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Essential metals, vitamins and antioxidant enzyme activities in COVID-19 patients and their potential associations with the disease severity

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Abstract The role of micronutrient deficiency in the pathogenesis of COVID-19 has been reviewed in the literature; however, the data are limited and conflicting. This study investigated the association between the status of essential metals, vitamins, and antioxidant enzyme activities in COVID-19 patients and disease severity. We recruited 155 patients, who were grouped into four classes based on the Adults guideline for the Management of Coronavirus Disease 2019 at King Faisal Specialist & Research Centre (KFSH&RC): asymptomatic ($N = 16$), mild ($N = 49$), moderate ($N = 68$), and severe ($N = 22$). We measured serum levels of copper (Cu), zinc (Zn), selenium (Se), vitamin D_3, vitamin A, vitamin E, total antioxidant capacity, and superoxide dismutase (SOD). Among the patients, 30%, 25%, 37%, and 68% were deficient in Se (< 70.08 µg/L), Zn (< 0.693 µg/mL), vitamin A (< 0.343 µg/mL), and vitamin D_3 (< 20.05 µg/L), respectively, and SOD activity was low. Among the patients, 28% had elevated Cu levels (> 1.401 µg/mL, KFSH&RC upper reference limit). Multiple regression analysis revealed an 18% decrease in Se levels in patients with severe symptoms, which increased to 30% after adjusting the model for inflammatory markers. Regardless of inflammation, Se was independently associated with COVID-19 severity. In contrast, a 50% increase in Cu levels was associated with disease severity only after adjusting for C-reactive protein, reflecting its possible inflammatory and pro-oxidant role in COVID-19 pathogenesis. We noted an imbalance in the ratio between Cu and Zn, with $\sim$ 83% of

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patients having a Cu/Zn ratio > 1, which is an indicator of inflammation. Cu-to-Zn ratio increased to 45% in patients with mild symptoms and 34%–36% in patients with moderate symptoms compared to asymptomatic patients. These relationships were only obtained when one of the laboratory parameters (lymphocyte or monocyte) or inflammatory markers (neutrophil-to-lymphocyte ratio) was included in the regression model. These findings suggest that Cu/Zn might further exacerbate inflammation in COVID-19 patients and might be synergistically associated with disease severity. A 23% decrease in vitamin A was seen in patients with severe symptoms, which disappeared after adjusting for inflammatory markers. This finding may highlight the potential role of inflammation in mediating the relationship between COVID-19 severity and vitamin A levels. Despite our patients' low status of Zn, vitamin D_3, and antioxidant enzyme (SOD), there is no evidence of their role in COVID-19 progression. Our findings reinforce that deficiency or excess of certain micronutrients plays a role in the pathogenesis of COVID-19. More studies are required to support our results.

Keywords COVID-19 · Essential metals · Vitamins · Antioxidant activity · Disease severity

Introduction

In December 2019, a severe acute respiratory syndrome caused by coronavirus-2 (SARS-CoV-2) emerged in Wuhan City, China and subsequently spread to many other countries around the globe. The disease was named COVID-19 by the World Health Organization (WHO). Coronaviruses are enveloped, positive single-stranded RNA viruses that infect humans and a wide range of animals and belong to different subfamilies: alpha, beta, gamma, and delta (Yu et al. 2020). Coronavirus-2 is a *betacoronavirus* and is similar to the coronaviruses that cause a severe acute respiratory syndrome in bats (Chan et al. 2020a, b). Due to its worldwide spread, WHO declared a pandemic on March 11th, 2020. By April 4th, 2020 (when this study was planned), there were 1,051,635 cases and 56,985 deaths from more than 180 countries. The total number of confirmed cases in Saudi Arabia was 2039 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports).

COVID-19 is a highly transmissible and pathogenic virus that may cause severe respiratory illness leading to intensive care unit admission and high mortality (Guan et al. 2020; Chan et al. 2020a, b). However, the disease may be asymptomatic or mildly symptomatic in more than 80% of patients, with the rest exhibiting severe or critical symptoms (Wu and McGoogan 2020). The immune system protects the body from infectious microorganisms (Sattler 2017). Viral factors (e.g., type, mutation, viral load, viral titer, and in vitro viability) and the individual's immune system (e.g., genetics, age, gender, nutritional status, neuroendocrine-immune regulation, and physical condition) contribute to both the duration and severity of the disease (Li et al. 2020a, b). Studies have emphasized the role of the innate immune response to coronavirus infection by inhibiting its replication, promoting its clearance, inducing tissue repair, and triggering a prolonged adaptive immune response (Li et al. 2020a, b).

Micronutrients such as vitamins A, D, C, E, B_6, and B_{12}, folate, zinc (Zn), iron, copper (Cu), and selenium (Se) play vital roles in maintaining a responsive immune system. Inadequate dietary intakes may increase the risk of infection (Gombart et al. 2020). In the absence of a treatment or vaccine for COVID-19 at its early onset, researchers have recommended nutritional interventions for COVID-19 patients with micronutrient deficiencies (Zhang and Liu 2020). According to Shi et al. (2020), the immune response to COVID-19 goes through two phases: (1) an immune defense-based protective phase and (2) an inflammation-driven damaging phase. Due to its lung protective properties, vitamin B_3 should be administered as soon as the patient begins to cough. At the time of this research, no studies confirmed our hypothesis that adequate serum levels of micronutrients in patients infected with COVID-19 might be associated with milder disease symptoms and optimal immune response. Several reviews have focused on nutrient status and COVID-19 severity, with vitamin D being the most studies (Oscanoa et al. 2021; Khatiwada and Subedi 2021; Domingo and Marquès 2021; Fedele et al. 2021); however, clinical studies based on laboratory analyses are limited and controversial (Campi et al. 2021; Skalny et al. 2021; Zeng et al. 2021b; Gonçalves et al. 2021).

Abnormalities in total white blood count, neutrophil, lymphocyte, monocyte, eosinophil, C-reactive protein and other hematological parameters were reported in COVID-19 patients. These abnormalities need to be monitored for potential disease progression (Henry et al. 2020). Researchers recommend the use of biomarkers of inflammation derived from differential counts such as ratios of neutrophil to lymphocyte, neutrophil to monocyte, and lymphocyte to monocyte as inflammatory prognostic markers for several diseases, including COVID-19 (Man et al. 2021; Anurag et al. 2020).

The objectives of our study were to (1) assess the levels of micronutrients (Cu, Zn, Se, vitamin A, vitamin E, and vitamin D_3) and antioxidant enzyme activities in COVID-19 patients; and (2) determine their potential association with disease severity after adjusting for specific laboratory parameters or inflammatory biomarkers. The results of our study might emphasize the importance of nutritional interventions as treatment strategies to control the spread and severity of COVID-19.

Materials and methods

Study participants

Between June 3rd and July 11th, 2020, we recruited 155 patients ($\geq$ 18 years old) that were infected with COVID-19 according to the Pathology and Laboratory Medicine Department at KFSH&RC. We obtained verbal and written consent from all patients. KFSH&RC Research Ethics Committee (RAC# 2200025) approved the study. Before treatment, we collected all samples. We obtained information on demographics, lifestyle, medical history, white blood cells (WBCs), differential counts (neutrophils [NEUT], lymphocytes [LYM], and monocytes [MONO]), immunology (ferritin [FER] and C-reactive protein [CRP]) from the patients' electronic medical reports. We calculated the ratios between NEUT and LYM (NLR), NEUT and MONO (NMR), and LYM and MONO (LMR). The Clinical Biochemistry, Pathology and Laboratory Medicine Department of KFSH&RC conducted the tests. Disease severity was classified into four groups based on the KFSH&RC Adult Guidelines for the Management of Coronavirus Disease 2019 (Table 1).

Metal assessment

We diluted (50 $\times$) of each serum sample (50 µL) with a diluent, a mixture of 0.5% nitric acid (Fisher Scientific, PA), 0.05% Triton-X (Sigma-Aldrich^{TM}, MO), 2% methanol (Fisher Scientific, PA; all v/v), rubidium as an internal standard at 1 µg/L for Zn and Cu and 0.1 µg/L for Se. We measured metals levels by inductively coupled plasma-mass spectrometry (ICP-MS; Perkin Elmer NexION® 2000). The diluent's intensity for each metal was subtracted from calibrator standards, quality control, and patient samples. We prepared calibration standards in serum with every batch of patient samples at 4–60 µg/L (Zn), 6–60 µg/L (Cu), and 0.5–10 µg/L (Se). These ranges showed satisfactory linearity, with linear correlation coefficients (r^2) of 0.9981 $\pm$ 0.0025 (Zn) and 0.9998 $\pm$ 0.0003 (Cu) from five independent runs. For Se, r^2 was 0.9998 $\pm$ 0.0001 from four independent runs. We evaluated the accuracy of the method using both external assurance reference materials and internal quality control samples. Our analytical results for UTAK 66,816 reference serum normal range (UTAK Laboratories Inc., CA) were 730.22 $\pm$ 61.822 µg/L (Zn), 1041.59 $\pm$ 31.42 µg/L (Cu), and 104.97 $\pm$ 0.347 µg/L (Se). These values were within $\pm$ 15% of the UTAK certified reference values of 650 µg/L (Zn), 1110 µg/L (Cu), and 105 µg/L (Se). Pooled serum samples spiked with three different Zn, Cu, and Se levels were analyzed in parallel with patient samples to check between-run precision. The average recoveries from five runs of pooled serum samples spiked with 10, 15, and 30 µg/L Zn were 98.8 $\pm$ 9.8%, 102.9 $\pm$ 4.6%, and 100.5 $\pm$ 2.4%, respectively. For spiked serum samples with 20, 40, and 50 µg/L Cu, the average recoveries from five runs were 106.0 $\pm$ 5.9%, 103.4 $\pm$ 3.4%, and 101.3 $\pm$ 2.8%, respectively. The average recoveries from four runs of serum samples spiked with 0.75, 1.5, and 3 µg/L Se were 106.2 $\pm$ 6.3%, 103.9 $\pm$ 3.5%, and 101.3 $\pm$ 1.7%, respectively. The within-run precision relative standard deviations (% RSD) values for ten replicates of pooled serum samples spiked with 10, 15, and 30 µg/L zinc were 9.0, 4.1, and 4.8%, respectively. From ten runs of 20, 40, and 50.0 µg/L Cu, % RSD was 4.2, 3.9, and 1.6%, respectively. Finally, % RSD for ten replicates of 0.75, 1.5, and 3.0 µg/L Se was 2.8, 3.2, and 2.2%, respectively. The method detection limit

Table 1 Classification of COVID-19 severity

Asymptomatic	Mild	Moderate	Severe
Patients with no signs or symptoms of infection	Patients with upper respiratory tract infection symptoms and other mild symptoms (including fever and gastrointestinal symptoms) without evidence of pneumonia	Patients with hypoxia with oxygen saturation less than 93% at rest or presence of pneumonia not requiring ICU admission	Patients with pneumonia requiring ICU admission or any of the following: Respiratory rate of 30 breaths/min Arterial oxygen partial pressure to fractional inspiratory oxygen ratio (PaO2/FiO2) Less than 300 More than 50% lung involvement on imaging within 24–48 h Critical respiratory failure requiring mechanical ventilation, septic shock or multiorgan dysfunction

(MDL) was calculated by multiplying the standard deviation (SD) of 10 replicates of the blank levels and the Student's *t*-value corresponding to $N - 1$ degrees of freedom and 99th percentile. Serum MDLs for Zn, Cu, and Se were 1.707, 0.767, and 0.079 μg/L, respectively.

Vitamin analysis

We quantitatively assessed vitamins A, E, and D_3 using the Chromsystems reagent kit (Chromsystems Instruments & Chemicals GmbH, Heimburgstrasse, Munich, Germany). The manufacturers provided all materials and reagents. The assays were performed following the supplier's protocols.

Vitamin A (retinol) and vitamin E (α-tocopherol)

In a light-protected reaction vial, we mixed 200 μL serum sample, 20 μL internal standard, and 25 μL precipitation reagent I using a vortex for 30 s. Following the addition of 400 μL precipitation reagent II, we mixed the solution for 30 s and centrifuged it at 9000 g for 10 min. An aliquot of supernatant was injected onto the C_{18} reverse-phase high-performance liquid chromatography (HPLC) column connected to the Alliance Waters HPLC 2695 system and a ultra-violet (UV) detector (Waters Corp., Milford, MA) at 325 nm switched after 3.5 min to 295 nm. The flow was maintained at 1.5 mL/min. The analysis time took $\sim$ 9 min. The calibration was performed using a lyophilized serum calibration standard with a known concentration of vitamin A (0.66 mg/L) and vitamin E (10.7 mg/L). We used level I and II vitamin A and E lyophilized serum samples as quality controls to monitor the accuracy and precision of the analytical method. From two independent runs, vitamin A concentrations were 0.485 and 0.515 mg/L (level I) and 1.055 and 1.12 mg/L (level II). Both were within the certified reference values provided by the manufacturer: 0.35–0.53 mg/L (level I) and 1.06–1.58 mg/L (level II). From two independent runs, vitamin E concentrations were 7.974 and 8.018 mg/L (level I) and 14.52 and 14.6 mg/L (level II). Both were within the certified reference values of 6.83–10.2 mg/L (level I) and 14.9–22.3 mg/L (level II).

Vitamin D_3 (25-OH-vitamin D_3)

In a reaction vial, we mixed 100 μL sample (serum/calibrator/quality controls), 25 μL precipitation reagent, and 200 μL internal standard for 20 s using a vortex and centrifuged the sample at $15{,}000 \times g$ for 5 min. An aliquot of supernatant (200 μL) was transferred into an auto-sampler vial,

and 50 µL was analyzed by ultra-performance liquid chromatography (UPLC)-tandem mass spectrometry (LC–MS/MS; Waters, Milford, MA, USA). Mobile phases (A and B) were independently used in isocratic mode. The total run time was 5 min. We performed the calibration using a lyophilized serum calibration standard of known concentration of vitamin D_3 (4.93, 31.8, and 60.4 µg/L). From two independent runs, the concentration of vitamin D_3 in lyophilized serum quality control concentrations was 9.57 and 10.54 µg/L (low), 30.81 and 32.3 µg/L (medium), and 108.82 and 116.44 µg/L (high). The three ranges were within the certified reference values provided by the company: 12.8–19.2 µg/L (low), 27.5–41.2 µg/L (medium), and 80.6–121 µg/L (high).

Antioxidant enzyme activities

We used the OxiSelect™ Total Antioxidant Capacity (TAC) assay kit (STA-360) from Cell Biolabs, Inc. (San Diego, CA, USA) according to the manufacturer's protocol. We diluted 20 µL of serum sample and standard with 1 × reaction buffer. We measured absorbance at 490 nm. Copper ion reagent was added and incubated for 5 min. The reaction was terminated with a 1 × stop solution, and absorbance was measured at 490 nm. We calculated TAC in samples by comparing their net optical density values to the uric acid standard curve (0–1 mM). Results were expressed as µM copper reducing equivalents.

We measured superoxide anions using the OxiSelect™ Superoxide Dismutase Activity (SOD) assay kit (STA-340) from Cell Biolabs, Inc. (San Diego, CA, USA). In a 96-well plate, following the addition of 10 µL serum and standard diluted with 1X xanthine oxidase, we mixed and incubated the sample for 1 h at 37 °C. The standard range was 0–5 U/µL. We measured enzyme activity as a function of optical density by the degree of inhibition at 490 nm and expressed as units/µL. One unit of activity was considered as the concentration that resulted in 50% inhibition of the reaction.

For both TAC and SOD, we measured absorbance in a Biotek™ EL × 800™ absorbance microplate reader (Winooski, VT, USA).

Statistical analysis

We presented continuous variables as mean, standard deviation (SD), median, minimum, and maximum and categorical variables as percentages. The analytes were transformed to the natural logarithm (ln) to approximate a normal distribution. Bivariate analyses such as the Mann–Whitney U test, Kruskal–Wallis test, or Chi-square statistic (χ^2 test or Fisher exact test) were used for categorical variables. We performed Spearman rank correlation analyses to assess associations between pairs of continuous variables to identify potential risk factors/confounders of analytes and COVID-19 severity. Separate multiple linear regression models were generated to examine the contribution of each analyte to COVID-19 severity. We created three dummy variables for the four classes of COVID-19 severity, in which one group became the reference group, and all other groups were compared to it. The asymptomatic category was used as a reference group. We adjusted each model for risk factors/confounders that were associated with COVID-19 severity and/or analytes ($p < 0.1$). Due to collinearity, each laboratory marker was entered individually in the model. We expressed the results as the value of β-standardized regression coefficients, 95% confidence intervals (CIs) as effect estimates, and p-values to assess statistical significance. β was presented as a percentage change. SPSS software (version 20; IBM, Armonk, NY, USA) was used for data analysis, and $p < 0.05$ was considered statistically significant. Due to the exploratory nature of this study, we also defined $p < 0.1$ as marginally significant (Weitkunat and Wildner 2002; Wilhelm et al. 2015).

Results

Basic characteristics of patients

In this study, we enrolled 155 patients (age range: 18–95 years with a median age of 50). The ratio of females to males was 78 to 77. The majority were Saudi ($N = 139$), and the rest ($N = 16$) were from different nationalities. Among the 155 patients, five were smokers, seven were former smokers, and 143 were nonsmokers. Approximately 89% ($N = 138$) had various health problems, some with severe clinical

Table 2 The levels of trace metals (Cu, Zn and Se), vitamins (A, E and D_3) and antioxidant enzymes (TAC and SOD) classified according to the severity of COVID-19

COVID-19 classification	Cu (μg/mL)	Zn (μg/mL)	Cu/Zn	Se (μg/L)	Vitamin A (mg/L)	Vitamin E (mg/L)	Vitamin D_3 (μg/L)	TAC (μM)	SOD (mU/L)
Asymptomatic									
N	16	16	16	16	15	15	16	15	14
Mean ± SD	1.30 ± 0.678	1.24 ± 1.41	1.44 ± 0.976	86.56 ± 18.95	0.744 ± 0.579	15.68 ± 8.13	10.93 ± 10.41	979.18 ± 358.21	695.01 ± 139.2
Median	1.11	0.885	1.17	86.99	0.597	15.90	8.13	993.4	704.4
Min–Max	0.18–2.78	0.463–6.42	0.183–4.055	50.23–115.8	0.223–2.39	4.59–29.9	0–39.19	440.4–1914.9	363.8–945.2
Reference limits[a]									
Low	1 < 0.701	4 < 0.693		4 < 70.08	4 < 0.343	1 < 5.5	13 < 20.03		
High	5 > 1.401	3 > 1.242		0 > 119.69	5 > 0.838	8 > 15.5	1 > 30.05		
Mild									
N	49	49	49	49	41	41	47	41	39
Mean ± SD	1.31 ± 0.351	0.986 ± 0.724	1.55 ± 0.554	78.36 ± 18.04	0.474 ± 0.277	14.30 ± 7.64	14.17 ± 12.52	1145.2 ± 505.7	686.5 ± 145.2
Median	1.29	0.844	1.445	77.83	0.436	13.81	10.25	1092.3	701.1
Min–Max	0.689–2.47	0.403–5.26	0.23–2.805	40.98–122.2	0.099–1.45	3.23–39.24	0–53.57	372.8–3263.8	281.1–943.5
Reference limits[a]									
Low	1 < 0.701	9 < 0.693		16 < 70.08	15 < 0.343	7 < 5.5	34 < 20.03		
High	14 > 1.401	5 > 1.242		1 > 119.69	3 > 0.838	17 > 15.5	7 > 30.05		
Moderate									
N	68	68	68	68	62	62	67	67	65
Mean ± SD	1.25 ± 0.341	1.04 ± 1.25	1.513 ± 0.604	87.51 ± 19.26	0.549 ± 0.397	15.31 ± 14.4	15.00 ± 13.17	1134.8 ± 388.3	698.4 ± 153.5
Median	1.19	0.859	1.433	86.43	0.390	12.14	12.41	1071.1	704.5
Min–Max	0.656–2.36	0.431–10.83	0.096–3.019	31.36–140	0.074–1.76	3.98–111.8	0–52.5	365.8–2635.6	277.9–1004.7
Reference limits[a]									
Low	2 < 0.701	18 < 0.693		13 < 70.08	23 < 0.343	5 < 5.5	45 < 20.03		
High	19 > 1.401	5 > 1.242		3 > 119.69	11 > 0.838	18 > 15.5	11 > 30.05		
Severe									
N	22	22	22	22	19	19	21	21	21
Mean ± SD	1.22 ± 0.370	1.30 ± 1.81	1.50 ± 0.603	76.6 ± 23.54	0.422 ± 0.275	13.92 ± 6.2	19.86 ± 20.35	1326.1 ± 387.9	639.7 ± 140.6
Median	1.2	0.772	1.56	66.73	0.374	14.05	12.45	1289.5	633.3
Min–Max	0.385–2.06	0.381–8.43	0.192–2.249	40.23–116.7	0.130–1.27	5.19–32.06	0–71.62	653.4–2018.5	294.0–859.5
Reference limits[a]									
Low	1 < 0.701	7 < 0.693		13 < 70.08	8 < 0.343	1 < 5.5	11 < 20.03		
High	6 > 1.401	4 > 1.242		0 > 119.69	1 > 0.838	6 > 15.5	4 > 30.03		

Table 2 continued

COVID-19 classification	Cu (µg/mL)	Zn (µg/mL)	Cu/Zn	Se (µg/L)	Vitamin A (mg/L)	Vitamin E (mg/L)	Vitamin D_3 (µg/L)	TAC (µM)	SOD (mU/L)
Total									
N	155	155	155	155	137	137	151	144	139
Mean ± SD	1.27 ± 0.392	1.08 ± 1.23	1.52 ± 0.631	82.97 ± 19.91	0.530 ± 0.381	14.86 ± 11.1	14.99 ± 13.99	1149.4 ± 427.1	685.8 ± 147.7
Median	1.21	0.844	1.436	83.05	0.413	13.21	12.45	1087.4	701.1
Min–Max	0.180–2.78	0.381–10.83	0.096–4.055	31.36–140	0.074–2.39	3.23–111.8	0–71.62	365.8–3263.8	277.9–1004.7
Reference limits[a]									
Low	5 < 0.701	38 < 0.693		46 < 70.08	50 < 0.343	14 < 5.5	103 < 20.03		
High	44 > 1.401	17 > 1.242		4 > 119.69	20 > 0.838	49 > 15.5	23 > 30.05		

[a]KFSH&RC reference ranges

conditions such as cancer ($N = 29$) and organ transplant ($N = 18$). The median body mass index (BMI) was 28.5 kg/m^2 (range: 11.1 to 51.7 kg/m^2). BMI was higher in females (31.2 kg/m^2) than in males (26.9 kg/m^2; $p = 0.004$). According to the World Health Organization's BMI classification, the prevalence of obesity (BMI $\geq$ 30 kg/m^2) and overweight (BMI $\geq$ 25 kg/m^2) and underweight (BMI < 18.5 kg/m^2) among our patients was 44.8, 75.3 and 1.29%, respectively https://www.who.int/data/gho/data/themes/theme-details/GHO/body-mass-index-(bmi). Among the 155 patients, 78 (50.3%) had no fever at diagnosis, 129 (83.2%) were admitted to the hospital for 1–113 days, and 26 (16.8%) were isolated at home. Sixteen patients (10.3%) died during the study. Ninety-nine (63.9%) patients took supplements including vitamin D (37) and minerals (15). Based on the COVID-19 symptoms exhibited at admission, 16 (10.3%), 49 (31.6%), 68 (43.9%), and 22 (14.2%) patients were classified as asymptomatic, mild, moderate, and severe, respectively.

There were 34 (21.9%), 4, 61, 7, 6, 10, and 6 patients with WBC, NEUT, LYM, MONO, CRP, and FER less than the lower KFSH&RC reference limit 3.9 × 10^9/L, 30%, 23%, 4%, 3 mg/L, and 30 µg/L, respectively. In contrast, 8, 47, 3, 44, 88, and 46 patients had WBC, NEUT, LYM, MONO, CRP, and FER higher than the upper KFSH&RC reference limit of 11 × 10^9/L, 70%, 60%, 12%, 3 mg/L, and 400 µg/L, respectively.

Trace element, vitamin, and antioxidant enzyme status

Table 2 shows the serum levels of Cu, Zn, Cu/Zn Se, vitamin A, vitamin E, vitamin D_3, TAC, and SOD based on COVID-19 severity. Out of 155 patients, 5 (3.2%), 38 (24.5%), and 46 (29.7%) had Cu, Zn, and Se levels below the lower KFSH&RC reference limits of 0.701 µg/mL, 0.693 µg/mL, and 70.08 µg/L, respectively. Two patients had the three metals below the reference limits. Forty-four (28.4%), 17 (11%), and four (2.6%) patients had Cu, Zn and Se levels above the upper reference limits of 1.401 µg/mL, 1.242 µg/mL, and 119.69 µg/L, respectively. There were 27 (17.4%) patients with Cu/Zn ratios less than 1. Among the patients, 50 (36.5%), 14 (10.2%), and 103 (68.2%) had vitamin A, E, and D_3 levels below the KFSH&RC lower reference limits of 0.343 mg/L,

Table 3 Clinical and demographic data classified according to the severity of COVID-19 and evaluated by Kruskal–Wallis test* and χ^2-test**

COVID-19 classification		Continuous variables											
		Age	BMI	Length of hospital stay	WBC ($\times 10^9$/L)	NEUT (%)	LYM (%)	MONO (%)	NLR	NMR	LMR	CRP (mg/L)	FRT (μg/L)
Asymptomatic	*N*	16	16	5	16	12	12	12	12	12	12	7	5
	Mean ± SD	38.7 ± 9.9	28.0 ± 6.3	4.6 ± 3.0	5.91 ± 2.39	56.0 ± 15.7	31.5 ± 13.5	10.4 ± 4.5	2.36 ± 1.57	6.92 ± 4.35	3.46 ± 1.75	15.4 ± 32.2	119.1 ± 56.8
	Median	36.5	27.6	6.0	5.69	60.250	28.1	10.3	2.20	5.57	3.14	1.5	109.0
	Min–Max	24–58	11.1–35.9	1–8	1.64–10.6	30.6–79.8	14.6–50.3	5–16.8	0.61–5.32	1.82–15.35	0.99–6.87	0.2–87.6	56.4–212
Mild	*N*	49	48	39	49	44	44	44	44	44	44	27	26
	Mean ± SD	48.3 ± 15.5	29.1 ± 5.7	11.4 ± 8.8	7.04 ± 12.6	56.14 ± 20.02	29.53 ± 15.39	12.59 ± 6.78	2.93 ± 2.53	6.41 ± 5.03	2.71 ± 1.68	50.38 ± 85.26	472.2 ± 735.5
	Median	50.0	28.4	8.0	5.12	60.6	25.6	11.5	2.45	5.44	2.26	13.8	171
	Min–Max	18–74	19.9–44.7	3–41	1.42–92.46	1–87.6	7.5–65.8	3–34.5	0.02–11.68	0.03–21.25	1.00–9.33	1.1–300	10.4–2944
Moderate	*N*	68	68	63	68	63	63	63	63	63	63	49	53
	Mean ± SD	51.4 ± 16.5	28.9 ± 6.5	13.9 ± 16.7	5.17 ± 2.01	63.61 ± 12.82	25.14 ± 11.1	10.11 ± 4.29	3.56 ± 2.89	7.93 ± 5.10	2.90 ± 2.36	56.01 ± 76.23	666 ± 644.8
	Median	51.0	27.8	10.0	5.06	63.7	26.6	9.3	2.44	6.92	2.43	18.4	435.0
	Min–Max	22–95	17.4–51.7	2–113	1.21–11.07	30.6–89.3	6–56	3–23.4	0.58–14.5	2.11–29.77	0.65–18.67	1.8–290.5	17.7–2723
Severe	*N*	22	22	22	22	17	17	17	17	17	17	15	16
	Mean ± SD	56.3 ± 17.1	32.4 ± 7.1	22.0 ± 13.0	7.89 ± 3.94	79.92 ± 10.47	11.52 ± 7.27	5.74 ± 3.05	13.48 ± 16.03	19.76 ± 17.73	2.79 ± 3.09	168.6 ± 120.8	800.5 ± 834.7
	Median	62.0	32.4	22.0	6.875	83.40	10.00	5.20	8.50	17.29	1.64	239.2	400.5
	Min–Max	21–83	22.1–45.7	6–55	3.16–18.93	56–93.9	1.5–25.3	1–15	2.65–62.6	4.13–84.0	0.33–13.0	4.8–293.4	41.8–2628
Total	*N*	155	154	129	155	136	136	136	136	136	136	98	100
	Mean ± SD	49.8 ± 16.2	29.4 ± 6.4	14.1 ± 14.2	6.22 ± 7.46	62.56 ± 17.08	25.42 ± 13.65	10.39 ± 5.49	4.49 ± 6.95	8.83 ± 8.75	2.87 ± 2.21	68.8 ± 94.44	609.8 ± 696.7
	Median	50.0	28.5	10.0	5.350	63.95	24.45	9.3	2.64	6.75	2.40	17.2	322
	Min–Max	18–95	11.1–51.7	1–113	1.21–92.46	1–93.9	1.5–65.8	1–34.5	0.02–62.6	0.03–84.0	0.33–18.67	0.2–300	10.4–2944
***p*-value**		0.005	0.182	< 0.001	0.008	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.209	< 0.001	0.006

COVID-19 classification	N (% of total)- categorical variables											
	Gender		Nationality		Medical history		Patients with cancer		Patients who underwent organ transplant		Supplements' intake	
	Male	Female	Non-Saudi	Saudi	No	Yes	No	Yes	No	Yes	No	Yes
Asymptomatic	7 (4.5%)	9 (5.8%)	3 (1.9%)	13 (8.4%)	1 (0.6%)	15 (9.7%)	11 (7.1%)	5 (3.2%)	14 (9%)	2 (1.3%)	5 (3.2%)	11 (7.1%)
Mild	20 (12.9%)	29 (18.7%)	4 (2.6%)	45 (29%)	4 (2.6%)	45 (29%)	37 (23.9%)	12 (7.7%)	43 (27.7%)	6 (3.9%)	18 (11.6%)	31 (20%)
Moderate	36 (23.2%)	32 (20.6%)	6 (3.9%)	62 (40%)	12 (7.7%)	56 (36.1%)	60 (38.7%)	8 (5.2%)	58 (37.4%)	10 (6.5%)	25 (16.1%)	43 (27.7%)
Severe	14 (9%)	8 (5.2%)	3 (1.9%)	19 (12.3%)	0 (0%)	22 (14.2%)	18 (11.6%)	4 (2.6%)	20 (12.9%)	2 (1.3%)	8 (5.2%)	14 (9%)
Total	77 (49.7%)	78 (50.3%)	16 (10.3%)	139 (89.7%)	17 (11%)	138 (89%)	126 (81.3%)	29 (18.7%)	135 (87.21%)	20 (12.9%)	56 (36.1%)	99 (63.9%)
***p*-value**	0.288		0.522[a]		0.099[a]		0.154[a]		0.951[a]		0.988[a]	

[a] χ^2 with fisher extract

5.5 mg/L, and 30.05 µg/L, respectively. However, there were 20 (14.6%), 49 (35.8%), and 23 (15.2%) patients who had vitamin A, E, and D_3 levels higher than the upper reference limits of 0.838 mg/L, 15.5 mg/L, and 30.05 µg/L, respectively.

Potential confounders/risk factors associated with the COVID-19 severity and tested analytes

Patients with severe symptoms had the highest levels of WBC ($p = 0.008$), NEUT ($p < 0.001$), CRP ($p < 0.001$), and FER ($p = 0.006$). Additionally, these patients were older ($p = 0.005$) and had longer hospitalizations ($p < 0.001$). In contrast, patients with severe symptoms had the lowest levels of LYM and MONO ($p < 0.001$, for both). Patients with severe symptoms had the highest NLR and NMR ($p < 0.001$). No significant differences in LMR were obtained among the four groups ($p = 0.209$). Results are displayed in Table 3.

Bivariate analyses showed that only TAC was positively and significantly correlated with age ($p < 0.001$) and length of hospital stay ($p = 0.002$). Females had significantly higher Cu ($p = 0.034$) but lower TAC ($r = 0.011$) than males. Patients with cancer had significantly lower Se and vitamin D_3 levels ($p < 0.01$). Low vitamin E and vitamin D_3 levels were obtained in patients who underwent organ transplants ($p = 0.003$ and $p = 0.006$, respectively). Patients taking supplements had significantly higher vitamin E levels ($p = 0.046$) but lower SOD ($p = 0.071$). While WBC was negatively associated with Cu/Zn, it was positively associated with TAC. NEUT was negatively associated with Zn and Se but positively associated with TAC.

In contrast, LYM was positively correlated with Zn, Cu/Zn, and Se but negatively correlated with TAC. An inverse relationship was obtained between MONO and Cu/Zn and TAC. While CRP was positively correlated with Cu, it was inversely associated with Zn, Se, and vitamin A. FER was only associated with TAC. NLR was inversely correlated with Zn and TAC but positively correlated with Cu/Zn. Only TAC was positively correlated with NMR, and LMR was positively correlated with Zn and Se but negatively correlated with TAC. The results are presented in Table 4.

Relationship between tested analytes and severity of COVID-19

We used separate linear regression models to evaluate the unadjusted (crude) and adjusted relationships between each analyte and COVID-19 severity in the four groups of patients. All models were adjusted for age and medical history that were significantly associated with COVID-19 severity and confounders significantly related with the analyte, including laboratory and inflammation markers in the bivariate analyses at $p < 0.1$. Table 5 shows that models adjusted for confounders but not for laboratory parameters showed a decrease in Se ($\beta = -0.203$, 95% CI $-0.308, -0.015$, $p = 0.074$) in patients with severe COVID-19 symptoms compared to asymptomatic ones. Additionally, we observed a significant decrease in the regression estimates of vitamin A in COVID-19 patients with severe ($\beta = -0.263$, 95% CI $-0.973, -0.034$, $p = 0.036$) and mild symptoms ($\beta = -0.262$, 95% CI $-0.78, 0.023$, $p = 0.064$).

Further adjustment of regression models was performed separately for only laboratory parameters and inflammatory markers that were significantly associated with the analyte to examine whether they were independently related to disease severity. Table 5 shows that only Cu, Cu/Zn, and Se had significant associations with COVID-19 severity.

In the Cu model, we obtained a significant increase in the regression estimates in patients with mild ($\beta = 0.427$, 95% CI 0.04, 0.467, $p = 0.021$) and moderate ($\beta = 0.402$, 95% CI 0.006, 0.42, $p = 0.044$) COVID-19 in comparison to asymptomatic patients.

When we included LYM, MONO, or NLR in the Cu/Zn model, we obtained a significant increase in the regression estimate in patients with mild symptoms ($\beta = 0.373$, 95% CI 0.076, 0.731, $p = 0.016$), ($\beta = 0.369$, 95% CI 0.066, 0.732, $p = 0.019$) and ($\beta = 0.371$, 95% CI 0.074, 0.729, $p = 0.017$), respectively. Even though the pattern was the same in patients with moderate symptoms, the statistical significance was marginal after including LYM ($\beta = 0.293$, 95% CI -0.032, 0.626, $p = 0.076$), MONO ($\beta = 0.309$, 95% CI -0.015, 0.642, $p = 0.061$) or NLR ($\beta = 0.303$, 95% CI -0.021, 0.634, $p = 0.061$).

When incorporating CRP in the Se model, we observed a significant decrease in the regression

Table 4 Bivariate analyses between the serum levels of tested analytes (trace metals, vitamins and antioxidant enzymes) in COVID-19 patients and various risk factors

Risk factors/ confounders	Cu (μg/mL)	Zn (μg/mL)	Cu/Zn	Se (μg/L)	Vitamin A (mg/L)	Vitamin E (mg/L)	Vitamin D_3 (μg/L)	TAC (μM)	SOD (mU/L)
Age (years)	0.011 (0.891)[a]	− 0.118 (0.143)[a]	0.071 (0.378)[a]	− 0.082 (0.313)[a]	− 0.040 (0.645)[a]	− 0.069 (0.426)[a]	0.119 (0.146)[a]	**0.295 (< 0.001)[a]**	− 0.107 (0.210)[a]
BMI (kg/m^2)	0.118 (0.144)[a]	− 0.089 (0.274[a]	**0.191 (0.018)[a]**	− 0.092 (0.256)[a]	− 0.006 (0.943)[a]	0.055 (0.523)[a]	0.047 (0.569)[a]	0.015 (0.858)[a]	− 0.031 (0.717)[a]
Length of stay in hospital (days)	0.060 (0.496)[a]	− 0.128 (0.147)[a]	**0.158 (0.074)[a]**	− 0.131 (0.140)[a]	0.029 (0.759)[a]	− 0.114 (0.227)[a]	0.123 (0.169)[a]	**0.273 (0.002)**[a]	− 0.061 (0.505)[a]
NEUT (%)	− 0.012 (0.887)[a]	**− 0.229 (0.007)[a]**	− 0.052 (0.521)[a]	**− 0.203 (0.018)[a]**	− 0.141 (0.125)[a]	− 0.039 (0.675)[a]	0.072 (0.410)a	**0.340 (< 0.001)[a]**	0.002 (0.979)[a]
LYM (%)	0.039 (0.650)[a]	**0.279 (0.001)a**	**− 0.319 (< 0.001)[a]**	**0.219 (0.010)[a]**	0.138 (0.135)[a]	0.017 (0.854)[a]	− 0.082 (0.347)[a]	**− 0.395 (< 0.001)[a]**	− 0.016 (0.861)[a]
NLR	− 0.035 (0.682)[a]	**− 0.270 (0.001)[a]**	**0.211 (0.014)[a]**	**− 0.210 (0.014)[a]**	− 0.140 (0.130)[a]	− 0.015 (0.874)[a]	0.079 (0.369)[a]	**0.388 (< 0.001)[a]**	0.002 (0.980)[a]
MONO (%)	− 0.035 (0.688)[a]	0.044 (0.614)[a]	**− 0.342 (< 0.001)[a]**	0.055 (0.525)[a]	0.101 (0.275)[a]	0.048 (0.605)[a]	− 0.019 (0.828)[a]	**− 0.210 (0.018)[a]**	0.098 (0.280)[a]
NMR	0.032 (0.714)[a]	− 0.124 (0.149)[a]	0.138 (0.110)[a]	− 0.119 (0.166)[a]	− 0.121 (0.189)[a]	− 0.040 (0.663)[a]	0.045 (0.604)[a]	**0.282 (0.001)[a]**	− 0.074 (0.419)[a]
LMR	0.056 (0.519)[a]	**0.253 (0.003)[a]**	− 0.165 (0.055)[a]	**0.174 (0.042)[a]**	0.067 (0.467)[a]	0.014 (0.879)[a]	− 0.084 (0.336)[a]	**− 0.261 (0.003)[a]**	− 0.052 (0.572)[a]
	136	**136**	136	**136**	119	119	133	**126**	123
CRP (mg/L)	**0.401 (< 0.001)[a]**	**− 0.241 (0.017)[a]**	− 0.122 (0.129)[a]	**− 0.332 (0.001)[a]**	**− 0.479 (< 0.001)[a]**	− 0.150 (0.176)[a]	− 0.004 (0.966)[a]	0.101 (0.340)[a]	0.031 (0.771)[a]
FER (μg/L)	− 0.080 (0.431)[a]	− 0.132 (0.192)[a]	− 0.020 (0.842)[a]	− 0.123 (0.222)[a]	− 0.198 (0.063)[a]	− 0.170 (0.111)[a]	0.013 (0.895)[a]	**0.294 (0.004)[a]**	− 0.170 (0.098)[a]
Gender (male/ female)	**− 2.122 (0.034)[b]**	− 1.077 (0.281)[b]	**− 2.137 (0.033)[b]**	− 1.396 (0.163)[b]	− 0.638 (0.524)[b]	− 1.068 (0.285)[b]	− 1.363 (0.173)[b]	**− 2.547 (0.011)[b]**	− 1.294 (0.196)[b]
Nationality (Saudi/non-Saudi)	− 1.647 (0.100)[b]	− 1.541 (0.123)[b]	− 2.517 (0.012)[b]	− 0.441 (0.659)[b]	− 1.535 (0.125)[b]	− 0.945 (0.345)[b]	− 0.654 (0.513)[b]	− 1.128 (0.259)[b]	− 0.991 (0.322)[b]
Medical history (yes/no)	− 0.252 (0.801)[b]	− 0.716 (0.474)[b]	− 0.218 (0.828)[b]	− 1.655 (0.098)[b]	− 0.524 (0.600)[b]	− 0.007 (0.995)[b]	− 0.333 (0.739)[b]	− 0.607 (0.544)[b]	− 1.715 (0.086)[b]
Patients with cancer (yes/no)	− 0.073 (0.941)[b]	− 1.097 (0.273)[b]	− 0.867 (0.386)[b]	**− 3.579 (< 0.001)[b]**	− 0.563 (0.574)[b]	− 0.067 (0.947)[b]	**− 2.721 (0.007)[b]**	− 1.044 (0.296)[b]	− 0.473 (0.636)[b]
Patients underwent organ transplant (yes/ no)	− 0.608 (0.543)[b]	− 1.612 (0.107)[b]	− 0.203 (0.839)[b]	− 0.573 (0.567)[b]	− 0.791 (0.429)[b]	**− 2.981 (0.003)[b]**	**− 2.752 (0.006)[b]**	− 0.354 (0.723)[b]	− 0.608 (0.543)[b]
Supplements intake (yes/no)	− 0.458 (0.647)[b]	− 0.283 (0.777)[b]	− 0.73 (0.465)[b]	− 0.443 (0.658)[b]	− 0.180 (0.857)[b]	**− 1.999 (0.046)[b]**	− 0.081 (0.935)[b]	− 1.410 (0.159)[b]	**− 1.808 (0.071)[b]**

Values between parentheses are the level of significance (p). Bold characters denoted significant associations

[a]Spearman rank correlation analysis

[b]Mann–Whitney-test

Table 5 The adjusted relationship (β coefficient and 95% CI) between ln-transformed analytes and COVID-19 severity

Analytes	Laboratory/ inflammatory parameters included in the model	Mild				Moderate				Severe				Laboratory/inflammatory parameters	
		β	*p*	95%CI		β	*p*	95%CI		β	*p*	95%CI		β	*p*
Cu-unadjusted		0.174	0.199	− 0.066	0.312	0.113	0.416	− 0.107	0.257	0.036	0.756	− 0.181	0.249		
Cu[a]		0.189	0.169	− 0.058	0.326	0.151	0.299	− 0.090	0.291	0.080	0.509	− 0.150	0.300		
Cu[a]	CRP	**0.427**	**0.021**	**0.040**	**0.467**	**0.402**	**0.044**	**0.006**	**0.420**	0.126	0.469	− 0.161	0.347	**0.371**	**0.001**
Zn-unadjusted		− 0.095	0.482	− 0.379	0.180	− 0.106	0.449	− 0.373	0.166	− 0.061	0.597	− 0.404	0.233		
Zn[b]		− 0.080	0.563	− 0.369	0.202	− 0.088	0.547	− 0.369	0.196	− 0.037	0.757	− 0.385	0.280		
Zn[b]	CRP	− 0.179	0.365	− 0.672	0.250	− 0.223	0.297	− 0.682	0.211	− 0.252	0.181	− 0.916	0.175	− 0.037	0.751
Zn[b]	NEUT	− 0.147	0.351	− 0.453	0.162	− 0.083	0.618	− 0.386	0.230	− 0.037	0.786	− 0.430	0.326	− 0.114	0.249
Zn[b]	LYM	− 0.138	0.377	− 0.443	0.169	− 0.074	0.657	− 0.376	0.238	− 0.018	0.896	− 0.403	0.353	0.154	0.117
Zn[b]	NRL	− 0.140	0.375	− 0.448	0.170	− 0.097	0.562	− 0.399	0.218	− 0.060	0.670	− 0.475	0.306	− 0.044	0.664
Zn[b]	LMR	− 0.139	0.379	− 0.449	0.172	− 0.098	0.558	− 0.401	0.218	− 0.081	0.536	− 0.479	0.250	0.010	0.911
Cu/Zn-unadjusted		0.192	0.157	− 0.087	0.532	0.164	0.239	− 0.120	0.477	0.077	0.505	− 0.234	0.472		
Cu/Zn[c]		0.170	0.215	− 0.117	0.514	0.155	0.288	− 0.144	0.481	0.033	0.791	− 0.325	0.426		
Cu/Zn[c]	WBC	0.166	0.228	− 0.122	0.510	0.156	0.285	− 0.143	0.484	0.028	0.818	− 0.333	0.421	0.046	0.576
Cu/Zn[c]	LYM	**0.373**	**0.016**	**0.076**	**0.731**	**0.293**	**0.076**	**− 0.032**	**0.626**	0.166	0.229	− 0.162	0.668	-0.100	0.300
Cu/Zn[c]	MONO	**0.369**	**0.019**	**0.066**	**0.732**	**0.309**	**0.061**	**− 0.015**	**0.642**	0.212	0.119	− 0.085	0.733	0.022	0.818
Cu/Zn[c]	NLR	**0.371**	**0.017**	**0.074**	**0.729**	**0.303**	**0.066**	**− 0.021**	**0.634**	0.153	0.282	− 0.194	0.661	0.101	0.303
Se-unadjusted		− 0.185	0.161	− 0.242	0.040	0.020	0.880	− 0.126	0.147	**− 0.196**	**0.084**	**− 0.304**	**0.019**		
Se[d]		− 0.192	0.138	− 0.243	0.034	− 0.032	0.814	− 0.154	0.121	**− 0.203**	**0.074**	**− 0.308**	**0.015**		
Se[d]	CRP	**− 0.367**	**0.049**	**− 0.411**	**− 0.001**	− 0.230	0.252	− 0.314	0.083	**− 0.357**	**0.045**	**− 0.492**	**− 0.006**	− 0.138	0.214
Se[d]	NEUT	**− 0.251**	**0.092**	**− 0.276**	**0.021**	− 0.014	0.931	− 0.157	0.144	− 0.213	0.101	− 0.336	0.030	-0.140	0.134
Se[d]	LYM	− 0.240	0.107	− 0.270	0.026	− 0.006	0.969	− 0.153	0.147	− 0.203	0.120	− 0.330	0.038	0.153	0.101
Se[d]	NLR	− 0.247	0.100	− 0.275	0.024	− 0.039	0.808	− 0.170	0.132	**− 0.277**	**0.043**	**− 0.389**	**− 0.007**	0.011	0.910
Se[d]	LMR	− 0.238	0.115	− 0.270	0.030	− 0.028	0.860	− 0.165	0.138	**− 0.266**	**0.035**	**− 0.367**	**− 0.014**	0.052	0.539
Vitamin A-unadjusted		**− 0.271**	**0.051**	**− 0.783**	**0.001**	− 0.219	0.127	− 0.664	0.084	**− 0.267**	**0.026**	**− 0.960**	**− 0.063**		
Vitamin A[b]		**− 0.262**	**0.064**	**− 0.780**	**0.023**	− 0.201	0.180	− 0.658	0.125	**− 0.263**	**0.036**	**− 0.973**	**− 0.034**		
Vitamin A[b]	CRP	− 0.236	0.231	− 0.932	0.228	− 0.207	0.336	− 0.823	0.284	− 0.160	0.407	− 0.970	0.398	**− 0.398**	**0.001**
Vitamin A[b]	FER	− 0.325	0.174	− 1.279	0.235	− 0.201	0.452	− 1.018	0.457	− 0.286	0.187	− 1.359	0.270	− 0.166	0.143
Vitamin E-unadjusted		− 0.080	0.571	− 0.437	0.242	− 0.071	0.624	− 0.405	0.244	− 0.040	0.744	− 0.453	0.325		
Vitamin E[e]		− 0.040	0.778	− 0.392	0.294	− 0.013	0.929	− 0.350	0.320	0.000	0.999	− 0.401	0.401		
Vitamin D_3-unadjusted		0.114	0.419	− 0.339	0.811	0.218	0.130	− 0.129	0.988	**0.210**	**0.080**	**− 0.073**	**1.272**		
Vitamin D_3[f]		0.079	0.576	− 0.415	0.744	0.142	0.346	− 0.305	0.864	0.162	0.188	− 0.230	1.157		
TAC-unadjusted		0.167	0.216	− 0.080	0.350	0.213	0.133	− 0.048	0.359	**0.312**	**0.009**	**0.082**	**0.563**		

Table 5 continued

Analytes	Laboratory/ inflammatory parameters included in the model	Mild				Moderate				Severe				Laboratory/inflammatory parameters	
		β	*p*	95%CI		β	*p*	95%CI		β	*p*	95%CI		β	*p*
TAC[a]		0.105	0.428	− 0.126	0.297	0.098	0.492	− 0.133	0.276	0.191	0.112	− 0.047	0.440		
TAC[a]	WBC	0.112	0.402	− 0.122	0.303	0.098	0.494	− 0.134	0.276	0.197	0.103	− 0.042	0.447	− 0.056	0.494
TAC[a]	NEUT	0.114	0.439	− 0.144	0.330	0.014	0.931	− 0.222	0.242	0.083	0.526	− 0.195	0.380	**0.288**	**0.003**
TAC[a]	LYM	0.089	0.544	− 0.164	0.309	0.008	0.959	− 0.226	0.237	0.075	0.569	− 0.205	0.371	**− 0.291**	**0.002**
TAC[a]	MONO	0.148	0.330	− 0.123	0.363	0.057	0.719	− 0.191	0.276	0.139	0.281	− 0.128	0.438	**− 0.231**	**0.017**
TAC[a]	FER	− 0.117	0.564	− 0.458	0.251	− 0.199	0.383	− 0.491	0.191	− 0.021	0.909	− 0.399	0.355	**0.176**	**0.083**
TAC[a]	NLR	0.087	0.569	− 0.174	0.315	0.055	0.734	− 0.197	0.279	0.148	0.286	− 0.139	0.469	0.109	0.274
TAC[a]	NMR	0.087	0.571	− 0.175	0.317	0.064	0.698	− 0.192	0.286	0.219	0.115	− 0.061	0.549	− 0.030	0.765
TAC[a]	LMR	0.074	0.629	− 0.186	0.306	0.051	0.754	− 0.201	0.277	0.200	0.122	− 0.060	0.505	− 0.091	0.315
SOD-unadjusted		− 0.029	0.835	− 0.170	0.137	− 0.004	0.976	− 0.147	0.143	− 0.127	0.309	− 0.258	0.082		
SOD[g]		− 0.018	0.902	− 0.167	0.147	− 0.004	0.982	− 0.154	0.150	− 0.105	0.415	− 0.249	0.103		
SOD[g]	FER	− 0.121	0.574	− 0.332	0.185	− 0.030	0.900	− 0.265	0.234	− 0.099	0.618	− 0.343	0.205	**− 0.203**	**0.059**

Bold characters denoted significant associations

[a]Age (years), medical history (yes/no), and gender (male/female)

[b]Age (years) and medical history (yes/no)

[c]Age (years), medical history (yes/no), and BMI (kg/m^2), gender (male/female)

[d]Age (years), medical history (yes/no), and patients with cancer (yes/no)

[e]Age (years), medical history (yes/no), patients underwent organ transplant (yes/no) and supplements intake (yes/no)

[f]Age (years), medical history (yes/no), patient with cancer (yes/no) and patients underwent organ transplant

[g]Age (years), medical history (yes/no), and supplements intake (yes/no)

estimates of patients with mild symptoms (β− 0.367, 95% CI − 0.411, − 0.001, $p = 0.049$) and severe symptoms (β = − 0.357, 95% CI − 0.492, − 0.006, $p = 0.045$). When incorporating NEUT in the Se model, we observed a decrease in the regression estimate of patients with mild symptoms (β = − − 0.251, 95% CI − 0.276, − 0.021; $p = 0.092$). Again the regression estimate decreased in patients with severe symptoms when incorporating NLR (β = − 0.277, 95% CI − 0.389, − 0.007, $p = 0.043$) or LMR (β = − 0.266, 95% CI − 0.367, − 0.014, $p = 0.035$) in the model.

However, the significant decrease in vitamin A observed in patients with severe or mild symptoms in crude and adjusted analyses for risk factors disappeared.

There were no changes in the relationship between COVID-19 severity and levels of Zn, vitamin E, vitamin D_3, TAC, and SOD after adjusting the model for laboratory parameters or inflammatory markers. However, NEUT, LYM, and MONO remained significantly associated with TAC levels with p-values of 0.003, 0.002, and 0.017, respectively, and marginally significant with FER ($p = 0.083$).

While the relationship between CRP and Se levels became non-significant ($p = 0.214$), it remained significant with Cu and vitamin A (both $p = 0.001$) after adjusting the model.

Discussion

Our study revealed that a decline in serum Se levels was independently associated with COVID-19 severity. An increase in Cu and Cu/Zn levels was associated with disease severity only after adjusting for specific individual laboratory parameters or inflammatory markers, suggesting their possible role in exacerbating inflammation in COVID-19 patients and might be synergistically associated with disease severity. A decrease in vitamin A was observed in patients with severe symptoms, which disappeared after adjusting for inflammatory markers. This result may highlight the potential role of inflammation in mediating the relationship between COVID-19 severity and vitamin A levels. Despite our patients' low status of Zn, vitamin D_3, and antioxidant enzyme (SOD), there is no evidence of their role in COVID-19 progression. Our findings reinforce that deficiency or excess of certain micronutrients plays an essential role in the pathogenesis of COVID-19. More studies are required to support our findings.

Several studies have shown that micronutrients play vital roles in maintaining tissue function, and their excess or deficiency can disturb metabolic functions that the immune system relies on to defend the body against infections (Pecora et al. 2020; Maggini et al. 2018). In this study, we assessed the levels of trace elements, vitamins, and antioxidant enzyme activity in four groups of COVID-19 patients and explored their associations with disease severity.

Trace elements (Se, Cu, and Zn)

Se levels were low in approximately 30% of patients, followed by Zn (∼ 25%) and Cu (3%). Lower levels of these metals have been observed in patients diagnosed with various infectious diseases (Skalny et al. 2021; Weiss and Carver 2018; Oh et al. 2019; Sepehri et al. 2017). Se is an essential trace element in humans that supports antioxidant defense systems (Burk 2002) and, consequently, plays a role in immune-related diseases (Huang et al. 2012; Hoffmann and Berry 2008). Studies have shown that Se deficiency during influenza aggravates viral infections (Beck et al. 2001; Nelson et al. 2001). In our study, 30% of COVID-19 patients were Se deficient (< 70.08 µg/L; mean value: 59.46 µg/L). Our study showed that patients with severe COVID-19 had an 18% decrease in Se levels after adjusting for risk factors. However, the decline increased to 30%, 24%, and 23% when CRP, NLR, and LMR, respectively, were separately included in the model. CRP, NLR, and LMR are inflammatory markers that correlate well with the progression of COVID-19 and/or other diseases (Danwang et al. 2020; Man et al. 2021). In our study, low CRP and NLR and high LMR were associated with elevated Se levels, which is indicative of the anti-inflammatory role of Se, particularly in viral infections (Guillin et al. 2019; Huang et al. 2012). Nonetheless, these relationships disappeared after adjusting the model for these inflammatory markers suggesting that Se is independently associated with COVID-19 severity, regardless of inflammation is a confounder or a result of Se deficiency. Both Majeed et al. (2021) and Skalny et al. (2021) found low Se levels in COVID-19 patients. Skalny et al (2021) reported that low Se levels were associated with lung

damage in COVID-19 patients. Moghaddam et al. (2020) concluded that Se levels were significantly lower in COVID-19 non-survivors (40.8 μg/L) than in survivors (53.3 μg/L). In our study, Se levels were lower in 16 deceased patients (75.41 μg/L) than in 139 survivors (83.84 μg/L), statistically, not significant ($p = 0.106$), which might be related to the small sample size of deceased patients. Se levels in survivors were similar to the Se levels (77.8 μg/L) reported by Younesian et al. (2021). Se deficiency might be a risk factor for COVID-19 mortality (Bae and Kim 2020). In China, Zhang et al. (2020) found an association between COVID-19 cure rates and regional Se status. Studies have shown an association between COVID-19 cure rates and regional Se status, suggesting an additional risk factor that might affect the human response to SARS-CoV-2 infections, particularly in populations where Se intake is sub-optimal or low (Bermano et al. 2021).

Both Zn and Cu are essential trace metals required for the appropriate function of the immune system, and nutritional deficiency of either mineral increases susceptibility to bacterial/viral infections (Wessels et al. 2017; Djoko et al. 2015). The antiviral properties of Zn have been investigated with coronaviruses, hepatitis C virus, and HIV (Barocas et al. 2019; Read et al. 2019). Researchers recommend Zn with antiviral medications to manage COVID-19 (Asl et al. 2021). While only 3% of our COVID-19 patients had Cu deficiency (< 0.18 μg/mL), a higher percentage of patients (25%) were Zn deficient (< 0.693 μg/mL). Despite the high percentage of patients with Zn deficiency, we found no association with COVID-19 severity, even after adjusting for inflammatory markers (CRP, NRL, or LMR) or individual laboratory parameters (NEUT or LYM). Vogel-González et al. (2021) observed poor COVID-19 outcomes, such as worse clinical presentation, longer time to reach stability, and higher mortality rates with serum Zn levels < 0.5 μg/mL (23% of patients). In comparison, only seven patients with Zn levels < 0.5 μg/mL, three of them with severe symptoms, were included in our study. A study by Gonçalves et al. (2021) showed that among 80% of patients who were Zn deficient (< 0.7 μg/mL), there was an association with the severity of respiratory distress. The lack of an association in our study was related to the low prevalence of patients with low Zn. The mean Zn value in our deficient patients was 0.584 μg/mL, lower than the value (0.745 μg/mL) reported by Jothimani et al. (2020) in patients who developed complications associated with a prolonged hospital stay and increased mortality. Serum Zn levels was lower in deceased COVID-19 patients (0.7 μg/mL) than in survivors (1.117 μg/mL) but with borderline significance ($p = 0.065$).

Data on serum Cu status in COVID-19 patients are limited, apart from hypothesizing that Cu potent antiviral activities may act as a preventive and therapeutic approach against COVID-19 by boosting innate and adaptive immunity (Raha et al. 2020). In line with Skalny et al. (2021) and Zeng et al. (2021a), the levels of Cu in serum were elevated (> 1.401 μg/mL) in 28% of our COVID-19 patients. In the present study, the positive association between Cu and CRP affected the relationship between Cu and COVID-19 severity. Patients with mild and moderate COVID-19 symptoms had a 50% and 53%, respectively, increase in Cu levels compared to asymptomatic patients. This association was observed only after controlling for CRP; however, the marker remained significantly and independently associated with Cu levels. This result may indicate the possible participation of Cu in inflammatory and pro-oxidant mechanisms in the pathogenesis of COVID-19 (Fernandes et al. 2020; Bo et al. 2008). Even though Cu is an essential micronutrient involved in various biological mechanisms, controlling its homeostasis is critical in maintaining the balance between absorption and distribution and biliary/urinary excretion (de Romaña et al. 2011; Peña et al. 1999). Both Cu deficiency and excess have been associated with specific clinical symptoms (Hordyjewska et al. 2014).

In our study, we noted an imbalance in the levels of Cu and Zn, which has been reported in inflammatory conditions. The normal ratio of Cu to Zn in adults is close to 1:1 (Malavolta et al. 2015). The Cu/Zn ratio in the current study was high (1.5 ± 0.63), with 128 ($\sim$ 83%) patients having a ratio above 1, which is an indicator of inflammation. The ratio was significantly correlated with CRP ($r = 0.44, p < 0.001$). It has been reported that Cu/Zn > 2 means severe bacterial infection (Bahi et al. 2017). There were 33 (21%) patients in our study with Cu/Zn ratio > 2. Skalny et al. (2021), who observed elevated Cu/Zn ratios in COVID-19 patients, reported that the ratio increased gradually with disease severity. Cu/Zn increased up to 45% in patients with mild symptoms and 34%–36% in

patients with moderate symptoms compared to asymptomatic patients. These relationships were only observed when one of the laboratory parameters (LYM or MONO) or inflammatory marker (NLR) was included in the regression model. Furthermore, none of these inflammatory markers remained significantly associated with Cu/Zn after adjusting the model. These findings suggest that Cu/Zn might further exacerbate inflammation in COVID-19 patients and be synergistically associated with disease severity.

Vitamins (D_3, A, and E)

Vitamin D_3 deficiency was observed in 68% of our patients. In general, the prevalence of vitamin D deficiency among Saudis is high ($\sim$ 64%) (Al-Alyani et al. 2018). Increasing evidence shows the relationship between vitamin D deficiency and a high risk of infectious diseases (Watkins et al. 2015) due to its immunomodulatory action in human respiratory epithelial cells infected with respiratory viruses (Greiller and Martineau 2015; Skrobot et al. 2018). The clinical connection between low vitamin D status and viral infections has prompted researchers to explore its potential link with SARS-CoV-2 infection severity and/or mortality (Peng et al. 2021; Charoenngam et al. 2021). In our study, even though asymptomatic patients had the lowest vitamin D_3 levels (10.9 μg/L) in comparison to those with mild (14.2 μg/L), moderate (15.0 μg/L), and severe symptoms (19.9 μg/L), there were no significant differences among patients with different symptoms because all were vitamin D deficient ($<$ 20.05 μg/L). The concept of using vitamin D to prevent or reduce the risk of COVID-19 infection or as an intervention strategy has been emphasized in the literature (Shah et al. 2021; Brenner 2021). Despite the evidence, other studies found that vitamin D sufficiency does not lower the risk of adverse clinical outcomes in COVID-19, such as duration of hospitalization and disease severity, and more research is required to support the potential benefits of vitamin D supplementation (Davoudi et al. 2021; Jolliffe et al. 2021; Grove et al. 2021; Hastie et al. 2020; Brandão et al. 2021). According to Brandão et al. (2021), susceptibility to SARS-CoV-2 infections might be related to several clinical, environmental, socioeconomic, and cultural factors rather than vitamin D status. Hastie et al. (2020) reported that assessing vitamin D_3 status would not be useful in clinical practice. AlSafar et al. (2021) showed that higher risks of COVID-19 and death were associated with vitamin D_3 levels $<$ 12 μg/L. In our study, 80 patients had vitamin D_3 levels $<$ 12 μg/L: 1/16 (asymptomatic), 27/47 (mild), 33/67 (moderate), and 10/21 (severe). Even though 12 out of the 16 deceased patients had vitamin D_3 levels $<$ 12 μg/L with a mean value of 5.09 μg/L, there were no significant differences with the vitamin D_3 levels in survivors (4.81 μg/L). In contrast, 15% of our patients had serum vitamin D_3 levels $>$ 30 μg/L with a mean value of 40.12 μg/L (30.96–71.62 μg/L): 1/16 (asymptomatic), 7/47 (mild), 11/67 (moderate), and 4/21 (severe). Four of our patients had vitamin D_3 levels $>$ 50 μg/L, two of which had severe COVID-19 symptoms who did not take vitamin D supplements. In contrast, the other two had mild and moderate symptoms, and both were taking vitamin D supplements. Vitamin D overdose may lead to hypercalcemia due to hypervitaminosis D (Marcinowska-Suchowierska et al. 2018).

Vitamin A is another micronutrient that plays a vital role in innate and acquired immunity and the body's response to inflammation (Rubin et al. 2017). Its deficiency has been associated with a high risk of infection (Huang et al. 2018; Mawson 2013). Due to its immunomodulatory properties, researchers recommend vitamin A as a potential adjuvant in COVID-19 therapy (Trasino 2020; Gaziano et al. 2021). To the best of our knowledge, there are no studies assessing vitamin A status in COVID-19 patients. In our study, 37% of our patients were vitamin A deficient ($<$ 0.343 mg/L). During infection, vitamin A is depleted, suppressing its levels in serum, particularly in COVID-19, which as a result, the immune defense mechanism switches from the congenital immune system to the adaptive immune system, where retinoic acids cannot be used (Sarohan 2020). We observed that patients with severe COVID-19 symptoms had a 23% decrease in vitamin A levels compared to asymptomatic patients, which disappeared after controlling for CRP or FER. CRP remained significantly and independently associated with vitamin A. It is possible that systemic inflammation could have mediated the relationship between vitamin A and disease severity. Barffour et al. (2019) and Maqsood et al. (2004) reported increased serum CRP levels in patients with viral infections and vitamin A deficiency. A study by Larson et al. (2018) recommended

adjusting for inflammation to avoid overestimating vitamin deficiency, particularly during the acute phase of infection (Mitra et al. 1998). Low levels of vitamin A in serum have been associated with liver damage, which is a clinical COVID-19 feature (Herta and Berg 2021). A study showed that patients with cirrhosis had serum vitamin A levels of 0.166 mg/L, while controls had serum vitamin A levels of 0.259 mg/L (Ukleja et al. 2002). Twelve of our patients had levels below 0.166 mg/L (0.074–0.162 mg/L). In contrast, high levels of vitamin A in serum (> 0.839 mg/L) were obtained in 15% of patients, with the majority of patients being asymptomatic (5/15). Mawson et al. (2021) proposed that liver damage due to SARS-CoV-2 leads to the release of retinoic acid and stored retinyl esters into the circulation that cause further damage to organs including the lungs, heart, blood vessels, and skin.

Vitamin E is another micronutrient that modulates immune and inflammatory responses leading to improved protection against infection and other immune-related diseases (Wu and Meydani 2019; Tang and Smit 1998). Though no study has evaluated the association between vitamin E and COVID-19, it has been hypothesized that the vitamin can amplify the immune system due to its antioxidant properties and its roles in maintaining the integrity of the T-cell membranes and reducing the duration of infection (BourBour et al. 2020). In our study, 10% of COVID-19 patients had vitamin E < 5.5 mg/L, and the levels were not significantly different among the four groups of patients. Surprisingly, 36% of our patients had vitamin E levels > 15.5 mg/L (15.6–111.77 mg/L): 8/15 (asymptomatic), 17/41 (mild), 18/62 (moderate), and 6/19 (severe). Elevated vitamin E levels were attributed to supplementation in 73% of patients. Studies have shown that high vitamin E intake might be associated with decreased levels of vitamin K-induced coagulation factors (Booth et al. 2004; Owen and Dewald 2021). A study found that patients with intracranial hemorrhages and who took vitamin E supplementation had vitamin E levels in serum that ranged between 23.3 and 46.7 mg/L (Le et al. 2020). In our study, 16 patients had vitamin E > 23.3 mg/L, and three of the deceased patients had the highest vitamin E levels (111.77 mg/L). Thirteen out of these patients with high vitamin E were taking supplements.

Our results suggest that the link between supplements intake and a patient's health conditions is underestimated and under-reported. It needs to be assessed to avoid overdosing, which might be behind the etiology of health conditions or the exacerbatation of existing conditions.

Antioxidant enzyme activity

Oxidative stress, which results from an imbalance between reactive oxygen species (ROS) and enzymatic and nonenzymatic antioxidants, plays a complex role in the pathogenesis of human diseases (Ďuračková 2010). A wide range of viral and bacterial infections trigger oxidative stress (Ivanov et al. 2017). Inflammation due to SARS-CoV-2 infection and oxidative stress plays roles in COVID-19 progression and response to therapy (Forcados et al. 2021; Beltrán-García et al. 2020). We evaluated the role of oxidative stress in the pathogenesis of COVID-19 by assessing the levels of SOD, which is an enzymatic antioxidant, and TAC, which represents the cumulative effect of all antioxidants present in serum rather than a single antioxidant (Ghiselli et al. 2000), that limit its benefits (Rubio et al. 2016). Among all four COVID-19 groups, there were no significant differences in TAC and SOD levels, even when laboratory and inflammatory markers were included in the model. An increase in NEUT and a decrease in LYM and MONO were independently associated with TAC levels. These parameters are predictors of inflammation, particularly in infection (Wu et al. 2021), and their association, particularly NEUT, with ROS generation has been documented (Banerjee et al. 2012). In a recent review, Goud et al. (2021) hypothesized that NEUT, eosinophils, MONO, macrophages, mitochondrial damage, and NADPH oxidase are the major sources of ROS generation at sites of inflammation. The authors emphasized that ROS contribute to the pathogenesis of COVID-19. Gadotti et al. (2021) were unable to confirm the interplay between COVID-19 severity and oxidative stress in patients with severe COVID-19. However, the lowest TAC values were found in asymptomatic patients (979.2 μM) and the highest in patients with severe COVID-19 symptoms (1326.1 μM), with a significant difference between the two ($p = 0.009$) based on bivariate analysis. In contrast to the results reported by Karkhanei et al. (2021), we observed that TAC levels and length of hospital stay were associated ($r = 0.273$, $p = 0.002$). Additionally, deceased patients had higher TAC levels

than survivors ($p = 0.047$). The low SOD activity in our patients reflects an increase in ROS production associated with SARS-CoV-2 infection (Wenzhong and Hualan 2021; Barciszewska 2021). Similar results were reported by Muhammad et al. (2021). The information in the literature on the antioxidant status in COVID-19 patients is both limited and conflicted.

In general, our findings showed that inflammation could be triggered by excess or deficiency of certain trace metals and vitamins. When their predictive role in COVID-19 severity is investigated, it is essential to adjust the regression model for inflammatory markers.

The present study had some limitations. First, the small sample size may have some impact on the statistical power. Second, the study recruited patients from a single hospital with multiple co-morbidities, which likely had adverse effects on the progression of COVID-19. Third, there were no data on healthy patients because the samples were collected during the highest peak of the pandemic when subject recruitment was difficult. Fourth, deficiencies in some micronutrients could be due to various chronic health conditions and/or behavioral factors that increase COVID-19 risk. Fifth, there were incomplete data for some patients because we used the remaining serum samples that were withdrawn to monitor the patients. Furthermore, we did not include immunological markers because they were performed after admission and not in all cases. Six, the laboratory parameters were limited during the hospital routine assessment. Seven, we did not measure levels of lipids, which are reliable indicators of vitamin E status (Winbauer et al. 1999). Finally, some confounding factors, such as socioeconomic, that might impact COVID-19 severity were not evaluated (Hawkins et al. 2020).

Despite these limitations, the data were reliable and homogenous because they were extracted from a centralized database where all patients were tested and treated under the same guidelines. Furthermore, our data provide valuable information on the role of micronutrients and antioxidant deficiency in the pathogenesis of COVID-19.

Conclusions

Our study findings showed a 18% decrease in Se levels in patients with severe symptoms, which increased to 30% after adjusting the model for inflammatory markers. Regardless of inflammation, Se was independently associated with COVID-19 severity. In contrast, a 50% increase in Cu was associated with disease severity only after adjusting for CRP, reflecting its possible inflammatory and pro-oxidant role in COVID-19 pathogenesis. We noted an imbalance in the ratio between Cu and Zn, with $\sim$ 83% of patients having a Cu-to-Zn ratio > 1, which is an indicator of inflammation. Cu-to-Zn ratio increased to 45% in patients with mild symptoms and to 34%–36% in patients with moderate symptoms when compared to asymptomatic patients. These relationships were only obtained when one of the laboratory parameters (lymphocyte or monocyte) or inflammatory markers (neutrophil-to-lymphocyte ratio) was included in the regression model. These findings suggest that Cu/Zn might further exacerbate inflammation in COVID-19 patients and might be synergistically associated with disease severity. Even though a 23% decrease in vitamin A was observed in patients with severe symptoms, it disappeared after adjusting for inflammatory markers. This result highlights the potential role of inflammation in mediating the relationship between COVID-19 severity and vitamin A levels. Despite our patients' low status of Zn, vitamin D_3, and antioxidant enzyme (SOD), there is no evidence of their role in COVID-19 progression. Our findings reinforce that the deficiency or excess of certain micronutrients plays an essential role in the pathogenesis of COVID-19. More studies are required to support our results.

Author contributions IAS- study design, data analysis, results interpretation and writing the manuscript. NA- collection of clinical data. HA- methodology/validation. RE- methodology/validation. MS- sampling design. FA- samples provision. RB- methodology. MA- resources.

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Data availability Not applicable.

Declarations

Conflict of interest The authors reported no potential conflict of interest.

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