ARTICLE

Nutrition in acute and chronic diseases

Low serum levels of zinc and 25-hydroxyvitmain D as potential risk factors for COVID-19 susceptibility: a pilot case-control study

Esmat Ghanei[1,2,5], Moein Baghani[3,5], Hamideh Moravvej[3], Atefeh Talebi[4], Ayda bahmanjahromi [3✉] and Fahimeh Abdollahimajd [2,3✉]

BACKGROUND AND AIMS: This study aimed to evaluate serum 25-hydroxyvitmain D and zinc levels in coronavirus disease 2019 (COVID-19) patients in comparison to healthy subjects.
METHODS: This was a single-center case-control study performed from March 20, 2020, to January 20, 2021, in Tehran, Iran. All patients diagnosed with COVID-19 based on a positive nasopharyngeal swab polymerase chain reaction (PCR) test were included in the case group. Controls were selected from patients referred for routine checkups who had a negative COVID-19 PCR test. Age, sex, marital and educational status, comorbidities, and serum 25-hydroxyvitmain D and zinc levels of patients were recorded.
RESULTS: Ninety patients in the case group and 95 subjects in the control group who were sex and age-matched were studied. 25-hydroxyvitmain D levels higher than 20 ng/ml were observed in 58 (64%) cases and 72 (76%) controls ($P = 0.09$). The median 25-hydroxyvitmain D level in the case group was significantly lower than controls (26 (interquartile range [IQR] = 24) ng/ml vs. 38 (IQR = 22) ng/ml, respectively, $P < 0.01$). The median zinc level in the case group was 56 (IQR = 23) mcg/dL, while it was 110 (IQR = 27) mcg/dL among the controls ($P < 0.01$). There was no significant difference in the level of 25-hydroxyvitmain D and zinc between cases with and without comorbidities ($P > 0.05$). Susceptibility to SARS-CoV-2 infection could be predicted by serum 25-hydroxyvitmain D levels below 25.2 ng/ml (81% sensitivity; 48% specificity) or zinc levels below 86.3 mcg/dL (93% sensitivity; 92% specificity).
CONCLUSIONS: Low serum zinc and 25-hydroxyvitmain D levels appear to be risk factors for COVID-19 affliction; thus, the treatment of individuals with such deficiencies is recommended.

INTRODUCTION

The world is currently experiencing the third leading pandemic of coronavirus (CoV) infections. The outbreak of the new CoV infection began in late 2019 in Wuhan, China. The agent responsible was named the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19) [1]. One of the most important issues about COVID-19 is the response of the patient's body to the virus, which can be influenced by various factors. During the COVID-19 pandemic, interest in dietary supplements to support immune function has increased. Micronutrients in the human body may also be involved [2–10].

25-hydroxyvitmain D is a fat-soluble steroid molecule that has hormonal-like effects [11, 12]. 25-hydroxyvitmain D deficiency is a major health problem worldwide [13]. Recent studies have shown a high frequency of 25-hydroxyvitmain D deficiency in the Iranian population [14, 15]. A potential link between 25-hydroxyvitmain D deficiency and systemic infection has been demonstrated [16, 17]. The immunomodulatory role of 25-hydroxyvitmain D affects the immune system. By inducing the secretion of antiviral peptides, 25-hydroxyvitmain D increases innate immunity and improves mucosal defenses [18–20]. The correlation of 25-hydroxyvitmain D levels with COVID-19 severity and mortality has been shown in retrospective studies on COVID-19 patients [21–24]. Also, 25-hydroxyvitmain D deficiency or insufficiency has been shown to be higher in patients hospitalized with COVID-19 [25]. Overall, poor 25-hydroxyvitmain D status appears to be associated with an increased risk of COVID-19 infection and severe disease; however, the causal relationship between 25-hydroxyvitmain D status and severe COVID-19 is not well-understood, as decreased 25-hydroxyvitmain D levels may be the consequent of severe disease instead of causing it [26].

[1]Department of Internal Medicine of Shohada Tajrish Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [2]Clinical Research Development Unit of Shohada-e Tajrish Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [3]Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [4]Colorectal Research Center, Rasool Akram Hospital, Iran University of Medical Sciences, Tehran, Iran. [5]These authors contributed equally: Esmat Ghanei, Moein Baghani. ✉email: a.bahmanjahromi@gmail.com; fabdollahimajd@sbmu.ac.ir
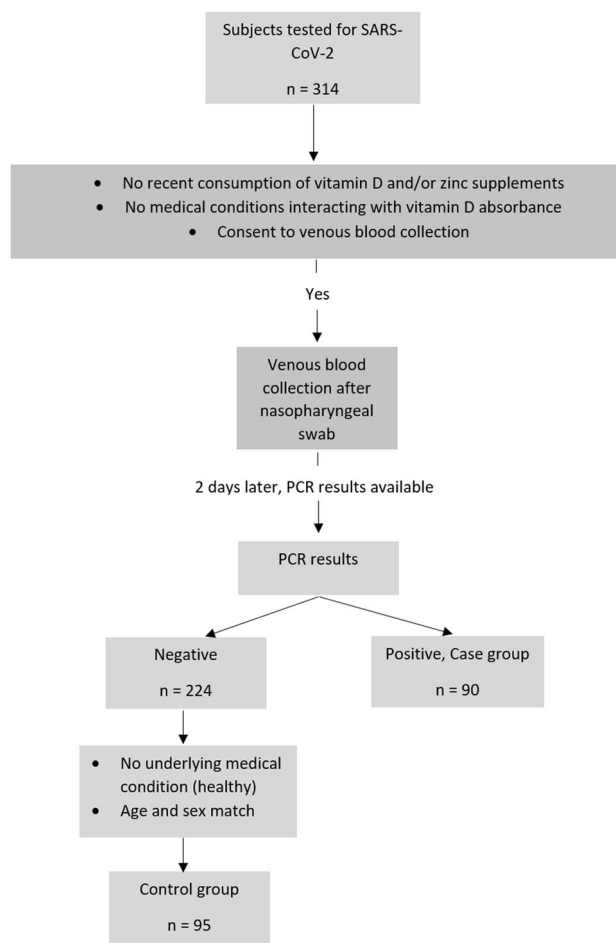
Fig. 1 Sample recruitment flow chart.

Zinc is another important micronutrient, fulfilling various roles in the human body. High concentrations of zinc and the addition of compounds that stimulate zinc entry into the cell have been shown to inhibit the replication of various viruses that contain RNA, including influenza viruses [27]. Besides, the inhibitory effect of zinc on the net activity of rhinoviruses and the hepatitis C virus has been reported [28, 29]. Provided that zinc deficiency accounts for 16% of deep respiratory infections worldwide, there can be a link between zinc deficiency and the risk of COVID-19 infection or progression to severe forms of the disease [30].

Although there are different opinions about the consequences of 25-hydroxyvitmain D deficiency in COVID-19, what is certain is that Iranian society is facing severe 25-hydroxyvitmain D deficiency, especially in certain populations. The relationship between the various aspects of the disease, as well as infection or severity, needs to be investigated to provide information concerning the micronutrition supplementations necessary for overcoming the COVID-19 pandemic. Also, studies have shown that patients hospitalized with COVID-19 who had low serum levels of zinc had more severe manifestations than patients with normal zinc levels [31]. Hence, the current study aimed to evaluate serum levels of 25-hydroxyvitmain D and zinc in COVID-19 patients in comparison with controls.

PATIENTS AND METHODS
Study design and settings
This was a single-center case-control study performed at Shohada-e-Tajrish Hospital of Shahid Beheshti University of Medical Sciences from March 20, 2020, to January 20, 2021. The study protocol was approved by our

institute's Ethics Committee in Biomedical Research with reference code IR. SBMU.SRC.REC.1399.007.

Participants
With $\alpha = 0.05$, $\beta = 0.1$, and a calculated effect size of 0.5, the minimum number of samples in each group was determined as 85. All patients who were diagnosed with COVID-19 based on a positive nasopharyngeal swab polymerase chain reaction (PCR) test and agreed to participate in the study were included in the case group. For the control group, with an approximately 1:1 recruitment ratio, healthy age- and sex-matched subjects with a negative COVID-19 nasopharyngeal swab test were included. The two groups were matched for age and sex due to age-related immunologic changes and sex differences in this regard [32]. Sampling continued till there were no significant differences among the groups in terms of sex and age. Affliction with some known medical conditions that potentially interact with 25-hydroxyvitmain D absorbance as well as malabsorption disorders, celiac disease, inflammatory bowel disease, end-stage renal disease (ESRD), or gastric bypass surgery were considered as exclusion criteria for both groups. Furthermore, pregnant or lactating subjects were not included, as well as subjects taking medications such as glucocorticoids, anticonvulsants, 25-hydroxyvitmain D/zinc supplements, calcium, or any drug that is known to influence the level of 25-hydroxyvitmain D or zinc or affects the coagulation system. Controls were selected from participants in a COVID-19 screening program conducted in the hospital area. Only those with no comorbidities were selected as we were not able to match comorbidities between the case and control groups. Referral bias or admission bias was not possible as the nasopharyngeal swab test was conducted in a COVID-19 screening area at the hospital and subjects with comorbidities had an equal chance of being included in the study as healthy subjects; nonetheless, comorbidities remained as a potential confounder (Fig. 1).

Variables
The main study outcomes in our study were evaluating serum 25-hydroxyvitmain D (25OHD) and zinc levels. 25-hydroxyvitmain D and zinc levels were assessed on the same day of the nasal swab test. We explained the details of the study objectives to the patients and recorded their information after obtaining informed consent. A minimum of 5 ml of venous blood was collected in clotted conditions from all enrolled patients. The serum 25-hydroxyvitmain D level was determined via the electro-chemical luminescence method (DiaSorin LIAISON, Dietzenbach, Germany). The same sample was used for zinc measurement by the endpoint method using the Randox zinc assay (made in England). Age, sex, marital and educational status, and comorbidities of patients were recorded through an interview by the researcher. The measurement of anthropometric indices was not undertaken to decrease the chance of COVID-19 transmission, and subjective responses about height and weight were not satisfactory. The medication history was also recorded.

Statistical methods
Categorical variables were described using frequency and percent, while mean and standard deviation (SD) or median and interquartile range (IQR) were used to describe continuous data. The results of 25-hydroxyvitmain D levels were interpreted and compared between groups categorically at a cut-off of 20 ng/dL, above which 25-hydroxyvitmain D levels are considered adequate in adults. Chi-squared or Fisher's test was used to examine the relationship between the qualitative variables. Parametric methods such as the independent t-test were used to compare the means of the two groups if the data were normally distributed after checking normality using the Kolmogorov-Smirnov test. Also, in the case of non-parametric data, equivalent non-parametric methods such as the Mann-Whitney test were performed. In addition, receiver operating characteristics (ROC) curve analysis was carried out to evaluate the performance of zinc and 25-hydroxyvitmain D in both case and control groups. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) software (version 25.0, Armonk, NY: IBM Corp., USA) at a significance level of 0.05.

RESULTS
In this study, 90 patients were evaluated in the case group alongside 95 subjects in the control group. There were 35 males (39%) and 55 females (61%) in the case group and 33 males (71%)

Table 1. Characteristics of study participants.

	Case *n* = 90		Control *n* = 95		P-value
	n/Mean	%/SD	n/Mean	%/SD	
Sex					
Male	35	39	33	71	0.59†
Female	55	61	62	29	
Age, year	52	16	48	19	0.06‡
Marital status, married	38	42	33	35	0.29†
Educational status					
Illiterate	18	20	15	16	0.46†
Literate	72	80	80	84	
Comorbidities*	72	80	0	0	–
Hypertension	30	22	0	0	
Diabetes mellitus	23	17	0	0	
Ischemic heart disease	12	9	0	0	
Heart failure	1	0.7	0	0	
Chronic kidney disease	8	6	0	0	
Chronic obstructive pulmonary disease	0	0	0	0	
Asthma	9	7	0	0	
Thyroid disorders	7	5	0	0	
Malignancy	9	7	0	0	
Dyslipidemia	7	5	0	0	
Depression	2	1	0	0	
Alzheimer's disease	5	4	0	0	
Coronary artery bypass graft	3	2	0	0	
25-hydroxyvitmain D > 20 ng/ml	58	64	72	76	0.09§

*Controls were all healthy and frequency of Medication history of cases is listed in Supplementary Table S1
†Chi-squared test
‡Independent t-test
§Mann–Whitney U test

and 62 females (29%) in the control group. The mean age was 52 ± 16 and 48 ± 19 years among the patients and controls, respectively. As shown in Table 1, 25-hydroxyvitmain D levels higher than 20 ng/ml were observed in 58 (64%) subjects in the case group and 72 (76%) subjects in the control group ($P = 0.09$).

Median 25-hydroxyvitmain D levels in the case and control groups were 26 (IQR = 24) ng/ml and 38 (IQR = 22) ng/ml, respectively ($P < 0.01$) (Fig. 2a). Seventy-two (80%) patients in the case group had comorbidities and were taking medications that may affect 25-hydroxyvitmain D levels who were excluded from the final analysis (Supplementary Table S1), while controls had a negative medical history. We considered two approaches to eliminate this confounding factor. First, data were analyzed based on three groups, namely COVID-19 patients with and without comorbidities and the control group. A significant difference was found among the three groups. The median 25-hydroxyvitmain D level in cases with comorbidities was 31 (IQR = 24) ng/ml, compared to 23 (IQR = 21) ng/ml in cases without comorbidities. As shown in Fig. 2b, there was no significant difference between the cases with and without comorbidities ($P = 0.57$), while healthy controls had significantly higher 25-hydroxyvitmain D levels than both groups ($P < 0.01$).

Median zinc levels in the case and control groups were 56 (IQR = 23) mcg/dL and 110 (IQR = 27) mcg/dL, respectively ($P < 0.01$) (Fig. 2c). A significant difference was found between the three groups previously mentioned (Fig. 2d). The median zinc level in cases with comorbidities was 54 (IQR = 23) mcg/dL, compared to 59 (IQR = 20) mcg/dL in cases without comorbidities and 110 (IQR = 26) mcg/dL in healthy subjects. There was no significant difference between the cases with and without comorbidities ($P = 0.07$), while healthy subjects had significantly higher zinc levels than both groups ($P < 0.01$).

Receiver operating characteristic (ROC) curves were drawn to determine the best cut-off for the zinc and 25-hydroxyvitmain D levels below which COVID-19 affliction was more probable. As shown in Fig. 3, the optimum cut-off of 25-hydroxyvitmain D was 25.2 ng/ml, meaning that 25-hydroxyvitmain D levels below 25.2 ng/ml could predict susceptibility to SARS-CoV-2 infection with a sensitivity of 81% and a specificity of 48%. The area under the ROC (AUROC) curve of 25-hydroxyvitmain D was 0.63 (CI 95% 0.55 to 0.718; $P = 0.03$). In addition, the best cut-off of serum zinc was 86.3 mcg/dL, meaning that serum zinc levels below this could predict COVID-19 susceptibility with 93% sensitivity and 92% specificity according to the AUROC curve of 0.95 (CI 95% 0.92 to 0.99; $P < 0.01$).

DISCUSSION

The present study compared healthy controls with COVID-19 cases in terms of serum 25-hydroxyvitmain D and zinc levels. Our results showed that median serum 25-hydroxyvitmain D and zinc levels in the case group were significantly lower than controls. However, there was no significant difference in the level of 25-hydroxyvitmain D and zinc between cases with and without comorbidities.

Various studies have been conducted to determine the association between COVID-19 and 25-hydroxyvitmain D. D'Avolio et al. compared COVID-19 PCR positive vs. negative cases and showed that plasma 25-hydroxyvitmain D levels were significantly lower in the PCR-positive patients [33]. However, their research was one of the initial studies conducted in this era, facing some limitations due to its timing and lacking some essential data about possible 25-hydroxyvitmain D supplementation. While our study results showed higher levels of 25-hydroxyvitmain D in PCR-negative cases, we only included patients who had no history of 25-hydroxyvitmain D supplementation. Nonetheless, sunshine-produced 25-hydroxyvitmain D and dietary 25-hydroxyvitmain D intake (expect supplementation medications) could have biased our results. Due to the customs of Iranian women's way of clothing (Hijab), sunshine-produced 25-hydroxyvitmain D could be lower in female cases [34], though our sex-matched case-control study design restricts the confounding effect of this issue to some extent.

In line with our findings, a recent study by Hurst et al. showed higher frequency of 25-hydroxyvitmain D insufficiency/deficiency in hospitalized COVID-19 patients [25]. The potential roles of 25-hydroxyvitmain D in reducing the risk for COVID-19 include decreasing the replication and viability of SARS-CoV-2 through induction of antimicrobial peptides and binding to the host cell surface receptors, and decreasing the cytokine storm through promoting the body's ability to reduce the production of inflammatory cytokines [35].

Meltzer et al.'s findings are also consistent with ours [36]. Their retrospective cohort study of 489 patients demonstrated that the relative risk of a positive COVID-19 PCR test in individuals who had insufficient 25-hydroxyvitmain D levels in the year before the COVID-19 pandemic was almost two-fold higher compared with individuals with sufficient 25-hydroxyvitmain D [36].

Moreover, it is well known that some nutrients, including 25-hydroxyvitmain D and zinc, play key roles in immune system
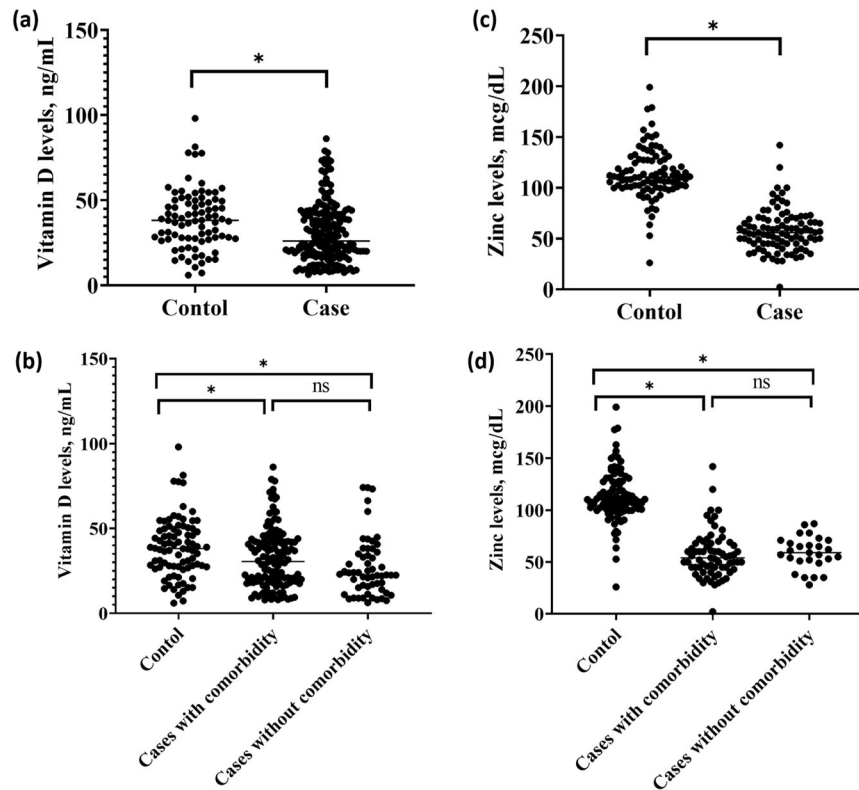
Fig. 2 Serum 25-hydroxyvitmain D and zinc levels in the study groups. *Significant difference; ns: not significant.*
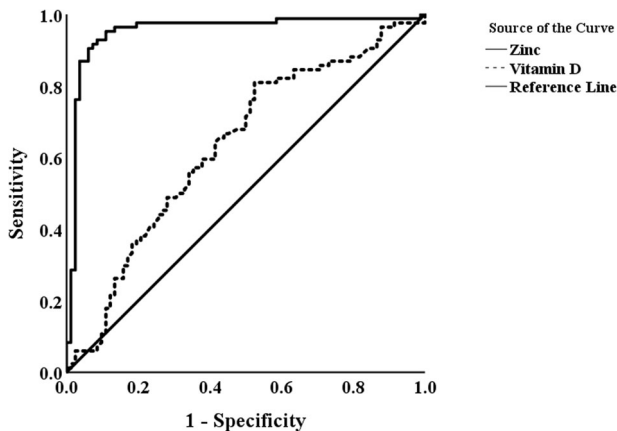

Fig. 3 Receiver operating characteristic (ROC) curves of serum 25-hydroxyvitmain D and zinc thresholds. *The straight diagonal line is the reference line.*

integrity and function and are believed to have immunomodulatory effects. It has also been proposed that 25-hydroxyvitmain D and zinc, along with vitamin C, may have synergistic actions in the maintenance of tissue barriers. Based on the literature review, 25-hydroxyvitmain D and zinc deficiency can compromise the immune system, making individuals more susceptible to viral infections such as COVID-19, and increasing the likelihood of a poor disease prognosis [37]. However, it is not yet clear whether 25-hydroxyvitmain D deficiency leads to severe COVID-19 disease or 25-hydroxyvitmain D is reduced as a result of severe disease, potentially due to its consumption [26]. On the other hand, Maghbooli et al. demonstrated that oral 25-hydroxyvitmain D could improve immune function in COVID-19 patients with 25-hydroxyvitmain D levels below 30 ng/ml, reflected by increased blood lymphocyte percentage [38].

As reviewed by Biesalski, there is clear evidence that in different comorbidities like hypertension and diabetes, reduced plasma levels of 25-hydroxyvitmain D are seen [39]. In our study, there was no correlation between comorbidities and serum 25-hydroxyvitmain D level of COVID-19 patients, as was the case in the study of Giannini et al. [40]. Also, Pinzon et al. showed that there is no difference in 25-hydroxyvitmain D deficiency status between patients with and without comorbidities [41].

Few previous studies have investigated the association of serum zinc levels with SARS-CoV-2 infection. Most studies have compared zinc levels between COVID-19 patients with poor outcomes and those with mild disease [42]. On the other hand, Jothimani et al. showed that COVID-19 patients have lower levels of zinc than healthy controls, and comorbidities had no significant effects on the zinc levels in their study groups [31]. Also, Abdolahi et al. demonstrated significantly lower serum zinc levels in COVID-19 patients compared with healthy subjects [43]. On the other hand, Carlucci et al. showed that zinc may play a role in the therapeutic management of COVID-19. They reported that hospitalized COVID-19 patients who took zinc sulfate were more frequently discharged from the hospital and had lower mortality [44]. Nevertheless, Thomas et al. demonstrated no significant reduction in the duration of symptoms in COVID-19 patients who received high-dose zinc [45]. Overall, as an important signaling molecule, zinc can alter host defense systems. Also, by regulating leukocyte immune responses and modulating the nuclear enhancer of activated B cells and consequently altering cytokine production, zinc has a positive role in inflammatory conditions [46].

Although lower levels of 25-hydroxyvitmain D may make individuals prone to COVID-19 affliction, the opposite may not be true. Respiratory diseases can reduce 25-hydroxyvitmain D levels. Based on the role of 25-hydroxyvitmain D and its receptor in immunomodulatory events, 25-hydroxyvitmain D is thought to

be consumed in respiratory infections; however, whether a similar trend in 25-hydroxyvitamin D reduction applies to COVID-19 infection is not known [26]. On the other hand, Souza et al. hypothesized that organisms like SARS-COV-2 could consume zinc for their own functions or for the modification of their receptors, which may decrease the serum levels of zinc [37]. On the other hand, some studies have shown the anti-SARS-CoV-1 effects of zinc [47].

Our study had some limitations. First, the severity of COVID-19 was not evaluated. However, we were exploring the link between 25-hydroxyvitamin D and zinc levels with susceptibility to SARS-CoV-2 infection, and disease severity was not our main objective. Moreover, we did not assess the duration of daily sunshine exposure and the dietary intake of 25-hydroxyvitamin D and zinc; these could have influenced the results. Furthermore, we did not take body mass index (BMI) into account as BMI can be a confounding factor of 25-hydroxyvitamin D and zinc status [48, 49]. Also, the 25-hydroxyvitamin D status of the participants prior to admission is not known and we did not measure 25-hydroxyvitamin D binding protein.

CONCLUSION
Our study showed extremely lower levels of 25-hydroxyvitamin D and zinc in COVID-19 patients, independent of age, sex, and comorbidities. 25-hydroxyvitamin D and zinc deficiency may be important factors in determining susceptibility to COVID–19. Therefore, we recommend the diagnosis and treatment of individuals with low serum 25-hydroxyvitamin D and zinc levels.

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AUTHOR CONTRIBUTIONS

EG and FA contributed to the conception of the work. HM, EG, MB, AB and FA contributed to the acquisition of data and AT contributed to the analysis, and interpretation of data for the work. AB, MB and FA drafted the manuscript. FA and EG and HM critically revised the manuscript. All authors gave final approval and agreed to be accountable for all aspects of the work ensuring integrity and accuracy. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL

The study protocol was approved by our institute's Ethics Committee in Biomedical Research with reference code IR.SBMU.SRC.REC.1399.007.

ADDITIONAL INFORMATION