Nutrition in acute and chronic diseases

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

Esmat Ghanie1,2,5, Moein Baghani3,5, Hamideh Moravje3, Atefeh Talebi4, Ayda bahmanjahromi3,5, and Fahimeh Abdollahimajd2,3,5

© The Author(s), under exclusive licence to Springer Nature Limited 2022, corrected publication 2022

BACKGROUND AND AIMS: This study aimed to evaluate serum 25-hydroxyvitamin 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-hydroxyvitamin 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-hydroxyvitamin D levels higher than 20 ng/ml were observed in 58 (64%) cases and 72 (76%) controls (P = 0.09). The median 25-hydroxyvitamin 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-hydroxyvitamin D and zinc between cases with and without comorbidities (P > 0.05). Susceptibility to SARS-CoV-2 infection could be predicted by serum 25-hydroxyvitamin 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-hydroxyvitamin D levels appear to be risk factors for COVID-19 affliction; thus, the treatment of individuals with such deficiencies is recommended.

European Journal of Clinical Nutrition (2022) 76:1297–1302; https://doi.org/10.1038/s41430-022-01095-5

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

| Characteristic                        | 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             | 80      | 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-hydroxyvitamin 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
†Independent t-test
‡Mann-Whitney U test
§Mann-Whitney U test

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-hydroxyvitamin D levels below which COVID-19 affliction was more probable. As shown in Fig. 3, the optimum cut-off of 25-hydroxyvitamin D was 25.2 ng/ml, meaning that 25-hydroxyvitamin 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-hydroxyvitamin 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-hydroxyvitamin D and zinc levels. Our results showed that median serum 25-hydroxyvitamin D and zinc levels in the case group were significantly lower than controls. However, there was no significant difference in the level of 25-hydroxyvitamin D and zinc between cases with and without comorbidities.

Various studies have been conducted to determine the association between COVID-19 and 25-hydroxyvitamin D. D’Avolio et al. compared COVID-19 PCR positive vs. negative cases and showed that plasma 25-hydroxyvitamin 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-hydroxyvitamin D supplementation. While our study results showed higher levels of 25-hydroxyvitamin D in PCR-negative cases, we only included patients who had no history of 25-hydroxyvitamin D supplementation. Nonetheless, sunshine-produced 25-hydroxyvitamin D and dietary 25-hydroxyvitamin 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-hydroxyvitamin 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-hydroxyvitamin D insufficiency/deficiency in hospitalized COVID-19 patients [25]. The potential roles of 25-hydroxyvitamin 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-hydroxyvitamin D levels in the year before the COVID-19 pandemic was almost two-fold higher compared with individuals with sufficient 25-hydroxyvitamin D [36].

Moreover, it is well known that some nutrients, including 25-hydroxyvitamin D and zinc, play key roles in immune system...
integrity and function and are believed to have immunomodulatory effects. It has also been proposed that 25-hydroxyvitamin D and zinc, along with vitamin C, may have synergistic actions in the maintenance of tissue barriers. Based on the literature review, 25-hydroxyvitamin D 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-hydroxyvitamin D deficiency leads to severe COVID-19 disease or 25-hydroxyvitamin 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-hydroxyvitamin D could improve immune function in COVID-19 patients with 25-hydroxyvitamin 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-hydroxyvitamin D are seen [39]. In our study, there was no correlation between comorbidities and serum 25-hydroxyvitamin 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-hydroxyvitamin 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-hydroxyvitamin D may make individuals prone to COVID-19 affliction, the opposite may not be true. Respiratory diseases can reduce 25-hydroxyvitamin D levels. Based on the role of 25-hydroxyvitamin D and its receptor in immunomodulatory events, 25-hydroxyvitamin 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.

REFERENCES

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382:272–33.
2. Gasmii A, Tippairote T, Mukwajidya PK, Peana M, Menzel A, Dadar M, et al. Micronutrients as immunomodulatory tools for COVID-19 management. Clin Immunol. 2020;2022:108545.
3. Carr AC. Micronutrient status of COVID-19 patients: a critical consideration. Crit Care. 2020;24:1–2.
4. Gorji A, Khalighi Ghadiri M. Potential roles of micronutrient deficiency and immune system dysfunction in the coronavirus disease 2019 (COVID-19) pandemic. Nutrition. 2021;91:11047.
5. McAliffle S, Ray S, Fallon E, Bradfield J, Eden T, Kohlmeyer M. Dietary micronutrients in the wake of COVID-19: an appraisal of evidence with a focus on high-risk groups and preventative healthcare. BMJ Nutr Prev Health. 2020;93.
6. Pecora F, Persico F, Argentiero A, Neglia C, Esposito S. The role of micronutrients in support of the immune response against viral infections. Nutrients. 2020;12:23198.
7. Richardson DP, Lovegrove JA. Nutritional status of micronutrients as a possible and modifiable risk factor for COVID-19: a UK perspective. Brit J Nutr. 2021;125:678–84.
8. Junaid M, Ejaz H, Abdalla AE, Alves AC, Dunn-Waters DK, et al. Vitamin D insufficiency in COVID-19 and influenza A, and critical illness survivors: a cross-sectional study. BMJ. 2021;11:e055435.
9. Lordan R. Notable developments for vitamin D amid the COVID-19 pandemic, but caution warranted overall: A narrative review. Nutrients. 2021;13:740.
10. Tang Q, Wang X, Gao G. The short form of the zinc finger antiviral protein inhibits influenza A virus protein expression and is antagonized by the virus-encoded NS1. J Virol. 2017;91:e01909–16.
11. Berg K, Bolt G, Andersen H, Owen TC. Zinc potentiates the antiviral action of human IFN-α and the interferon response to the SARS-CoV-1 envelope protein. J Interferon Cytokine Res. 2001;21:471–4.
12. Tellinghuisen TL, Marcorugiano J, Rice CM. Structure of the zinc-binding domain of an essential component of the hepatitis C virus replicase. Nature 2005;435:374–9.
13. Wessels I, Rolles B, Rink L. The potential impact of zinc supplementation on COVID-19 pathogenesis. Front Immunol. 2020;11:1712.
14. Jothimani D, Kalasam E, Danielraj S, Nallathambi B, Ramachandran H, Sekar P, et al. COVID-19: Poor outcomes in patients with zinc deficiency. Int J Infect Dis. 2020;100:343–9.
15. Gasmii A, Noor S, Tippairote T, Dadar M, Menzel A, Bjerklund G. Individual risk management strategy and potential therapeutic options for the COVID-19 pandemic. Clin Immunol. 2020;215:108409.
16. D’Avolio A, Avataneo V, Manca A, Cusato J, De Nicolò A, Lucchini R, et al. 25-hydroxyvitamin D concentrations are lower in patients with positive PCR for SARS-CoV-2. Nutrients. 2020;12:1359.
17. Buyskia N, Eini K, Hilì H, Sunal N, Yigit P, Garipagaoğlu M. Clothine preference affects vitamin D status of young women. Nutr Res. 2014;34:688–93.
18. Grant WB, Lordan R. Vitamin D for COVID-19 on trial: An update on prevention and therapeutic application. Endocr Pract. 2021;27:1266–8.
19. Meltzer DO, Best TJ, Zhang H, Yokes T, Avora V, Solway J. Association of vitamin D status and other clinical characteristics with COVID-19 test results. JAMA. 2020;334:209722.
20. Souza ACR, Vasconcelos AR, Prado PS, Pereira CPM, Zinc, Vitamin D and Vitamin C: perspectives for COVID-19 with a focus on physical tissue barrier integrity. Front Nutr. 2020;7:295.
21. Maghboobi Z, Sahraiai MA, Jamal-Moghaddam SR, Asadi A, Zendeheh A, Vaziri T, et al. Treatment with 25-hydroxyvitamin D3 (calcifediol) is associated with a reduction in the blood neutrophil-to-lymphocyte ratio marker of disease severity in patients hospitalized with COVID-19: A pilot, multicenter, randomized, placebo-controlled double blind clinical trial. Endocr Pract. 2021;27:1242–51.
22. Biesalski HK. Vitamin D deficiency and co-morbidities in COVID-19 patients—A fatal relationship? NFS J 2020;20:10.
23. Giammini S, Passeri G, Tripepi G, Sella S, Fusaro M, Ardiconio G, et al. Effectiveness of in-hospital cholecalciferol use on clinical outcomes in comorbid COVID-19 patients: a hypothesis-generating study. Nutrients. 2021;13:219.
24. Pinzon RT, Pradana AW. Vitamin D deficiency among patients with COVID-19: case series and recent literature review. Trop Med Health. 2020:48:1–7.
25. Yao JS, Pagueo JA, Dee EC, Tan HC, Moulick A, Milazzo C, et al. The minimal effect of zinc on the survival of hospitalized patients with COVID-19: an observational study. Chest. 2021;159:108–11.
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

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41430-022-01095-5.

Correspondence and requests for materials should be addressed to Ayda bahmanjahromi or Fahimeh Abdollahimajd.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.