted	299.03 ± 255.13	276.36 ± 244.83	319.44 ±264.15	315.86 ± 277.64	280.33 ± 228.31
Vitamin D (ng/mL)	26.18 ± 13.99	23.81 ± 13.55 *	28.13 ± 14.09	22.29 ± 13.42 *	31.03 ± 13.20
Adjusted	26.77 ± 14.47	23.73 ± 13.62	29.51 ± 14.77	22.25 ± 13.84	31.79 ± 13.57
CRP (mg/L)	19.91 ± 32.61	18.64 ± 28.53	20.97 ± 35.73	20.63 ± 36.94	19.06 ± 26.76
Adjusted	12.78 ± 21.21	15.13 ± 24.30*	10.66 ± 17.90	13.79 ± 22.83*	11.66 ± 19.39
D-Dimer (ng/mL)	247.21 ± 484.87	301.16 ± 574.74	206.92 ± 404.29	239.90 ± 407.58	255.92 ± 566.641
Adjusted	271.35 ± 577.86	352.83 ± 683.55	209.87 ± 481.04	256.19 ± 494.75	287.53 ± 660.47
Hb (g/dL)	13.81 ± 1.96	13.84 ± 2.08	13.78 ± 1.85	13.95 ± 1.97	13.63 ± 1.94
Adjusted	14.05 ± 1.92	14.05 ± 1.97*	14.06 ± 1.88	14.24 ± 1.84*	13.85 ± 2.00

BUN, blood urea nitrogen; Cr, creatinine; Alb, albumin; CRP, C-reactive protein; Hb, hemoglobin. Qualitative variables are reported as frequency (percent). Quantitative variables are reported as mean ± SD. P-value is reported based on one-way ANOVA test.

**P-value is <0.05 and considered as statistically significant.*

*Data are presented as x ± s. Model 1: The P-values were controlled by inpatient, gender, age, history and current onset of diabetes, hypertension, hyperlipidemia, ischemic heart disease, cerebrovascular accident, lung disease, kidney failure, psychiatry disease, obesity, immune deficiency disease, cardiovascular disease, transplant, chronic kidney disease, coronary artery disease, hypothyroid, asthma, congestive heart failure, osteoarthritis, Alzheimer's disease, rheumatoid arthritis, chronic obstructive pulmonary disease, end-stage renal disease, coronary heart disease, Parkinson's, hepatitis B, deep vein thrombosis, meningitis and taking hydroxychloroquine, azithromycin, heparin, oseltamivir, Kaletra, ribavirin, favipiravir, remdesivir, atazanavir, intravenous immune globulin, ivermectin, anticoagulants, psychotropic drugs body mass index, hemoglobin, nutrition overall score, existence of respiratory support, fever, blood oxygen level, partial pressure of carbon dioxide, albumin, vitamin D, and c-reactive protein which were included as covariates. P-value is reported based on one-way ANOVA test. *P-value is <0.05 and considered as statistically significant.*

it difficult to extrapolate these results to the general population (47). The study reviewed vitamin D supplementation in the last 3 months, but our study provides data on both recent (2 months ago) and long-term (1 year) intakes of all supplements involved in the immune system (47). Our findings from this study were in line with a clinical trial study, which showed that supplementation did not affect the number of days of hospitalization of COVID-19 patients (48, 49). In contrast, a clinical study demonstrated a favorable association between supplementation and hospital length of stay (50). Naturally, the results obtained from taking a particular supplement may differ from taking different types of supplements and their relationship to the length of hospital stay. Previous studies have examined the association of adjuvant supplementation with clinical symptoms. For instance, recently, a clinical trial showed that high doses of intravenous vitamin C significantly increased the percentage of oxygen saturation and decreased the respiratory rate and the percentage of lung involvement (48). The beneficial effects of vitamin C supplementation are exerted by correcting the disease-induced deficiency, reducing inflammation, increasing interferon production, and supporting the anti-inflammatory action of glucocorticosteroids in acute respiratory infections and critical COVID-19 patients (9). However, some studies did not find a significant effect of vitamin C supplementation on the duration of symptoms and length of hospital stay (51–53). Furthermore, several studies have investigated the association between vitamin D supplementation and clinical symptoms. Vitamin D supplementation improved blood oxygen levels, reduced oxygen requirement, reduced hospital length, and reduced mortality (33). A clinical trial study showed that following vitamin D supplementation, the ratio of arterial oxygen saturation to the inspired fraction of oxygen improved further (54). The vitamin D group also had a shorter average length of stay, less need for ICU transfers, and fewer deaths and readmissions (54). In addition, high-dose vitamin D supplementation normalized serum 25-OH vitamin D levels and was associated with shorter hospital stays, fewer oxygen requirements, and reduced inflammatory markers (33). Although vitamin D's exact mechanism of action in reducing the risk of infection is yet to be determined, the existing mechanisms are classified into three groups: physical barrier, natural cellular immunity, and adaptive immunity. Vitamin D can play a role in maintaining the physical barrier by improving tight junctions, gap junctions, and junction adhesions (55). Moreover, vitamin D induces antimicrobial peptides, such as human cathelicidin, LL-37, and defensins, thereby enhancing innate cellular immunity (56–58). Finally, vitamin D reduces cytokine storms, which results in enhanced cellular immunity

TABLE 4 | Clinical and paraclinical parameters in patients with COVID-19 in different supplement intake categories.

	Total	Recent supplement intake		Long-term supplement intake	
		No	Yes	No	Yes
Systolic BP (mmHg)	119.51 ± 15.82	121.19 ±15.61	117.75 ± 15.99	120.22 ± 16.41	118.62 ± 15.16
Adjusted	118.33 ± 15.20	121.94 ± 13.84	113.93 ± 15.86	118.88 ± 14.16	117.71 ± 16.54
Diastolic BP (mmHg)	69.82 ± 19.81	72.44 ± 17.67	67.31 ± 21.54	69.54 ± 21.39	70.17 ± 17.93
Adjusted	65.90 ± 23.70	73.15 ± 17.43	58.65 ± 27.08	66.44 ± 25.37	65.32 ± 22.27
Headache	60 (20%)	25 (18.1%)	35 (21.6%)	27 (19.6%)	33 (29.5%)
Adjusted	-	-	-	-	-
Fever	28 (9.3%)	16 (11.5%)	12 (7.4%)	17 (12.3%)	11 (9.8%)
Adjusted	-	-	-	-	-
Duration of fever (days)	1.67 ± 7.3	2.35 ± 8.75	1.05 ± 5.63	2.64 ± 9.21 *	0.49 ± 3.54
Adjusted	1.83 ± 7.65	3.07 ± 9.96*	0.70 ± 4.41	2.80 ± 9.46*	0.74 ± 4.70
Respiratory rate (breaths/minute)	19.3 ± 9.87	18.31 ± 5.03	20.21 ± 12.74	19.51 ± 13.05	19.05 ± 2.97
Adjusted	18.54 ± 4.52	18.31 ± 5.91*	18.75 ± 2.78	18.21 ± 5.43*	18.91 ± 3.24
Spo2 (%)	89.52 ± 15.24	87.98 ± 18.10	90.95 ± 11.91	87.44 ± 18.71*	92.09 ± 8.78
Adjusted	89.01 ± 16.43	85.89 ± 21.06*	91.83 ± 10.03	86.77 ± 19.83*	91.50 ± 11.20
Ventilator					
Yes	105 (35%)	58 (48.3%)	57 (43.8%)	68 (49.3%)	47 (42%)
Adjusted	-	-	-	-	-
No	135 (45%)	62 (51.7%)	73 (56.2%)	70 (50.7%)	65 (58%)
Adjusted	-	-	-	-	-
Duration of ventilator (days)	2.69 ± 4.12	2.38 ± 3.71	2.98 ± 4.47	2.78 ± 4.12	2.58 ± 4.14
Adjusted	2.61 ± 3.80	2.50 ± 3.75*	2.71 ± 3.86	2.56 ± 3.88*	2.66 ± 3.72
Invasive respiratory support	7 (2.3%)	5 (3.6%)	2 (1.2%)	7 (5.1%)*	0 (0%)
Adjusted	-	-	-	-	-
Non-invasive respiratory support	104 (34.6%)	50 (36.2%)	54 (33.3%)	58 (42%)	46 (41.1%)
Adjusted	-	-	-	-	-
Ocular congestion	8 (2.7%)	2 (1.4%)	6 (3.7%)	3 (2.2%)	5 (4.5%)
Adjusted	-	-	-	-	-
Pulse rate (beats/minute)	81.91 ± 21.85	80.15 ± 24.37	83.53 ± 19.19	79.79 ± 26.37	84.51 ± 14.17
Adjusted	81.98 ± 21.28	78.98 ± 25.59*	84.68 ± 16.20	81.11 ± 25.34*	82.94 ± 15.76
Sinusoidal heart rhythm	217 (72.3%)	99 (71.7%)	118 (72.8%)	127 (92.7%)	102 (91.9%)
Adjusted	-	-	-	-	-
Acute failure syndrome	5 (1.7%)	2 (1.4%)	3 (1.8%)	3 (2.2%)	2 (1.8%)
Adjusted	-	-	-	-	-

*SBP, systolic blood pressure; DBP, diastolic blood pressure; Spo2, saturation of peripheral oxygen. Qualitative variables are reported as frequency (percent). Quantitative variables are reported as mean ± SD. P-value is reported based on one-way ANOVA test. *P-value is <0.05 and considered as statistically significant.*

*Data are presented as $\bar{x} \pm s$. Model 1: The P-values were controlled by inpatient, gender, age, history and current onset of diabetes, hypertension, hyperlipidemia, ischemic heart disease, cerebrovascular accident, lung disease, kidney failure, psychiatry disease, obesity, immune deficiency disease, cardiovascular disease, transplant, chronic kidney disease, coronary artery disease, hypothyroid, asthma, congestive heart failure, osteoarthritis, Alzheimer's disease, rheumatoid arthritis, chronic obstructive pulmonary disease, end-stage renal disease, coronary heart disease, Parkinson's, hepatitis B, deep vein thrombosis, meningitis and taking hydroxychloroquine, azithromycin, heparin, oseltamivir, Kaletra, ribavirin, favipiravir, remdesivir, atazanavir, intravenous immune globulin, ivermectin, anticoagulants, psychotropic drugs body mass index, hemoglobin, nutrition overall score, existence of respiratory support, fever, blood oxygen level, partial pressure of carbon dioxide, albumin, vitamin D, and c-reactive protein which were included as covariates. P-value is reported based on one-way ANOVA test. *P-value is <0.05 and considered as statistically significant.*

(38). However, findings in this regard are inconsistent and some studies have shown that vitamin D supplementation has no effect on ventilator requirement, length of hospital stay, or mortality (59–62). Several studies have examined the association between zinc and clinical parameters. The effect of zinc supplementation on improving survival rate and reducing the length of hospital stay has been shown (63). Similarly, findings from a meta-analysis of 28 clinical trials in patients unlikely to be zinc deficient showed that zinc supplementation orally or intranasal reduced the symptoms of respiratory tract infections and improved the symptoms earlier (64). Likewise, zinc sulfate supplementation, as adjunctive therapy in critically ill patients, reduces 30-day mortality and has a potentially beneficial effect on kidney health (63). Zinc mediates mentioned desired effects by enhancing the anti-infective properties of basophils, eosinophils, and neutrophils (65). Moreover, according to *in vitro*

TABLE 5 | Length of hospitalization in different supplement intake categories.

	Total	Recent supplement intake		Total	Long-term supplement intake	
		Yes	No		Yes	No
Crude	6.93 ± 4.99	6.99 ± 4.79	6.85 ± 5.22	6.93 ± 4.99	6.94 ± 5.12	6.91 ± 4.88
Model 1	6.42 ± 4.52	6.54± 4.20	6.30 ± 4.84	6.42 ± 4.52	6.43 ± 4.48	6.41 ± 4.57
Model 2	6.67 ± 4.57	6.68 ± 4.23	6.67 ± 4.91	6.67 ± 4.57	6.68 ± 4.52	6.67 ± 4.62
Model 3	6.53 ± 3.82	6.36 ± 3.32*	6.71 ± 4.33	6.53 ± 3.82	6.29 ± 4.13*	6.74 ± 3.55

*Data are presented as $\bar{x} \pm s$. Model 1: The P-values were controlled by gender, age, history and current onset of diabetes, hypertension, hyperlipidemia, ischemic heart disease, cerebrovascular accident, lung disease, kidney failure, psychiatry disease, obesity, immune deficiency disease, cardiovascular disease, transplant, chronic kidney disease, coronary artery disease, hypothyroid, asthma, congestive heart failure, osteoarthritis, Alzheimer's disease, rheumatoid arthritis, chronic obstructive pulmonary disease, end-stage renal disease, coronary heart disease, Parkinson's, hepatitis B, deep vein thrombosis, meningitis and taking hydroxychloroquine, azithromycin, heparin, oseltamivir, Kaletra, ribavirin, favipiravir, remdesivir, atazanavir, intravenous immune globulin, ivermectin, anticoagulants, and psychotropic drugs which were included as covariates. Model 2: The P-values were controlled by body mass index, hemoglobin, and nutrition overall score which were included as covariates additionally. Model 3: The P-values were controlled by existence of respiratory support, fever and blood oxygen level, partial pressure of carbon dioxide, albumin, vitamin D, and c-reactive protein in addition to the above variables which were included as covariates. P-value is reported based on a one-way ANOVA test. *P-value is <0.05 and considered as statistically significant.*

research, it may inhibit RNA polymer, which needs further study (66, 67).

Various vitamins are involved in different stages of the immune response by the following possible mechanisms: Vitamin A plays a key role in boosting the function of innate immune cells, such as neutrophils, macrophages, NK cells, and boosting the antibody response (68, 69). Vitamins B6 and B12 also have a positive effect on the adaptive immune response by increasing the number of lymphocytes and enhancing the maturation of lymphocytes (70, 71). Vitamin C stimulates phagocytes and T lymphocytes and protects them against oxidative stress (68, 72). Studies have also shown that vitamins A, B, C, and E prevent cell tissue damage from the virus, and a deficiency in these vitamins can cause NK cells to malfunction (73, 74).

The results of our study showed that people with higher education have significantly more vitamin and mineral supplement intakes in the last 2 months. This is in agreement with Poland cross-sectional study which found that vitamin and mineral supplements, including vitamin D and zinc, were significantly higher in people with a medical degree (75).

Possible confounding variables were adjusted in three phases. One of the factors that may affect the laboratory indices and duration of hospitalization is medication. We examined the interaction of drugs with supplements in such a way that in a separate model, we performed statistical adjustments. If the effect of the interaction of drugs with supplements was significant, then the effect would disappear. In model 1, where the variables of gender, age, history and the current onset of diseases, and history and current medications were adjusted, no significant relationship was observed between supplementation and length of hospital stay. Furthermore, in model 2, no significant association was found following the adjustment of the covariates body mass index, hemoglobin, and nutrition overall score, additionally. Finally, after performing multiple variable adjustments in model 3, we could not find a practical significant favorable relationship between recent and long-term use of supplements with the length of hospital stay in COVID-19 patients. As we know, the nutritional status of the hospitalized person has a significant relationship with the duration of the patient's hospitalization; however, in our study, supplementation has not reduced the number of hospitalization days by improving the nutritional status. Overall, further studies appear to be needed to examine the association of recent and long-term adjuvant supplementation with the length of hospital stay in COVID-19 patients.

The main strength of this study was that it thoroughly investigated the relationship between the intakes of various immune-modulating supplements instead of one particular supplement with the laboratory indices of COVID-19 and the length of hospital stay. Another strength of this study is the complete study of the relationship between the use of supplements in short term and long term with various laboratory indicators of COVID-19 patients. In this cross-sectional study, some limitations should be considered in interpreting our results. According to the cross-sectional design of our study, the cause and effect relationship cannot be deduced from this study. However, it should be noted that a detailed analysis of the findings of the cross-sectional study can be used as a first step to determine the relationship between supplementation and length of hospital stay. Another limitation of this study was that it was conducted in a specific hospital. However, this limitation has been modified due to appropriate random selection and a sample size of 300 people. Furthermore, each questionnaire has its own biases that should be considered. For instance, due to the retrospective nature of our questionnaires and reliance on patients' memory, in some cases, the use of some supplements in the last 2 months and during the year before hospitalization may not be reported accurately. However, as we studied the intake of a large number of supplements, the effect of this error on our results is minimized. Similar studies may show different results due to the time-consuming process of our study and the rapid emergence of new strains of the COVID-19 virus.

CONCLUSION

In conclusion, although practically the length of hospital stay was the same in both groups of supplement consumers and others, immune-boosting supplements were associated with improved several laboratory indices. The COVID-19 pandemic constantly raises the question of whether we need to take immune-boosting supplements. Another ambiguity is whether people who

take immune-boosting supplements are less likely to develop the disease, which in our study 54% and 46% of COVID-19 patients had used the supplements in the last 2 months and year, respectively. Apart from the possibility of contracting the disease, there is another question whether patients with a history of supplementation are in a better condition in terms of complications and symptoms, length of hospital stay, laboratory, and paraclinical parameters, which this study can answer all these questions.

However, due to the cross-sectional nature of our study which cannot reveal causal relationships and given that new strains of COVID-19 are developing constantly, further longitudinal studies, concentrating on different types of supplements, seem to be essential.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Tehran University of Medical Sciences. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LA provided the idea for the study and supervised the study. LA, RH, and ZS designed the study settings. ZS drafted the proposal for this project and contributed to preparing the questionnaires. RH contributed to sampling, data collection, completing all the questionnaires related to this research, extracting data from the medical records, and entering the data into the software. LA and MM performed the statistical analysis. MM drafted the article and wrote the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.834826/full#supplementary-material

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Vitamin D for Recovery of COVID-19 in Patients With Chronic Kidney Disease

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The severity of coronavirus disease 2019 (COVID-19) is determined not only by viral damage to cells but also by the immune reaction in the host. In addition to therapeutic interventions that target the viral infection, immunoregulation may be helpful in the management of COVID-19. Vitamin D exerts effects on both innate and adaptive immunity and subsequently modulates immune responses to bacteria and viruses. Patients with chronic kidney disease (CKD) frequently have vitamin D deficiency and increased susceptibility to infection, suggesting a potential role of vitamin D in this vulnerable population. In this paper, we review the alterations of the immune system, the risk of COVID-19 infections and mechanisms of vitamin D action in the pathogenesis of COVID-19 in CKD patients. Previous studies have shown that vitamin D deficiency can affect the outcomes of COVID-19. Supplementing vitamin D during treatment may be protective against COVID-19. Future studies, including randomized control trials, are warranted to determine the effect of vitamin D supplementation on the recovery from COVID-19 in CKD patients.

Keywords: chronic kidney disease, COVID-19, immune system, treatment, vitamin D

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INTRODUCTION

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused the major devastating coronavirus disease 2019 (COVID-19) pandemic. Although the symptoms of most infected patients are mild to moderate, some may quickly progress to a life-threatening condition (1). SARS-CoV-2 can cause lung tissue damage, resulting in acute respiratory distress syndrome (ARDS), which is usually accompanied by sepsis and septic shock, both of which are leading causes of mortality (2). ARDS is a consequence of uncontrolled inflammatory cytokine production and oxidative stress in the lungs following viral infection (3). Chronic kidney disease (CKD) is characterized by the retention of uraemic toxins and cytokines, leading to the coexistence of immunoactivation and immunodepression, and is a major risk factor for poor prognosis of COVID-19. Therefore, it is crucial to discern the immunopathologic process underlying SARS-CoV-2 infection to identify appropriate management strategies for COVID-19 in this vulnerable population.

Although specific antiviral agents against SARS-CoV-2 have been approved by the US Food and Drug Administration, an effective cure remains lacking. Thus, there is an urgent need to seek alternative and timely treatments for the disease. Since patients with CKD usually have suboptimal nutrient intake and chronic systemic inflammation, supplementing certain nutrients may be helpful

in recovery from COVID-19. Vitamin D, a widely available, inexpensive and harmless supplement, may potentially have a significant effect on reducing COVID-19 severity in CKD patients. This review was designed to discuss the pathophysiology that may occur in CKD patients with COVID-19 and how vitamin D administration can contribute to modulating the immune system and alleviating the pathological consequences of COVID-19.

ALTERATIONS OF THE IMMUNE SYSTEM IN CKD PATIENTS

Normal Immune Reaction

There are two major subsystems of the immune system, the innate and adaptive immune systems. The innate immune system is a primary defense mechanism against invading organisms and consists of cellular and humoral defenses against pathogens. The cellular components involve a variety of different types of leukocytes, including monocytes, macrophages, neutrophils, dendritic cells, endothelial cells, and humoral components such as C-reactive protein, lysozymes, and complement. Innate immune cells can recognize invading microorganisms via pathogen-recognition receptors (PRRs), namely, Toll-like receptors (TLRs), which then stimulate immune cells to release cytokines and various antimicrobial peptides (AMPs) (4, 5). The adaptive immune system acts as a second line of host defense and comprises the immune response through the activation of antigen-presenting cells (APCs), such as dendritic cells, and the antigen recognition cells, T and B lymphocytes. Adaptive immune cells learn to recognize foreign molecules the first time they are encountered, retain their memory and identify these molecules in subsequent encounters. APCs use major histocompatibility complex (MHC) molecules to present antigens and interact with naïve T cells that are converted to activated effector T cells. There are three subsets of effector T cells: cytotoxic T cells and T helper 1 and 2 cells. T helper 2 cells can stimulate B cells to produce antibodies.

CKD and Immune Dysfunction

In addition to removal of metabolic waste materials and medicines from the body, the kidneys play an important role in the clearance of circulating cytokines and bacterial toxins and in the continuous sampling of blood-borne proteins, contributing to homeostasis of the immune system. A decline in renal function that persists for >3 months is referred to as CKD and is associated with profound alterations in immune function, including immune activation, marked by systemic inflammation and acquired immunosuppression (6). Systemic inflammation leads to atherosclerosis, cardiovascular disease, cachexia and anemia, whereas immunosuppression contributes to poor vaccination response and increased incidence and susceptibility to severe infections. In CKD, neutrophils and monocytes display an exaggerated response to stimulation, an increase in TLR expression, and defective phagocytic function. The number of dendritic cells is reduced, as is the expression of MHC class I and class II and costimulatory molecules, leading to an impaired capacity to activate T cells. CKD is also associated with a decreased number of naïve T cells together with an increase in the number of terminally differentiated T cells, which represent a proinflammatory phenotype (7). The number of naïve B cells in CKD patients also decreases due to an increased rate of apoptosis, leading to impaired humoral immunity (8). Collectively, these alterations contribute to compromised innate and adaptive immune responses and chronic-low grade inflammation in CKD patients.

The Risk of COVID-19 Infection in CKD Patients

Many underlying medical conditions have been linked to increased severity and mortality of COVID-19. Male sex, older age, current smoking habit, obesity, elevated D-dimer level, diabetes, hypertension, chronic obstructive pulmonary disease, malignancies, cardiovascular disease, and CKD have been reported to be risk factors associated with fatal outcomes of COVID-19 (9). CKD patients have increased susceptibility to infections due to an attenuated response of both the innate and adaptive immune systems, consequently leading to worse outcomes of COVID-19 compared with those of patients without CKD. Patients with CKD have an elevated risk of hospitalization, severe disease course and COVID-19-related death (10). In systematic reviews comparing CKD subgroups, a higher risk of COVID-19 mortality was observed with higher stages of CKD (10). In addition, patients with end-stage kidney disease are especially susceptible to SARS-CoV-2 infection and severe COVID-19 (11). CKD has emerged as the most prevalent risk factor for severe COVID-19, preceded only by age (12).

IMPACT OF VITAMIN D ON THE IMMUNE SYSTEM AND RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM (RAAS)

Vitamin D and the Immune System

Vitamin D is a fat-soluble essential vitamin that has a substantial role in the modulation of both innate and adaptive immune responses (13). Vitamin D receptors and 1α-hydroxylase are present in several immune cells, including neutrophils, macrophages and dendritic cells, suggesting the effects of vitamin D on the immune system beyond the musculoskeletal system (14). TLR binding enhances the expression of both 1-α-hydroxylase and the vitamin D receptor, leading to the production of AMPs, including cathelicidin and β-defensin 4 (15). Cathelicidins and β-defensins are important AMPs that enhance immune responses by not only the elimination of pathogenic microbes but also the release of chemoattractants, inducing the recruitment of neutrophils, monocytes, and other immune cells to inflammation sites. Vitamin D is also involved in reducing the cytokine storm induced by the innate immune system. Moreover, vitamin D decreases the maturation and antigen-presenting ability of dendritic cells, leading to alterations of the profiles of T helper cells (Th1, Th2, Th9, Th17) and regulatory T cells, leading to overall suppression of the adaptive immune pathway (16). Regarding B cells, vitamin D is involved

in inhibition of the generation of both memory and plasma cells, as well as a reduction in immunoglobulin production by inducing apoptosis of immunoglobulin-producing B cells (17).

Vitamin D and RAAS

Renin is secreted from the kidneys and cleaves angiotensinogen secreted from the liver to the inactive form angiotensin I. Angiotensin I is subsequently cleaved by angiotensin-converting-enzyme (ACE) into active angiotensin II, which binds to two target receptors, angiotensin receptor 1 or 2 (AT1R or AT2R), to exert its effects (18). As a key component of the RAAS, ACE2 cleaves angiotensin I into angiotensin 1–9, which exerts cardioprotective effects by binding to AT2R, and angiotensin II into angiotensin 1–7, which acts on the MAS receptor pathway, counterbalancing the effect of angiotensin (19). Patients with vitamin D deficiency have increased RAAS activity and angiotensin II levels (20, 21). This finding has been confirmed by several studies showing that vitamin D is a negative regulator of the renin gene, leading to reduced renin synthesis independent of angiotensin II (22, 23). Other studies have shown that vitamin D can enhance the expression of ACE2 and inhibit the activation of NF-κB, a transcription factor that regulates multiple aspects of the immune response in infection (19, 24).

VITAMIN D ACTION IN THE PATHOGENESIS OF COVID-19 AMONG CKD PATIENTS

COVID-19 Pathogenesis

SARS-CoV-2 binds to target cells via ACE2, which is present in the epithelial cells of the lungs, kidneys, intestines, and blood vessels (25). Once SARS-CoV-2 enters target cells, the virus can trigger an innate or adaptive immune response. TLRs expressed on immune cells have the capacity to recognize viruses, leading to the production of interferon (INF) (26). SARS-CoV-2 may dampen antiviral IFN responses by immune evasion, resulting in uncontrolled virus replication, the subsequent infiltration by neutrophils and monocytes/macrophages and the increased release of proinflammatory cytokines. Additionally, the antigen of SARS-CoV-2 is presented by MHC and then recognized by cytotoxic T lymphocytes (26). Activation of specific lymphocyte T helper cells (Th1/Th17) may also lead to aggravated inflammatory responses. Viral antigens can also be recognized by B cells/plasma cells, which are activated to produce specific antibodies to neutralize SARS-CoV-2 and provide systemic immunity in different organs. Collectively, the overall immune response involves the sustained production of proinflammatory cytokines such as TNF-α, IL-6, and IFN-α/-γ, resulting in "cytokine storm," accompanied by a reduction in anti-inflammatory cytokine levels (27). Overwhelming viral replication together with cytokine storm induces considerable damage to bodily tissues with endothelial injury and thrombotic microangiopathy, leading to ARDS, respiratory failure, sepsis, heart failure and thrombotic complications, which have been reported as the most common causes of death.

ACE2 not only serves as the point of cellular entry for SARS-CoV-2 but also might be involved in COVID-19 pathogenesis. Upon infection, SARS-CoV-2 downregulates ACE2 expression, leading to decreased downstream conversion of angiotensin II to angiotensin 1–7 and angiotensin 1–9 (28). This imbalance in RAAS regulation increases angiotensin II concentrations and upregulates the AT1R pathway, resulting in excessive production of proinflammatory cytokines and chemokines and the subsequent initiation of cytokine storm (29).

Vitamin D Deficiency in CKD Patients

Vitamin D is produced predominantly in skin exposed to ultraviolet B radiation, and only 10% is absorbed from the diet. The kidneys play an important role in the metabolism of vitamin D in the body. It is hydroxylated first to 25-hydroxyvitamin D in the liver and then to its active form, 1,25-dihydroxyvitamin D, in the kidney by the enzyme 1α-hydroxylase. Cholecalciferol/ergocalciferol synthesis in the skin is reduced in patients with uraemia due to skin discolouration or hyperpigmentation and reduced exposure to sunlight. Additionally, dietary restriction and protein-energy wasting may also lead to decreased vitamin D intake. CKD patients also have reduced 25-hydroxyvitamin D levels because of a lack of its precursor, urinary loss in nephrosis, and sequestration in the body fat compartment due to a higher percentage of obese patients in the CKD population (30). Furthermore, the 1,25-dihydroxyvitamin D level is reduced in CKD patients because hyperphosphataemia, metabolic acidosis, and elevated levels of fibroblast growth factor 23 can suppress 1α-hydroxylase activity. Finally, a decrease in the number of functioning renal tubules results in lower 1,25-dihydroxyvitamin D production in patients with advanced CKD (31).

Vitamin D and Respiratory Tract Infections

Several studies have shown that vitamin D deficiency is associated with higher susceptibility to serious viral respiratory tract infections (13). The potential benefits of vitamin D on immune modulation are due to crosstalk between vitamin D metabolism and signaling and both innate and adaptive immunity. Recent evidence has shown that vitamin D promotes immune sensing of respiratory viral infections, including influenza A and B, parainfluenza 1 and 2, and respiratory syncytial virus (32). A systematic review of clinical studies showed evidence linking low vitamin D levels and increased risk of both upper and lower respiratory tract infections, suggesting a role of vitamin D in the prevention of respiratory tract infections (33). Additionally, vitamin D deficiency can amplify the risk of non-COVID respiratory tract infections (34–37) and has been associated with worse outcomes (38). However, randomized controlled trials addressing the hypothesis that vitamin D could reduce the risk of respiratory tract infections have obtained conflicting results, possibly due to the different dosing regimens of vitamin D administered and serum levels of vitamin D at baseline (33).

Vitamin D and COVID-19

Vitamin D may provide protection against COVID-19 infection and modulate the severity of its outcome (39, 40). Previous

studies have demonstrated that SARS-CoV-1 inhibits type 1 IFN receptors, which can attenuate host innate immune responses (41). Calcitriol can bind to the vitamin D receptor to enhance the type 1 IFN response and improve the innate immune response (42). The production of AMPs in airway epithelial cells is induced by vitamin D, making infection with SARS-CoV-2 and the development of severe COVID-19 less likely (43). Vitamin D might help to reduce the inflammatory response to infection with SARS-CoV-2 (13). As a binding protein for viral entry into host cells, the expression of ACE2 decreased during SARS-CoV-2 infection. Vitamin D can modulate the RAAS pathway and increase ACE2 expression, thus potentially protecting against severe lung injury (44). In addition, vitamin D acts as a negative acute phase reactant in most acute and chronic inflammatory diseases (45). Vitamin D can acidify endolysosomes and enhance autophagy, and thus, it might promote SARS-CoV-2 degradation (46, 47). Notably, calcium signaling plays important roles in virus entry and gene expression, and the alteration of calcium homeostasis in host cells can benefit virus lifecycles (48). Several studies have reported that hypocalcaemia is commonly observed among COVID-19 patients with severe disease (49, 50). In the setting of chronic vitamin D deficiency, the presence of acute hypocalcaemia in COVID-19 contributes to a unique osteometabolic phenotype (51). Altogether, the aforementioned findings have led to the hypothesis that vitamin D deficiency may increase the risk of COVID-19 infection and predispose patients to worse outcomes.

EFFECT OF VITAMIN D SUPPLEMENTATION ON COVID-19

Vitamin D Levels

Several observational studies have assessed the correlation between vitamin D levels and outcome of COVID-19. Evidence has suggested that vitamin D deficiency is associated with susceptibility to COVID-19 infection (52–55). Most studies suggested that low vitamin D levels among COVID-19 patients increase the probability of hospitalization, severity of disease, and risk of mortality (56–61). In contrast, other studies did not find any correlation between vitamin D and COVID-19 (62–64). These findings suggest that vitamin D supplementation may be a potential adjunct treatment to reduce the risk for SARS-CoV-2 infection and COVID-19. Of note, several factors may influence the assessment of vitamin D levels, such as acute critical illness. 25-hydroxyvitamin D levels are usually lower in patients hospitalized with COVID-19, and blood samples collected during critical illness may be prone to spurious correlations. Additionally, studies using retrospectively obtained vitamin D levels before hospitalization may have bias, as these patients have increased interaction with healthcare providers (65).

Clinical Studies in the General Population

Currently, the number of clinical trials investigating the effects of vitamin D supplementation on recovery from COVID-19 infection is limited but increasing. The results of observational studies assessing the role of vitamin D supplementation in COVID-19 patients remain a matter of debate, wherein there is a higher risk of mortality among patients with calcifediol supplementation (66, 67), a lack of association between vitamin D supplementation and clinical outcomes (64), and improved clinical outcomes among patients supplemented with vitamin D (68–71). Some clinical trials also showed improved survival of COVID-19 patients who were supplemented with vitamin D (72, 73). To date, only 11 randomized controlled trials assessing the effect of therapeutic vitamin D supplementation on COVID-19 patients have been published; however, the role of vitamin D in disease severity remains controversial (42, 74–83). Most of these trials showed beneficial effects on a reduction in inflammatory markers, improvements in immune function, SARS-CoV-2 viral clearance, recovery of symptoms, and reductions in the severity of the disease and in-hospital mortality (42, 74, 75, 77–80). Nevertheless, other studies demonstrated contradictory data that vitamin D supplementation did not improve inflammatory markers or reduce the length of hospital stay, admission to the intensive care unit or mortality (76, 81–83). Most of these trials were limited by variable vitamin D absorption and sun exposure because of different ethnic groups, seasons, and geographic latitudes in the study population. Additionally, the supplemented dosage of vitamin D varies in these studies, as do the assays used for laboratory measurement of vitamin D levels. Regarding systematic reviews and meta-analyses, a previous review that included three studies did not show the effectiveness of vitamin D supplementation on mortality in COVID-19 patients (84). However, a recent meta-analysis found that vitamin D supplementation may be beneficial for clinical outcomes of COVID-19, especially when treatment begins following the diagnosis of COVID-19 (85).

Clinical Studies in CKD Patients

Currently, clinical studies evaluating the effects of vitamin D supplementation on COVID-19 in patients with CKD are extremely limited because this specific population is often excluded from ongoing clinical trials. To our knowledge, only four observational studies have been published (**Table 1**). In a case series of non-vaccinated haemodialysis patients with SARS-CoV-2 infection, chronic active vitamin D treatment was associated with a reduced risk of severe COVID-19 (88). Another retrospective observational study conducted on haemodialysis patients with COVID-19 showed that those who received paricalcitol, calcimimetics or the combination of both exhibited improved survival (87). In a large population-based cohort study, calcitriol supplementation showed a beneficial effect on a reduction in disease severity and mortality of COVID-19, particularly in patients with advanced CKD (86). Last, a recent retrospective cohort study showed that there was a trend toward a reduction in COVID-19 mortality among patients with advanced CKD supplemented with calcifediol (89). Among these studies, three showed significant beneficial effects of calcitriol or vitamin D receptor activator analog (paricalcitol) while one study demonstrated the potential role of calcifediol. In CKD, hypocalcaemia and 1,25-dihydroxyvitamin D deficiency contribute to the development of secondary hyperparathyroidism and consequently mineral and bone disorders that are frequently treated with active

TABLE 1 | Clinical studies evaluating vitamin D effects on COVID-19 in CKD patients.

Reference	Design, country	Participants	Mean age, sex	Conclusions
Oristrell et al. (86)	Observational, Spain	Overall cohort: 6252 subjects on calcitriol and 12,504 matched controls Subgroup analysis: 2296 stage 4 or 5 CKD subjects on calcitriol and 3407 matched controls	70.2 ± 15.6 years old v.s. 70.7 ± 14.7 years old, female 57.5 v.s. 57.5%	Calcitriol use reduce risk of SARS-CoV2 infection, severe COVID-19, and mortality in stage 4 or 5 CKD
Arenas Jimenez et al. (87)	Observational, Spain	288 HD patients with COVID-19 Vitamin D treatment includes calcifediol, calcitriol, and paricalcitol. Calcimimetic includes cinacalcet and etelcalcetide	72.4 ± 12.6 years old, female 29.2%	Paricalcitol, calcimimetics or combination reduce mortality rate
Tylicki et al. (88)	Case series, Poland	85 nonvaccinated HD patients with COVID-19	69.74 ± 13.19 years old, female 47.06%	Chronic active vitamin D treatment reduce the risk of severe pneumonia
Oristrell et al. (89)	Observational, Spain	Overall cohort: 134,703 patients on calcifediol and 269,406 matched control Subgroup analysis: 130,323 stage 1–3 CKD subjects on calcifediol and 263,873 matched controls; 4380 stage 4 or 5 CKD subjects on calcifediol and 5533 matched controls	68.8 ± 14.9 years old v.s. 68.8 ± 15.1 years old, female 78.1 v.s. 77.9%	Calcifediol supplementation reduce risk of SARS-CoV2 infection and severe COVID-19 and a trend toward a reduction in mortality in stage 4 or 5 CKD

CKD, chronic kidney disease; COVID-19, coronavirus disease 2019; HD, haemodialysis.

vitamin D (90). However, the use of active vitamin D decreases the 1α-hydroxylase and 25-hydroxylase activity and increases the 24-hydroxylase activity, leading to increased production and decreased degradation of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D (91). Because 1-hydroxylase and ACE2 are both expressed in renal proximal tubular cells, SARS-CoV-2 infection may decrease generation of 1,25-dihydroxyvitamin D which cannot be replenished with nutritional vitamin D supplementation (cholecalciferol or ergocalciferol) (92). Additionally, calcitriol has a higher affinity for the vitamin D receptor and greater potency to exert its biological activity, including immune modulation and RAAS regulation. Collectively, supplementing active vitamin D or its analogs restores the physiologic levels of the active vitamin D hormone and may be more effective than nutritional vitamin D in CKD patients with COVID-19. Further randomized, interventional trials are warranted in patients with CKD to clarify which vitamin D supplementation would be beneficial for recovery from COVID-19.

CONCLUSIONS

As a respiratory infection, COVID-19 may affect multiple organ systems and contribute to infection-related tissue damage. Vitamin D is an immunomodulator hormone and has been shown to have protective effects against respiratory infections. Accumulating evidence has shown that vitamin D is associated with improved clinical outcomes in terms of disease severity and/or mortality during the COVID-19 pandemic, although current data from the CKD population are still limited. Patients with CKD are characterized by the coexistence of immune activation and immune deficiency and are at increased risk for vitamin D deficiency. Supplementing vitamin D in this vulnerable population may be a critical form of support and is helpful for the recovery from COVID-19. Current observational data provide avenues for further research, including randomized controlled trials, to determine the effect of vitamin D supplementation on COVID-19 among CKD patients.

AUTHOR CONTRIBUTIONS

W-FC wrote the manuscript. P-JH conceived and organized the structure of the review. J-SC contributed to the critical revision of the paper. All authors approved the final version of the manuscript and ensured the accuracy and integrity of the work.

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REVIEW
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A Review of the Potential Effects of Melatonin in Compromised Mitochondrial Redox Activities in Elderly Patients With COVID-19

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Melatonin, an endogenous indoleamine, is an antioxidant and anti-inflammatory molecule widely distributed in the body. It efficiently regulates pro-inflammatory and anti-inflammatory cytokines under various pathophysiological conditions. The melatonin rhythm, which is strongly associated with oxidative lesions and mitochondrial dysfunction, is also observed during the biological process of aging. Melatonin levels decline considerably with age and are related to numerous age-related illnesses. The signs of aging, including immune aging, increased basal inflammation, mitochondrial dysfunction, significant telomeric abrasion, and disrupted autophagy, contribute to the increased severity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. These characteristics can worsen the pathophysiological response of the elderly to SARS-CoV-2 and pose an additional risk of accelerating biological aging even after recovery. This review explains that the death rate of coronavirus disease (COVID-19) increases with chronic diseases and age, and the decline in melatonin levels, which is closely related to the mitochondrial dysfunction in the patient, affects the virus-related death rate. Further, melatonin can enhance mitochondrial function and limit virus-related diseases. Hence, melatonin supplementation in older people may be beneficial for the treatment of COVID-19.

Keywords: COVID-19, melatonin, mitochondria, mitophagy, oxidative stress, SARS-CoV-2

INTRODUCTION

The new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for causing coronavirus disease (COVID-19). Regardless of the patient's age, SARS-CoV-2 increases the clinical morbidity and mortality of individuals with underlying chronic diseases or poor immunity. This is especially true for the elderly. Although the virulence of SARS-CoV-2 is high, pathogen–host

interactions result in significant differences in the severity of the illness, its complications in humans, and survival outcomes. Previous studies have proposed that components of aging, which are interactive with COVID-19, can affect the health of older people through mechanisms that regulate the immune system (1, 2).

Aging is a complex and multifactorial process (3). As cells age, their ability to cope with external and internal stress and their functionality gradually diminish, whereas disease morbidity and mortality gradually increase. Age-related immune system degeneration, known as immune senescence, increases the sensitivity of the elderly to infectious diseases, autoimmune diseases, and cancer. As a part of the normal physiological function for fighting pathogens, the mitochondria generate low levels of reactive oxygen species (ROS) (4). However, excessive ROS production can be as damaging as coronavirus infection (5). Many viruses also induce mtROS production, which can aid viral replication and the release of progeny (6). The increase in ROS production is related to age-related diseases and a reduced lifespan (7). Furthermore, the ROS detoxification system significantly weakens with age. A reverse correlation between glutathione peroxidase (GSH-Px) and age has also been found (8). Aging damages the mitochondrial electron transport chain (ETC). The induction of stress in the endoplasmic reticulum (ER) damages the ETC in human mitochondria, which are in close contact with the ER. ER stress gradually increases with age and leads to mitochondrial dysfunction. Hence, the reduction of ER stress is a potential strategy for enhancing the function of the cardiac mitochondria in the elderly (9). Autophagy is also an important protein degradation process in normal cellular metabolism. Therefore, the reduction in autophagy owing to aging can aggravate the severity and prognosis of COVID-19 in elderly patients, since worsening age-related autophagy accelerates viral infections by reducing virus degradation and mitigating innate and adaptive immunity (10).

Impaired cellular respiration reduces ATP production, increases ROS production, decreases cell detoxification, and impairs autophagy and immune dysfunction, which seem to play critical roles in the inflammatory state and disease severity in elderly patients with COVID-19. Melatonin, associated with almost all aging-related processes, is primarily released from the pineal gland and regulates the circadian rhythm. The retina, lacrimal glands, Harderian gland, gastrointestinal tract, thrombocytes, and bone marrow are other sources of melatonin (11). Thus, melatonin is a widespread physiological mediator (12). It is also an effective free radical scavenger that influences the immune system considerably (13). However, melatonin synthesis and its levels in the pineal gland and plasma gradually decrease with aging. In animals, treatment with exogenous melatonin may postpone aging and the onset of age-related illnesses (14).

The review primarily focuses on how melatonin can decrease the severity or accelerate the recovery of older adults with COVID-19 by protecting damaged mitochondria.

THE RELATIONSHIP OF SARS-CoV-2 WITH AGING, IMMUNE SYSTEM, AND OXIDATIVE STRESS

It is common knowledge that asymptomatic patients with COVID-19 can increase the risk of virus spread. This poses a serious threat to older people, who may suffer from severe complications owing to virus–host interactions (15), including sudden stroke or cardiovascular events (16). Hence, awareness of the adaptability of the immune response to SARS-CoV-2 infections can lead to better clinical care and management for older people (2, 17).

During aging, the basal metabolism and physiological function largely depend on the mitochondria. The mitochondria are the major source of ATP and are crucial for various processes in cell survival, such as β-oxidation of fatty acids, calcium signaling, generation of ROS, phospholipid biosynthesis, and apoptosis (18). Intracellular mitochondria are the main sites for oxygen consumption and ROS production; therefore, they are the prime targets of ROS-dependent cell damage. Mitochondrial respiration can also affect a cell's longevity by modifying the ROS equilibrium (19). Aging is characterized by the increased production of ROS. However, despite the current interest in antioxidant supplementation, such as CoQ10 supplementation, there is insufficient evidence to recommend CoQ10 supplementation as an anti-aging and antioxidant therapy (20). However, CoQ10 supplementation improves respiratory viral infections and the critical inflammatory status (21). With age, the mitochondrial phosphorus capacity and ATP production decline. Hence, the significant increase in energy consumption due to a cytokine storm establishes a non-adaptive state in which the metabolic reserve of the mitochondria significantly increases in elderly patients with COVID-19.

COVID-19 and Aging

Normal senescence involves the degeneration of cells, tissues, and organs, which increases mortality and morbidity in the elderly. The various aspects of aging include immune aging, inflammation and inflammasome formation, mitochondrial dysfunction, oxidative stress, telomere shortening, and impaired autophagy. Such changes cause the decline of various reserves and adaptability, all aspects of system function, in addition to poor response to the mutual influence of pressure of infection and interference with cells. The various aspects of aging also play a critical role in patients with chronic diseases (22, 23) and may affect SARS-CoV-2 infection (**Figure 1**). Hence, with the COVID-19 pandemic still at large, it is necessary to consider these characteristics when treating infected elderly patients (24).

SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) receptor and penetrates the host cell through endocytosis. Foreign nucleic acids from the invading viruses are detected by multiple intracellular pattern recognition receptors (PRRs). The PRRs include DNA and RNA receptors located in the cytoplasm as well as specific Toll-like receptors (TLRs) expressed in endolysosomal compartments. The coronavirus spike protein

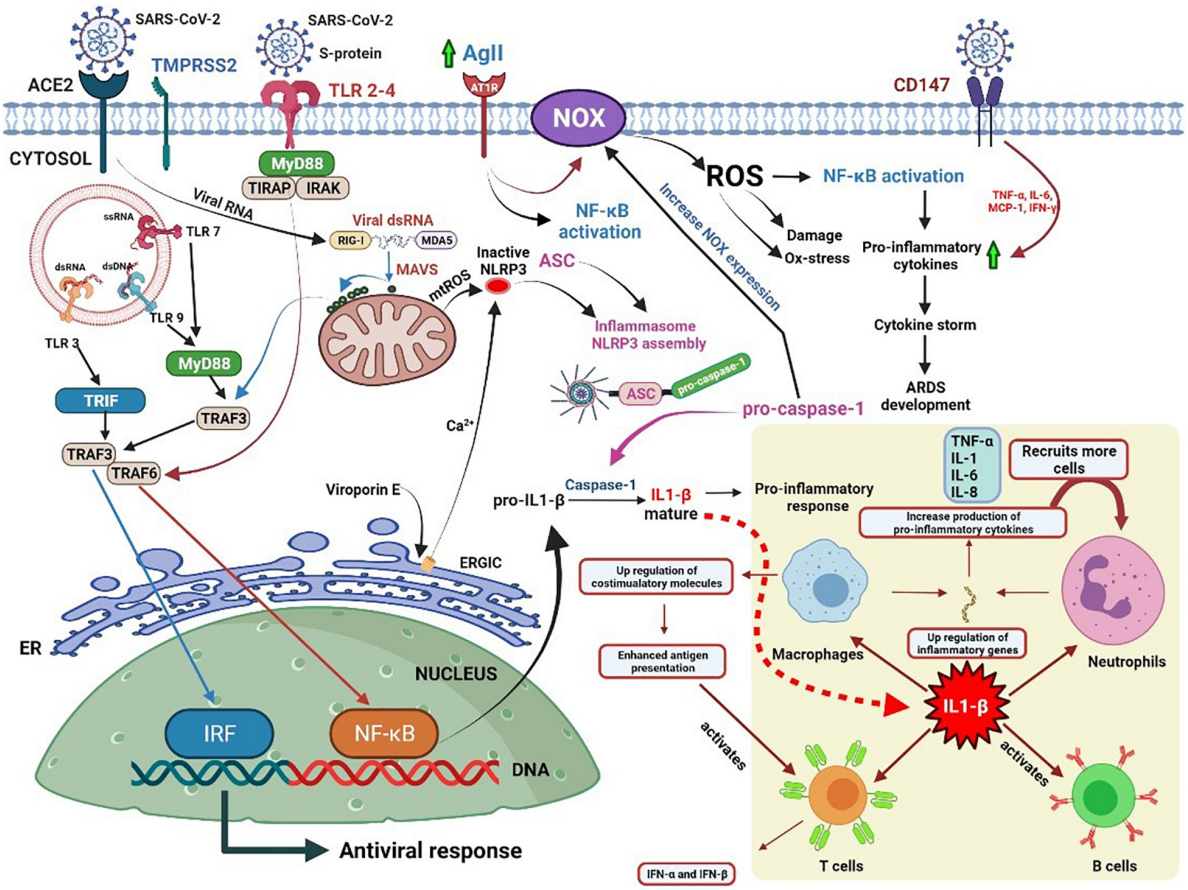

FIGURE 1 | Potential pathways of oxidative stress and the role of IL-1β in eliciting immune responses against coronavirus disease.

is recognized by TLR2/4, and the single-stranded RNA (ssRNA) is recognized by TLR7/8. Additionally, double-stranded RNA (dsRNA), which exists as an RNA virus replication intermediary, is recognized by RIG-I/melanoma differentiation-associated protein 5 (MDA5). The detection of dsRNA leads to coordination with mitochondrial outer membrane antiviral signal protein (MAVS) followed by oligomerization, attracting a variety of adaptor proteins and ultimately activating nuclear factor kappa light chain enhancer of activated B cells (NF-κB) and interferon regulatory factors (IRFs). TLR detection leads to the recruitment of adaptor proteins, which can form myddosomes to facilitate the activation of NF-κB. NF-κB signaling is closely associated with the expression of IL precursors. The activation of the nucleotide-binding oligomerization domain-like receptor (NLR) family pyrin domain-containing 3 (NLRP3) inflammasome facilitates the oligomerization and recruitment of apoptosis-associated speck-like proteins with a caspase-recruitment domain. The resultant inflammasome transforms pro-caspase-1 into active caspase-1 for further processing of pro-ILs.

Further, the stimulation of NF-κB signaling is related to an increase in NADPH oxidase (NOX) expression, which can also be induced by angiotensin II (AGII), followed by the production of ROS. However, ROS itself can induce NF-κB signal transduction. Therefore, excessive ROS production mediates excessive inflammation and the induction of cytokine storms, which might lead to the development of acute respiratory distress syndrome (ARDS) and worsen the severity of the disease (16).

The bottom right corner of **Figure 1** illustrates the role of IL-1 in initiating an immune response to infection. IL-1 enhances the extensive pro-inflammatory activity of various immune cells. It not only induces the rapid recruitment of neutrophils to the site of infection, the activation of endothelial adhesion molecules, and the induction of chemokines, but also promotes the release of various cytokines. Furthermore, it induces the proliferation of T helper cells and B lymphocytes and improves the antigen presentation capability of APCs (25).

Viroporin E, an integral ion membrane channel protein of SARS-CoV-2, can modify the ionic flux of Ca^{2+}, K^{+}, and H^{+} in the ER, which induces ROS production, autophagy, and ER stress, and drives NLRP3 activation. IL-1β induces the expression of adhesion molecules and integrins on leukocytes, endothelial cells, and other cell types, promoting cell infiltration and inflammation. IL-1 regulates neutrophil recruitment by inducing the expression of adhesion molecules (ICAM-1 and β2 integrins) and the production of local chemokines in the endothelium. It upregulates the production of IL-8, which acts as

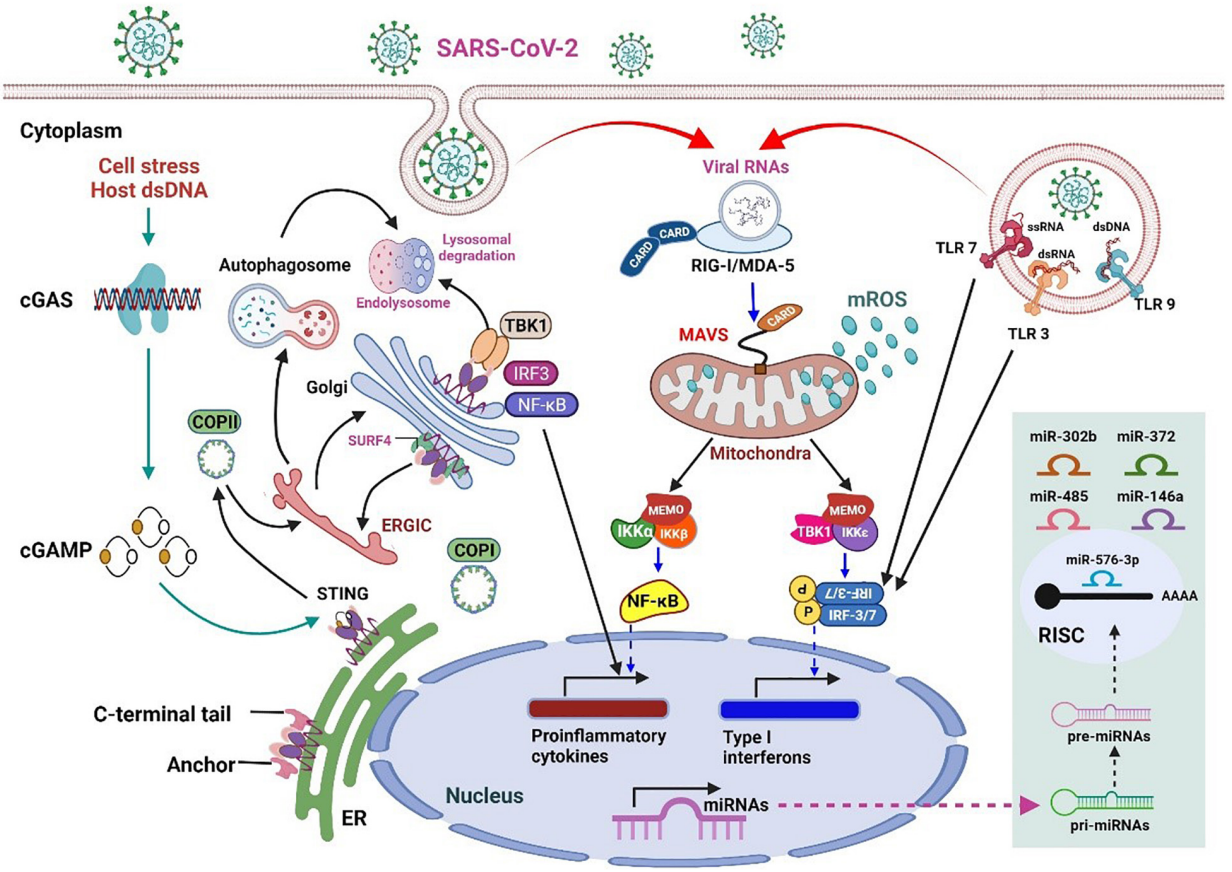

FIGURE 2 | An overview of innate immunity against RNA virus infection and the stress response to host cell infection in mammals.

a chemotactic and activating factor for neutrophils, endothelial cells, and macrophages. SARS-CoV-2 infection enhances the expression of AGII, which activates NOX through the AGII type 1 receptor, and in turn, triggers the production of ROS and subsequent damage (26). SARS-CoV-2 may recognize the CD147 receptor (a glycoprotein that can cause a cytokine storm in lung epithelial cells) on the surface of the host cell by binding the viral nucleocapsid protein to the CD147 ligand cyclophilin A (27). However, studies have shown that melatonin can reduce tissue damage by blocking the production of pro-inflammatory cytokines associated with CD147 activity in patients with COVID-19 (28).

Innate and Adaptive Immunosenescence, Inflammation, and Inflammasomes

A prominent structural protein of SARS-CoV-2 is the spike glycoprotein (S), which contains two major subunits, S1 and S2. Other coronavirus proteins include the membrane protein (M) from the host ER or Golgi apparatus, mainly responsible for virus assembly; the nucleocapsid (N), involved in genome replication; and the envelope protein (E). SARS-CoV-2 is similar to SARS-CoV-1, and the S-protein amino acids of both the viruses are nearly 80% similar (29). Furthermore, the concordance of N, M, and 3a proteins in SARS-CoV-1 and SARS-CoV-2 suggests that they share the same pathogenicity (**Figure 2**).

RNA virus infection in mammalian cells is first detected by host RRPs (RIG-I and MDA5), which initiates the retinoic acid–inducible gene-I (RIG-I)-like receptor (RLR) signaling pathway. MAVS acts as a linker for PRRs, which then activates NF-κB or IRF3/7, leading to the rapid and effective production of pro-inflammatory cytokines and IFN-I (early). During this process, the mitochondria act as a signaling platform on the outer membrane to activate various downstream molecules. The dynamic mitochondrial properties under the mitochondrial outer membrane (MOM), such as inner membrane potential ($\Delta\psi m$), oxidative phosphorylation activity, and mitochondria-related metabolites, also help fine-tune signaling events. The topological gap between mitochondria-related metabolites and MAVS is filled with the prohibitin complex, which establishes a bridge between the MOM and the mitochondrial inner membrane (MIM) when a virus infects (30). Approximately 24 h after infection (late stage), some miRNAs are significantly upregulated (**Figure 2**, light green box on the lower right), which mediate various regulatory immune responses. miRNAs, such as miR-146a and miR-576-3p, negatively regulate the antiviral

response after viral infection, which is essential to terminate RLR over-signaling.

In addition, the mitochondria are involved in the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) signaling pathway, activated by viral infection-induced host cell stress and dsDNA (31). The schematic on the left in **Figure 2** details the activation of cGAS induced by the dsDNA of the host cell owing to pathogenic infection or host cellular stress. cGAS dimers assemble upon binding to dsDNA, leading to the activation of the cGAS enzyme and the synthesis of 2′,3′ cyclic GMP–AMP (cGAMP). cGAMP binding to the STING dimer located on the ER membrane significantly changes the conformation, triggers STING oligomerization, induces release from anchoring factors such as stromal interaction molecule 1, and, along with the transport factor, gets incorporated into the coat protein complex II vesicles. While passing through the ERGIC and Golgi apparatus, STING not only recruits the TNF receptor-associated factor (TRAF) family member-associated NF-κB activator-binding kinase 1 (TBK1) and IRF3 but also promotes the phosphorylation of TBK1 and Ser366 of STING. The phosphorylation of IRF3 by TBK1 leads to the dimerization and translocation of IRF3 to the nucleus to induce the expression of IFN1, interferon-stimulated genes (ISGs), and genes encoding several other inflammatory mediators, pro-apoptotic enzymes, and chemokines. The activation of STING also activates NF-κB and the formation of light chain (LC)3+ vesicles (autophagosomes). Finally, STING in the autophagosome and those from the Golgi apparatus to the lysosome are involved in STING degradation (32).

Animal and human cells mainly use PRRs to recognize pathogen-associated molecular patterns (PAMPs) and endogenous danger-associated molecular patterns (DAMPs). There are various prominent PRRs, such as TLR, NLR, and cytoplasmic RLR, which are essential regulators of inflammatory and innate immune responses. Viral ssRNA is primarily recognized by TLR, such as TLR-7 (33), which subsequently stimulates the production of pro-inflammatory cytokines and type I and III IFNs (34). The first step in limiting viral entry or replication is the synthesis of IFNs and their release from virus-infected cells, which upregulates ISGs (35). IFNs can boost the activity of macrophages, natural killer (NK) cells, and T and B lymphocytes to inhibit virus assembly, spread, and disturbances in the immune system (36). Some studies have suggested that the coronavirus may not only antagonize the production of IFNs but also reduce the transmission of IFN signals to escape immune surveillance (37) (**Figure 1**).

The RIG-I receptor, present on the MOM, can recognize viral RNA and activate MAVS proteins. However, these proteins can also increase pro-inflammatory cytokine production by activating the NF-κB signaling pathway (38). The overall increase in baseline inflammation during general aging contributes to advanced age-related chronic diseases (39). Furthermore, viral infection can exacerbate age-related immune response injury by stimulating the inflammatory pathways. With aging, decreased innate immunity against viral infections weakens IFN synthesis from neutrophils, monocytes, macrophages, and NK cells and their subsequent response to IFN signaling (40, 41). In addition, costimulatory signals are attenuated by different APCs, which activate T cells in advanced age (42). Hence, old age leads to functional changes in these immune cells, which can result in deviant innate immune responses.

The NLRP3 inflammasome is an oligomeric complex that may promote the secretion of IL-1β and IL-18 and induce pyroptosis by defending the host from microorganisms. It has been demonstrated that coronaviruses vitalize the NLRP3 inflammasome and the NF-κB pathway (43), resulting in increased levels of TNF-α conversion enzyme (TACE), TNF receptors, and ACE2. ACE2 enhances the production of angiotensin 1–7 (44) (**Figure 1**). An earlier report revealed that ACE2 and TACE levels are associated with poor prognosis in patients with heart disease (45). However, ACE2 expression during human aging and its activity following SARS-CoV-2 infection remain unclear. COVID-19 has led us to focus on the vulnerability of aging populations to emerging diseases. This predisposition to illness and death is also a major challenge in the development of vaccines and immunotherapeutics. The efficacy of vaccines decreases significantly with age because of the gradual age-related decline in innate and adaptive immunity (46).

The adaptive immune response targets non-self-pathogens, but it may occasionally attack host cells erroneously. Adaptive immunity includes humoral and cellular immunization, which identify and respond to specific pathogens *via* B cells and T cells, respectively. After infection, B lymphocytes are activated, differentiated into plasma cells, and produce immunoglobulins. This property can be induced by cytokines generated by $CD4^+$ cells (47). As people age, there is a significant shift from naïve to memory B cells and a decline in the antigen recognition capacity and antibody production. Moreover, the long-lived plasmocyte population is reduced in response to vaccination (48). The number of naïve T cells and the synergy between T-cells and APCs, which can transform naïve T cells into memory cells, are reduced (49). Notably, an age-related gender gap has been observed in the immune system, with a higher incidence of COVID-19 in men than in women (50). The epigenetic and transcriptomic variance associated with age-dependent immune cell dysfunction may also underlie the age-related differences in the severity of COVID-19 symptoms (51).

Genomic Instability and Telomere Attrition

The accumulation of mutations in various tissues and cell types in aging humans can lead to the age-related breakdown and death of cells. Since the somatic mutation rate is considerably higher than the germline mutation rate, and the base replacement load in somatic cells is adequately high, it may affect the function of immune cells (52). By extending the cell cycle, p53 allows additional time for the repair machinery to restore genome stability and plays a leading role as a promoter of DNA repair. In the context of DNA repair mechanisms, p53 lowers stress levels and protects cells from oxidative lesions (53). In contrast, higher levels of oxidative stress (such as viral infection) will cause the continuous activation of p53 and increase the permeability of the MOM, which increases the release of cytochrome c,

ultimately causing apoptosis (1, 54). Although p53 can reduce the replication of coronaviruses by prolonging the cell cycle, papain-like protease (PLpro) produced by the coronavirus itself can degrade p53, enabling the virus to continue replicating in infected cells (55, 56). Many viruses damage the DNA of host cells, resulting in genetic instability throughout their replication cycle. DNA viruses activate and manipulate the DNA damage response. Even if they only replicate in the cytoplasm, many RNA viruses can cause severe DNA damage in host cells. DNA damage can increase the risk of RNA virus pathogenicity by triggering apoptosis, stimulating inflammatory immune responses, and introducing harmful mutations that increase tumor risk (57). Notably, some SARS-CoV accessory proteins regulate the IFN signaling pathway and the production of pro-inflammatory cytokines. For example, the coronavirus accessory protein 7a can mediate subsequent cell apoptosis by inhibiting Bcl-X_L (an anti-apoptotic protein) (58).

Telomeres (repetitive TTAGGG DNA sequence at the end of linear chromosomes) form a part of the 3D spatial organization of the nuclear genome. They stabilize the genome with high fidelity throughout early adulthood, but their effects weaken after the reproductive age (59). They also play a crucial role in maintaining the stability of the genome and regulating innate immunity while combatting viral infections. Regions close to telomeres, namely the sub-telomeres, contain GC-enriched genes that regulate innate immunity (60). Varying lengths of telomeres (61) are present in various aging cells, which may be the basis of different severities of responses to viral attacks (60). During aging, a decrease in telomerase activity occurs with the gradual shortening of telomeres; shorter telomeres are associated with more severe diseases (62). Previous studies have shown that the telomere/telomerase system can alleviate the harmful effects of aging on lymphocytes, which may help identify high-risk individuals (63). Thus, viral infection can cause telomere attrition, resulting in serious clinical consequences for the elderly with COVID-19, and telomere attrition may guide future treatments against SARS-CoV-2 and other viral pathogens.

SARS-CoV-2 AND THE MITOCHONDRIA

Under normal physiological conditions, the mitochondria play pivotal roles in metabolic oxidation (through the tricarboxylic acid cycle), ATP production (through an efficient ETC), and complete β-oxidation of fatty acids. In mammals, the mitochondria are also involved in the innate immune response against various microorganisms or toxic environmental stimuli (31). The mitochondria produce ROS at low levels, which may aid immune cell maturation and function; however, at high levels, ROS may increase oxidative damage (4). All components of the mitochondrial ETC, including complexes I, II, and III, produce ROS in the matrix or inner membrane, and mtROS may regulate NF-κB- and TNF-α-mediated cell death and aid cellular transformation. Many signaling pathways, such as hypoxia and PI3-kinase-induced mtROS production, control mtROS production, and FOXOs regulate mtROS production under oxidative stress (64). Importantly, some viral proteins may interact with the mitochondrial ETC and produce ROS, which may promote virus replication and release (6). Therefore, mtROS overproduction may be enhanced upon virus infection and aid replication to promote the survival of the virus.

The mitochondria also regulate innate and adaptive immunity, which stimulate NF-κB, the NLRP3 pathways, and IRFs. Mitochondria-mediated innate immunity is induced by activating the signaling pathways of NLRP3 inflammasomes and RLRs. MAVS and the mitochondrial components are critical for signal transduction (31). Mitochondrial DNA (mtDNA) can be regarded as a DAMP and serves as a crucial platform for signaling molecules, such as MAVS, in RIG-I signal transduction and NLRP3 inflammasomes (65).

Mitochondrial biogenesis, fusion, and fission play a role in activating immune cells. In addition, the mitochondria mediate cytotoxic responses to cellular stress (65). The combination of respiratory failure, decreased ATP production and detoxification capacity, increased ROS production, and immune dysfunction appears to contribute to the onset of fulminant inflammation and severe COVID-19 (66).

Chaperone–Protease Networks

Most mitochondrial proteins are produced in the cytoplasmic ribosomes and pass through one or two mitochondrial membranes into the intermembrane space or mitochondrial matrix (67). These proteins are unfolded, which allows them to effectively translocate across the mitochondrial membrane. Translocations catalyze these highly dynamic machines induced by potential membrane reactions, ATP, or redox reactions. They coordinate with chaperones and assembly complexes to steer mitochondrial proteins to the correct position. Translocation is carried out through tight pores formed by closely synchronized translocases, including the translocases of the outer and inner membrane complexes (TOM and TIM, respectively) (**Figure 3A**) (68). Adequate mitochondrial protein homeostasis is based on endogenous enzyme components comprising a diverse set of chaperones and proteases forming an interconnected functional network (69).

SARS-CoV-2 uses the normal protein translocation mechanism of the mitochondria to suppress the antiviral cellular immune response and effectively maintain viral replication. SARS-CoV-2 can interact with TOM components to prevent the synergistic effect between Tom70 and MAVS, suppressing the activity of mitochondrial IRF3 and subsequently reducing the induction of antiviral signal response (70). SARS-CoV polyproteins exhibit a deubiquitinating activity that attenuates the ubiquitination capacity of infected cells. The SARS-CoV nucleocapsid protein can interact with the proteasome and suppress its proteolytic activity, contributing to the inhibition of viral protein proteolysis and suppression of host misfolded protein degradation (71). **Figure 3B** illustrates the relative positions of the mitochondrial electron transport complex (CI–CV), which forms the ETC involved in oxidative phosphorylation, ultimately generating energy in the form of ATP. When electrons leak and reduce adjacent oxygen molecules to superoxide anion radicals ($O_2^{\bullet}$-), free radicals are generated. CIII secretes electrons into the matrix and inner membrane

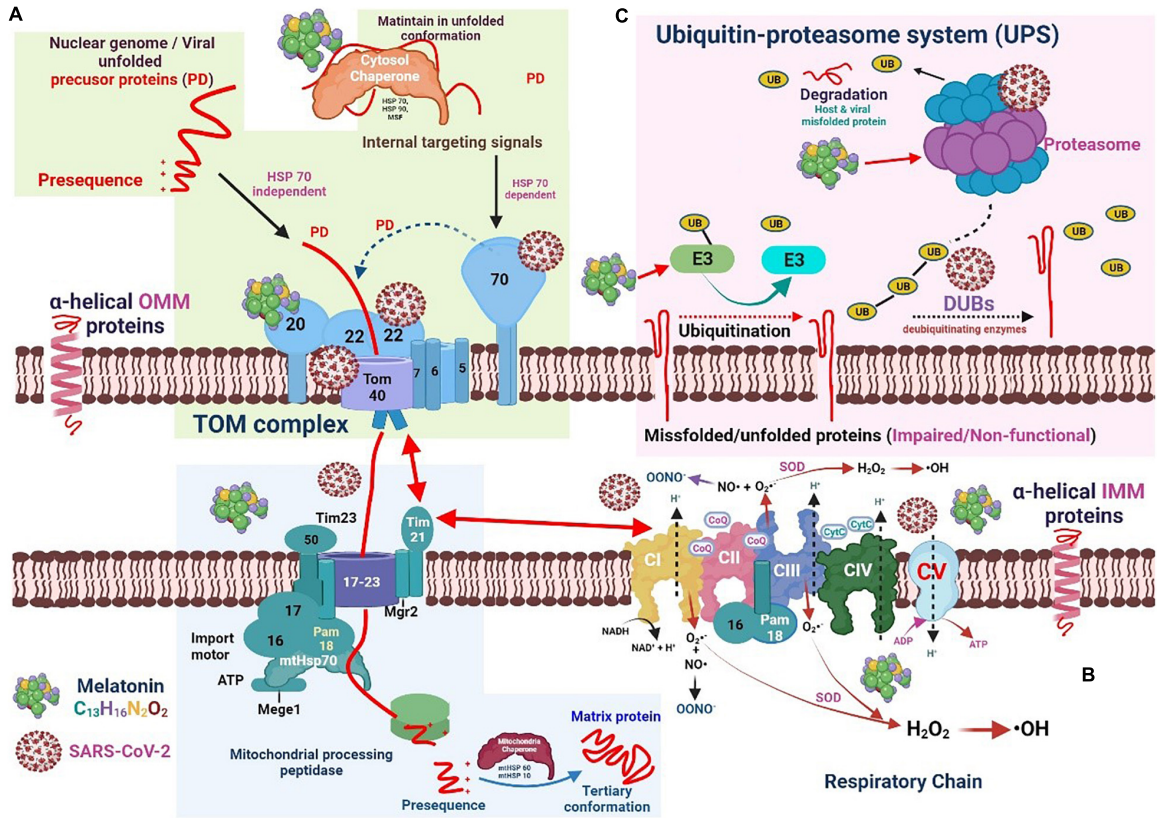

FIGURE 3 | The interaction of severe acute respiratory syndrome coronavirus 2 and melatonin with the chaperone-protease system and the respiratory electron transport chain machinery. **(A)** Viral unfolded precursor proteins translocate across mitochondrial membranes into the intermembrane space or mitochondrial matrix. Melatonin may regulate HSPs and proteasome activity which can improve mitochondrial function and restrict viral replication. **(B)** The mitochondrial electron transport complex (CI-CV) involved in oxidative phosphorylation, generating ATP. Free radicals are generated when electrons leak and reduce adjacent oxygen molecules to superoxide anion radicals ($O_2^{\bullet-}$). The pyrrole ring in melatonin may be suitable for capturing $O_2^{\bullet-}$ and •OH free radicals. **(C)** In the mitochondrial outer membrane, the ubiquitin-proteasome system (UPS) drives the quality control of proteins. Melatonin may decrease the level of downregulated protein 4-1 (E3 ligase).

space, whereas CI secretes electrons into the mitochondrial matrix. In addition, superoxide dismutase (SOD) can transform $O_2^{\bullet}$- into hydrogen peroxide by converting it to hydroxyl radicals (•OH). $O_2^{\bullet}$- can also combine with nitric oxide to generate peroxynitrite anions.

Under normal conditions, the mitochondrial precursor protein exposes the hydrophobic area, leading to unwanted protein interactions and aggregation. Thus, before TOM translocation, these unfolded precursor proteins must bind to a cytoplasmic chaperone to enter the mitochondria (72). TOM is a transport protein containing the surface receptors that recognize pre-proteins and is the main entry point of the mitochondria. Mitochondrial protein biogenesis usually involves complex folding and assembly processes for the attainment of enzymatically active states. Molecular chaperone proteins, such as heat shock protein (Hsp) 70, promote the translocation and folding of mitochondrial proteins (69). The chaperone activity of heat shock cognate protein 70 (Hsc70), Hsp70, and Hsp90 is derived from the collaborative cycle of ATP hydrolysis and substrate binding and is regulated by numerous accessory chaperone proteins. These chaperones transfer the precursor protein to the Tom70 receptor by recognizing the signal embedded in the membrane-targeted precursor (73). Mitochondrial precursor proteins can be attached by an Hsp70-dependent (Tom20 and Tom70) or Hsp70-independent (Tom20 only) manner and then pass through the Tom40 tunnel (74). At this point, the cytosolic chaperon mitochondrial import stimulant factor binds with Tom70 and transfers the precursor protein to Tom20 and Tom22, which uses energy from ATP hydrolysis (74). When transferred through TOM, the pre-sequence protein is directed toward the Tim23 complex, essential for importing cleavable pre-proteins into the mitochondria. Tim23 (the Tim21 and Mgr2 subunits) is involved in the interaction between the TOM and respiratory chain complex. Pre-proteins contain amino-terminal targeting sequences removed through mitochondrial processing peptidase and/or internal membrane protease (IMP). The cytosolic precursor of Mgr2 contains a carboxy-terminal sequence that promotes mitochondrial targeting, whereas Mgr2 is treated by IMP (75).

The presequence translocase-associated motor (PAM) transports precursor proteins into the mitochondrial matrix,

mostly in an ATP-dependent manner. Tim44 is a subunit protein in the mitochondrial matrix that can bind to the Tim17–Tim23 complex core. In addition, Tim44 interacts with mitochondrial heat shock 70 kDa protein (mtHsp70) and recruits chaperones and their associated proteins into the translocation channel (76). Pam18 and Pam16 subunits form a stable heterodimer, which may stimulate the ATPase activity of mtHsp70 and are connected to multiple import channels (77). Hydrophobic proteins use the Hsp70/90 complex and TOMM34 to prevent misfolding in the cytoplasm. Once the protein enters the mitochondria, the mitochondrial chaperone protein system (composed of Hsp10 and Hsp60) bends the polypeptide chain in a precise tertiary configuration (78).

In the MOM, the ubiquitin-proteasome system (UPS) drives the quality control of proteins (**Figure 3C**). In general, when conventional methods cannot bend proteins into their natural configuration, these proteins are targeted by the UPS for degradation (79). The UPS complex can efficiently eliminate damaged and/or malfunctioning proteins. Under normal circumstances, the UPS and autophagy-lysosomal pathway are two important mechanisms for the repair or removal of abnormal proteins. The UPS is responsible for eliminating mitochondrial proteins, whereas the autophagy-lysosome pathway is responsible for the degradation of the entire organelle by lysosomes (80).

Foreign and native proteins are digested into small peptides, triggering an adaptive immune response. In eukaryotic cells, the proteasome is responsible for almost all ATP-dependent proteolytic processes. Ubiquitin is a protein with 76 amino acids, and its binding to the substrate protein residues (monoubiquitination) is responsible for the degradation of small proteins mediated by the proteasome. The polyubiquitin chain is constructed by linking each subsequent ubiquitin molecule to the lysine residue present in the preceding ubiquitin (81). Ubiquitination is reversed by the deubiquitinating enzyme (DUB), which removes or alters the ubiquitin chain to counteract the E3 ligase activity (82). Alterations in proteasome activity lead to mitochondrial dysfunction. Additionally, different components of the UPS and DUB are present in the mitochondria simultaneously. These findings show the close relationship between mitochondrial proteins and the UPS. As the mitochondria are the major source of ROS, they may also be damaged by oxidation. The UPS uses other quality control mechanisms to eliminate damaged mitochondrial proteins. Excessive ROS can oxidize and destroy proteasome subunits, thereby reducing their catalytic activity (83).

Utilization of the Chaperone–Protease Complex System by SARS-CoV-2

The virus can generate various components and virions by activating the host cell machinery. Viruses use ongoing scalable strategies to interfere with infected cells and create an environment conducive to their replication and survival (84). MAVS is a central regulator of antiviral and inflammatory responses (85), and viruses have developed strategies to suppress the antiviral response of host MAVS signaling. Viruses can also damage mitochondrial reproduction by altering their dynamic morphology (86). In addition, a host of viral proteins can be found in the mitochondria during viral replication, where they can interact with mitochondrial proteins and manipulate various mitochondrial function and components, such as oxidative homeostasis maintenance, MPTPs, $\Delta\psi m$, electron transport, and ATP production (84).

Various proteins are synthesized in a relatively short time during viral replication. Therefore, adequate protein folding cannot be performed in a timely and effective manner. Consequently, most viruses require chaperones throughout their life cycle for the proper folding of proteins (**Figure 3**). For instance, the Hsp70 levels elevate after viral infection, which is critical for virus proliferation. In addition to issues related to protein folding, viruses interfere with cellular signal transduction, cell cycle regulation, and apoptosis induction, creating an environment favorable for their proliferation and preventing/delaying the premature death of host cells (87). SARS-CoV-2 ORF9b suppresses the IFN-1 response by targeting Tom70 (88). Intracellular RNA viruses are detected by RIG-I/MDA5, which triggers the formation of the MAVS signaling complex in the mitochondria. When infecting cells forming MAVS, Tom70 interacts with RNA viruses and induces antiviral responses by enhancing IRF3-mediated gene expression (70). Therefore, joining SARS-CoV-2 ORF9b with Tom70 suppresses the IFN-I response by disrupting the MAVS signal (88). RNA-Seq profiling studies of paired ribosomes demonstrated that SARS-CoV-2 non-structural protein (NSP) 1 inhibits the translation of Tom22 and Tom40 in host cells, consequently altering the mitochondrial import of precursor proteins (88). Further, the SARS-CoV-2 NSP4 protein interacts with components of the TIM complex and affects the import of MIM proteins (89). The ORF9b protein can interfere with the nuclear translocation of NF-κB or p65 by disrupting the polyubiquitination of K63-related NF-κB essential regulators [inhibitors of NF-κB kinase subunit (IKK) γ], thereby inhibiting the RIG -I-MAVS signal and weakening the IFN-I response (90) (**Figure 4**).

The SARS-CoV-2 infection progresses mostly in two stages. Studies have confirmed significant immunosuppression in the early stages of COVID-19 (91). Most patients with mild or asymptomatic COVID-19 demonstrate mild immunosuppression, which may promote the shedding and spread of a large number of viruses. ORF9b and other SARS-CoV-2 proteins (such as NSP13, NSP14, NSP15, and ORF6) are effective IFN antagonists in these early stages. For example, SARS-CoV-2 uses the early protein ORF9b to mask antiviral defense and inflammatory responses in newly infected patients.

However, when COVID-19 progresses to later stages, most patients with the severe disease develop ARDS with a highly inflammatory state. Numerous pro-inflammatory cytokines are produced at this time point, and the lymphocyte population is reduced. The levels of IL-6, IL-10, and C-reactive protein increase substantially, which is a reliable indicator of severe COVID-19 (92, 93). Although the inflammatory response is vigorous, the production of IFN in patients at advanced stages of the disease is hindered. The delayed IFN expression and unbalanced host response may be partly attributed to impaired

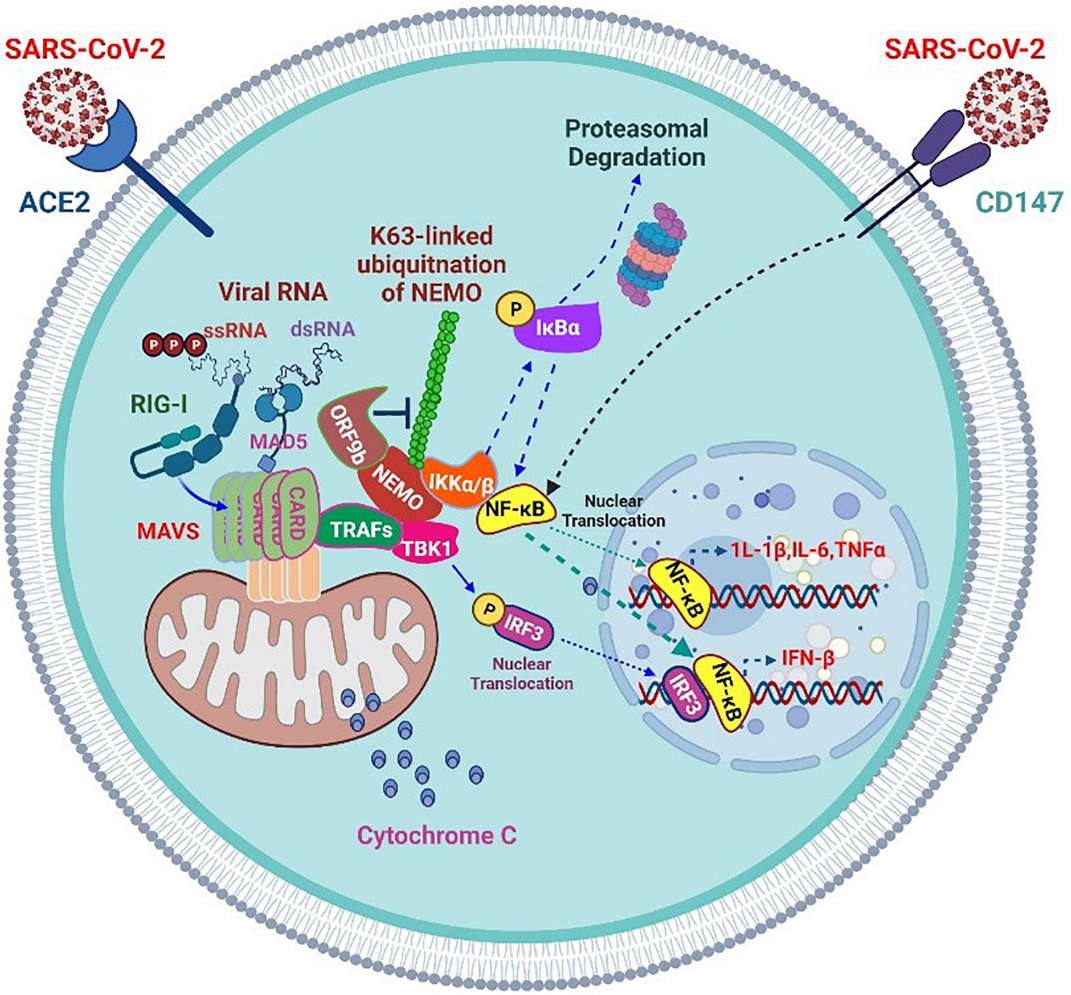

FIGURE 4 | Severe acute respiratory syndrome coronavirus 2 accessory proteins inhibit RIG-I/MAD5-MAVS antiviral signaling but enhance CD147 inflammatory signaling.

IRF3 signal transduction, which is also the target of other SARS-CoV-2 accessory proteins, such as ORF6. Previous studies have confirmed that SARS-CoV-2 NSP1, NSP3, NSP12, NSP13, NSP14, ORF3, ORF6, and M proteins may inhibit the virus-induced activation of the IFN-β promoter. In-depth analysis shows that ORF6 not only inhibits the production of type I IFN but also reduces the downstream signal transduction of IFN itself (94). SARS-CoV-2 ORF6 has been confirmed to exert the most obvious inhibitory effect on primary IFN production and the IFN signaling pathway (95).

SARS-CoV-2 ORF9c is a 73-amino acid protein that interacts with membrane proteins from several cellular organelles and alters antiviral processes. It targets the path of IFN through interaction with negative MAVS controllers (NLRX1 and NDFIP2) (96). SARS-CoV-2 NSP13, NSP14, NSP15, and ORF6 are strong IFN antagonists (95) (**Figure 4**). NSP13 targets the trajectory of IFN by interacting with the MAVS TBK1 effector (89). The SARS-CoV-2 M glycoprotein impairs the aggregation of MAVS and its recruitment of downstream signaling components, such as TRAF3, TBK1, and IRF3, resulting in the inhibition of downstream IFN-1 antiviral genes. In addition, it inhibits the stimulatory effects of RIG-I, MDA5, MAVS, and TBK1 on the IFN-I promoter (97). Furthermore, it inhibits the production of IFN-I by preventing the formation of a functional complex containing TRAF3, mediated by its first transmembrane domain (TM1). Mechanistically, TM1 is capable of binding with RIG-I, TRAF3, TBK1, and IKKε and prevents the interaction of TRAF3 with its downstream effectors (98).

In addition to effectively removing incorrectly folded proteins from cells, the ubiquitin-proteasome (UPS) system also acts as a host defense mechanism for removing viral components. Viruses can disrupt or manipulate the cellular mechanism underlying the functioning of the UPS to promote their reproduction and block the host immune response. In some cases, viruses encode proteins with E3 activity or even form a part of the E3 cell complex to degrade cellular proteins that limit the spread of the virus (99). The coronavirus genes include two open-reading frames (ORFs), ORFs 1a and 1b, which produce two viral

polyproteins, pp1a and pp1ab, respectively, which are further cleaved by viral proteases to produce functional NSPs (100). Two coronavirus proteases, papain-like protease (PLpro; released from NSP3) and 3C protease (3CLpro), are especially critical for coronavirus replication (100). The 3CLpro processes the C-terminus of the viral polyproteins pp1a and pp1ab, whereas PLpro processes its N-terminus. In addition, SARS-CoV PLpro exerts a deubiquitinating effects on host cell proteins (101), which inhibits the breakdown of poorly folded viral and host proteins. Moreover, the nucleocapsid protein of SARS-CoV interacts with the p42 proteasome subunit, resulting in altered viral proteolysis and escape of SARS-CoV from immune surveillance (102, 103). The inhibition of the proteolytic activity of proteases may also affect the degradation of poorly folded host proteins.

In docking analysis conducted in drug research, the binding mode of some proposed compounds with the SARS-CoV-2 PLpro protein has been used to establish a basic intermolecular interaction spectrum. Comparative analysis with known standard inhibitors also indicates that these proposed compounds may serve as potential active chemical entities that regulate the SARS-CoV-2 PLpro protein. Therefore, targeting PLpro may be a crucial therapeutic strategy for COVID-19 (104).

Effect of Melatonin in the Mitochondrial Chaperone-Protease Complex System

Melatonin can affect the molecular chaperone–protease network by regulating the expression of HSPs, Tom20, and Tim23, and interacting with the mechanism of action of the UPS (105, 106). In cold stress-induced immunosuppression studies, exogenous melatonin treatment not only improved cold-induced immunosuppression but also upregulated the expression of HSF-1 and HSP-70 in immune cells, which prevented protein unfolding and cell death (107). The increase in the expression of HSPs is beneficial for promoting the anti-apoptotic effect and sirtuin-1 (SIRT1) expression and suppressing the activation of inflammatory p38 MAPK and NF-κB pathways (108). Melatonin exhibited neuroprotective effects by reducing oxidative stress and HSP70 expression in ovariectomized rats with chronic brain hypoperfusion (109). The overexpression of HSP70, induced by changes in antioxidant defense, is considered to exert a protective effect by directly preventing inflammation and pathways of cell destruction, such as apoptosis and necrosis. However, melatonin can reverse the increased oxidative stress-induced HSP70 expression by suppressing the altered antioxidant status (109). In cardiomyocyte hypoxia experiments, melatonin improved mitochondrial metabolism, reduced excessive mitochondrial oxidative stress, induced effective mitochondrial fusion, and prevented mitochondrial apoptosis. Further, melatonin can enhance the expression of Tim23 and Tom20, resulting in the enhanced expression of adenosine monophosphate (AMP)-activated protein kinases, such as 5′ AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) (110).

In hematological malignancies, melatonin can inhibit the proteasome, which is closely related to the regulation of major signal transduction proteins, including p53, NF-κB, caspase-3/9, apoptosis factors BAX and BIM, and anti-apoptotic factors BCL-2, TRAIL, nuclear factor erythroid 2-related factor 2 (NRF2), and β-catenin (111). Melatonin also regulates various cellular functions by interacting with the UPS. In pathological hypoxia with high proteasome levels, melatonin decreases the level of downregulated protein 4-1 (E3 ligase in neuronal cells) expressed by neural precursor cells, thereby weakening proteasome activity. However, melatonin does not directly affect UPS (111). Direct evidence shows that melatonin inhibits proteasomes in human kidney cancer cells. Additionally, melatonin therapy induces apoptosis and regulates the expression of the pro-apoptotic protein BCL-2-mediating cell death (BIM) in renal cancer cells (112).

In aging animal models, melatonin may enhance protease activity to support cellular health. Aging exerts a significant negative effect on proteasome activity in various tissues, which leads to the increased vulnerability of cells to oxidative stress and inflammation. This is because the inhibition of the protein clearance mechanism is directly related to aging. Under these circumstances, melatonin may inhibit the buildup of abnormal proteins in cells by increasing proteasome activity (105). During aging, circadian disturbances cause a decrease in melatonin production, which is also linked to reduced proteasome viability (106). The regulatory effect of melatonin on HSPs and proteasome activity can improve mitochondrial function and restrict the replication of SARS-CoV-2 (**Figure 3A**). Because COVID-19 is more severe in older patients, and melatonin and proteasome levels gradually decrease with age, melatonin supplementation may be helpful in older patients undergoing COVID-19 treatment.

EFFECTS OF SARS-CoV-2 ON MITOCHONDRIAL DYNAMICS AND AUTOPHAGY

Autophagy is used by cells to eliminate invasive pathogens, such as SARS-CoV-2. Autophagy is involved in several critical functions of protein complexes. In general, the mammalian rapamycin complex 1 (mTORC1)/unc-51-like autophagy activating kinase (ULK)1/2 complex inhibits autophagy in the absence of stress. Stress, including that resulting from viral infections, inhibits mTORC1, which subsequently activates a series of proteins, leading to autophagy and viral particle packaging and degradation (113). Autophagy also regulates the link between the innate and adaptive immune responses to viral infections by inducing antigen-presenting cells (APCs, such as macrophages). APCs present viral antigens to CD4+ T cells to release cytokines and subsequently regulate adaptive immune responses. In early autophagy, $CD4^+$ T cells are induced to release interferon gamma (IFN-γ), which promotes the response of $CD8^+$ T cells, NK cells, and macrophages in the elimination of virus particles (114, 115). In addition, autophagy can moderate the inflammatory response by promoting IL-1β expression for lysosomal degradation to prevent the inflammatory pathway and ROS accumulation (116). Even though some viruses may escape surveillance related to autophagy, autophagy-related immune

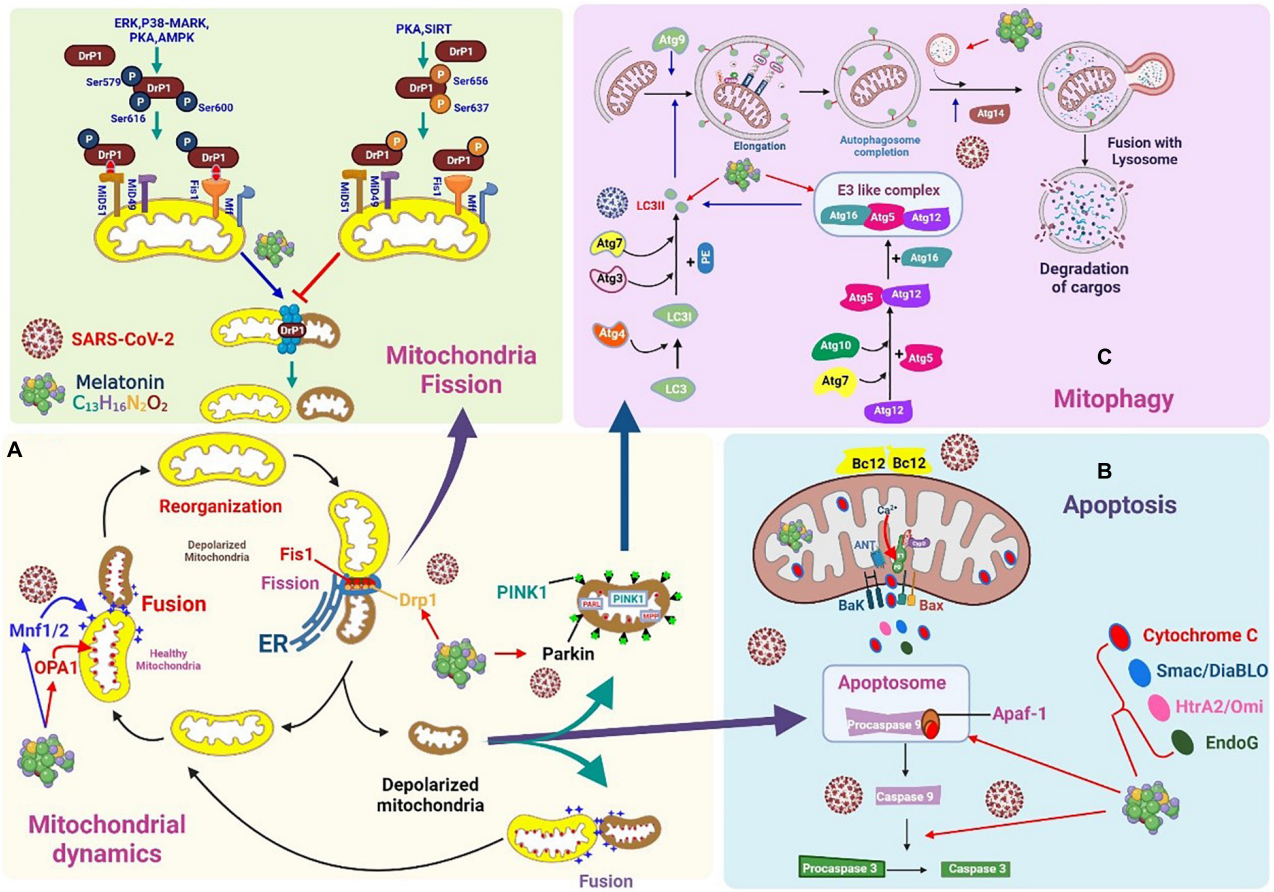

FIGURE 5 | Severe acute respiratory syndrome coronavirus 2 affects mitochondrial dynamics, mitophagy, and apoptosis. **(A)** Mitochondrial fusion is mediated by mitochondrial dynamin-related guanosine triphosphatases, which include mitochondrial fusion proteins 1 and 2. Melatonin downregulates Drp1 expression and suppresses mitochondrial fission. **(B)** Viral protein and open reading frame may induce mitochondria apoptosis. Melatonin may modulate processes associated with apoptosis, which reduces viral spread. **(C)** Coronaviruses can alter mitochondrial dynamics and the status of mitochondrial autophagy to promote interference with the normal antiviral signaling pathway of cells and maintain infection. Melatonin may promote mitophagy via LC3II, autophagosome-lysosome fusion, and E3-like complex.

effects can still be used to combat viral infection and prevent inflammation-induced organ injury.

Mitochondrial Fission–Fusion

Mitochondrial dynamics involve repeated fusion and fission cycles within the mitochondrial system. Studies on the monitoring of individual mitochondria during fusion and fission revealed that both events are matched, and fusion triggers fission. The balance between these two opposing processes regulates the shape of the mitochondria and affects mitochondrial quality and homeostasis (117). Previous studies have shown that fusion events coupled to fission event can accelerate the removal of damaged mitochondrial components by autophagy. The fusion process involves mitochondrial membrane components, matrix metabolites, and complete copies of mtDNA that can be rapidly exchanged and equilibrated between adjacent mitochondria. Fusion also repairs and reconstitutes damaged mitochondrial functions. The frequency and selectivity of mitochondrial fusion are essential for maintaining quality (118). In mammals, mitochondrial fusion is mediated by mitochondrial dynamin-related guanosine triphosphatases (GTPases), which include mitochondrial fusion proteins 1 and 2 (Mfn1 and Mfn2, respectively), responsible for fusion of the MOM, and optic atrophy 1 (OPA1), responsible for the fusion of the MIM. In mammalian cells, Mfn2, located both on the MOM and ER surface, has been proposed as a physical bridge between the two organelles. Mfn2 prevents excessive and potentially toxic proximity between both organelles (119) (**Figure 5A**). OPA1 is located on the MIM, facing the intermembrane space and controlling the fusion and remodeling of the MIM. OPA1 is processed by proteases dependent on ATP levels and $\Delta\psi m$, suggesting that the mitochondrial energy status is an important regulator of OPA1 processing. The cleavage of OPA1 induces mitochondrial dysfunction (120). The mitochondria are rich in the deacetylase SIRT3, which can directly deacetylate OPA1 and activate the protein by increasing its GTPase activity, thereby regulating mitochondrial dynamics under stress (121). Mfn2 in the MOM may mediate the recruitment of parkin to

damaged mitochondria. Parkin binds Mfn2 in a phosphatase and tensin homolog-induced kinase 1 (PINK1)-dependent manner. PINK1 (a mitochondrial serine/threonine protein kinase) phosphorylates Mfn2 and subsequently favors parkin-mediated ubiquitination. Experiments have confirmed that the ablation of Mfn2 in mouse cardiomyocytes significantly prevented mitochondrial depolarization-induced parkin translocation and effectively inhibited mitochondrial autophagy (122). Mitochondrial fusion proteins can also be ubiquitylated with multiple E3 ligases and degraded by proteases, which inhibits fusion (123). Mitochondrial depolarization enhances the expression of protease OMA1, which leads to the cleavage and decay of Long-OPA1. This results in a reduction in mitochondrial fusion and the autophagic degradation of mitochondrial organelles (124).

MPTPs are involved in an inducible activity that regulates solute exchange between the mitochondrial matrix and the surrounding cytoplasm. However, this exchange can cause a sharp decline in $\Delta\psi m$, which eventually leads to cell death, whereby the organ is severely swollen, ruptured, and disabled (**Figure 5B**). The MPTP primarily consists of BAX/BAK on the MOM for inducing membrane permeability. The F1FO ATP synthase on the MIM is regulated by CypD in the matrix and can be used in the response to stimuli for initiating the opening and outflow of Ca^{2+}, loss of $\Delta\psi m$, and the swelling and rupture of the mitochondria (125).

Mitochondrial fission is initiated by the recruitment of dynamin-related protein 1 (Drp1) and its anchor points on the MOM. Drp1 is post-translationally modified by phosphorylation, which affects its localization to the cytoplasm or MOM. Extracellular signal-regulated kinases, p38-MAPK, protein kinase A, AMPK, and SIRT are capable of phosphorylating Drp1. The phosphorylation of Drp1 at Ser637 and Ser656 significantly inhibits mitochondrial fission, whereas phosphorylation at Ser616, Ser579, and Ser600 enhances mitochondrial fission (126). Fusion is mediated by mitogens (Mfn1 and Mfn2) on the MOM, and OPA1 plays a major role in MIM. Fission begins when the ER is recruited to the mitochondrial contracting sites, where it is tagged with mtDNA. Next, a variety of MOM-binding proteins (FIS1, MFF, MiD49, and MiD51) recruit Drp1 to the surface of the mitochondria, aiding the contraction response mediated by the ER (127).

SARS-CoV-2 can efficiently inhibit mitochondrial fusion by targeting mitochondrial deubiquitination enzymes to inhibit the deubiquitination of Mfn1 and Mfn2 (128). After SARS-CoV-2 enters the infected cell, viral RNA and proteins are found in the mitochondria. Following infection, non-coding RNA may also regulate mitochondrial dynamic host proteins, such as ubiquitin carboxy-terminal hydrolase 30 (USP30). SARS-CoV-2 may divert the host mitochondria by regulating mitochondrial dynamics, mitochondrial physiological functions, and the release of mtDNA to help suppress the host's immune response. This diversion is crucial for SARS-CoV-2 infection (128). Drp1 is a key contributor to mitochondrial fission, since its phosphorylation regulates mitochondrial fission (129). Altered mitochondrial autophagy mediated by PINK1/parkin is the molecular basis for mitochondrial anomalies in Parkinson's disease. Recent studies have shown that PINK1 directly phosphorylates Drp1 at S616. Mitochondrial fission mediated by PINK1 is related to the phosphorylation of Drp1S616 (130). RNA viruses support the proteasomal degradation of Drp1, which contributes to the induction of optimal mitophagy (70). Some coronaviruses augment the ratio of LC3-II to LC3-I, causing autophagosomes to accumulate in infected cells (131). SARS-CoV-2 can target the USP30 protein and inhibit PINK1-mediated mitophagy (132). The different effects of various coronaviruses on host cell autophagy can be attributed to the fact that autophagy inhibits the replication of certain viruses but is beneficial to the replication of other viruses (133).

In other words, fusion-based functional complementarity and mitochondria-derived vesicles can serve as the first line of protection against mitochondrial injury. As the degree of damage to the mitochondria increases, the damaged compartment is separated from the mitochondrial system by fission, followed by mitochondrial autophagy. Depending on the type of stressor, there are many molecular mechanisms responsible for mitochondrial autophagy (127). For example, PINK1/parkin contributes to mitochondrial autophagy when the mitochondria are depolarized. Moreover, when mitochondrial autophagy is induced during hypoxia or erythropoiesis, the connection between damaged mitochondria and new autophagosomes is formed by mitochondrial receptors (for example, NIX, BNIP3, FUNDC1, and BCL2L) interacting with LC3.

Mitophagy

In the process of mitochondrial depolarization, PINK1 and parkin physically participate and collaborate to identify damaged mitochondria for degradation (134). The completion of specific mitochondrial autophagy (mitophagy) involves several consecutive steps, which are closely regulated by more than 35 genes linked to autophagy (ATGs) and their corresponding proteins (**Figure 5C**). The first step is the induction of phagophore nucleation, which is affected by a complex of ULK1 (a mammalian homolog of Atg1), Atg13, Atg10, RB1CC1/FIP200, Vps34 (a type III PI 3-kinase), and p150. During the development of mitophagy, the ULK1 complex participates in the initiation of autophagy and the activation of class III PI 3 complexes through the phosphorylation of Beclin-1, which then induces phagophore nucleation (135). The second step is the formation of phagophores and autophagosomes, which is primarily regulated by the Atg9 protein. Thereafter, mitophagy completes the merging of autophagosomes and lysosomes. ATG14 binds directly to the STX17-SNAP29 binary complex on autophagosomes and favors the fusion of autophagosomes and lysosomes by STX17-SNAP29-VAMP8 (136). The final step is the degradation of the cargo trapped in the autophagosome by lysosomal hydrolase. To cope with various conditions of stress, the degraded molecules are transported into the cytoplasm, recycled, and reused to synthesize basic cellular components.

Autophagosome formation is negatively controlled by mTORC1. The inhibition of mTORC1 may lead to the activation of Vps34, which is essential for inducing phagophore nucleation. The translocation of damaged mitochondria to phagophores occurs through the MOM covering the protein, which acts as an

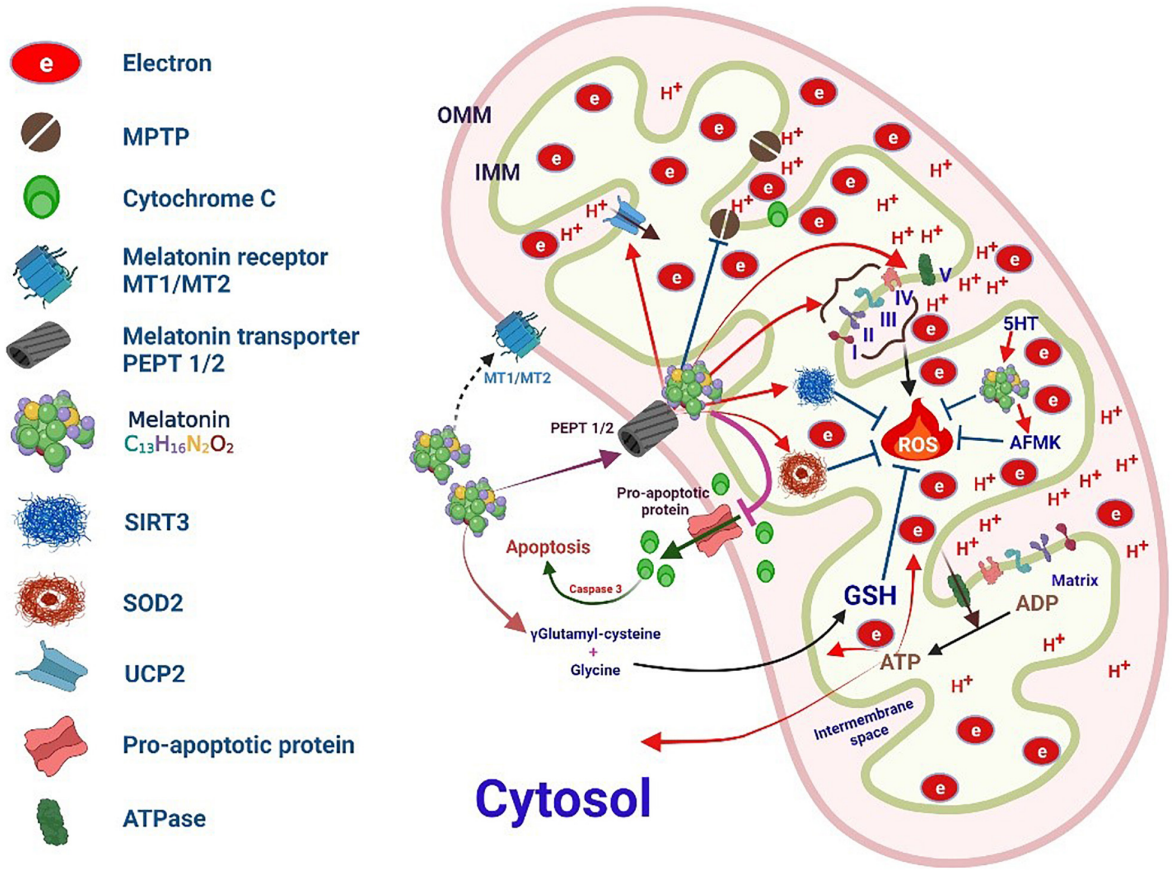

FIGURE 6 | Melatonin targets mitochondrial oxidative stress.

LC3-II receptor (137) (**Figure 5C**). PINK1 and parkin promote the mitochondrial autophagy-mediated degradation of damaged mitochondria (138).

Effects of SARS-CoV-2 on Mitochondrial Dynamics and Mitophagy

Coronaviruses can alter mitochondrial dynamics and the status of mitochondrial autophagy to promote interference with the normal antiviral signaling pathway of cells and maintain infection. The SARS-CoV-2 ORF3a protein targets USP30, a mitochondrial deubiquitinase that normally binds to the MOM. Because USP30 can counter the effects of parkin, it is a key inhibitor of mitochondrial autophagy. It hydrolyzes the attached ubiquitin and, through parkin, acts on target proteins such as mitochondrial Rho GTPase 1 and Tom20, thereby inhibiting the ability of parkin to drive mitochondrial autophagy (139–142). Additionally, USP30 promotes mitochondrial fusion by deubiquitinating Mfn1 and Mfn2. The expression of mitochondrial ribosomes, respiratory complex I, and mitochondrial fission-promoting genes (such as SOCS6 and MTFP1 genes) is reduced considerably in cells infected with SARS-CoV-2. However, reducing the expression of SOCS6 and MTFP1 limits mitochondrial fission and leads to excessive mitochondrial fusion. This frequently occurs in cells infected with SARS-CoV-2 (143).

Several studies have shown the basic functions of autophagy during a viral infection. Autophagy can induce innate immune responses to inhibit the proliferation of viruses; in contrast, viruses evolve various strategies to defend and escape the destructive effects of autophagy and even use it to promote their proliferation. SARS-CoV-2 rearrangement involves the formation of ER-derived double-membrane vesicles (DMVs) as sites for RNA replication. Phosphatidylinositide 3-kinase, a cytokine with a biosensor, is activated in virus-infected cells to produce phosphatidylinositol 3-phosphate (PI3P), which is closely related to viral replication and the formation of viral DMV. The PI3P-binding protein DFCP1 is recruited to omegasomes during the early stages of autophagosome formation and participates in virus replication and DMV formation. Thus, SARS-CoV-2 uses components of the autophagy machinery to create replicating organelles (144). Similar to porcine epidemic diarrhea virus (PEDV), it can induce an ER stress response, and the activation of NF-κB signaling also contributes to PEDV replication (145, 146). In a study of porcine intestinal epithelial cells, rapamycin-induced autophagy effectively limited the infectivity of PEDV. Therefore, inducing complete autophagy may be an effective strategy for preventing viral infections

(131). However, experimental infection with transmissible gastroenteritis coronavirus (TGEV) resulted in an increase in the ratio of LC3-II to LC3-I, leading to the accumulation of autophagosomes in these cells. These results indicate that TGEV infection activates autophagy, and autophagy subsequently inhibits the replication of TGEV (133). Since pharmacological suppression of autophagy can lead to increased replication of TGEV, autophagy induction can be an efficient method to prevent TGEV from causing disease in infected cells. In addition, in TGEV-infected cells, the decrease in total mitochondrial mass, upregulation of mitochondrial p62/SQSTM1 levels, increase in the LC3-II/LC3-I ratio, and expression of Beclin 1 implies that TGEV may induce mitochondrial autophagy to attenuate oxidative stress and cell apoptosis, which will further promote cell survival and possible viral infection. In simple terms, mitochondrial autophagy in TGEV infection can counteract oxidative stress and apoptosis and help the virus to replicate (147). Since autophagy is critical for SARS-COV2 mediated COVID-19, we can design drugs that target autophagy to inhibit and treat viral infections (148).

SARS-CoV-2 is also detrimental to other special characteristics of autophagy in infected cells (**Figure 5C**). Its ORF9b protein localizes in the mitochondria, which accelerates the degradation of Drp1, thereby prolonging the lifespan of the mitochondria. ORF9b may also target and degrade important mitochondria-binding molecules, such as MAVS, TRAF3, and TRAF6, which severely restricts the antiviral IFN response in the host cell (90, 149).

The host USP30 protein is pivotal for mitochondrial homeostasis and collaborates with parkin-mediated mitochondrial autophagy. The ORF3a protein of SARS-CoV-2 can interact with USP30 to alter mitochondrial ubiquitination, causing a mitochondrial collapse in infected cells and premature death (90). Previous studies have reported that infection with SARS-CoV-2 upregulates the expression of PINK1, which contributes to the development of mitophagy (150). However, the expression of the E3 ubiquitin ligase Skp2 is significantly reduced in cells infected with SARS-CoV-2, which prevents the degradation of Beclin 1, which may induce significant autophagic flow (128, 151). The similarity in the amino acid sequence of ORF9b in SARS-CoV-2 and SARS-CoV shows that SARS-CoV-2 ORF9b may inhibit mitochondrial breakage and induce autophagosome formation to promote cell survival and increase virus infection and replication. However, co-expression network analysis shows that SARS-CoV-2 reduces the autophagic flux by upregulating glycogen synthase kinase 3 beta or downregulating autophagy genes and lysosomal acidification genes (128, 143).

Effect of Melatonin on Mitochondrial Dynamics and Mitophagy

Melatonin downregulates Drp1 expression and suppresses mitochondrial fission (**Figure 5A**). This effect can be mediated *via* the activation of the SIRT1–PGC-1α pathway because the combination of PGC-1α and its promoter decreases the expression of Drp1. During viral infection, the activation of SIRT1 increases $\Delta\psi m$ and reduces ROS production. Since melatonin is absorbed and synthesized in the mitochondria, this is the best method to suppress ROS production (152). Melatonin improves mitochondrial function and biogenesis by inducing the expression of SIRT1 (153) and can even inhibit neural damage caused by prions by regulating mitochondrial function and dynamics. Melatonin corrects the imbalance in mitochondrial dynamics by enhancing OPA1 and reducing Drp1 expression (154). Further, it decreases the translocation of Drp1 in mitochondria, thus attenuating the co-localization of Drp1 and Tom20 proteins (155). Melatonin also inhibits mitochondria-mediated cell death by preventing the opening of MPTP and activating PINK1/parkin, thereby inhibiting mitochondrial Drp1-mediated fission.

AMPK directly promotes autophagy by enhancing the phosphorylation of autophagy-related proteins (mTORC1, ULK1, and PIK3C3/VPS34 complexes). Further, it can indirectly promote autophagy by regulating transcription factors such as BRD4, FOXO3, and TFEB. AMPK causes the fragmentation of damaged mitochondria and favors the translocation of autophagic machines to damaged mitochondria to achieve effective mitophagy (156). A porcine intestinal epithelial cell study (IPC-J2) has shown that AMPK-mediated mitophagy is needed to attenuate the lesions of the intestinal epithelial barrier caused by oxidative stress and dysfunction of mitochondrial energy metabolism (146). In addition, AMPKα can inhibit mitochondrial fission by inhibiting Drp1 (157). The inhibitory effect on Drp1 activation and mitochondrial translocation of melatonin may be owing to the antioxidant effect of melatonin and its prevention of cytosolic calcium overload. Melatonin can also activate AMPKα, which inhibits the opening of the MPTP (158). Moreover, under oxidative stress, melatonin restores the reduced expression of Mfn2 and OPA1 by activating the AMPK/PGC-1α pathway to achieve efficient mitochondrial fusion (110) (**Figure 5A**).

APOPTOSIS AND MITOCHONDRIAL QUALITY MONITORING

The fusion of healthy and defective mitochondria can help repair dysfunctional mitochondria. However, when mitochondrial damage is severe, dysregulated mitochondria can be separated by fission, and these separated organelles are eventually eliminated by mitophagy. Defective mitochondria may rupture if quality control is compromised or mitochondrial damage exceeds the reparative capability of fission/fusion and mitophagy routes (159). When the mitochondria are damaged, apoptotic factors are released from the damaged mitochondria into the cytoplasm, which triggers the death of apoptotic cells by inducing caspase activation, chromosomal condensation, and rupture. These apoptotic factors are released when members of the pro-apoptotic Bcl-2 family destroy the integrity of the MOM. When these pro-apoptotic proteins, including Bax and Bak, are activated, they form the MPTP, which leads to the penetration of the MOM (160). This process releases various apoptotic proteins and cytochrome c from the MIM space into the cytoplasm (159). In the cytoplasm, cytochrome c interacts with the

activator of apoptotic protease 1 and procaspase-9 to produce apoptosome complexes. Apoptotic bodies are subjected to the autocatalytic activation of caspase-9, followed by activation of caspase-3. Once activated, caspase-3 cleaves vital cellular proteins and subsequently induces cell death (161). Caspase-3 is a critical intracellular proteolytic enzyme that cleaves several key substrates during apoptosis, causing DNA fragmentation and nucleoprotein degradation and promoting apoptotic body formation (**Figure 6**) (162). In addition to caspases, cell death factors released by the mitochondria can activate caspase-independent cell apoptosis. Furthermore, the apoptosis-inducing factor, a mitochondrial flavoprotein, is translocated into the nucleus to bind to chromosomal DNA and cause chromatin condensation, which causes DNA fragmentation (163). In addition, Bax/Bak promotes Drp1 phosphorylation, inducing a stable membrane-associated form of Drp1 during apoptosis.

Effects of SARS-CoV-2 Infection on Mitochondrial Apoptosis

Intracellular BCL-2-related proteins (BCL-2s) promote the mitochondrial release of pro-apoptotic signaling molecules to regulate apoptosis. These factors activate cysteine proteases of the caspase family, which help propagate the apoptotic cell death signal (164). Correspondingly, the inhibition of the apoptosis of infected cells can increase the risk of viral infection. For example, during mouse hepatitis viral infection, the expression of the pro-apoptotic gene BNIP3 is downregulated in cultured astrocytes (165).

The caspase recruitment domain (CARD) is involved in the cascade of apoptotic and inflammatory signals. Upon activation, Nod-like receptors assemble into multi-protein complexes, such as NODosomes and inflammasomes (166). When activated by RNA viruses, MAVS initiates type 1 IFN signaling by activating the nuclear translocation of NF-κB and IRF3. MAVS, a mitochondrial antiviral-signaling protein, possesses an N-terminal CARD domain, which indicates that the mitochondrial localization of MAVS is related to the induction of apoptosis (167). Therefore, the inhibition of MAVS function is an effective strategy for some viruses to inhibit the death of host cells (166). The NSP15 protein of SARS-CoV assists in the replication of SARS-CoV, and can completely inhibit MAVS-induced apoptosis (166). NSP15 of murine coronavirus inhibits RIG-I and MDA5 sensors for the efficient monitoring of viral dsRNA, thereby delaying IFN activation and inhibiting apoptosis in infected macrophages (168).

Many viral gene products can effectively trigger apoptosis by interfering with cellular signaling cascades. Previous studies have shown various pro-apoptotic proteins in the SARS-CoV genome, including ORF3a, ORF3b, ORF4, ORF6, ORF7a, and ORF7b, which can induce apoptosis in specific infected cells (169, 170). In cells infected with SARS-CoV, the ORF3a protein is found in the ER, which can induce chromatin condensation as well as DNA fragmentation, leading to host cell apoptosis (171), which favors mitochondrial apoptosis by activating p38 MAPK signaling (172). SARS-CoV-2 ORF3a can effectively induce host cell apoptosis by promoting caspase-3 activation, Bid truncation, caspase-9 lysis, and cytochrome c expression (173). SARS-CoV 3b protein (ORF4) is present both in the mitochondria and nucleus during viral infection, and its overexpression through transfection can induce cell cycle arrest in the G0/G1 phase (170). Studies have confirmed that transient transfection of green fluorescent protein (GFP)–ORF6 in different cells induces host cell apoptosis by promoting the activation of caspase-3 and c-Jun N-terminal kinase (JNK)/MAPK pathways, which are elicited by the exacerbation of ER stress. In addition, the level of the ER chaperone protein GRP94 was significantly upregulated (169). In the ER, ORF7a can act on the anti-apoptotic protein Bcl-XL, causing the isolation of Bcl-XL in the ER-Golgi apparatus and subsequently promoting apoptosis (174), associated with the activation of p38 MAPK (175). The ORF7a protein may also interact with human Ap4A hydrolase, triggering the activation of the cascade mechanism that causes cell cycle arrest and apoptosis (176). The SARS-CoV M protein induces apoptosis by lowering Akt pro-survival signaling and inducing the release of mitochondrial cytochrome c (177). ORF8a is primarily located in the mitochondria and not only promotes virus replication, but also accelerates mitochondrial hyperpolarization, ROS production, and subsequent caspase 3-dependent apoptosis (**Figure 5B**) (178).

SARS-CoV-2 also induces apoptosis in various lymphocytes, which is related to the uncontrolled production of inflammatory cytokines, leading to excessive inflammation and eventually causing damage to visceral organs (179, 180). In patients with acute COVID-19, non-clonal T cell populations are characterized by the upregulated expression of voltage-dependent anion channels and mitochondrial membrane (mtM) proteins. These mtM proteins are involved in enhanced permeabilization of the MOM, which induces infected cell death. The increase in mtM protein and anion channel expression is also related to mitochondrial dysfunction, which leads to the release of cytochrome c and caspase activation. Finally, caspase-induced T cell apoptosis can lead to decreased lymphocyte count, which is observed in patients with COVID-19 (181, 182).

Melatonin and Mitochondrial Apoptosis

Melatonin plays an entirely different role in healthy cells than in tumor cells. Melatonin interfaces with cancer cells to promote apoptosis (183) while preventing apoptosis in healthy cells (184). Melatonin has the ability to improve immune surveillance, recover free radicals, which significantly reduces associated molecular harm, and modulate processes associated with apoptosis, which reduces viral spread (185) (**Figure 5B**).

The anti-apoptotic properties of melatonin are primarily related to the inhibition of MPTP opening and Ca^{2+} overload induced by oxidative stress. The overloading of Ca^{2+} subsequently inhibits the depolarization of the mitochondrial membrane, facilitates the opening of the MPTP, and induces the release of Ca^{2+} (186) (**Figure 6**). In addition, melatonin can stabilize the level of Ca^{2+} buffer proteins such as paralbumin and hippocampal calcineurin, which prevents a large increase in intracellular Ca^{2+} levels and possibly induces apoptosis

(187). Melatonin can also donate or accept electrons from the mitochondrial ETC complex, which enhances the activity of respiratory complexes and helps maintain effective oxidative phosphorylation and ATP synthesis (188). Melatonin influences these processes to improve mitochondrial respiration and energy production, thereby reducing oxygen consumption and superoxide anion production, depolarizing mitochondria, causing MPTP opening, and releasing apoptotic proteins and cytochrome c from the MIM space to the cytoplasm to reduce oxidative damage (189). Melatonin antagonizes injury-induced apoptosis by interacting with MT-1 and MT-2 plasma membrane receptors. In response to apoptotic stimuli, melatonin allows mitochondrial translocation of the pro-apoptotic protein Bax but impairs its activation/dimerization and prevents the formation of MPTP in downstream apoptotic events. Melatonin also induces the strong relocalization of Bcl-2, a major Bax antagonist in mitochondria, thereby antagonizing the intrinsic pathway of apoptosis (190). Further, it can downregulate the phosphorylation of JNK and p-38 MAPK by activating the membrane melatonin receptors MT1 and MT2 to inhibit p53 phosphorylation (191). In addition, melatonin binds to the MT1 receptor located on the MOM and activates the MT1/Gαi signaling in the mitochondrial intermembrane space, effectively inhibiting the activity of adenylate cyclase and Ca^{2+}-mediated cytochrome c release and preventing caspase activation and subsequent apoptosis (192).

A previous report showed that melatonin exerts a significant anti-apoptotic effect in cells with certain viral infections, such as the Venezuelan equine encephalitis (VEE) infection (193). Melatonin also inhibits hepatocyte apoptosis induced by rabbit hemorrhagic disease virus, primarily owing to the upregulation of Bcl-2 and Bcl-XL expression, downregulation of Bax expression, reduction of cytochrome c release, and restriction of caspase-9 activation (194). Moreover, melatonin restores mitochondrial function in coxsackievirus B3 (CVB3)-infected cardiomyocytes. Further, melatonin significantly improved CVB3-induced myocarditis, inflammatory cell infiltration, necrosis, and edema, and decreased the expression of autophagy-related proteins, thus inhibiting apoptosis (195).

THE EFFECT OF MELATONIN ON THE REDOX STATE OF THE MITOCHONDRIA

Because of its lipophilic and hydrophilic properties, melatonin can easily diffuse into cells and intracellular organelles and cross the blood-brain barrier. The pyrrole ring in melatonin makes it highly suitable for capturing $O_2^{\bullet -}$ and $^{\bullet}OH$ free radicals. Previous studies have demonstrated that melatonin is five times more effective at scavenging free radicals than GSH, an endogenous free radical scavenger, and 15 times more effective than mannitol, an exogenous free radical scavenger. Its antioxidant effect is primarily mediated by the capture of $O_2^{\bullet -}$ radicals by indole cation radicals formed by the reaction of melatonin and $^{\bullet}OH$ radicals (14, 196). In particular, melatonin is abundant in subcellular compartments, such as the nucleus and mitochondria (197, 198).

Further, mitochondrial dysfunction is extremely critical for the processes of cellular self-destruction, including apoptosis, autophagy, and necrosis, during various diseases (199). mtDNA is more fragile than nuclear DNA because it lacks histones. Therefore, mtDNA damage in the elderly is ten times more severe than nuclear DNA damage (199). The mitochondria are well-known as the prime source of ROS formation but are also the prime targets of attack by ROS (2) (**Figure 6**).

A previous report showed that melatonin treatment prevents the age-dependent increase in mitochondrial oxidative stress (200). Melatonin is also essential for mitochondrial homeostasis as it optimizes the transfer of electrons to the ETC in MIM (201, 202). Another study showed that melatonin treatment restored mitochondrial ATP production in cardiomyocytes. Further, long-term melatonin therapy protects against mitochondrial dysfunction and oxidative stress without any side effects (203).

Under normal circumstances, organisms possess powerful defense mechanisms against oxidative stress, one of which involves the use of antioxidant enzymes. However, the potency of antioxidant enzymes gradually declines with age (204, 205). An age-dependent increase in oxidative stress reduces the mitochondrial GSH pool in the brain by 50% and simultaneously increases oxidative GSH levels (206, 207). A study in rat lymphocytes and brain showed that reductions in components related to free radical scavenging, such as catalase and SOD activities and GSH levels, were associated with aging (208). Although the mitochondrial antioxidant defense mechanisms are dependent on GSH, the mitochondria cannot synthesize GSH (209). Instead, GSH is imported into the mitochondria from the cytosol through a multicomponent transport system (210). Melatonin increases GSH-Px, GSR, SOD1, and CAT gene expression and enzymatic activity at pharmacological and physiological doses (211). The antioxidant enzymes GSH-Px and SOD are critical for protecting the body from age-associated oxidative stress. GSH-Px activity is known to increase with age, whereas melatonin therapy was shown to decrease GSH-Px activity in elderly rats (56). However, melatonin treatment augmented the mitochondrial SOD levels in older rats and prevented the reduction of the SOD/GSH-Px and GR/GSH-Px ratios (212). Melatonin treatment in rats with renal ischemia/reperfusion lesions increases SOD activity and GSH levels and decreases nitrogen oxide levels (2, 213).

The mitochondrial respiratory chain primarily uses oxygen as raw material and produces energy as ATP. Free radicals and ROS are formed when electrons transferred between consecutive complexes chemically reduce adjacent oxygen molecules. The complexes involved in oxidative phosphorylation in the ETC (CI–CV) are then formed, which produces the energy necessary for the cell in the form of ATP. Melatonin enters the mitochondria *via* the peptide transporter (PepT 1/2) in a reverse gradient. Further, we found that the melatonin concentration in mitochondria is considerably higher than that in other subcellular compartments (152).

Melatonin plays a multifaceted role in the mitochondria. In particular, it strengthens the tolerance of key mitochondrial molecules to oxidative damage and preserves the function of healthy organelles. ROS generated *via* electron leakage

from the ETC can be directly eliminated by melatonin and its metabolite [N1-acetyl-N2-formyl-5-methoxykynurenure (AFMK)]. Indeed, ROS can also be metabolized by mitochondrial superoxide dismutase (SOD2) and subsequently scavenged by GSH and SIRT3. In addition, melatonin can effectively modulate uncoupling protein 2, maintain optimal MIM potential, and effectively limit the opening of MPTP. In addition to its rapid entry into the mitochondria, melatonin can also be synthesized in the mitochondria, where it can be metabolized to AFMK; therefore, it has optimal properties for eliminating toxic oxidative substances (152).

MELATONIN SUPPLEMENTATION FOR COVID-19

A retrospective review of intubated patients with COVID-19 revealed that melatonin reduced the risk of death considerably, indicating a significant anti-inflammatory effect. Melatonin can upregulate the expression of SIRT1, a deacetylase known to effectively inhibit the activity of pro-inflammatory NF-κB, and can also upregulate Nrf2, which in turn increases antioxidant protein transcription (214, 215). Recent epidemiological studies have shown that melatonin supplementation can reduce the risk of COVID-19 infection detected serologically by 28%, a significant reduction; among Black Americans, this risk is reduced by 52% (216). The basis for this risk reduction may be related to the enhancement of SIRT1 activity by melatonin and the subsequent transcriptional upregulation of ACE2 expression (217).

SIRT1 induced by melatonin can enhance virus-mediated MAVS activation (218). Through mitochondrial membrane receptors, melatonin induces the nuclear translocation of the transcription factor retinoid-related orphan receptor alpha (RORα). RORα boosts the transcription of the gene encoding the clock transcription factor brain and muscle aryl hydrocarbon receptor nuclear transporter-like 1 (Bmal1). Bmal1 transcription significantly upregulates the expression of SIRT1 and Nrf2 (214) (**Figure 4**). Nrf2 plays a pivotal role in the innate immune system by inhibiting ROS and directly inhibiting the pro-inflammatory cytokines IL-1β and IL-6 to limit the development of inflammation. In macrophages, Bmal1 regulates IL-1β *via* Nrf2 (219). The MAVS protein is a key mediator in the dsRNA sensing pathway, which leads to the activation of IRF3 and the induction of type 1 IFNs (220). TRIM31 is an E3 ubiquitin ligase of the TRIM protein family and is mainly used as a regulator of MAVS aggregation. TRIM31 is recruited to the mitochondria following viral infection and specifically regulates antiviral signal transduction mediated by RLR PRRs. TRIM31 interacts with MAVS and catalyzes its Lys63 (K63)-linked polyubiquitination at Lys10, Lys311, and Lys461. This modification leads to the formation of MAVS prion-like aggregates following viral infection, promotes the activation and phosphorylation of IRF3, and induces type 1 IFN (221) (**Figure 4**). However, ovarian tumor ubiquitinating enzyme 3 (OTUD3) suppresses this activation by deubiquitinating MAVS (222). In response to a viral infection, acetylated Lys129 is deacylated and eliminated by SIRT1, which rapidly inactivates OTUD3, inducing innate antiviral immunity by upregulating the viral activation of MAVS and inducing type 1 IFN (222).

The effect of SIRT1 on IFN-induced antiviral immunity is complex, as SIRT1 may inhibit NF-κB transcriptional activity. NF-κB also works downstream of MAVS to promote type 1 IFN induction (223). The cell's response to RNA viruses often activates IRF3, NF-κB, ATF2, and c-Jun, which can bind to the promoter of the IFN-β gene IFNB1 and promote its transcription (219).

Nrf2 is a sensor of oxidative stress that maintains a normal physiological state by inducing redox balance. Melatonin mediates various beneficial biological and therapeutic effects, such as antioxidant, anti-inflammatory, anti-tumor, anti-diabetic, and cardioprotective effects. The Nrf2 signaling pathway explains some of the therapeutic and biological effects of melatonin (224).

In most vertebrates, melatonin synthesis gradually decreases with age. This decrease may be attributed to a decrease in β-adrenergic receptors in the pineal gland and in the expression of aralkylamine N-acetyltransferase (AANAT) (225). Older cells are prone to produce more reactive nitrogen species (RNS) and ROS, but the antioxidant effect of endogenous melatonin counteracts the damage caused by RNS and ROS in senescent cells (3). Previous studies have shown that melatonin can retard the aging of the gastric mucosa by stimulating telomerase activity and effectively inhibiting lipid peroxidation and cell proliferation (226). Melatonin can also reduce oxidative damage induced by angiotensin by inhibiting the production of ROS and RNS, inflammatory cytokines, and advanced glycation end products and preventing telomere shortening (26) (**Figure 7**).

Respiratory epithelial cells, macrophages, monocytes, and dendritic cells can produce IFN. RIG-1/MDA-5 can recognize viral dsRNA in the infected cytoplasm and help TRAF-3 activate IRF3. Aging is related to the degradation of TRAF-3 and reduction in the phosphorylation of IRF3. IRF3 acts as an intermediary for the transcription of IFN-I and IRF8. IRF8 enhances and amplifies IFN-I expression. The deubiquitination of MAVS by OTUD3 can inhibit the activation of IRF3, thus blocking the innate antiviral immune response (222). SIRT1 can remove the OTUD3 acetyl group, switch off OTUD3 activity, and thus upregulate the viral activation of MAVS and type 1 IFN (222). In response to inflammatory signals or certain viral infections, the DAMP protein high-mobility group box-1 (HMGB1) is over-acetylated, causing it to be exported from the nucleus and released from the cell (227). After reversing the acetylation, SIRT1 tends to confine HMGB1 to the nucleus and promote the transcription of antiviral genes stimulated by type 1 IFNs in the nucleus (228). Oxidative stress causes Keap1 to release Nrf2, following which Nrf2 is phosphorylated and translocated into the nucleus. Melatonin may inhibit Nrf2 ubiquitination, thereby suppressing its proteasomal degradation. In the nucleus, Nrf2 binds to Maf, which binds to the antioxidant response element (ARE). This leads to the transcription of many phase 2 enzymes, which convert ROS and RNS into inactive products, thus reducing oxidative stress (219). Phase 2 enzymes traditionally refer to enzymes that catalyze the conjugation reaction, such as GSH S-transferase. However, the scope of the term has gradually expanded to include several enzymes that

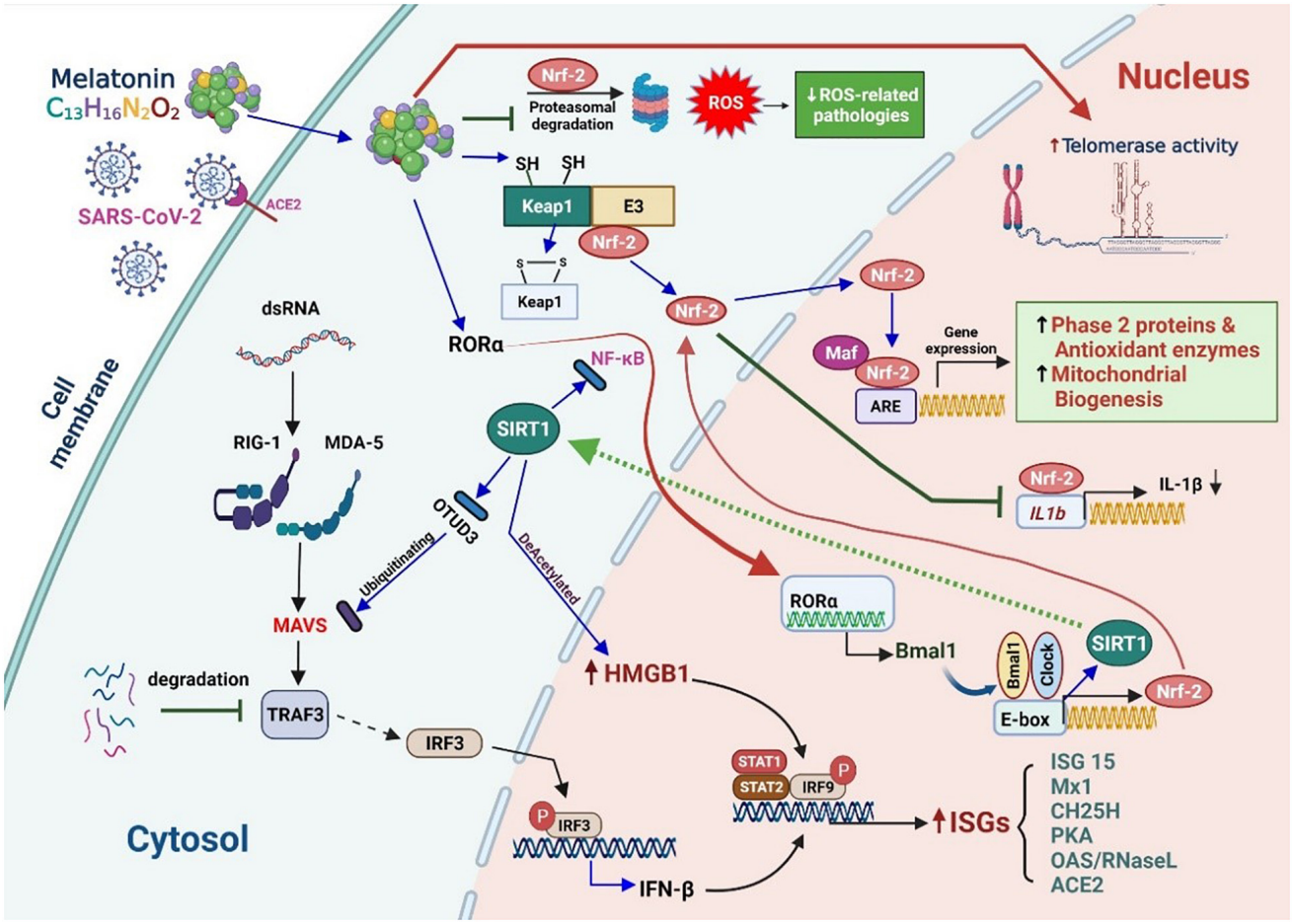

FIGURE 7 | Melatonin supplementation reduces the risk of coronavirus disease (COVID-19) by rescuing the repression IFN/ISG production. Melatonin supplementation restores the suppressed production of IFN/ISG, increases telomerase activity, upregulates SIRT1 expression, enhances virus-mediated MAVS activation, promotes nuclear translocation of the transcription factor RORα, enhances Bmal1 expression, and further upregulates SIRT1 and Nrf2 expression to reduce the risk of COVID-19. Cellular responses to RNA viruses tend to activate IRF3, NF-κB, ATF2, and c-Jun, which may bind to the promoter of the IFNB1 gene and promote its transcription. Overall, melatonin can help suppress processes that promote inflammation, such as NO production, cyclooxygenase 2 activity, NLRP3 inflammasome formation, and cytokine release, in elderly patients with COVID-19. It also activates processes in the anti-inflammatory network, including SIRT1 activation, Nrf2 upregulation, NF-κB downregulation, and release of the anti-inflammatory cytokines IL-4 and IL-10.

catalyze the phase 1 reaction, such as heme oxygenase-1 and NAD(P)H:quinone oxidoreductase type 1.

In short, melatonin can regulate the induction of type 1 IFN and ISGs by inhibiting OTUD3 and promoting the nuclear retention of HMGB1. It also inhibits NF-κB activity and enhances Nrf2 signaling. Furthermore, melatonin improves Keap/Nrf2/ARE signaling and can inhibit the metabolic pathways of ubiquitin and proteasomes (111, 229).

MELATONIN REVERSES BIOLOGICAL AGING

Melatonin and the Mitochondria

The mitochondria play a key role in calcium homeostasis, apoptosis, and the regulation of multiple physiological and pathological processes (230). They are responsible for energy production (ATP) *via* glycolysis in the cytosol and oxidative phosphorylation in the MIM. Glycolysis produces pyruvate, which is actively transported to the mitochondrial matrix, where it is metabolized to acetyl-CoA (231). The mitochondrial ETC uses a series of electron transfer reactions to generate cellular ATP by oxidative phosphorylation. One consequence of electron transfer is the generation of ROS, which contribute to both homeostatic signaling as well as oxidative stress during pathology (232). Acetyl CoA is also a significant co-factor of AANAT, which converts serotonin to N-acetylserotonin, a precursor of MT (233). The presence of nitric oxide synthase (mtNOS) in the mitochondria indicates the production of nitric oxide (NO) and peroxynitrite ($ONOO^-$), which are generated in these organelles during inflammation (234, 235). Additionally, the mitochondria produce precursors of DNA/RNA, proteins, and lipids (236), and participate in the maintenance of cellular homeostasis by Ca^{2+} (237). Conversely, the mitochondria regulate apoptosis

by releasing apoptotic factors (cytochrome C) and activating caspases (230, 238).

Melatonin synthesized in the mitochondria provides protection against oxidative stress and the reprogramming of altered intracellular metabolism to levels higher than those in other subcellular organelles and in the absence of circadian rhythms (197, 239). Mitochondria synthesize melatonin *de novo* and release it, which controls the release of cytochrome C in an autocrine-regulated manner through the MT1 receptor on the membrane (240). Melatonin is present in mitochondrial membranes and passes into the mitochondria through the oligopeptide transporters PEPT1 and PEPT2 located on the membrane (241). The Mitochondrial Free Radical Theory of Aging proposes that mitochondrial free radicals, produced as by-products during normal metabolism, cause oxidative damage (242). The effects of aging include mitROS production, lipid unsaturation, autophagy, mtDNA repair, and others, such as apoptosis and protease or telomeric shortening, corresponding to the various theories of aging (243). These changes exert severe effects on mitochondrial and cell physiology and are common in mitochondrial diseases (244, 245). High levels of melatonin and its multiple actions as an antioxidant provide strong protection to the organelles exposed to free radicals (152). Within the mitochondria, melatonin acts as a direct scavenger of free radicals and related non-radical products, stimulates antioxidant enzymes, including SOD2, catalase, and glutathione reductase, and simultaneously inhibits pro-oxidative enzymes (13, 246, 247).

Melatonin, *via* its receptor-independent activities, may suppress NO synthesis and lipoxygenase activity, which aids the formation of superoxide anion (248). By controlling lipoxygenase activity, melatonin protects cells from the hydroperoxidation of polyunsaturated fatty acids (249). It also modulates reactions associated with ER stress (250), sirtuin activity (251), mitophagy, and autophagy (252).

Melatonin also possesses a significant ability to enhance the efficiency of the ETC and improve the production of ATP (253). The marked improvement in mitochondrial function in response to melatonin reduces ROS production, thereby preventing a deleterious reduction in the mitochondrial membrane potential (189, 254). Low melatonin levels may lead to serious consequences in terms of increased oxidative stress and reduced ATP production, followed by increased ROS formation (152). Melatonin also confers multiple layers of protection to the mitochondria *via* its metabolites, such as cyclic 3-hydroxymelatonin (C3-OHM) and AFMK; these allow melatonin to extend the metabolic cascades necessary for mitochondrial protection during mROS- and mCa^{2+}-mediated MPTP-associated apoptotic stress, and may provide therapeutic benefits in neurodegenerative diseases (255).

Melatonin Reverses Mitochondrial Biological Aging

Aging and various age-related diseases are associated with multiple factors, including reduced secretion of hormones such as melatonin (256), reduced activities of aging-related proteins, such as SIRT1 (239, 257), deterioration of circadian oscillator system activity (258), multiple alterations in the immune system with frequent shifts toward the proinflammatory arm (259), and several other deviations from regular biological functions of cells. Notably, the changes are largely based on the pleiotropy of melatonin (260) and the circadian system (261).

In general, melatonin is an immune stimulant, and the direction in which changes in balance have turned out to be highly conditional. The effect of melatonin on the expression of SIRT1 also revealed downregulation or upregulation, in which case a marked difference was observed between tumor and non-tumor cells (258). The activities of sirtuin are not primarily determined by their protein levels but by the NAD^+ concentration, which depends on the activity of nicotinamide phosphoribosyltransferase (NAMPT) (262, 263). SIRT1 activity, but not its level, was found to be reduced because of lowered NAD^+ levels in the course of aging (264). The high SIRT1 expression levels in several age-related diseases highlight the importance of epigenetics in healthy aging (264). A number of studies have shown that melatonin increases the expression of SIRT1, which could be suppressed by sirtuin inhibitors (265, 266). Pretreatment with melatonin reversed the dexamethasone-induced negative effects on matrix degeneration in chondrocytes. The significant decrease in the NAD^+/NADH ratio and NADPH concentration in the dexamethasone group was reversed upon pretreatment with melatonin (266).

Overall, melatonin inhibits inflammation-promoting processes during aging, such as NO release; cyclooxygenase 2, NLRP3inflammasome, gasdermin D, TLR4, and mTOR signaling; senescence-associated secretory phenotype induction; cytokine release; and beta-amyloid toxicity. It also activates processes in the anti-inflammatory network, which involve the activation of SIRT1, upregulation of Nrf2, downregulation of NF-κB, and the release of the anti-inflammatory cytokines IL-4 and IL-10. Another key role may be to promote the polarization of macrophages or microglia, which favors the anti-inflammatory phenotype of M2 macrophages.

Clinical network analyses, which compare medications used to treat SARS-CoV-2 in humans, have also predicted that melatonin would be the most effective agent to prevent/treat COVID-19. Importantly, melatonin use is associated with a 52% reduction in the likelihood of a positive laboratory test result for SARS-CoV-2 (216). When patients severely infected with COVID-19 were treated with melatonin, the severity of infection, mortality, and length of hospitalization were reduced. Of note, melatonin has a high safety profile at a broad dose range and is not highly toxic (267). Consequently, we encourage the consideration of the use of melatonin as a countermeasure to SARS-CoV-2 infection. Melatonin plays a crucial role in maintaining normal mitochondrial function and energy production in cells by directly scavenging ROS in the mitochondria, activating antioxidant production, and protecting membrane integrity, especially in elderly patients. Further research into the effects of melatonin on the mitochondrial function in patients with COVID-19 as well as mitochondria-targeted melatonin studies will help provide insights into the mechanisms underlying its protective effects and suitability for use in the treatment of various diseases.

POTENTIAL ADVERSE EFFECTS OF MELATONIN IN THE ELDERLY

Dual Immune Effects of Melatonin in Viral Infection

Melatonin is an immunomodulating agent with pro- and anti-inflammatory properties. Its pro-inflammatory properties have been well-documented in several studies of leukocyte-derived cell lines; therefore, melatonin may be detrimental in autoimmune diseases (215), rheumatoid arthritis (268), and multiple sclerosis (269). The immune effects of melatonin are condition-based, although the conditions are not fully understood (270, 271). Melatonin exhibits pro-oxidant, pro-apoptotic, and SIRT1-downregulating properties in cancer cells. However, melatonin is well-known as an antioxidant and anti-apoptotic agent in most non-tumor cells and acts as a SIRT1-upregulating agent under senescent conditions (239, 258). Chronic low-grade inflammation is a hallmark of aging (272). It is generally accepted that melatonin primarily exhibits anti-inflammatory activities (215). As a result, its usage in an aging population is reasonable.

Beneficial Effects of Melatonin in COVID-19

Recent reports suggest that melatonin has been proposed to overcome sepsis-related hyper-inflammation with extensive oxidative damage and cytokine storm in SARS-CoV-2 infection (273, 274). During SARS-CoV-2 infection, viral ORF3a induces HIF-1α activation, which in turn aggravates viral infection and inflammatory responses (275). Melatonin may attenuate the damage resulting from COVID-19-mediated septicemia by mollifying HIF-1α, suppressing NF-κB, inhibiting the inflammasome, converting pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages, and reversing Warburg-type metabolism (276–278). After administration as an adjuvant therapy, melatonin was found to attenuate Th1 and Th2 inflammatory cytokines by downregulating the expression of Th1 and Th2 regulatory genes in patients with COVID-19 (279). A correlation between the levels of circulating secreted Group IIA phospholipase-A2 (SPLA2-IIA) and the severity of COVID-19 was recently reported (280). Activated sPLA2-IIA is an inflammatory agent that is particularly damaging to cellular bio-membranes owing to its ability to hydrolyze fatty acids (281). The damaged membrane releases arachidonic acids that are then transformed through cyclooxygenase into different eicosanoids. These eicosanoids stimulate inflammation and oxidative stress in multiple organs (282, 283). Given the known properties of sPLA2-IIA, it may prolong and exacerbate tissue and organ damage in COVID-19. Commonalities between the symptoms of different conditions associated with sPLA2-IIA may explain some of the beneficial effects of melatonin observed in various diseases.

Adverse Effects of Melatonin

The maximal effective doses of melatonin range from 0.5 to 10.0 mg (284). The European Food Safety Authority (EFSA) recommends maximum doses of 0.3 to 1.0 mg (285). Although pharmacopeias and the EFSA recommend administering melatonin 1–2 h before bedtime, several studies have shown that melatonin is not always effective if administered according to this recommendation (285). It is mainly metabolized in the liver (90%), in a process primarily involving CYP1A2. The most common side effects are headaches, nausea, and dizziness (286). Exogenous melatonin can reduce blood pressure and cause hypothermia in the elderly (286). A large study reported the side effects associated with the use of melatonin, including neurological disorders in 43%, anxiety and depression in 24%, rashes in 19%, and digestive problems in 19% of the study participants (287).

Additionally, drug interactions with melatonin are a source of concern. Cardiovascular diseases are more common in the elderly, and beta-adrenergic receptor blockers are often used for treatment (288). Beta-blockers reduce melatonin production by significantly inhibiting β-1 adrenergic receptors (289). Melatonin intake before bedtime can prevent this common side effect in beta-blocker users (290). Previous studies have reported that melatonin inhibits glucocorticoid synthesis (291). Melatonin exerts inhibitory effects on adrenocorticotropic hormone secretion in the anterior pituitary and on adrenal cortisol production (292). Melatonin also reduces glucocorticoid-induced toxicity by reducing glucocorticoid receptor nuclear translocation (293).

CONCLUSION

This review discusses the benefits of melatonin intake in elderly patients with COVID-19. After the viral protein enters the mitochondria, it uses various mitochondrial protein delivery systems and host cell accessory proteins to stabilize itself. The mitochondria may also complete the steps required to replicate the virus through the viral protein translocation machinery, UPS, and mitochondrial electron distribution system, thereby severely aggravating oxidative stress and inflammation. In addition, COVID-19 affects mitochondrial fusion and fission, which enlarges the mitochondria. Damaged mitochondria undergo mitophagy or apoptosis. However, melatonin plays an important role in these processes and can adequately mitigate these conditions.

Melatonin has the potential to reduce inflammation and possibly curb cytokine storms caused by SARS-CoV-2. Melatonin can directly scavenge various toxic oxygen- and nitrogen-based reactants, stimulate antioxidative enzymes, increase the efficiency of the ETC (thereby limiting electron leakage and free radical generation), and promote ATP synthesis (209). Melatonin is also anti-inflammatory and has special medicinal value in conditions associated with severe inflammation, such as sepsis and ischemia/reperfusion, and low-grade inflammation during aging (215).

Further, the effects of melatonin seem to be closely related to the upregulation of SIRT1. Melatonin also interferes with mTOR and Notch signaling and reduces the expression of pro-inflammatory lncRNA-CCL2 (218). Compared with younger adults, the elderly are more likely to contract severe COVID-19, and the degree of inflammation and aberrant

immune response is closely related to the mitochondrial function. Because the elderly are in a basic state of mild inflammation, COVID-19 appears to be particularly fatal. Melatonin can improve the normal physiological functions of the mitochondria and reduce free oxygen radical production. Therefore, we believe that melatonin use in the elderly is a safe and effective adjunct therapy. Melatonin may also play a crucial role in the treatment of novel viral variants. Further, enhancing an individual's immunity and reducing basal inflammation using melatonin could be a useful strategy against COVID-19.

AUTHOR CONTRIBUTIONS

W-LS, M-TL, C-CW, C-LL, and K-CL drafted the manuscript and obtained revision documents. S-FW, M-CL, and C-CW performed data analysis. W-LS, M-TL, C-LL, and K-CL conducted the research. C-CW and W-LS were responsible for collecting examination data. W-LS, K-CL, M-TL, C-LL, and S-FW designed the study. All authors have read and agreed to the published version of the manuscript.

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GLOSSARY

ACE, angiotensin-converting enzyme; AGII, angiotensin II; Akt, protein kinase B; AMPK, AMP-activated protein kinase; BAX, Bcl2-associated X; Bcl2, B-cell lymphoma 2; Bcl-xL, Bcl-2-like protein 1; BIM, Bcl2-interacting mediator of cell death; CAT, catalase; cGAS, cytoplasmic ring GMP-AMP synthase; COVID-19, coronavirus disease; DAMP, danger-related molecular pattern; ER, endoplasmic reticulum; ERGIC, endoplasmic Reticulum-Golgi intermediate compartment; ETC, electron transport chain; GSH-Px, glutathione peroxidase; GSH, glutathione; HMGB1, high-mobility group box-1; ICAM, intracellular adhesion molecule; IMP, internal membrane protease; IRF, interferon regulatory factor; MAVS, mitochondrial antiviral-signaling proteins; MDA-5, melanoma differentiation-associated gene 5; Mfn, mitochondrial melting factor; MOM, mitochondrial outer membrane; MPTP, mitochondrial permeability transition pore; mtHSP70, mitochondrial heat shock 70 kDa protein; mTORC1, mechanistic target of rapamycin receptor complex 1; NF-κB, nuclear factor kappa light chain enhancer of activated B cells; NICD, intracellular domain of Notch; NLRP3, NLR family pyrin domain containing 3; Nrf2, nuclear factor erythroid 2-related factor 2; OPA1, optical atrophy 1; PAMPs, pathogen-associated molecular patterns; PRR, pattern recognition receptor; RIG-I, retinoic acid-inducible gene I; RLR, retinoic gene I-analog receptor; ROS, reactive oxygen species; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; SIRT, sirtuin; SOD, superoxide dismutase; TACE, TNF-α conversion enzyme; TBARS, thiobarbituric acid reaction products; TBK1, TANK-binding kinase 1; TGEV, transmissible gastroenteritis coronavirus; TLR, Toll-like receptor; TNF, tumor necrosis factor; TOM, outer membrane translocase; UPS, ubiquitin-proteasome system; $\Delta\psi m$, mitochondrial membrane potential.

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BRIEF RESEARCH REPORT
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Vitamin D 25OH Deficiency and Mortality in Moderate to Severe COVID-19: A Multi-Center Prospective Observational Study

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Introduction: Several studies and meta-analyses suggested the role of vitamin D 25OH in preventing severe forms of coronavirus disease 2019 (COVID-19). However, the evidence on the clinical benefits of vitamin D 25OH adequacy in patients hospitalized for COVID-19 remain conflicting and speculative. We aimed to investigate the association between vitamin D 25OH serum levels and mortality in hospitalized patients with moderate to severe COVID-19.

Method: This prospective observational multicentre study included 361 consecutive patients with moderate to severe COVID-19 admitted to the Italian hospitals involved in the NUTRI-COVID19 trial from March to August 2020. For each patient, serum vitamin D 25OH levels were assessed 48 h since admission and classified as deficient (<20 ng/mL) or adequate (≥20 ng/mL). We built a propensity score for low/adequate vitamin D 25OH levels to balance the clinical and demographic properties of the cohort, which resulted in 261 patients with good common support used for the survival analysis.

Results: Two Hundred-seventy-seven (77%) of the 361 enrolled patients (207 [57%] males, median age 73 ± 15.6 years) had vitamin D 25OH deficiency. Fifty-two (20%) of the 261 matched patients died during the hospital stay, corresponding to a hazard ratio of 1.18 for vitamin D 25OH deficiency (95% confidence interval: 0.86–1.62; $p = 0.29$).

Discussion: The prevalence of vitamin D 25OH deficiency was confirmed to be very high in hospitalized patients with COVID-19. The use of a propensity score demonstrate an absence of significant association between vitamin D deficiency and mortality in hospitalized patients.

Keywords: COVID-19, vitamin D 25OH, hospitalized patients, mortality, propensity score (PS)

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INTRODUCTION

Several observational studies and meta-analyses suggested the protective role of vitamin D 25OH in coronavirus disease 2019 (COVID-19) (1, 2), due to its immunomodulatory and anti-inflammatory properties and its ability to modulate endothelial functions (3, 4). However, the clinical benefits of vitamin D 25OH adequacy in patients hospitalized for COVID-19 remain conflicting and speculative (5–8). In a recent paper published by our group, we surprisingly observed that vitamin D 25OH levels were proportionally associated with mortality in patients with COVID-19 (9). However, the limited sample and the severity of the patients' clinical conditions could have influenced the observed results. Hence, we decided to increase the sample size to investigate further the association between vitamin D 25OH levels and mortality in patients hospitalized for moderate to severe COVID-19. For this purpose, we conducted the present prospective observational study, which included patients admitted to the Italian hospitals involved in the multicentric NUTRI-COVID19 trial (10).

METHODS

The multicentre cohort consisted of 361 consecutive COVID-19 patients (nasopharyngeal reverse transcriptase-polymerase chain reaction positive swab) admitted to six Italian hospitals

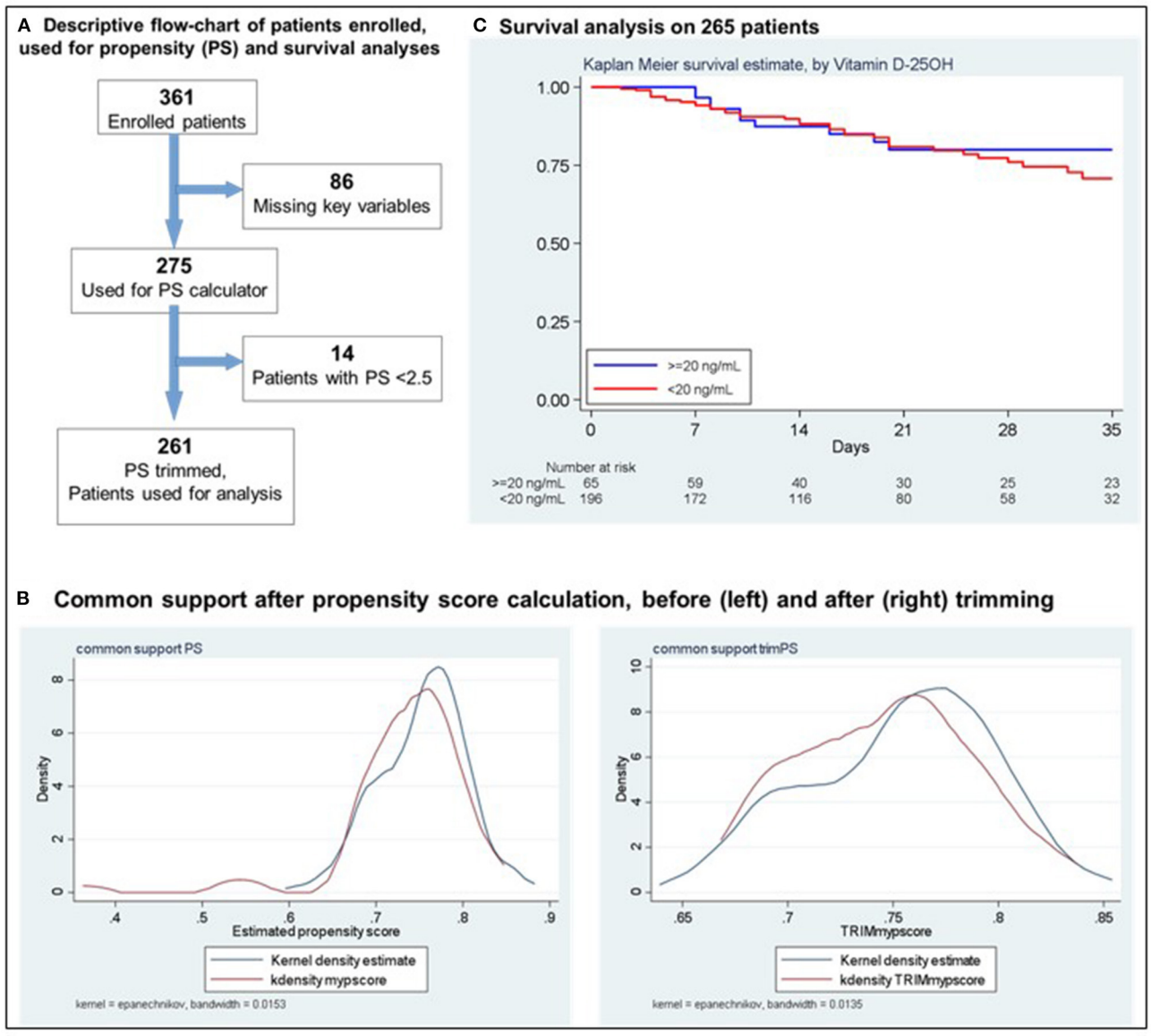

FIGURE 1 | (A) Descriptive flow-chart of patients enrolled used for propensity (PS) and Survival analyses. **(B)** Common support after propensity score calculation, before (left) and after (right) trimming. **(C)** Survival analysis on 265 patients.

TABLE 1 | Clinical and demographic characteristics of the enrolled patients.

Feature	All patients in the cohort *N* = 361			Patients used for PS building *N* = 275		
	25(OH)Vitamin D≥20 ng/mL *N* = 84 (23%)	25(OH)Vitamin D<20 ng/mL *N* = 277 (77%)	P-value	25(OH)Vitamin D≥20 ng/mL *N* = 69 (25%)	25(OH)Vitamin D<20 ng/mL *N* = 206 (75%)	P-value
Male, *N* (%)	41 (480.8)	166 (590.9)	0.04	36 (230.4)	118 (760.6)	0.27
Age, Median (IQR)	710.7 (660.5–810.5)	690.5 (590.0–820.0)	0.67	710.7 (640.0–810.0)	700.3 (600.0–840.0)	0.77
Body mass index (kg/m^2), Median (IQR)	240.45 (220.7–270.3)	250.5 (220.5–280.1)	0.21	240.3 (220.7– 270.3)	250.2 (220.2–270.7)	0.38
COPD, *N* (%)	13 (150.5)	34 (120.3)	0.28	11 (150.9)	26 (120.6)	0.30
Diabetes, *N* (%)	21 (25)	78 (280.3)	0.33	18 (260.1)	63 (300.6)	0.29
Hypertension, *N* (%)	49 (580.3)	172 (620.3)	0.29	40 (58)	134 (65)	0.18
Ischemic heart disease, *N* (%)	23 (270.4)	86 (310.2)	0.30	19 (270.5)	65 (310.5)	0.32
Cancer, *N* (%)	17 (200.2)	37 (130.4)	0.09	13 (180.8)	31 (150.1)	0.29
Chronic kidney disease, *N* (%)	14 (160.7)	48 (170.3)	0.51	11 (150.9)	42 (200.4)	0.26
Number of comorbidities, Median (IQR)	2 (1–3)	2 (1–3)	0.64	2 (1–3)	2 (1–3)	0.69
Lactate dehydrogenase (U/L), Median (IQR)	338 (203–345)	305 (210–369)	0.67	3360.14 (2030.−343)	3030.93 (261–359)	0.54
C–reactive protein (mg/dL), Median (IQR)	110.04 (10.71–90.29)	80.98 (10.88–140.50)	0.07	110.72 (10.52– 80.12)	90.64 (20.25– 140.93)	0.02
Severe pneumonia [a], *N*(%)	41 (490.4)	143 (510.8)	0.39	32 (460.4)	96 (460.6)	0.54
Pavia Hospital	30 (350.7)	99 (350.7)	0.55	29 (42)	94 (450.6)	0.35
Other Hospitals	54 (640.3)	178 (640.2)	0.55	40 (570.9)	112 (540.4)	0.35

***COPD**, chronic obstructive pulmonary disease; **c–PAP** continuous Positive Airway Pressure; [a]According to the American Thoracic Society guidelines0. PS, propensity score0.*

between March and August 2020, not included in experimental treatment protocols. The local Institutional Ethics Committees approved the study, and written informed consent was obtained from every patient. Each patient was tested within 48 h since admission for serum vitamin D 25OH status [chemiluminescent immunoassay (Abbott Diagnostics, Lake Forest, IL, USA)] and, based on the results, was classified as adequate (≥20 ng/mL) or deficient (<20 ng/mL). Of the 361 hospitalized patients, complete data on key variables for mortality (age, sex, C-reactive protein [PCR], lactic acid dehydrogenase [LDH], body mass index [BMI], major comorbidities, and severe pneumonia) were available for 275 patients. Considering the significant clinical and laboratory differences between patients admitted to our and the other hospitals and the heterogeneity of the key variables in the enrolled patients, we fitted a Cox model to assess the association of vitamin D 25OH and in-hospital mortality, weighting the analysis by the inverse propensity score. This weight was derived from a propensity score for low/adequate vitamin D 25OH levels, including the key clinical and demographic properties. After trimming the upper and lower 2.5th percentiles of the score, the procedure yielded 261 patients with good common support (**Figures 1A,B**). All the analyses were performed using Stata 16 (StataCorp. College Station, TX, USA).

RESULTS

Two Hundred-seventy seven (77%) of the 361 enrolled patients (207 [57%] males, median age 73 ± 15.6 years) had vitamin D 25OH deficiency. No significant differences were observed in demographic and clinical features according to vitamin D 25OH status. In the 275 patients considered for the propensity score, a statistically significant difference in PCR values between those with adequate and deficient vitamin D 25OH serum levels [11.72 (1.52−8.12) vs. 9.64 (2.25–14.93), respectively; $p = 0.02$] was detected (**Table 1**). After a median follow-up of 20 days (95% confidence interval [CI]:10–33), in the propensity score-matched and trimmed group ($N = 261$), 52 patients died during the hospital stay. Thirteen had vitamin D 25OH adequate levels (mortality rate 5.3 per 100 per year, 95% CI: 3.1–9.6), 39 had vitamin D 25OH deficiency (mortality rate 6. per 100 per year, 95% CI: 4.8–8.9), which resulted in a hazard ratio [HR] of 1.18 (95% CI: 0.86–1.62; $p = 0.29$) (**Figure 1C**). A sensitivity analysis addressing severe deficiency (vitamin D 25OH <10 ng/mL, $p = 0.64$; <5 ng/mL, $p = 0.14$) yielded similar results. A further model using vitamin D 25OH on a continuous scale (linearity checked using fractional polynomials) confirmed the lack of association with the outcome (HR = 1.01, 95%CI 0.99–1.04; $p = 029$).

DISCUSSION

This study confirms that vitamin D 25OH deficiency is prevalent in hospitalized COVID−19 patients, with close to 80% prevalence rates. However, this deficiency is not associated with increased mortality. Our results contrast with some other studies, such as a recent retrospective observational study from Saudi Arabia, which detected an association between severe

deficiency and mortality (11). On the other hand, in line with our findings, a recent meta–analysis confirmed the absence of a correlation between vitamin D 25OH supplementation and clinical outcomes (admission to Intensive Care Units and mortality) in COVID−19 patients (12). Moreover, in a recent randomized trial conducted on 240 patients, a single dose (200.000 IU) of vitamin D 25OH did not significantly reduce in–hospital mortality compared to placebo (13); nor did small doses of 25OH vitamin D administered daily reduce mortality, although it shortened recovery symptoms (14). In the recent spread of articles attempting to correlate vitamin D 25OH levels with clinical outcomes in COVID−19, our choice to use a propensity score to limit confounding factors may have helped clarify this issue. Although vitamin D 25OH has an acknowledged immunomodulatory function which could reduce the risk of COVID−19 infection, it probably cannot limit its progression in hospitalized and severe patients, in whom the extensive activation of innate effectors and the ineffective adaptive T lymphocytes–mediated response may have a decisive role in determining the disease outcomes (15). Considering the high prevalence of vitamin D 25OH deficiency observed, in line with a recent and updated meta–analysis (16), our confirmatory results may reinforce the hypothesis that adequate vitamin D 25OH levels could be protective in reducing the risk of hospitalization for COVID−19, but not for the survival in hospitalized patients with moderate to severe disease. However, although our propensity score–based study design allowed us to perform a more robust analysis of a multivariable model, avoiding the risk of overfitting, it should be acknowledged that it could be associated with bias due to potential unmeasured confounders. It is also important to specify that no pre–admission vitamin D values were available. It is therefore difficult to establish whether the vitamin D deficiency depends on the infection or on an earlier status. Given the age of the population and the known epidemiological data on endemic vitamin D deficiency, as well as the relative stability of the 25–hydroxy form, a pre–existing deficit could be hypothesized. On the other hand, a sustained inflammatory burden–as the one characterizing COVID−19–could be responsible for a shorter half–life of vitamin–binding proteins and an increase in total body water, thus resulting in low serum concentrations (17). In light of these limitations, our results confirm that even though 25OH vitamin D may have a protective role in preventing severe COVID−19, its deficiency is not associated with increased mortality in hospitalized patients with moderate to severe disease.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comitato Etico referente Area di Pavia, Fondazione IRCCS Policlinico San Matteo, Pavia. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LB, EC, and RC had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of data analysis. RC is chief investigators and act as guarantors for this work. Concept and design: LB, CK, RC, and EC. Acquisition, analysis, or interpretation of data: RC, EC, LB, CK, LD, FL, SM, SC, SB, SD, AM, NC, AM, and SC. Drafting of the manuscript: LB, RC, EC, CK, and LD. Statistical analysis: CK. All authors contributed to the article and approved the submitted version.

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Major dietary patterns in relation to disease severity, symptoms, and inflammatory markers in patients recovered from COVID-19

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Background: COVID-19 is a highly transmissible viral infection with high morbidity. Few studies have been done about dietary intakes in patients with COVID-19. This study aimed to evaluate the association between major dietary patterns before COVID-19 diagnosis in recovered patients and the risk of disease severity and symptoms after the disease begins.

Methods: Overall, 250 recovered cases with both genders completed study questionnaires providing data on demographic characteristics, self-reported web-based 168-item food frequency questionnaire (FFQ), and COVID-19 outcomes in Shahid Beheshti Hospital, Kashan. PCR was used to determine a positive diagnosis of COVID-19. We used multivariable logistic regression models to assess the association between major dietary patterns and study outcomes. All statistical analyses were done by SPSS version 16.

Results: We identified three major dietary patterns—unhealthy, traditional, and healthy dietary patterns. Serum levels of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were significantly higher in patients with unhealthy and traditional dietary patterns and lower in those with healthy dietary patterns. There was a significant direct relationship between unhealthy and traditional patterns with risk of severe COVID-19 and hospitalization duration and a significant direct association between an unhealthy pattern and the odds ratio (OR) of convalescence duration. A significant inverse relationship was found between healthy pattern and risk of severe COVID-19 and OR of convalescence duration. We found a significant direct association between unhealthy pattern and OR of cough, fever, chilling, weakness, myalgia, nausea and vomiting, and sore throat and between traditional pattern and OR of cough, fever, and chilling. In contrast, a significant inverse association was seen between healthy pattern and OR of dyspnea, weakness, and sore throat.

Conclusion: This study showed that high adherence to an healthy pattern was associated with lower CRP and ESR levels and lower risk of severe COVID-19, hospitalization, and convalescence duration in patients who recovered from COVID-19. More adherence to unhealthy or traditional dietary patterns was associated with higher CRP and ESR levels and a higher risk

of severe COVID-19 and hospitalization duration. A direct association was found between unhealthy and traditional patterns and the risk of some COVID-19 symptoms, while an inverse association was found for a healthy dietary pattern.

KEYWORDS

dietary patterns, COVID-19, inflammation, symptoms, disease severity

Introduction

Coronaviruses are a large family of viruses that can cause respiratory infections in animals and humans (1). COVID-19 is an infectious disease caused by a new coronavirus and was first observed in Wuhan, China (2). The disease was unknown before it began to spread in Wuhan in December 2019 (2). So far, it has affected more than 190 countries around the world (3, 4). The clinical and laboratory features of COVID-19 are similar to the severe acute respiratory syndrome (SARS), which was first observed in China, and Middle East respiratory syndrome (MERS), which was first observed in Saudi Arabia (5).

Acute respiratory syndrome in COVID-19 is the main cause of hospitalization in the intensive care unit (ICU) and death (6). Cytokine storm is the main cause of organ dysfunction among these patients (7). Among environmental factors, dietary intake is an important factor affecting inflammation in the body (8–10). Therefore, it seems that the dietary intake of patients with COVID-19 before the beginning of the disease might influence the disease outcomes (11). So far, numerous studies have indicated that deficiency in vitamins and minerals might influence susceptibility to infectious diseases (12). In addition, some studies have shown the special role of some vitamins such as vitamin D in the immune function through infectious diseases (13, 14). However, it must be kept in mind that interactions between nutrients might confound the association of a specific nutrient with COVID-19. Therefore, the dietary pattern can be used as a new direction in nutritional epidemiology to find diet–disease relationships.

A recent population-based case–control study among six countries indicated that consumption of a plant-based diet was associated with a lower odds ratio (OR) of moderate to severe COVID-19 (15). Another study about COVID-19 symptoms and habitual food intake in adult outpatients indicated that an increase in habitual intake of legumes and grains, bread, and cereals was associated with reduced overall symptom severity in patients with COVID-19 (11). A recent study about diet and duration of recovery from COVID-19 showed that adherence to a healthy diet was associated with a shorter duration of recovery from COVID-19 (16).

Although the association of several nutrients with COVID-19 outcomes has received great attention, we are unaware of any study linking major dietary patterns to COVID-19 outcomes. Therefore, this study is conducted to determine the relationship between major dietary patterns and COVID-19 outcomes in Kashan, Iran.

Methods

This retro-prospective study was performed among 250 recovered cases of COVID-19 aged 18–65 years of both genders, who were selected using a simple random sampling method from Shahid Beheshti Hospital, Kashan, Iran. This study was performed from June to September 2021. The study protocol was approved by the Ethics Committee of Kashan University of Medical Sciences (Registration No. IR.KAUMS.MEDNT.REC.1400.048). All participants were requested to complete informed consent.

All patients with COVID-19 who had medical records in the Shahid Beheshti Hospital with a maximum of 3 months from the beginning of their COVID-19 diagnosis were included. Patients were excluded if any of the following conditions existed: 1- Patients with other diseases than COVID-19. 2- those who had a history of chronic diseases such as diabetes and heart disease as well as diseases that affect the severity of COVID-19. 3- patients with a body mass index of more than 40. 4- pregnant or breastfeeding women; 5- current smokers. 6- patients who were consuming dietary supplements more than two times in a week before the first diagnosis of COVID-19. 7- patients who were on specific diets. 8- patients who were consuming medicines that influence respiratory function including fluticasone and flunisolide; and 9- subjects with insufficient data in their medical records (Figure 1).

Assessment of dietary intake

A 168-item food frequency questionnaire (FFQ) was obtained from patients through a web-based online questionnaire to collect information on their dietary intakes during the past year before the diagnosis of COVID-19. Participants were asked to report their dietary intakes as daily, monthly, or annually. Finally, we converted their intakes of food

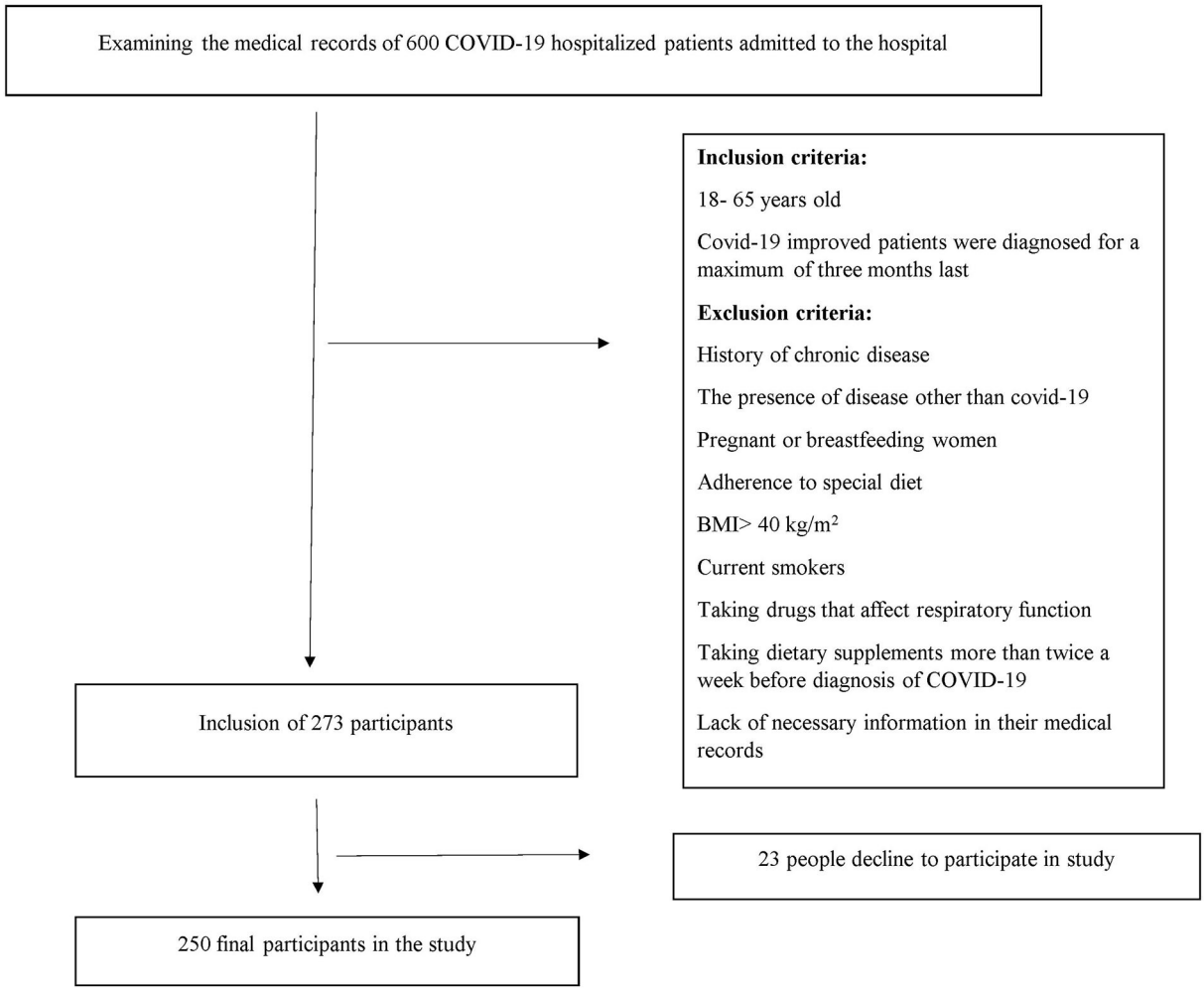

FIGURE 1
Flowchart of the study's participants.

items into grams per day using "household measures." Dietary intakes of micro- and macro-nutrients were calculated by the use of the Nutritionist 4 (N4) software.

Measurement of COVID-19 severity

COVID-19 severity was assessed by the COVID-19 Treatment Guidelines (CTG) (17), updated on 19 October 2021. According to the CTG, the severity of COVID-19 was categorized into five levels. Asymptomatic or presymptomatic infection: individuals with a positive test for SARS-CoV-2 using a virologic test (i.e., a nucleic acid amplification test [NAAT] or an antigen test) but without the symptoms of COVID-19. Mild illness: individuals with any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, and loss of taste and smell) but without breath shortness, dyspnea, or abnormal chest imaging. Moderate illness: individuals with evidence of lower respiratory disease during clinical assessment or imaging and who have an oxygen saturation (SpO2) ≥94% on room air at sea level. Severe illness: individuals with SpO2 <94% on room air at sea level, a ratio of arterial partial oxygen pressure to the fraction of inspired oxygen (PaO2/FiO2) <300 mm Hg, and a respiratory rate >30 breaths/min or lung infiltrates >50%. Critical illness: individuals who had respiratory failure, septic shock, and/or multiple organ dysfunctions. We considered mild and moderate illnesses as a non-severe illness.

Measurement of COVID-19 symptoms

We asked patients to fulfill a general questionnaire including a question about the presence of each common symptom

of COVID-19. These symptoms were dyspnea, cough, fever, chilling, weakness, myalgia, sore throat, nausea, and vomiting.

Assessment of inflammatory markers

Data on erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were obtained from medical records. First measurements of CRP and ESR at the beginning of the disease were obtained.

Assessment of other variables

Required information on demographic characteristics, physical activity, convalescence duration, supplements intake, corticosteroids use, antiviral drug use, and participants' height and weight were obtained for each subject by a general questionnaire.

Statistical analysis

Normal distribution of data was explored by the Kolmogorov-Smirnov test. We classified 168 food items in FFQ into 21 predefined food groups to identify dietary patterns. The similarity of nutrients in food items was the basis for this classification: eggs, processed meat, sweets and desserts, sweetened drinks, meats, solid oils, junk foods, liquid oils, salt, refined grains, whole grains, flavors and pickles, chicken and fish, caffeine-containing drinks, red meat, vegetables, fruits and juices, low-fat dairy products, nuts, high-fat dairy products, and legumes (18–20). We conducted varimax rotation to generate a simple and differential varimax. The scree plot test and eigenvalues>1 were used to determine major dietary patterns. Adherence score for each dietary pattern was obtained and participants were categorized as tertiles based on these scores. We used an independent *t*-test to compare quantitative variables between categories of dietary patterns. A chi-square test was used to compare qualitative variables between categories. The correlation between adherence to each dietary pattern with outcomes of interest by considering confounding variables was assessed by the multivariable regression test. All statistical analyses were performed by the Statistical Package for Social Sciences software (SPSS Inc., version 16). A p-value of <0.05 was considered statistically significant.

Results

Major dietary patterns

We identified three major dietary patterns – unhealthy, traditional, and healthy dietary patterns (21). The unhealthy pattern was mainly characterized by a high intake of processed meats, sweets and desserts, energy drinks, red meats, solid oils, and junk foods. Participants in the traditional pattern had a high load of sweets and desserts, solid oils, salt, refined cereals, caffeine, and high-fat dairy product consumption. The healthy pattern was highly characterized by the intake of eggs, liquid oils, flavors and pickles, chicken and fish, vegetables, fruits and fruit juices, and nuts and legumes. Factor loadings of food groups in these major patterns are shown in Table 1.

TABLE 1 Factor loadings of food groups in major dietary patterns.

Food group	Major dietary pattern		
	Unhealthy	Traditional	Healthy
Egg			0.427
processed meat	0.726		
Sweets and desserts	0.543	0.466	
Energy drinks	0.833		
Visceral meats			
Solid oils	0.702	0.423	
Junk foods	0.679		
liquid oils			0.550
Salt	0.459	0.526	
Refined cereal		0.657	
Whole grain		−0.684	
Flavor and pickle	0.482		0.541
Chicken and fish	−0.606		0.555
Caffeine		0.579	
Red meats	0.432		
Vegetables	−0.675		0.461
Fruits and juice	−0.702		0.439
Low fat dairy products	−0.471	−0.445	
Nuts			0.765
High fat dairy products		0.521	
Legume	−0.561		0.421

Characteristics of participants according to tertiles of major dietary patterns

The characteristics of participants according to tertiles of major dietary patterns are shown in Table 2. We found significant differences in age (43.80 ± 11.52 vs. 47.26 ± 11.33, $p = <0.01$), body mass index (BMI) (29.27 ± 3.56 vs. 26.20 ± 3.01, $p = <0.001$), likelihood of overweight or obesity (75 vs. 51, $p = <0.001$), supplements intake (83 vs. 79, $p = <0.01$), and corticosteroids use and antiviral drug use (83 vs. 77, $p = <0.01$) in the highest vs. lowest tertiles of unhealthy dietary pattern. However, there were no significant differences in physical activity level and gender between tertiles of the

TABLE 2 General characteristics of participants across tertiles of dietary patterns.

Variables		Dietary patterns								
		Unhealthy			Traditional			Healthy		
		T1	T3	*p*–value	T1	T3	*p*–value	T1	T3	*p*–value
Age (year)		47.26 ± 11.33	43.80 ± 11.52	<0.01	42.21 ± 12.28	43.04 ± 12.41	0.02	46.49 ± 11.66	41.62 ± 12.46	0.036
BMI (kg/m^2)		26.20 ± 3.01	29.27 ± 3.56	<0.001	25.16 ± 2.77	27.96 ± 3.48	<0.001	27.95 ± 3.4	26.24 ± 3.62	<0.01
Physically active (%)	sedentary	11(13.3)	11(13.3)	0.932	6(7.2)	9(10.8)	0.171	12(14.5)	6(7.2)	0.157
	moderate	67(80.7)	65(78.3)		71(85.5)	67(80.7)		64(77.1)	69(83.1)	
	intense	5(6)	7(8.4)		6(7.2)	7(8.4)		7(8.4)	8(9.6)	
Gender (female) (%)		42(50.6)	44 (53.0)	0.93	45(54.2)	44(53.0)	0.81	48(57.8)	37(44.6)	0.21
Overweight or obese (%)		51 (61.4)	75 (90.4)	<0.001	46 (55.4)	64 (77.1)	0.01	65 (78.3)	47 (56.6)	0.012
Supplements intake (%)		79 (95.2)	83 (100)	<0.01	75 (90.4)	81 (97.6)	0.08	78 (94)	77 (92.8)	0.34
Corticosteroids use (%)		77 (92.8)	83 (100)	<0.01	70 (84.3)	81 (97.6)	<0.001	77 (92.8)	74 (89.2)	0.49
Antiviral drugs use (%)		77 (92.8)	83 (100)	<0.01	70 (84.3)	81 (97.6)	<0.001	77 (92.8)	74 (89.2)	0.49

unhealthy pattern. With regard to traditional pattern, significant differences were observed in age (43.04 ± 12.41 vs. 42.21 ± 12.28, p = 0.02), BMI (27.96 ± 3.48 vs. 25.16 ± 2.77, p = <0.001), likelihood of overweight or obesity (64 vs. 46, p = 0.01), and corticosteroids use and antiviral drug use (81 vs. 70, p = <0.001) between the highest and lowest tertiles of the traditional pattern. There were no significant differences in physical activity level, gender, and percentage of participants with supplement use across tertiles of the dietary pattern. Finally, significant differences were observed in age (41.62 ± 12.46 vs. 46.49 ± 11.66, p = 0.03), BMI (26.24 ± 3.62 vs. 27.95 ± 3.4, p = <0.01), and the likelihood of overweight or obesity (47 vs. 65, p = 0.01) between the highest and lowest tertiles of healthy pattern, while no significant differences were found in physical activity level, gender, supplements intake, and corticosteroids or antiviral drug use.

Dietary intake of nutrients across tertiles of major dietary patterns

Dietary intake of nutrients across tertiles of major dietary patterns is shown in Table 3. Comparing the highest to the lowest tertile of unhealthy pattern, significant differences were observed in total energy (2926.94 ± 447.75 vs. 2653.89 ± 429.51, p = <0.001), carbohydrate (431.69 ± 43.66 vs. 408.33 ± 54.36, p = <0.001), fat (120.83 ± 21.49 vs. 87.65 ± 20.69, p = <0.001), dietary fiber (19.44 ± 1.8 vs. 26.57 ± 3.9, p = <0.001), vitamins B_1 (2.5 ± 0.3 vs. 2.56 ± 0.37, p = <0.01), B_6 (1.59 ± 0.27 vs. 1.78 ± 0.3, p = <0.001), B_9 (357.78 ± 40.25 vs. 466.44 ± 85.29, p = <0.001), B_{12} (3.72 ± 0.62 vs. 4.74 ± 1.24, p = <0.001), C (111.5 ± 13.5 vs. 165.5 ± 31.64, p = <0.001), E (8.5 ± 2.24 vs. 6.8 ± 1.6, p = <0.001), D (2.59 ± 0.72 vs. 2.01 ± 0.58, p = <0.001), and A (1,175 ± 166 vs. 1,438 ± 322, p = <0.001), and calcium (867.2 ± 76.08 vs. 951.11 ± 145.57, p = <0.001) and magnesium (306.79 ± 25.68 vs. 354.68 ± 55.12, p = <0.001) intakes. In addition, dietary intakes of energy (2,894.68 ± 416.27 vs. 2,586.39 ± 503.03, p = <0.001), carbohydrate (437.58 ± 45.31 vs. 385.43 ± 59.31, p = <0.001), fat (106.80 ± 23.60 vs. 90.6 ± 28.53, p = <0.001), vitamins D (2.45 ± 0.64 vs. 2.14 ± 0.79, p = 0.019), E (8.1 ± 1.99 vs. 5.8 ± 1.46, p = <0.001), and B_1 (2.65 ± 0.3 vs. 2.3 ± 0.37, p = <0.001), and calcium (945.5 ± 134.4 vs. 906.7 ± 164.05, p = <0.01) were significantly different between the highest in contrast to the lowest tertile of traditional pattern. Comparing the highest to the lowest tertiles of healthy pattern, we found significant differences in total energy (2,892.26 ± 414.30 vs. 2,551.5 ± 529.97, p = <0.001), protein (121.23 ± 11.15 vs. 92.84 ± 15.86, p = <0.001), carbohydrate (422.07 ± 49.72 vs. 391.52 ± 62.2, p = <0.001), fat (108.41 ± 16.91 vs. 84.73 ± 30.8, p = <0.001), dietary fiber (26.38 ± 4.7 vs. 20.05 ± 4.4, p = <0.001), vitamins B_1 (2.6 ± 0.32 vs. 2.35 ± 0.37, p = <0.001), B_6 (1.9 ± 0.15 vs. 1.39 ± 0.25, p = <0.001), B_9 (484.76 vs. 347.86 ± 85.53, p = <0.001), B_{12} (4.8 ± 0.91 vs. 3.39 ± 1.14, p = <0.001), C (162.04 ± 29.34 vs. 113.55 ± 31.36, p = <0.001), E (7.06 ± 1.4 vs. 6.64 ± 2.33, p = <0.01), D (2.32 ± 0.57 vs. 2.1 ± 0.97, p = 0.032), and A (1,461 ± 271 vs. 1,139 ± 281, p = <0.001), and zinc (11.6 ± 1.1 vs. 8.6 ± 1.6, p = <0.001), calcium (998.9 ± 111.8 vs. 823.93 ± 136.49, p = <0.001), and magnesium (370.16 ± 37 vs. 282.4 ± 49.23, p = <0.001) intakes.

The association between major dietary patterns, CRP, and ESR

The association between major dietary patterns and inflammatory markers after adjustment for sex, age, BMI, physical activity, and energy intake in patients with COVID-19 is shown in Table 4. Serum levels of CRP and ESR were

TABLE 3 Daily nutrient intake of all subjects across tertiles of dietary patterns.

Nutrients	Major dietary pattern								
	Unhealthy			Traditional			Healthy		
	T1	T3	*p*–value	T1	T3	*p*–value	T1	T3	*p*–value
Energy (Kcal/day)	2653.89 ± 429.51	2926.94 ± 447.75	<0.001	2586.39 ± 503.03	2894.68 ± 416.27	<0.001	2551.5 ± 529.97	2892.26 ± 414.30	<0.001
Protein (g/day)	111.59 ± 18.54	105.20 ± 11.12	0.052	106 ± 21.85	111.21 ± 15.36	0.122	92.84 ± 15.86	121.23 ± 11.15	<0.001
Carbohydrate (g/day)	408.33 ± 54.36	431.69 ± 43.66	<0.001	385.43 ± 59.31	437.58 ± 45.31	<0.001	391.52 ± 62.2	422.07 ± 49.72	<0.001
Fat (g/day)	87.65 ± 20.69	120.83 ± 21.49	<0.001	90.6 ± 28.53	106.80 ± 23.60	<0.001	84.73 ± 30.8	108.41 ± 16.91	<0.001
Dietary fiber (g/day)	26.57 ± 3.9	19.44 ± 1.8	<0.001	23.46 ± 4.44	23.33 ± 5.33	0.58	20.05 ± 4.4	26.38 ± 4.7	<0.001
Vitamin D	2.01 ± 0.58	2.59 ± 0.72	<0.001	2.14 ± 0.79	2.45 ± 0.64	0.019	2.1 ± 0.97	2.32 ± 0.57	0.032
Vitamin A	1438 ± 322	1175 ± 166	<0.001	1346 ± 424	1341 ± 274	0.299	1139 ± 281	1461 ± 271	<0.001
Vitamin E	6.8 ± 1.6	8.5 ± 2.24	<0.001	5.8 ± 1.46	8.1 ± 1.99	<0.001	6.64 ± 2.33	7.06 ± 1.4	<0.01
Vitamin C	165.5 ± 31.64	111.5 ± 13.5	<0.001	144.32 ± 30.94	138.14 ± 42.86	0.282	113.55 ± 31.36	162.04 ± 29.34	<0.001
Vitamin B_1	2.56 ± 0.37	2.5 ± 0.3	<0.01	2.3 ± 0.37	2.65 ± 0.3	<0.001	2.35 ± 0.37	2.6 ± 0.32	<0.001
Vitamin B_6	1.78 ± 0.3	1.59 ± 0.27	<0.001	1.69 ± 0.33	1.66 ± 0.32	0.222	1.39 ± 0.25	1.9 ± 0.15	<0.001
Vitamin B_9	466.44 ± 85.29	357.78 ± 40.25	<0.001	426.53 ± 95.3	411.69 ± 96.76	0.47	347.86 ± 85.53	484.76	<0.001
Vitamin B_{12}	4.74 ± 1.24	3.72 ± 0.62	<0.001	3.99 ± 1.5	4.28 ± 1.02	0.273	3.39 ± 1.14	4.8 ± 0.91	<0.001
Zinc	10.45 ± 1.74	10.01 ± 1.16	0.24	10.35 ± 2.37	10.28 ± 1.53	0.923	8.6 ± 1.6	11.6 ± 1.1	<0.001
Calcium	951.11 ± 145.57	867.2 ± 76.08	<0.001	906.7 ± 164.05	945.5 ± 134.4	<0.01	823.93 ± 136.49	998.9 ± 111.8	<0.001
Magnesium	354.68 ± 55.12	306.79 ± 25.68	<0.001	321.76 ± 60.5	336.83 ± 54.62	0.187	282.4 ± 49.23	370.16 ± 37	<0.001

TABLE 4 Inflammatory biomarkers across tertiles of dietary patterns.

	Dietary patterns											
	Unhealthy				Traditional				Healthy			
	T1	T3	*P*–value	Ad.P	T1	T3	*p*–value	Ad.P	T1	T3	*p*–value	Ad.P
CRP (mg/dl)	10.61 ± 14.85	35.62 ± 24.29	<0.001	<0.001	12.63 ± 18.43	25.3 ± 24.44	<0.01	<0.001	26.14 ± 25.5	11.89 ± 12.42	<0.001	<0.001
ESR (mm/hr)	15 ± 16	41.65 ± 29.83	<0.001	<0.001	16.96 ± 16.57	31.33 ± 30.66	<0.01	<0.001	33.9 ± 29.88	15.62 ± 11.69	<0.001	<0.001

Adjusted for sex, age, BMI, physical activity and energy intake.

significantly higher in patients at top tertiles of unhealthy (35.62 ± 24.29 vs. 10.61 ± 14.85, $p = <0.001$ and 41.65 ± 29.83 vs. 15 ± 16, $p = <0.001$, respectively) and traditional (25.3 ± 24.44 vs. 12.63 ± 18.43, $p = <0.001$ and 31.33 ± 30.66 vs. 16.96 ± 16.57, $p = <0.001$, respectively) dietary patterns than those at the bottom. In contrast, our analysis indicated lower levels of CRP and ESR in those at the third tertile vs. those at the first tertile of the healthy dietary pattern (11.89 ± 12.42 vs. 26.14 ± 25.5, $p = <0.001$ and 15.62 ± 11.69 vs. 33.9 ± 29.88, $p = <0.001$, respectively).

The relationship between major dietary patterns and the risk of severe COVID-19

Multivariable binary logistic regression for the relationship between major dietary patterns and risk of severe COVID-19 is indicated in Table 5. In the crude model, we found a significant direct association between adherence to unhealthy and traditional dietary patterns and risk of severe COVID-19 (OR: 4.34; 95% confidence interval (CI): 2.26, 8.34, $p = <0.001$ and 3.37; 95% CI: 1.77, 6.42, $p = <0.001$, respectively). Such relationship was also observed after controlling for age, sex, and energy intake (OR: 5.06; 95% CI: 2.51, 10.21, $p = <0.001$ and 3.61; 95% CI: 1.81, 7.19, $p = <0.001$, respectively). Additional adjustments for other potential confounders including physical activity, supplement use, corticosteroids use, and antiviral drug use had no effect on the association (OR: 4.57; 95% CI: 2.34, 9.64, $p = <0.01$ and 3.13; 95% CI: 1.55, 6.3, $p = <0.01$, respectively). In the fully adjusted model, this association also remained significant (OR: 3.23; 95% CI: 1.53, 6.81, $p = <0.01$ and 2.17; 95% CI: 1.03, 4.54, $p = 0.04$, respectively). We found a significant inverse relationship between adherence to healthy pattern and risk of severe COVID-19 (OR: 0.25; 95% CI: 0.13, 0.49, $p = <0.001$). After adjustments for the potential confounders in three models, the association remained as statistically significant

TABLE 5 Odds ratio (95% CI) of severe disease in relation to major dietary patterns.

	Dietary patterns								
	Unhealthy			Traditional			Healthy		
	T1	T3	*p*–value	T1	T3	*p*–value	T1	T3	*p*–value
Crude	1	4.34 (2.26, 8.34)	<0.001	1	3.37 (1.77, 6.42)	<0.001	1	0.25 (0.13, 0.49)	<0.001
Model 1	1	5.06 (2.51, 10.21)	<0.001	1	3.61 (1.81, 7.19)	<0.001	1	0.22 (0.1, 0.45)	<0.001
Model 2	1	4.57 (2.34, 9.64)	<0.01	1	3.13 (1.55, 6.3)	<0.01	1	0.21 (0.1, 0.45)	<0.01
Model 3	1	3.23 (1.53, 6.81)	<0.01	1	2.17 (1.03, 4.54)	0.04	1	0.31 (0.14, 0.68)	<0.01

Model 1, Adjusted for sex, age and energy intake; Model 2, Further adjusted for physical activity, supplement use, corticosteroids use, and antiviral drugs use; Model 3, Further adjusted for BMI.

(model 1: 0.22, 95% CI: 0.1, 0.45, $p = <0.001$; model 2: 0.21, 95% CI: 0.1, 0.45, $p = <0.01$; model 3: 0.31, 95% CI: 0.14, 0.68, $p = <0.01$).

The association between major dietary patterns and the risk of each COVID-19 symptom

Multivariable binary logistic regression for the association between major dietary patterns and the risk of each COVID-19 symptom is shown in Table 6. After controlling for potential confounders, we found a significant direct association between unhealthy pattern and OR of cough (6.21, 95% CI: 2.5, 15.45, $p = <0.01$), fever (9.07, 95% CI: 2.83, 28.98, $p = <0.001$), chilling (12.21, 95% CI: 3.34, 44.64, $p = <0.001$), weakness (2.25, 95% CI: 1.01, 5, $p = 0.04$), myalgia (2.91, 95% CI: 1.41, 6, $p = <0.01$), nausea and vomiting (5.71, 95% CI: 1.74, 18.71, $p = <0.01$), and sore throat (9.6, 95% CI: 3.88, 23.77, $p = <0.001$). A significant direct association was also found between traditional pattern and OR of cough (2.79, 95% CI: 1.32, 5.92, $p = <0.01$), fever (2.36, 95% CI: 1.03, 5.39, $p = 0.03$), and chilling (2.55, 95% CI: 1.1, 5.92, $p = 0.02$) at the fully adjusted model. In contrast, a significant inverse association was seen between healthy pattern and OR of dyspnea (0.27, 95% CI: 0.11, 0.63, $p = <0.01$), weakness (0.22, 95% CI: 0.1, 0.51, $p = <0.001$), and sore throat (0.36, 95% CI: 0.16, 0.8, $p = 0.01$) at the third model of adjustment.

The association of dietary patterns and OR of lasted hospitalization and convalescence duration

Multivariable binary logistic regression for the association of each dietary pattern and OR of lasted hospitalization and convalescence duration is presented in Table 7. There were significant associations between unhealthy and traditional patterns with OR of long-term hospitalization after adjusting for confounder variables (2.87, 95% CI: 1.28, 6.45, $p = 0.01$ and 2.97, 95% CI: 1.23, 7.15, $p = 0.01$, respectively). After controlling for the potential confounders, there was no significant association between healthy pattern and OR of lasted hospitalization (0.44, 95% CI: 0.18, 1.06, $p = 0.07$). Furthermore, we did not find a significant association between the traditional pattern and OR of convalescence duration after adjustment for the potential confounders (1.04, 95% CI: 0.51, 2.11, $p = 0.9$). However, a significant direct association was found between the unhealthy pattern and OR of convalescence duration in the final model (1.06, 95% CI: 0.97, 1.16, $p = <0.01$). Moreover, a significant inverse association was seen between healthy pattern and OR of convalescence duration (0.32, 95% CI: 0.15, 0.68, $p = <0.01$).

Discussion

This study aimed to evaluate the association between major dietary patterns before COVID-19 diagnosis in recovered patients and the risk of disease severity and symptoms after the disease begins.

More adherence to the healthy dietary pattern was associated with lower concentrations of CRP and ESR. In contrast, more adherence to unhealthy and traditional patterns was associated with higher concentrations of those pro-inflammatory markers. To the best of our knowledge, there is no study investigating the relationship between dietary patterns and inflammatory markers in patients with COVID-19. Findings from a case–control study showed a positive association between the dietary inflammatory index and serum levels of CRP and ESR in patients with COVID-19 (22). Other studies evaluated the relationship between an individual nutrient and the levels of CRP and ESR. For example, a meta-analysis suggested that consumption of healthy foods rich in antioxidant vitamins and phytochemicals was associated with lower CRP levels in men (23).

Our study indicated a positive relationship between unhealthy and traditional dietary patterns and the risk of severe COVID-19, while an inverse association was found for the healthy dietary pattern. A cross-sectional study on 236 patients

TABLE 6 Odds ratio (95% CI) for symptoms of COVID−19 according to major dietary patterns.

	Dietary patterns								
	Unhealthy			Traditional			Healthy		
	T1	T3	*p*–value	T1	T3	*p*–value	T1	T3	*p*–value
Dyspnea									
Crude	1	3.37 (1.83, 7.75)	<0.001	1	3.2 (1.69, 6.19)	<0.001	1	0.28 (0.14, 0.55)	<0.001
Model 1	1	3.8 (1.81, 8.2)	<0.01	1	3.25 (1.62, 6.52)	<0.01	1	0.21 (0.1, 0.46)	<0.001
Model 2	1	3.46 (1.61, 7.4)	<0.01	1	2.79 (1.36, 5.7)	<0.01	1	0.2 (0.08, 0.45)	<0.001
Model 3	1	2.4 (1.08, 5.36)	0.53	1	2.07 (0.98, 4.37)	0.51	1	0.27 (0.11, 0.63)	<0.01
Cough									
Crude	1	8.31 (3.56, 19.38)	<0.001	1	4.04 (2.09, 7.82)	<0.001	1	0.44 (0.23, 0.83)	0.01
Model 1	1	8.74 (3.62, 21.08)	<0.001	1	4.05 (2.02, 8.1)	<0.001	1	0.35 (0.17, 0.7)	<0.01
Model 2	1	8.7 (3.61, 21.32)	<0.001	1	3.83 (1.89, 7.79)	<0.001	1	0.36 (0.17, 0.73)	<0.01
Model 3	1	6.21 (2.5, 15.45)	<0.01	1	2.79 (1.32, 5.92)	<0.01	1	0.55 (0.259, 1.19)	0.15
Fever									
Crude	1	10.6 (3.52, 31.9)	<0.001	1	3.21 (1.53, 6.73)	<0.01	1	1 (0.49, 2.36)	0.85
Model 1	1	11.44 (3.71, 35.29)	<0.001	1	3.24 (1.49, 7.08)	<0.01	1	1.1 (0.47, 2.57)	0.79
Model 2	1	10.92 (3.52, 33.8)	<0.001	1	2.9 (1.3, 6.47)	<0.01	1	0.65 (1.22, 0.51	0.65
Model 3	1	9.07 (2.83, 28.98)	<0.001	1	2.36 (1.03, 5.39)	0.03	1	1.71 (0.69, 4.27)	0.17
Chilling									
Crude	1	14.32 (4.15, 49.37)	<0.001	1	3.52 (1.65, 7.5)	<0.01	1	1 (0.45, 2.2)	1
Model 1	1	16.43 (4.63, 58.21)	<0.001	1	3.65 (1.65, 8.07)	<0.01	1	1.06 (0.45, 2.47)	0.85
Model 2	1	15.68 (4.41, 55.73)	<0.001	1	3.26 (1.44, 7.36)	<0.01	1	1.16 (0.48, 2.78)	0.71
Model 3	1	12.21 (3.34, 44.64)	<0.001	1	2.55 (1.1, 5.92)	0.02	1	1.75 (0.69, 4.43)	0.15
Weakness									
Crude	1	2.69 (1.35, 5.36)	<0.01	1	0.88 (0.44, 1.75)	0.73	1	0.21 (0.1, 0.44)	<0.001
Model 1	1	2.96 (1.42, 6.19)	<0.01	1	0.75 (0.36, 1.57)	0.45	1	0.19 (0.08, 0.41)	<0.001
Model 2	1	3.08 (1.44, 6.56)	<0.01	1	0.73 (0.34, 1.56)	0.41	1	0.18 (0.08, 0,41)	<0.001
Model 3	1	2.25 (1.01, 5)	0.04	1	0.5(0.22, 1.12)	0.087	1	0.22 (0.1, 0.51)	<0.001
Myalgia									
Crude	1	0.25 (0.13, 0.48)	<0.001	1	1.05 (0.56, 1.96)	0.87	1	0.47 (0.25, 0.88)	0.019
Model 1	1	3.63 (1.83, 7.2)	<0.001	1	0.95 (0.48, 1.85)	0.87	1	0.47 (0.24, 0.95)	0.03
Model 2	1	3.5 (1.76, 6.99)	<0.001	1	0.85 (0.43, 1.68)	0.632	1	0.49 (0.24, 0.97)	0.04
Model 3	1	2.91 (1.41, 6)	<0.01	1	0.61 (0.29, 1.26)	0.182	1	0.64 (0.31, 1.3)	0.23
Nausea and vomiting									
Crude	1	0.15 (0.05, 0.43)	<0.001	1	2.6 (0.94, 7.1)	0.07	1	0.24 (0.08, 0.711)	<0.01
Model 1	1	7.25 (2.31, 22.69)	<0.001	1	1.06 (0.458, 2.49)	0.17	1	0.16 (0.05, 0.49)	<0.01
Model 2	1	6.84 (2.18, 21.47)	<0.001	1	1.88 (0.66, 5.37)	0.29	1	0.16 (0.05, 0.16)	<0.01
Model 3	1	5.71 (1.74, 18.71)	<0.01	1	1.29 (0.43, 3.89)	0.75	1	0.22 (0.07, 0.71)	0.48
Sore throat									
Crude	1	9.2 (4.1, 20.96)	<0.001	1	1 (0.5, 1.99)	1	1	0.3 (0.14, 0.63)	<0.01
Model 1	1	11.08 (4.71, 26)	<0.001	1	0.9 (0.44, 1.86)	0.76	1	0.27 (0.12, 0.58)	<0.01
Model 2	1	12.3 (5.1, 29.8)	<0.001	1	0.92 (0.44, 1.9)	0.78	1	0.26 (0.12, 0.58)	<0.01
Model 3	1	9.6 (3.88, 23.77)	<0.001	1	0.62 (0.28, 1.37)	0.2	1	0.36 (0.16, 0.8)	0.01

Model 1, Adjusted for sex, age and energy intake; Model 2, Further adjusted for physical activity, supplement use, corticosteroids use, and antiviral drugs use; Model 3, Further adjusted for BMI.

with COVID-19 showed that an increment in habitual intake of legumes, grains, and bread and cereals decreased overall symptom severity in patients with COVID-19 (11). Moreover, a population-based case–control study in six countries indicated that individuals who consumed plant-based diets with a higher intake of vegetables, legumes, and nuts, and a lower intake

TABLE 7 Odds ratio (95% CI) for hospital stay and convalescence duration according to major dietary patterns.

	Dietary patterns								
	Unhealthy			Traditional			Healthy		
	T1	T3	*p*–value	T1	T3	*p*–value	T1	T3	*p*–value
Hospital duration (days)									
Crude	1	2.74 (1.42, 5,29)	<0.01	1	3.59 (1.74, 7.39)	<0.01	1	0.39 (0.19, 0.77)	<0.01
Model 1	1	3.65 (1.69, 7.92)	<0.01	1	4.04 (1.75, 9.34)	<0.01	1	0.37 (0.16, 0.84)	0.017
Model 2	1	3.23 (1.49, 7)	<0.01	1	3.64 (1.55, 8.57)	<0.01	1	0.36 (0.15, 0.83)	0.016
Model 3	1	2.87 (1.28, 6.45)	0.015	1	2.97 (1.23, 7.15)	0.011	1	0.44 (0.18, 1.06)	0.068
Convalescence duration (days)									
Crude	1	2.7 (1.38, 5.29)	<0.01	1	1.22 (0.65, 2.26)	0.52	1	0.24 (0.12, 0.47)	<0.01
Model 1	1	3.48 (1.7, 7.1)	<0.01	1	1.3 (0.67, 2.5)	0.44	1	0.26 (0.13, 0.53)	<0.01
Model 2	1	3.64 (1.77, 7.48)	<0.01	1	1.35 (0.69, 2.65)	0.39	1	0.25 (0.12, 0.52)	<0.01
Model 3	1	1.06 (0.97, 1.16)	<0.01	1	1.04 (0.51, 2.11)	0.91	1	0.32 (0.15, 0.68)	<0.01

Model 1, Adjusted for sex, age and energy intake; Model 2, Further adjusted for physical activity, supplement use, corticosteroids use, and antiviral drugs use. Model 3, Further adjusted for BMI.

of poultry and red or processed meats had lower OR of severe COVID-19-like illness (15). Furthermore, the results of a prospective cohort study supported the finding of our study, in which adherence to plant-based foods was associated with lower risk and severity of COVID-19 (24). Due to common food items in such dietary patterns, it seems that more detailed studies are needed to illustrate which food items are more important and which are the main anti-COVID-19 micronutrients or bioactive components in those foods.

With regard to the symptoms of COVID-19, we found a significant increment in the risk of dyspnea, cough, fever, chilling, weakness, myalgia, nausea and vomiting, and sore throat with more adherence to the unhealthy pattern. Adjustment for BMI disappeared the association for dyspnea. It seems that BMI is an important confounding factor in the relationship between dietary intake and the risk of COVID-19 symptoms. A recent systematic review and meta-analysis including 46 studies involving 625,153 patients indicated a greater risk of infection, hospitalization, clinically severe disease, mechanical ventilation, ICU admission, and mortality due to COVID-19 in patients with obesity (25). A significant positive relation was found between the traditional dietary pattern and dyspnea, cough, fever, and chilling. The association for dyspnea disappeared after adjustment for BMI. A lack of significant association for some symptoms might be due to the effect of other confounding factors that need to be taken into account in future investigations. For instance, patients' physical activity before COVID-19 diagnosis, their medical history, and family history of different diseases are among the most important confounders that should be attended to with more detail. In contrast to the two aforementioned dietary patterns, higher adherence to the healthy dietary pattern was associated with a lowered risk of dyspnea, weakness, and sore throat. To the best of our knowledge, this study is the first investigation into the association between dietary patterns and symptoms of COVID-19. A recent case–control study indicated that more intake of legumes, grains, and bread and cereals was associated with a reduction in overall symptom severity in patients with COVID-19 (11).

More adherence to unhealthy or traditional patterns was associated with increased duration of hospitalization in patients with COVID-19. Although an inverse association was seen between adherence to the healthy dietary pattern and hospitalization time in our study, the association was removed after additional adjustment for BMI in the third model. Similar to what we said for COVID-19 symptoms, it can be suggested that BMI is an important confounder in the relationship between dietary intake and hospital duration. Findings from a retrospective cohort study indicated that subjects with obesity who were affected by COVID-19 required longer hospitalization and more intensive and longer oxygen treatments (26).

Finally, we found a direct association between more adherence to the unhealthy dietary pattern and convalescence duration, while an inverse association was found for more adherence to the healthy dietary pattern. However, no significant association was found between the traditional dietary pattern and convalescence duration in patients with COVID-19. In line with our findings, a cross-sectional study on COVID-19 survivors in Saudi Arabia indicated that more adherence to a healthy diet was associated with a shorter duration of recovery from COVID-19 (16). Further studies about different common known dietary patterns are needed to expand the current finding.

The exact mechanisms through which dietary patterns might affect COVID-19 severity and symptoms are unknown. It is

suggested that micro-nutrients in a diet might affect COVID-19 prognosis (27). Vitamin A has various functions in the body's immune system (28). Growth, development, and function of neutrophils, monocytes and macrophages, apoptosis, and gene expression of B and T lymphocytes are examples of vitamin A functions in the immune system (29). Vitamin B6 is another important factor in a diet that strengthens the immune system and increases the production of white blood cells including IL-2 and T cells (30). In addition, numerous studies have indicated the effective roles of vitamin C in the prevention of infections, such as SARS coronavirus (31, 32). A recent meta-analysis indicated that low serum vitamin D concentration was associated with more risk of in-hospital mortality among patients with COVID-19 (13). Roles of vitamin D in immune responses and protecting the body against various viruses have been reported previously (33).

For example, a recent meta-analysis indicated that vitamin D supplementation was associated with a reduction in the ICU admission rate, a reduction in the need for mechanical ventilation, and a reduction in mortality from COVID-19 (34).

Furthermore, vitamin E deficiency has been associated with lipid peroxidation (35), and omega-3 fatty acid has protective roles against infectious diseases by removing body inflammation (36). Cytokine storm in response to viral infections can lead to multi-organ failure in patients with COVID-19 (37). Furthermore, fibers are fermented by the gut flora to produce short-chain fatty acids, which have anti-inflammatory functions (38).

This study is the first investigation into the association of major dietary patterns with the risk of COVID-19 symptoms and severity. However, some limitations should also be taken into account when interpreting the findings of this study. This is a single-center study. Although the study population included adults, it would be prudent to consider their sample size and the fact that they were all drawn from the same center when determining their generalizability to the general population. We did not examine the socioeconomic status of participants, which may influence their dietary intake. Our study had a limited sample size, which highlights the need for larger studies. In addition, differences in virus variants can affect the severity and symptoms of COVID-19 (39, 40). Insufficient information in the medical records of some patients was another limitation of our study. Furthermore, we excluded patients with acute and very high severe diseases from our study. This was because of a lack of information about their dietary intake before the disease diagnosis and also due to their inability to fill out the questionnaires. We assessed the dietary intakes of participants with a self-reported web-based 168-FFQ. Therefore, recall bias and misclassification of participants by the dietary intakes should not be neglected. Finally, due to the cross-sectional design of this study, it is impossible to confer causality.

In conclusion, this study showed that high adherence to a healthy pattern was associated with less CRP and ESR and lower risk of severe COVID-19, and hospitalization and convalescence durations in patients who recovered from COVID-19. However, more adherence to unhealthy or traditional dietary patterns was associated with higher CRP and ESR, risk of severe COVID-19, and hospitalization duration. A direct association was found between adherence to the unhealthy pattern and risk of cough, fever, chilling, weakness, myalgia, nausea and vomiting, and sore throat, and between the traditional pattern with risk of dyspnea, cough, fever, and chilling. A healthy dietary pattern was inversely associated with the risk of dyspnea, cough, weakness, myalgia, nausea and vomiting, and sore throat.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Kashan University of Medical Sciences. The patients/participants provided their written informed consent to participate in this study.

Author contributions

AE: conceptualization, formal analysis, writing–original draft, writing–review and editing, and data collection. AM: supervision, conceptualization, methodology, investigation, funding acquisition, formal analysis, writing–original draft, and writing–review and editing. MT: supervision, conceptualization, formal analysis, writing–original draft, and writing–review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Supplementary material

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Association of fruits, vegetables, and fiber intake with COVID-19 severity and symptoms in hospitalized patients: A cross-sectional study

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Background and aims: Fruits and vegetables are rich in fiber and a good source of anti-inflammatory and immune-boosting vitamins, minerals, and antioxidants. We investigated the association between fruits, vegetables, and fiber intake and severity of COVID-19 and related symptoms in hospitalized patients.

Methods: A total of 250 COVID-19 hospitalized patients aged 18 to 65 years were recruited for this cross-sectional study in Kashan, Iran, between June and September of 2021. Dietary intakes were assessed using an online validated 168-item food frequency questionnaire (FFQ). COVID-19 severity and symptoms were evaluated using the National Institutes of Health (NIH) COVID-19 Treatment Guidelines. Moreover, we examined COVID-19 symptoms, inflammatory biomarkers, and additional factors.

Results: The mean age of participants was 44.2 ± 12.1 years, and 46% had severe COVID-19. Patients with higher consumption of fruits (OR: 0.28; 95% CI: 0.14-0.58, *P*-trend <0.001), vegetables (OR: 0.33; 95% CI: 0.16-0.69, *P*-trend <0.001), and dietary fiber (OR: 0.25; 95% CI: 0.12-0.53, *P*-trend <0.001) had lower odds of having severe COVID-19. In addition, they had shorter hospitalization and convalescence periods, lower serum C-reactive protein (CRP), and a reduced risk of developing COVID-19 symptoms such as sore throat, nausea and vomiting, dyspnea, myalgia, cough, weakness, fever, and chills.

Conclusion: Higher consumption of fruits, vegetables, and fiber was inversely linked with COVID-19 severity, clinical symptoms, hospitalization and convalescence duration, and CRP concentrations. The results should be interpreted with caution in light of the limitations, and prospective cohort studies are required to further evaluate these findings.

KEYWORDS

fruits, vegetables, dietary fiber, COVID-19, severe disease, infectious disease

Introduction

The coronavirus disease 2019 (COVID-19), which was caused by the novel coronavirus SARS-CoV-2, had a major impact on the lives of people around the world (1). Since its discovery in late 2019, it has resulted in significant mortality and morbidity worldwide (2). By 28 December 2021, the WHO had reported more than 279 million cases and 5.3 million deaths (3). Over 6.1 million confirmed COVID-19 cases and 131,400 COVID-19 deaths were reported in Iran as well (4). COVID-19 infection manifests from clinically asymptomatic to critical and life-threatening illness (5). COVID-19 also affects all organ systems in the human body, including the hematological system (alterations of the hemostasis), the pulmonary system (acute hypoxic respiratory failure, pneumonia, pulmonary embolism, and pulmonary fibrosis), the cardiovascular system (coronary artery atherosclerosis and myocardial infarction), the nervous system (stroke, impaired consciousness and encephalopathy, convulsions, visual impairment, and ataxia), and the gastrointestinal system (6). In addition, SARS-CoV-2 infection placed a great financial burden to the global economy and healthcare systems (7–9).

Diet and other lifestyle factors are well-known risk factors for obesity (10), hypertension (11, 12), and diabetes (13–15), all of which have been identified as potential risk factors for severe COVID-19 (16, 17). Adherence to low in fiber, fruits, and vegetables dietary patterns has been associated with an increased risk of these chronic diseases (18–21). In contrast, adherence to a healthy diet rich in fruits and vegetables is essential for proper immune function (22, 23). In a systematic review and meta-analysis of 83 studies, Hosseini et al. showed that high fruits and vegetables consumption is associated with lower levels of pro-inflammatory mediators and enhanced immune responses (24).

The Middle Eastern diet is characterized by a greater intake of carbohydrate (mostly derived from refined grains), trans and saturated fatty acids, a lower intake of fruits and vegetables, and absence of alcohol intake (25). To the best of our knowledge, no study has been conducted to examine the association between dietary fiber, vegetables, and fruit intake with the risk of severe COVID-19 and its symptoms. Therefore, we aimed to investigate the potential association between dietary fiber intake, fruit, and vegetables and the odds of severe COVID-19 and its symptoms in a cross-sectional study.

Materials and methods

Participants

This cross-sectional study included 250 COVID-19 patients aged 18 to 65 years who had recovered from the disease. Simple random sampling was used to select the study population from the Shahid Beheshti Hospital in Kashan, Iran, between June and September 2021. The study protocol was approved by the ethics committee of Kashan University of Medical Sciences (registration no. IR.KAUMS.MEDNT.REC.1400.048). The study was discussed with all participants and written consent was obtained from them.

Initially, the medical records of 600 patients with COVID-19 were reviewed, with consideration given to patients diagnosed within the past three months. In total, 350 of them were excluded due to the following exclusion criteria. Patients with diseases other than COVID-19, medical history of chronic diseases such as diabetes mellitus and cardiovascular disease, as well as conditions with potential impact on COVID-19 severity, body mass index (BMI) > 40 kg/m^2, pregnant or breastfeeding women, active smokers, those were taking dietary supplements more than twice a week before the initial diagnosis of COVID-19; followed specific diets; taking medications with potential effects on the respiratory system, e.g., fluticasone and flunisolide, and those with missing information in their medical records were not included. Consequently, 250 patients were analyzed and included in this study.

Dietary assessment tool

Participants' dietary intake was assessed using an online 168-items food frequency questionnaire (FFQ) whose reliability and validity has been approved previously (26). This questionnaire was used to collect data on dietary intake 1 year prior to the COVID-19 diagnosis in each patient. Participants initially reported their dietary intakes as daily, monthly, or annually.

These data were then converted to grams per day (g/d) using household measures (27). The Nutritionist IV software was used to calculate energy and micro-and micronutrient intakes.

COVID-19 severity assessment

The National Institutes of Health (NIH) COVID-19 Treatment Guideline (CTG) (28), updated on 19 October 2021, was used to determine the patients' COVID-19 severity. CTG classified the disease into five levels of severity; (1) Asymptomatic or presymptomatic infection: Individuals with a positive test for SARS-CoV-2 using either a nucleic acid amplification test (NAAT) or an antigen test but with no COVID-19 manifestations; (2) Mild illness: patients with any of the COVID-19 symptoms (e.g., loss of taste and smell, fever, headache, malaise, myalgia, nausea, vomiting, diarrhea, cough, sore throat) but no dyspnea or abnormal chest imaging; (3) Moderate illness: individuals with clinical evidence of lower respiratory tract involvement or chest imaging and an oxygen saturation (SpO_2) of at least 94% on room air at sea level; (4) Severe illness: individuals with a SpO_2 <94% on room air at sea level, a PaO_2/FiO_2 ratio <300 mmHg, a respiratory rate >30 breaths/min, or lung infiltrates >50%; and (5) Critical illness: individuals suffering from respiratory failure, septic shock, and/or multiple organ dysfunction. We assumed those who had mild and moderate diseases as non-severe.

Assessment of COVID-19 symptoms

Participants were asked to complete a general questionnaire to obtain information about the presence of each common clinical manifestation of COVID-19 (i.e., fever, rigors, weakness, myalgia, dyspnea, cough, sore throat, nausea, and vomiting).

Assessment of inflammatory markers

The initial measurement of C-reactive protein (CRP) at hospital admission was obtained from the medical records.

Assessment of other variables

Data were collected on demographic characteristics, height, weight, physical activity, duration of convalescence, supplements intake, corticosteroids, or antiviral medications for each participant using the general questionnaire.

Statistical analysis

The Kolmogorov–Smirnov test was used to assess the normal distribution of data (29). We divided participants into tertile groups based on their dietary intake of fruits, vegetables, and fiber. The energy-adjusted dietary intake of fruits, vegetables, and fiber using the residual method were calculated for this purpose (30). The means and SDs for continuous variables and the percentages for categorical variables were compared across tertiles of fruits, vegetables, and fiber intake using the one-way ANOVA and chi-square analysis, respectively. The inflammatory marker, i.e., CRP, were compared between tertiles of dietary intake of fruits, vegetables, and fiber using covariance analysis (ANCOVA) after being adjusted for age, gender, BMI, and physical activity. These variables were considered based on previous evidence (31, 32) and differences between tertiles of exposures of interest. Binary logistic regression was used in two models to explore the association between fruits, vegetables, and dietary fiber intake with odds of severe COVID-19 and also with the risk of each COVID-19 symptom. In the multivariable-adjusted model, age (continuous), energy intake (continuous), physical activity (sedentary/moderate/intense), and BMI (continuous) were adjusted. The Statistical Package for the Social Sciences (SPSS) was used for all data analyses (SPSS Inc., version 25). A p-value <0.05 was considered statistically significant.

Results

A total of 250 patients were included in the final analysis, comprising 119 men and 131 women. The prevalence of mild disease was 5.6% ($n = 14$), the moderate disease was 48.4% ($n = 121$), and severe illness was 46% ($n = 115$) among subjects with a mean age of 44.2 years.

The general characteristics of study participants across tertiles of fruits, vegetables, and fiber intake are depicted in Table 1. Participants with higher intake of fruits, vegetables, and fiber were male with a lower BMI, and were less likely to be overweight or obese. In addition, they were less likely to use supplements or been prescribed corticosteroids or antiviral medications. After controlling for age, gender, BMI, and physical activity, participants in the top tertile of fruit intake had significantly lower CRP levels than those in the bottom tertile (10.51 ± 2.17 vs. 27.05 ± 2.25 mg/L). Similarly, participants in the highest tertile of vegetable and fiber intake had lower levels of CRP than those in the lowest tertile (12.92 ± 2.11 vs. 33.10 ± 2.10 mg/L, 10.95 ± 2.02 vs. 33.86 ± 2.12 mg/L, respectively). Furthermore, participants who consumed more fruits, vegetables, and fibers had a considerably shorter duration of hospitalization and convalescence.

Crude and multivariable-adjusted odds ratios and 95% CIs for severe COVID-19 according to tertiles of dietary

TABLE 1 Demographic characteristics of the study participants across tertiles of dietary fruits, vegetables, and fiber.

	Tertiles of fruits				Tertiles of vegetables				Tertiles of dietary fiber			
	T1	T2	T3	P[a]	T1	T2	T3	P[a]	T1	T2	T3	P[a]
Participants (*n*)	83	84	83		83	84	83		83	84	83	
Age (years)	44.6 ± 12.0	45.3 ± 12.2	42.4 ± 12.2	0.26	43.8 ± 12.1	42.8 ± 13.1	45.8 ± 11.1	0.26	45.4 ± 12.4	43.0 ± 11.9	44.0 ± 12.2	0.43
Males (%)	42.2	41.7	59.0	0.04	44.6	42.9	55.4	0.21	43.4	45.2	54.2	0.33
BMI (kg/m^2)	29.0 ± 3.8	26.0 ± 3.5	26.9 ± 3.7	<0.001	28.7 ± 3.5	26.8 ± 4.2	25.3 ± 2.6	<0.001	29.2 ± 3.6	25.8 ± 3.6	25.9 ± 3.0	<0.001
Physical activity				0.42				0.66				0.57
Sedentary (31)	15.7	14.3	7.2		12.0	15.5	9.6		16.9	11.9	8.4	
Moderate (202)	79.5	77.4	85.5		80.7	76.2	85.5		75.9	82.1	84.3	
Intense (17)	4.8	8.3	7.2		7.2	8.3	4.8		7.2	6.0	6.8	
Overweight or obese (%)	85.5	58.3	57.8	<0.001	86.7	67.9	47.0	<0.001	86.7	58.3	56.6	<0.001
Supplements intake (%)	98.8	96.4	89.2	0.01	95.2	96.4	92.8	0.56	98.8	95.2	90.4	0.04
Corticosteroids use (%)	98.8	92.9	84.3	0.003	94.0	91.7	90.4	0.68	98.8	89.3	88.0	0.02
Antiviral Drugs use (%)	98.8	92.9	84.3	0.003	94.0	91.7	90.4	0.68	98.8	89.3	88.0	0.02
CRP (mg/L)[b]	27.05 ± 2.25	21.37 ± 2.14	10.51 ± 2.17	<0.001	33.10 ± 2.10	13.01 ± 2.00	12.92 ± 2.11	<0.001	33.86 ± 2.12	14.21 ± 2.02	10.95 ± 2.02	<0.001
Duration of hospitalization (day)	7.6 ± 3.0	6.6 ± 2.8	5.4 ± 2.5	<0.001	7.4 ± 3.0	6.2 ± 3.1	6.0 ± 2.5	0.005	7.7 ± 2.9	6.2 ± 2.9	5.8 ± 2.7	<0.001
Convalescence duration (day)	11.3 ± 4.4	8.7 ± 3.3	8.3 ± 2.7	<0.001	10.5 ± 3.8	9.6 ± 4.3	8.2 ± 2.6	<0.001	11.3 ± 4.5	8.7 ± 3.2	8.3 ± 2.6	<0.001

All values are mean ± SD or percent.

[a]Obtained from ANOVA or chi-square test, where appropriate.

[b]Presented as mean ± SE, and values were adjusted for age, energy intake, BMI, and physical activity using ANCOVA.

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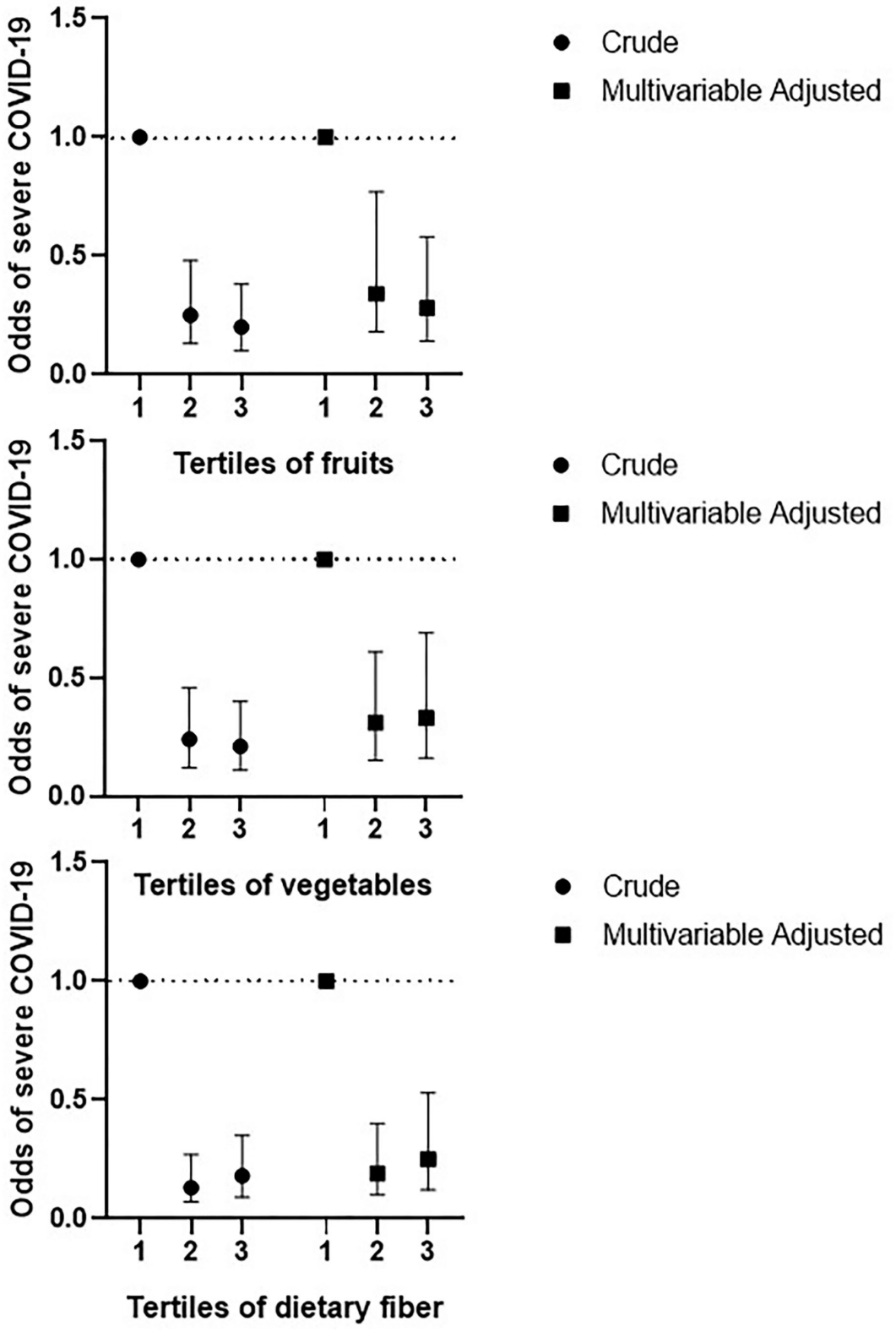

FIGURE 1
Crude and multivariable-adjusted odds ratios and 95% CIs of severe COVID-19 disease according to tertiles of dietary fruits, vegetables, and fiber. Multivariable adjusted for age, BMI, energy intake, and physical activity.

TABLE 2 Crude and multivariable-adjusted odds ratios and 95% CIs for symptoms of COVID-19 according to tertiles of dietary fruits, vegetables, and fiber.

	Tertiles of fruits				Tertiles of vegetables				Tertiles of dietary fiber			
	T1	T2	T3	P^{*}_{trend}	T1	T2	T3	P^{*}_{trend}	T1	T2	T3	P^{*}_{trend}
Dyspnea												
Crude	1.00	0.26 (0.13, 0.53)	0.21 (0.10, 0.43)	<0.001	1.00	0.40 (0.20, 0.79)	0.25 (0.13, 0.50)	<0.001	1.00	0.11 (0.05, 0.25)	0.18 (0.08, 0.39)	<0.001
Model 1	1.00	0.34 (0.15, 0.74)	0.28 (0.13, 0.60)	0.002	1.00	0.48 (0.23, 1.00)	0.37 (0.17, 0.77)	0.01	1.00	0.14 (0.06, 0.33)	0.23 (0.10, 0.54)	0.004
Cough												
Crude	1.00	0.11 (0.05, 0.25)	0.12 (0.06, 0.26)	<0.001	1.00	0.27 (0.14, 0.53)	0.20 (0.10, 0.39)	<0.001	1.00	0.07 (0.03, 0.17)	0.11 (0.05, 0.24)	<0.001
Model 1	1.00	0.16 (0.07, 0.36)	0.17 (0.07, 0.38)	<0.001	1.00	0.34 (0.16, 0.70)	0.35 (0.17, 0.75)	0.009	1.00	0.10 (0.04, 0.24)	0.15 (0.06, 0.35)	<0.001
Fever												
Crude	1.00	0.13 (0.05, 0.35)	0.12 (0.05, 0.37)	<0.001	1.00	0.09 (0.03, 0.27)	0.10 (0.03, 0.31)	<0.001	1.00	0.09 (0.03, 0.26)	0.11 (0.04, 0.34)	<0.001
Model 1	1.00	0.15 (0.05, 0.50)	0.15 (0.05, 0.43)	0.001	1.00	0.10 (0.03, 0.31)	0.13 (0.04, 0.42)	<0.001	1.00	0.10 (0.03, 0.32)	0.13 (0.04, 0.41)	0.002
Chilling												
Crude	1.00	0.07 (0.02, 0.26)	0.07 (0.02, 0.24)	<0.001	1.00	0.07 (0.02, 0.23)	0.08 (0.02, 0.27)	<0.001	1.00	0.04 (0.01, 0.18)	0.05 (0.01, 0.22)	<0.001
Model 1	1.00	0.09 (0.02, 0.34)	0.09 (0.02, 0.31)	<0.001	1.00	0.08 (0.02, 0.27)	0.10 (0.03, 0.38)	0.002	1.00	0.05 (0.02, 0.23)	0.06 (0.02, 0.28)	<0.001
Weakness												
Crude	1.00	0.65 (0.34, 1.22)	0.34 (0.17, 0.69)	0.003	1.00	0.40 (0.20, 0.77)	0.25 (0.12, 0.50)	<0.001	1.00	0.34 (0.17, 0.65)	0.26 (0.13, 0.51)	<0.001
Model 1	1.00	1.00 (0.48, 2.10)	0.54 (0.24, 1.16)	0.11	1.00	0.44 (0.22, 0.89)	0.31 (0.14, 0.69)	0.003	1.00	0.46 (0.22, 0.94)	0.35 (0.17, 0.74)	0.006
Myalgia												
Crude	1.00	0.66 (0.36, 1.22)	0.29 (0.14, 0.55)	<0.001	1.00	0.49 (0.27, 0.92)	0.35 (0.18, 0.66)	0.001	1.00	0.55 (0.30, 1.01)	0.31 (0.16, 0.59)	<0.001
Model 1	1.00	0.78 (0.39, 1.53)	0.41 (0.20, 0.83)	0.01	1.00	0.60 (0.31, 1.16)	0.44 (0.21, 0.90)	0.02	1.00	0.78 (0.39, 1.53)	0.41 (0.20, 0.83)	0.01
Nausea and vomiting												
Crude	1.00	0.84 (0.39, 1.82)	0.05 (0.01, 0.36)	<0.001	1.00	0.16 (0.06, 0.43)	0.12 (0.04, 0.38)	<0.001	1.00	0.16 (0.06, 0.43)	0.12 (0.04, 0.38)	<0.001
Model 1	1.00	1.21 (0.48,0.05)	0.06 (0.01, 0.52)	0.005	1.00	0.15 (0.05, 0.44)	0.15 (0.05, 0.51)	<0.001	1.00	0.18 (0.06, 0.54)	0.13 (0.04, 0.45)	<0.001
Sore throat												
Crude	1.00	0.56 (0.30, 1.05)	0.15 (0.07, 0.34)	<0.001	1.00	0.51 (0.27, 0.95)	0.15 (0.07, 0.32)	<0.001	1.00	0.25 (0.13, 0.49)	0.12 (0.06, 0.26)	<0.001
Model 1	1.00	0.32 (0.16, 0.66)	0.16 (0.07, 0.36)	<0.001	1.00	0.59 (0.30, 1.14)	0.19 (0.08, 0.45)	<0.001	1.00	0.32 (0.16, 0.66)	0.16 (0.07, 0.35)	<0.001

Adjusted for age, BMI, energy intake, and physical activity.
*Obtained from Binary logistic regression.

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fruits, vegetables, and fiber intakes are illustrated in Figure 1. Participants in the highest tertile of fruits intake had a lower risk of severe COVID-19 than those in the lowest tertile either in the crude model (OR: 0.20; 95% CI: 0.10, 0.38, *P*-trend < 0.001) or after adjustment for potential confounders (OR: 0.28; 95% CI: 0.14, 0.58, *P*-trend < 0.001). In terms of vegetable intake, participants at the highest tertile had a lower risk of severe COVID-19, both in the crude model (OR: 0.21; 95% CI: 0.11, 0.40, *P*-trend < 0.001) and after adjusting for potential confounders (OR: 0.33; 95% CI: 0.16, 0.69, *P*-trend < 0.001). Similarly, participants at the highest tertile of fiber intake had a lower risk of severe COVID-19 in both crude model (OR: 0.18; 95% CI: 0.09, 0.35, *P*-trend < 0.001) and after adjusting for potential confounders (OR: 0.25; 95% CI: 0.12, 0.53, *P*-trend < 0.001).

Crude and multivariable-adjusted odds ratios and 95% CIs for COVID-19 symptoms according to tertiles of dietary fruits, vegetables, and fiber are reported in Table 2. After controlling for potential confounders, there was a significant inverse relationship between fruit consumption and the likelihood of experiencing respiratory COVID-19 symptoms such as dyspnea and cough, digestive symptoms such as nausea and vomiting, systemic-neurologic symptoms such as fever, chilling, and myalgia, and rhino pharyngeal symptoms such as sore throat. Participants in the highest tertile of vegetable consumption were significantly less likely to exhibit respiratory, digestive, systemic-neurologic, and rhino pharyngeal COVID-19 symptoms. Similar associations were found between dietary fiber consumption and COVID-19 symptoms.

Discussion

Our study investigated the association between dietary intake of fruits, vegetables, and fiber and odds of severe COVID-19 and also COVID-19 symptoms among 250 hospitalized Iranian adults. According to our findings, the severity of COVID-19 and related symptoms was lower in those with a greater consumption of fruits, vegetables, and fibers. In addition, the requirement for corticosteroids and antiviral medications, the days of hospitalization and convalescence, and level of inflammation marker, i.e., CRP, were notably reduced in participants who consumed more fruits, vegetables, and fibers.

Health benefits of a rich diet in vegetables, fruits and fiber have long been investigated in previous studies. The Mediterranean diet, which contains high quantities of the aforementioned dietary elements, has been shown to have anti-inflammatory and antioxidant potential, as well as to ensure optimal immune activity, and its use has been recommended as a preventive strategy in the development of cardiometabolic diseases, namely, obesity, cardiovascular disease, type 2 diabetes mellitus, malignancies, asthma, allergies, and respiratory tract infections (28, 33). Our findings support this hypothesis, as subjects who followed a dietary pattern similar to the Mediterranean diet had fewer symptoms and less severe forms of COVID-19, and also lower levels of inflammatory markers. On the other hand, it has been demonstrated that the Western diet, which is high in fats and carbohydrates and low in fibers and antioxidants, is pro-inflammatory, stimulates innate immunity and the production of reactive oxygen species, and diminishes the efficacy of the adaptive immune response (34). Micronutrients and bioactive natural compounds, such as vitamins A, C, D, and/or E, minerals (iron, selenium, and zinc), probiotics, fibers, polyphenols, and omega-3 polyunsaturated fatty acids, have been shown to enhance the immune system's antiviral response and prevent the development of respiratory tract infections (35–37).

There is no association between the risk of upper respiratory tract infections and other dietary patterns, such as the Nordic dietary pattern issued by a joint committee of experts from the Nordic countries (38). Although the potential benefits of a Mediterranean-style diet in protecting against COVID-19 have been hypothesized, there is a lack of clinical evidence to support these theories. This is primarily due to the diet's antioxidant and anti-inflammatory effects, which can counteract the pro-oxidant and pro-inflammatory status triggered by the SARS-CoV-2 infection (3). The results of our cross-sectional study have, to the best of our knowledge, revealed for the first time an association between a higher intake of vegetables, fruits, and fiber and milder symptoms and less severe forms of COVID-19. In addition, patients who consumed more of these foods had shorter hospital stays, required fewer days to recover, and had lower CRP levels as inflammatory markers. Similarly, Merino et al. (4) investigated the dietary pattern of nearly 32,000 COVID-19 subjects and delineated that individuals who followed a plant-based nutritional approach were less likely to get infected with SARS-CoV-2 or may have a milder form of this respiratory infection (4). In addition, Salazar-Robles (39) concluded that the consumption of vegetables and grains was associated with COVID-19 symptoms that were less severe. According to an evaluation of 236 outpatients from Mexico, 44% tested positive for SARS-CoV-2 (39).

Elevated oxidative stress and inflammation levels in COVID-19 can result in a dysfunctional immune system response (40). Phytochemicals and antioxidants from fruits and vegetables can eliminate pro-oxidant and pro-inflammatory molecules associated with viruses. In addition, it can affect the interaction between oxidative stress and the NF-kB and/or Nrf-2 pathways (41). The mechanisms mentioned earlier could explain who consumed a lot of vegetables, fruits, and fibers in our study had lower levels of inflammation markers and were infected with a milder form of SARS-CoV-2. Furthermore, nutrients in fruits and vegetables may interact favorably with the gut microbiota to counteract excessive inflammation (42). The intestinal microbiome converts dietary fibers into short-chain fatty acids, which has lower inflammatory activity (41).

Therefore, it is hypothesized that a diet rich in fruits and vegetables may protect against the hyperinflammation and cytokine storm observed in severe COVID-19 forms (43, 44). As a result, the COVID-19 lockdowns and other events associated with the SARS-CoV-2 pandemic may have influenced the food choices of our participants, as our study was based on data derived from the hospital records of patients with COVID-19 hospitalized between June and September 2021.

Our study has several strengths and limitations. To the best of our knowledge, this is the first cross-sectional study reporting the effect of diet on COVID-19 symptomatology, severity, and laboratory parameters. The findings of our study can be used to issue dietary recommendations by experienced dietitian, researchers, physicians, and other clinical nutrition experts during the COVID-19 pandemic. Another strength is that we recruited people up to the age of 65, representing the great majority of hospitalized patients with SARS-CoV-2 infection. The elderly may be challenging to recollect their dietary consumption precisely; hence, their inclusion may affect our judgments. However, several limitations must be considered. First, due to the study's cross-sectional nature, causality cannot be determined. Future research should evaluate the association between vegetables, fruits, and fiber consumption and COVID-19 symptomatology and severity; ideally in prospective cohort studies with larger study samples. Second, we used a validated and reliable questionnaire to assess the dietary intake; however, misclassification could not be completely excluded. Third, despite accounting for a number of potential confounding variables, residual confounding cannot be excluded. Fourth, the study sample was relatively small considering the number of patients diagnosed with COVID-19 and was only limited to one city in Iran, thus affecting the generalization of the results for entire Iran or other countries. Fifth, the population of the study was restricted to healthy adults, so the findings cannot be generalized to other groups. For a better understanding of this relationship, it is suggested that additional research be conducted on populations with underlying diseases such as diabetes and cardiovascular disease. Finally, dietary alterations might have been induced by the COVID-19 pandemic, and the fear of contracting this infectious disease, and, thus, the investigated patients might only temporarily adhere to healthy eating habits.

Conclusion

We found that patients with a higher intake of fruits, vegetables, and fibers had a decreased likelihood of severe form of COVID-19 and related symptoms. In addition, the need for corticosteroids and antiviral medications, the length of hospitalization, and convalescence, and also the levels of the inflammatory marker, i.e., CRP, were significantly lower in patients who consumed more fruits vegetables, and fibers. Due to the limitations highlighted earlier, our findings should be regarded with caution, and it is recommended that this association be evaluated in large prospective studies and randomized trials.

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 Ethics Committee of Kashan University of Medical Sciences reviewed and authorized studies involving human participants. The patients/participants provided their written informed consent to participate in this study.

Author contributions

AM and MT conceived, designed, and supervised the study. AE and CA contributed to data collection. KT, NZ, SM, and M-AG performed statistical analyses, data interpretation, and drafting of the manuscript. PMob, PMok, and HR contributed to the manuscript drafting and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

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