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Conflict of Interest

The authors declare that there are no conflicts of interest.

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Abbreviations:

Intensive-care unit (ICU), oxygen (O_2), body mass index (BMI), coronavirus 19 disease (COVID-19), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), interferon-gamma (IFN-g), tumor necrosis factor-alpha (TNF-a), interleukin-6 (IL-6), acute physiologic assessment and chronic health evaluation (APACHE score), computed tomography scan (CT scan), magnesium (Mg), zinc (Zn)

Highlights

- Low serum levels of B9, B12, C, and D vitamins, and Mg, Iron, was seen in COVID-19 patients
- Lower levels of vitamin D, Zn, and Mg can induce more severe disease
- We recommend addressing the deficiency of micronutrients for preventing severe COVID-19

Abstract

Background and aim: To compare the serum level of micronutrients with the normal amount and assess the association among them and the severity of disease and inflammatory cytokines in patients with COVID-19. **Methods:** Present cross-sectional study included 60 ICU-admitted patients with COVID-19. We recorded data on demographics, anthropometric, and medical history. Serum levels of inflammatory markers (ESR, CRP, IFN-g, TNF-a, IL-6), vitamins (A, B_9, B_{12}, C, D, E), and minerals (Mg, Zn, Iron) were measured. A Radiologist assessed the severity of lung involvement according to their CT scan. The severity of illness was evaluated with APACHE score, O2 saturation, and body temperature. Independent associations among the serum levels of micronutrients with the severity of the COVID-19 were measured. **Results:** The median (IQR) of patient age was 53.50 (12.75). Except for vitamin A and Zn, serum levels of other micronutrients were lower than minimum normal. Patients with APACHE score ≥ 25 had higher BMI (p= 0.044), body temperature (0.003), ESR (0.008), CRP (0.003), and had lower O_2 saturation (0.005), serum levels of vitamin D (<

0.001) and Zn (< 0.001), compared to patients with APACHE score < 25. We found that the lower serum levels of vitamin D, Mg, and Zn were significantly and independently associated with higher APACHE scores (p= 0.001, 0.028, and < 0.001, respectively) and higher lung involvement (p= 0.002, 0.045, and < 0.001, respectively). **Conclusions:** Conclusions: Lower serum levels of vitamin D, Zn, and Mg were involved in severe COVID-19.

Abbreviations:

Intensive-care unit (ICU), oxygen (O_2), body mass index (BMI), coronavirus 19 disease (COVID-19), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), interferon-gamma (IFN-g), tumor necrosis factor-alpha (TNF-a), interleukin-6 (IL-6), acute physiologic assessment and chronic health evaluation (APACHE score), computed tomography scan (CT scan), magnesium (Mg), zinc (Zn)

Key words: COVID-19, Serum level, Cytokine, Micronutrients, Vitamin, Mineral

Introduction

COVID-19 is a recently discovered virus with a high rate of transmission and various clinical manifestations from asymptomatic contamination to a severe disease requiring ICU admission (1). It has been shown that the severity of COVID-19 can be related to not only the viral load but also the regulated immune responses in patients. The rapid replication of the virus due to improper and unregulated function of the immune system results in a destructive inflammatory response characterized by increased serum levels of inflammatory markers such as CRP, IL-6, TNF, and ESR (2-4). The data so far available have shown that unregulated responses of the immune system could be responsible for the multiorgan failure, which is the leading cause of death in severely ill cases (5, 6). Micronutrients, including vitamins and minerals, have been reported to play a vital role in the regulation and integrity of the immune system (7, 8). The epigenetic aspects of the mechanisms controlling the immune system responses and inflammatory processes, such as methylation and modification of DNA, and its proteins depend on the sufficient storage of some vitamins and minerals (7-9); thus, low levels of micronutrients may influence the severity of infectious diseases, such as COVID-19 [9-11]. Due to the high mortality rate in critically ill patients with COVID-19, one of the challenges facing researchers is to identify the mechanisms involved in virulence and severity of illness in patients with COVID-19 (9-11). Due to the high mortality rate in critically ill patients with COVID-19, one of the challenges facing researchers is to identify the mechanisms involved in virulence and severity of illness in patients with COVID-19 (12). Therefore, considering the crucial role of micronutrients in the regulation of immune responses, this study assessed the serum level of micronutrients and its relationship with the severity of COVID-19.

2. Material & Methods

2.1. Study design, participants, personal history, and physical examinations

This cross-sectional study included middle-aged and older PCR-confirmed COVID-19 patients admitted in ICU of Imam Khomeini Hospital (Tehran, Iran) from March to June 2020. The study protocol was according to the “Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)” Statement (13) and approved by the Ethical Committee of Tehran University of Medical Sciences. The subjects who had undergone chemotherapy in the previous 3 months, those with immunosuppressed diseases such as human immunodeficiency virus (HIV), and those who had taken vitamin or mineral supplements within 3 months before enrollment, were excluded from the study. Data on demographics and previous underlying diseases were collected. Physical examination was performed to measure weight, height, vital signs, and oxygen saturation. Body mass index (BMI) was calculated.

All patients were informed about the purpose and method of the study as well as written informed consent was obtained from them.

Inclusion criteria: Age more than 20 years old; both sex; definitive diagnosis of COVID-19; the satisfaction of cooperation in the study.

Exclusion criteria: The subjects who had undergone chemotherapy in the previous 3 months, those with immunosuppressed diseases such as human immunodeficiency virus (HIV); those who had taken vitamin or mineral supplements within 3 months before enrollment.

2.2. Laboratory measurements

All included patients underwent blood sampling from the antecubital vein for laboratory measurement at admission. Complete blood cell count and differential count were determined via a standardized automatic cell counter. CRP was measured using the ELISA method. ESR was measured using an automated erythrocyte sedimentation rate analyzer. Serum levels of IL6, TNF-α, and IFN-g were measured using an enzyme-linked immunosorbent assay. We measured blood concentrations of vitamins A, B9, B 12, C, E, and D by high-performance liquid chromatography (HPLC). Minerals, including Mg, Zn, and Iron were measured using coupled plasma mass spectrometer.

2.3. The methods of disease severity assessment

An intensivist physician through www.mdcalc.com measured the Acute Physiology and Chronic Health Evaluation (APACHE), known as a commonly used severity-scoring system, for patients at admission. The APACHE score of 25 and more was considered the high score for mortality (14). A Lung CT scan was performed and analyzed for the existence of ground-glass opacity or consolidation patterns by a Radiologist. The involvement of each lobe was scored as follows: no involvement (score 0); 1%–25% involvement (score 1); 26%-49%

involvement (score 2); 50%-75% involvement (score 3); and 76%-100% involvement (score 4). After that, the individual lobar scores were summed to obtain the overall severity score of lungs in each patient. The severity of lung involvement in each patient was classified on the basis of overall severity score as follows: no involvement (score 0); minimal involvement (score range of 1 to 5); mild involvement (score range of 6 to 10); moderate involvement (score range of 11 to 15); severe involvement (score range of 16 and more) (15, 16).

2.4. Statistical analysis

Data analyses were performed via IBM SPSS Statistics software (version 17). The normality of variables was assessed by the Kolmogorov-Smirnov test. Results were presented as mean (standard deviation) or median (interquartile range) for continuous variables and frequency (percentage) for categorical variables. Continuous data with normal distribution were compared using the independent t-test and data without a normal distribution were compared using the Mann–Whitney U test. Categorical variables were compared using a $\chi 2$ test. The correlation between serum level of micronutrients and the severity of disease was analyzed in different models. Multivariate linear regression analysis was performed to identify an independent association of serum level of micronutrients with APACHE score and CT involvement severity. $P < 0.05$ was considered for statistical significance.

3. Results

3.1. Comparison of demographic data, clinical characteristic and inflammatory markers between two groups according to APACHE score

Table 1 summarizes the demographic data, clinical characteristics, and inflammatory markers of patients that are grouped according to the APACHE score. In total, 60 patients were enrolled and categorized into two groups at analysis: APACHE scores ≥ of 25 (n = 20) and APACHE score < of 25 (N = 40).

The median (IQR) age of patients was 53.50 (12.75). Age and gender distributions were comparable between the study groups. The mean (SD) of BMI and temperature were significantly higher in the group with an APACHE score ≥ of 25 than the other group (p = 0.044 and 0.003, respectively). Oxygen saturation with or without respiratory aid was significantly lower in the group with an APACHE score ≥ of 25 than the other group (p = 0.005 and < 0.001, respectively). The group with an APACHE score ≥ of 25 used more invasive respiratory aid than the other group, and vice versa (p= 0.001). There was no significant difference in the frequency of previous underlying diseases between the study groups. Among the inflammatory markers, the serum level of ESR and CRP was significantly higher in the group with an APACHE score ≥ of 25, than the other group (p= 0.008 and 0.003, respectively). The other inflammatory markers including IL-6, TNF-a, and IFN-g showed no significant differences between the study groups. Regarding lung CT involvement, the group with an APACHE score ≥ of 25 had a higher severity score, and a higher prevalence rate of severe involvement compared to the group with an APACHE score < of 25 (P < 0.001).

3.2. Comparison of the serum level of micronutrients between two groups according to APACHE score

Table 2 summarizes the data on serum levels of vitamins and minerals between the two groups. The serum levels of vitamin D and Zn were significantly lower in the group with an APACHE score ≥ of 25, compared to the group with an APACHE score < 25 (p < 0.001).

3.3. Association of micronutrients with Apache score and CT involvement severity

Table 3 illustrates the association between serum levels of micronutrients and severity of the disease, according to APACHE and lung involvement scores. In the multivariate model, the serum levels of vitamin D, Zn, and Mg had an independent and an inverse association with APACHE score and lung involvement score, after adjustment for confounders.

3.4. Comparison of the serum level of micronutrients with the minimum of serum reference values

This study showed that the mean or median of serum levels of micronutrients was significantly lower than the expected minimum serum levels, except for Zn and vitamin A (table 4).

4. Discussion

We evaluated and compared the serum levels of micronutrients to the minimum normal range and demonstrated that except for vitamin A and Zn, serum levels of other micronutrients were significantly lower than normal in patients with COVID-19 admitted to ICU. Furthermore, we assessed the association between them and the severity of disease and our results showed that lower serum levels of vitamin D, Zn, and Mg were significantly and inversely correlated with a higher severity score, independent of confounding factors. A crucial challenge for researchers and experts is to identify the predictive factors involved in the higher severity of COVID-19, in terms of helping determine therapeutic goals for improving survival, especially in critically ill patients.

Critical ill patients experience several metabolic changes to rise the provision of nutrients to the vital tissues and survive; such as the release of pituitary hormones, the stimulation of the sympathetic nervous system, and antioxidant depletion (17, 18).

Micronutrients, classified as vitamins or minerals, have an essential role in intermediaries in metabolism, wound healing, immune function, antioxidant activity, cellular differentiation and proliferation, antioxidant activity, and blood coagulation. Micronutrient needs are elevated during acute illness because of increased demands and losses (19).

Various factors including advancing age (20-22), rising inflammatory markers such as CRP (21, 23), and comorbidities such as DM and cardiovascular disease (21, 22) have been shown to be related to higher severity and mortality rate for COVID-19.

The role of nutritional status in the modulation of immune system function has been previously elucidated, as it affects outcomes in infectious diseases (24). A few numbers of studies focused on the role of micronutrient deficiencies in the prognosis of patients with COVID-19. The result of a systematic review and meta-analysis showed that vitamin D deficiency, especially in elderly subjects, is associated with higher severity of disease and mortality among patients with COVID-19 (25). This result has been hypothesized to be related to the higher inflammatory response in vitamin D-deficient patients, which confronts the patient with severe disease (26).

Our results reinforced this hypothesis and found, however, the serum level of vitamin D was not lower than normal the lower serum levels of vitamin D are independently associated with higher severity of COVID-19, according to APACHE score and lung CT involvement score.

Vitamin C deficiency is another nutritional status that has received little attention as a potential cause of disease severity. A pilot study by Arvinte et al. (27) found that vitamin C deficiency, besides the advancing age, is a potential predictor of mortality among ICU-admitted COVID-19 patients. Also, the beneficial effects of vitamin C administration on critically ill patients have been previously elucidated, probably due to its potential immunomodulatory and anti-inflammatory effects (28). However, the levels of vitamin C was lower than normal in our patients; results did not show an independent association between lower serum level of vitamin C and higher severity scores.

Although the regulatory effects of vitamin A, different types of vitamin B and vitamin E on the immune system have been proven (7, 29), no study is so far available, which has been specifically focused on the association between the levels of these vitamins and prognosis of patients with COVID-19. We evaluated the serum levels of these vitamins and found no significant values for them in predicting disease severity.

The immunomodulatory effects of minerals in infectious disease have been shown in several studies (7, 29); however, few studies have focused on patients with COVID-19. Several studies found that Zn deficiency in patients with COVID-19 is associated with a higher rate of complications, hospitalizations, and mortality, compared to patients with a sufficient level of Zn (30-33). We also showed that Zn deficiency is independently correlated with higher severity of the COVID-19, based on both APACHE and CT involvement scores. Studies on deficiencies of other minerals in patients with COVID-19 are scarce. We showed an independent and inverse association of serum Mg level with a higher severity score, but no association was found between Iron deficiency and severity of COVID-19.

Several potential limitations in this study should be considered. First, this study is a single-center with a small number of cases, which limits further analysis with controlling more confounders; second, given the cross-sectional design of our study, the causality of the association cannot be detected. On the other hand, some conditions such as the use of vasoactive drugs, the hypermetabolism of critically ill patients, the need for mechanical ventilation that increases required micronutrients are other limitations of the study.

Overall, our results reinforce the evidence that the serum levels of vitamin D, Zn, and Mg are independently associated with the severity of the COVID-19. Randomized clinical trials are recommended to identify the effectiveness of micronutrient supplements on improving the prognosis of severe cases of patients with COVID-19.

Evidence has reported that intake of some nutrients may require increasing in particular conditions such as infection compare to regular conditions (34-36). In this way, some evidence found that supplementation may modify the severity of immune response and inflammatory process, and improve the survival of infectious disease (7, 37, 38), though due to insufficient studies, no definite conclusion can be made in this regard.

Conclusion: Low serum levels of some micronutrients such as B9, B12, C, and D vitamins, and Mg, Iron, were observed in patients with COVID-19. Furthermore, lower levels of vitamin D, Zn, and Mg were correlated with more severe disease. We recommend identifying and addressing the deficiency of micronutrients that could be involved in immune system response for preventing severe COVID-19 disease.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table1. Comparison of demographic and baseline characteristics between groups

variables	Total	Apache score < 25 (N=40)	Apache score ≥ 25 (N=20)	P-value
Age (year)	53.50 (12.75)	50.00 (16.5)	56.00 (8.50)	0.141
Gender, n				
Male	39	24	15	0.390
Female	21	16	5	
BMI (kg / m^2)	25.90 (2.70)	25.40 (2.58)	26.89 (2.73)	0.044
Temperature (Centigrade)	37.53 (0.55)	37.38 (0.46)	37.82 (0.61)	0.003
Respiratory aids, n				
Mask with reserve	16	13	3	
Simple mask	11	10	1	
Nasal aid	5	5	0	0.001
CPAP	5	4	1	
Noninvasive ventilation	2	2	0	
Invasive ventilation	21	6	15	
Underlying disease, n				
DM	5	5	0	
Asthma	12	5	7	
Thyroid diseases	6	6	0	0.057
Malignancy	19	13	6	
DM and HTN	18	11	7	
O2Sat				
With Aid	96.00 (2)	96.00 (2.5)	95.00 (2)	0.005
Without Aid	89.00 (4.75)	90.00 (3)	87.00 (3)	< 0.001
WBC count, × 10^9/L	6.95 (7.00)	6.70 (7.98)	7.05 (7.22)	0.931
Neutrophil count, × 10^9/L	80.20 (11.40)	80.65 (9.22)	79.90 (22.90)	0.456
Lymphocyte count, × 10^9/L	12.35 (11.00)	12.40 (10.60)	12.25 (15.88)	0.925
ESR (mm/hr)	63.50 (46)	54.00 (55.25)	85.50 (50)	0.008
CRP (mg/L)	87.50 (115.25)	82.50 (87.25)	118.50 (124.75)	0.003
IL-6 (pg/mL)	189.65 (191.85)	177.70 (164.57)	229.2 (233.97)	0.272
TNF-α (pg/mL)	207.7 (211.67)	207.6 (174.04)	248.2 (292.10)	0.410
IFN-γ (pg/mL)	118.15 (122.50)	82.80 (108.00)	141.7 (237.10)	0.293
CT involvement score	13.00 (9.00)	9.50 (6.00)	18 (3.00)	< 0.001

CT involvement severity, n				
No involvement	0	0	0	< 0.001
Minimal	9	9	0	
Mild	17	17	0	
Moderate	27	14	13	
Severe	7	0	7	

* Normally distributed variables; BMI, body mass index; CPAP, continuous positive airway pressure; DM, diabetes mellitus; HTN, hypertension; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IL6, Interleukin 6; TNF-α, tumor necrosis factor-α; IFN-γ, Interferon gamma

Table2. Comparison of serum levels of micronutrients between groups

Variables	Total	Apache score < 25	Apache score ≥ 25	P-value
Vitamin A (mcmol/L)	0.25 (0.40)	0.30 (0.37)	0.20 (0.39)	0.841
Vitamin B9 (mcg/L)	8.20 (8.37)	9.10 (8.87)	6.05 (7.75)	0.147
Vitamin B12 (pg/mL)	374.3 (541.05)	429.40 (564.3)	260.75 (546.25)	0.117
Vitamin C (mg/dL)	0.40 (0.60)	0.40 (0.50)	0.25 (0.27)	0.063
Vitamin D (ng/mL)	28.95 (13.39)	33.38 (13.26)	20.08 (8.49)	< 0.001
Vitamin E (mcg/mL)	7.45 (6.65)	7.75 (7.22)	7.30 (6)	0.406
Mg (mg/dL)	1.90 (0.40)	2 (0.85)	1.80 (0.10)	0.060
Zinc (mcg/dL)	70.00 (44.5)	80.00 (32.75)	50.50 (18)	< 0.001
Iron (mcg/dL)	48.00 (36.75)	52.00 (38.75)	38.00 (27)	0.249

Table3. Multivariate linear regression analysis to identify the independent association of serum level of micronutrients with APACHE score and the severity of CT involvement

Micronutrients	Apache score		CT involvement severity score	
	ß (95% CI)	P value	ß (95% CI)	P value
Vitamin A (mcmol/L)	3.673 (−1.553, 8.899)	0.164	1.742 (−2.025, 5,509)	0.357
Vitamin B9 (mcg/L)	0.150 (−0.072, 0.373)	0.181	0.073 (−0.088, 0.233)	0.367
Vitamin B12 (pg/mL)	0.004 (0.000, 0.008)	0.056	0.002 (−0.004, 0.005)	0.096
Vitamin C (mg/dL)	1.481 (−1.975, 4.937)	0.394	0.933 (−1.535, 3.401)	0.451
Vitamin D (ng/mL)	−0.157 (−0.251, −0.063)	0.001	−0.111 (−0.178, −0.044)	0.002
Vitamin E (mcg/mL)	0.004 (−0.040, 0.049)	0.849	0.012 (−0.237, 262)	0.921
Mg (mg/dL)	−2.578 (−4.868, −0.287)	0.028	−1.688 (−3.334, −0.042)	0.045
Zinc (mcg/dL)	−0.096 (−0.147, −0.045)	< 0.001	−0.082 (−0.119, −0.046)	< 0.001
Iron (mcg/dL)	0.021 (−0.040, 0.083)	0.491	0.001 (−0.043, 0.045)	0.971
BMI (kg / m^2)	0.123 (−0.264, 0.511)	0.526	0.072 (−0.212, 0.355)	0.355

*Adjusted for BMI, temperature, respiratory aids, O2Sat, ESR, CRP, vitamin D (except for vitamin D model), zinc (except for zinc model) and micronutrients including Vitamin C, D, Zinc, magnesium (only for BMI model) CI, confidence interval

Table4: Comparison of serum levels of micronutrients to the minimum level of normal range

Variables	Total	Normal range	p-value*
Vitamin A (mcmol/L)	0.25 (0.40)	0.3 - 0.8	0.203
Vitamin B9 (mcg/L)	8.20 (8.37)	3 - 17	< 0.001
Vitamin B12 (pg/mL)	374.3 (541.05)	160 - 950	< 0.001
Vitamin C (mg/dL)	0.40 (0.60)	0.6 - 2	0.003
Vitamin D (ng/mL)	28.95 (13.39)	> 20	< 0.001[&]
Vitamin E (mcg/mL)	7.45 (6.65)	5 – 18	< 0.001
Mg (mg/dL)	1.90 (0.40)	1.7 - 2.2	< 0.001
Zinc (mcg/dL)	70.00 (44.5)	70 - 120	1.00
Iron (mcg/dL)	48.00 (36.75)	60 - 70	< 0.003

*Sign test / [&] one sample t-test/