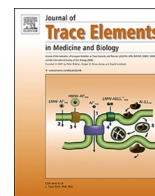


Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

The concentrations of essential/toxic elements in serum of COVID-19 patients are not directly related to the severity of the disease

Montse Marquès^{a,b}, Simona Iftimie^c, Jordi Camps^d, Jorge Joven^d, José L. Domingo^{a,b,*}

^a Universitat Rovira i Virgili, Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, 43201 Reus, Spain

^b Center of Environmental, Food and Toxicological Technology - TecNATox, Universitat Rovira i Virgili, Spain

^c Universitat Rovira i Virgili, Department of Internal Medicine, Hospital Universitari de Sant Joan, IISPV, 43204 Reus, Spain

^d Universitat Rovira i Virgili, Unitat de Recerca Biomèdica, Hospital Universitari de Sant Joan, IISPV, 43201 Reus, Spain

ARTICLE INFO

Keywords:

SARS-CoV-2
 COVID-19 patients
 Serum samples
 Essential elements
 Toxic elements

ABSTRACT

Background: In recent months, the current COVID-19 pandemic has generated thousands of studies directly or indirectly related with this disease and/or the coronavirus SARS-CoV-2 causing the infection. On August 22, 2022, the database PUBMED included 287,639 publications containing the term COVID-19. However, in spite of the importance of trace elements in human health, including the immune system, data on the levels of metals/metalloids in COVID-19 patients is very limited.

Methods: The concentrations of As, Cd, Cr, Cu, Hg, Fe, Mg, Mn, Pb, Se, V and Zn were determined by inductively coupled plasma-mass spectrometry (ICP-MS) in 126 serum samples of individuals infected with SARS-CoV-2, as well as in 88 samples of non-infected individuals. Participants were divided into four groups: i) individuals COVID-19 positive (COVID-19 +) with an asymptomatic infection course; ii) individuals suffering mild COVID-19; iii) individuals suffering severe COVID-19, and iv) individuals COVID-19 negative (COVID-19-) (control group). The occurrence of the analyzed metals/metalloids was evaluated along with the biochemical profile, including blood cell counts, lipids, proteins and crucial enzymes.

Results: Serum levels of Mg, V, Cr, Cu, Cd, and Pb were higher in COVID-19 positive patients than those in the control group. Although no significant differences were observed between the different groups of patients, the concentrations of Cd, Pb, V and Zn showed a tendency to be higher in individuals with severe COVID-19 than in those showing mild symptoms or being asymptomatic. Arsenic and Hg were rarely detected, regardless if the subjects were infected by SARS-CoV-2, or not. The current results did not show significant differences in the levels of the rest of analyzed elements according to the severity of the disease (asymptomatic, mild and severe).

Conclusions: In spite of the results here obtained, we highlight the need to reduce the exposure to Cd, Pb and V to minimize the potential adverse health outcomes after COVID-19 infection. On the other hand, although a protective role of essential elements was not found, Mg and Cu concentrations were higher in severe COVID-19 patients than in non-infected individuals.

1. Introduction

In February 2020, Wu et al. [1] published the first paper regarding human infection by SARS-CoV-2. The authors reported the genome sequence of that coronavirus, associated with the respiratory disease outbreak in China, which was isolated from a patient in Wuhan. That patient was a worker at the market of that city, who had been admitted to the Central Hospital of Wuhan on 26 December 2019. This coronavirus has been the cause of a pandemic of acute respiratory disease, named “coronavirus disease 2019” (COVID-19). The World Health

Organization (WHO) declared -on March 11, 2020- the novel coronavirus (COVID-19) outbreak as a global pandemic [2].

In the more than 2.5 years elapsed since the first cases of COVID-19 were reported, the advances on the knowledge of COVID-19 and SARS-CoV-2, have been very significant. These advances involve a number of areas such as diagnostic, prognostic factors, as well as the possibilities of treatment and prevention, including vaccination, among the most important. However, there are still some weak points on that knowledge, being the origin of SARS-CoV-2 one of the most notorious [3,4]. In turn, during this time, the severity of COVID-19 has shown to be highly

* Corresponding author at: Universitat Rovira i Virgili, Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, 43201 Reus, Spain.

E-mail address: jose Luis.domingo@urv.cat (J.L. Domingo).

<https://doi.org/10.1016/j.jtemb.2023.127160>

Received 23 August 2022; Received in revised form 3 February 2023; Accepted 24 March 2023

Available online 27 March 2023

0946-672X/© 2023 The Author(s).

Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

variable [5,6]. Thus, while some infected individuals were/are being asymptomatic, others were/are affected by moderate and/or serious symptoms. The most frequent symptoms of COVID-19 include fever, cough, fatigue, sputum and dyspnea, being others nausea, vomits and diarrhea less common [7]. However, in the first waves, up to 20% of COVID-19 patients resulted in severe disease, frequently leading to pneumonia, acute respiratory distress syndrome, organic dysfunctions, and death [8–11].

Taking into account that the respiratory system is the main target of the SARS-CoV-2 infection, respiratory comorbidities have been associated with COVID-19 severity [12]. Moreover, obesity, diabetes, cardiovascular disease, immune system depression and cancer -among other diseases- are also risk factors for the severity of COVID-19 [13–16]. In turn, it has been suggested that long-term exposure to certain environmental pollutants exacerbate COVID-19 health outcomes [17–20]. Anyway, the age is the most important variable determining the severity and mortality due to COVID-19 infection [21]. It is a well-known fact that the immune system weakens over the life course [22] becoming less resistant to infections. Consequently, the disease burden increases [23].

On the other hand, the role of nutrition in the immune system function is a well-known key factor [24]. In relation to this, a considerable number of clinical studies have demonstrated that essential minerals are responsible for regulating cellular homeostasis, humoral and cellular immune responses, acting as cofactors for a number of antioxidant enzymes and molecules [25–27]. However, abnormal levels of micronutrients may cause adverse health effects, including COVID-19 severity [28,29]. While some trace elements are essential for normal biological functions, certain metalloids and metals such as arsenic, cadmium, lead or mercury -among others- have no known functionality, being their potential toxicity associated with serious diseases, including cancer, neurological disorders, immunotoxicity, and adverse respiratory or skin effects [30–32].

Taking the above into account, the present study was aimed at assessing the potential role of various essential and toxic elements on COVID-19 health outcomes. The concentrations of As, Cd, Cr, Cu, Hg, Fe, Mg, Mn, Pb, Se, V and Zn were determined in serum samples of individuals infected with SARS-CoV-2, as well as in samples of non-infected individuals. The occurrence of these trace elements was evaluated along with the biochemical profile, including blood cell counts, lipids, proteins and crucial enzymes. Former chronic diseases and medication were also considered.

2. Materials and methods

2.1. Study design

A retrospective observational study was conducted with 210 individuals (>18 years old; 124 individuals infected with SARS-CoV-2 and 86 non-infected individuals), who were outpatients or admitted to the Hospital Sant Joan, Reus (Catalonia, Spain). Exclusion criteria were the following: pregnancy, altered liver function, and life expectancy of less than 24 hr. Participants were divided into four groups: i) individuals COVID-19 positive (COVID-19 +) with an asymptomatic infection course; ii) individuals suffering mild COVID-19; iii) individuals suffering severe COVID-19, and iv) individuals COVID-19 negative (COVID-19-) (control group). Mild and severe COVID-19 individuals were admitted to the hospital due to COVID-19 infection, being classified according to the WHO Guidelines [33]. Asymptomatic COVID-19 + and control groups entailed subjects attending the hospital for other purposes, and being tested in the qPCR, positively and negatively, respectively. The control group was formed according to sex and age distribution, and medication of the COVID+ groups. For this reason, serum samples from healthy individuals, which were stored at Pere Virgili Health Research Institute Biobank (Tarragona, Catalonia, Spain), were also included.

2.2. Clinical data

An ad hoc database including data on age, sex, toxic habits, comorbidities, medication and clinical outcomes, was constructed. Data were anonymized and recorded following legal provisions of the personal data protection in Spain, as well as European Union Regulations (EU) 2016/6799 on the physical protection of the treatment of personal data. The study was conducted in accordance with the Declaration of Helsinki, being approved by the Ethics Committee of the Pere Virgili Health Research Institute (Ref. CEIm 040/2018, amended on 16 April 2020).

2.3. Blood extraction

Fasting venous blood samples were extracted from each individual in BD Vacutainer® serum tubes with separating gel and no added anticoagulant. Subsequently, samples were centrifuged at 2500g for 15 min. Up to 250- μ L of serum were collected and stored individually at -80°C until further analysis.

2.4. Analyses of trace elements

One-hundred μ L of serum were digested with 100 μ L of HNO_3 (65% Suprapur, Merck, Darmstadt, Germany) in a Milestone Start D Microwave digestion system (Milestone S.r.l., Sorisole, Italy). The ramp temperature was set from room temperature to 200°C during 40 min, and held for 25 min. Subsequently, extracts were cooled down to room temperature, filtered, and made up to 2 mL with ultra-pure water. The concentrations of As, Cd, Cr, Cu, Fe, Hg, Mg, Mn, Pb, Se, V and Zn were determined by inductively coupled plasma-mass spectrometry (ICP-MS) (Perkin-Elmer Elan 6000, Woodbridge, ON, Canada) following instructions by the manufacturer. The accuracy of the analytical procedures was checked by performing the analyses in duplicate. Quality control was assured by the analysis of a certified reference material (DOLT-5 Dogfish Liver Certified Reference Material for Trace Metals and other Constituents) and Seronorm™ Trace elements whole blood L-2 (SERO, Billingstad, Norway). Recoveries of the elements here analyzed were between 79% and 108%. The limits of detection (LOD) were the following: 0.0025 $\mu\text{g/L}$ (Cd and Pb), 0.005 $\mu\text{g/L}$ (Cr and V), 0.01 $\mu\text{g/L}$ (Mn and Hg), 0.025 $\mu\text{g/L}$ (Cu), 0.05 $\mu\text{g/L}$ (Se), 0.1 $\mu\text{g/L}$ (As), 0.25 $\mu\text{g/L}$ (Zn), 0.5 mg/L (Fe), and 10 mg/L (Mg).

2.5. Statistical analysis

Statistical analyses were performed using the GraphPad Prism version 5.01 for Windows, GraphPad Software (La Jolla, CA, USA, www.graphpad.com). The descriptive analysis was performed with continuous variables estimated as median and interquartile range (IQR), and categorical variables summarized as frequencies. The distributions of the trace elements were tested by the D'Agostino-Pearson omnibus normality test. As the distribution of the elements was not normal, nonparametric tests were used in the data analyses. Hence, Kruskal-Wallis's test followed by Dunn's post-test correction were applied to compare the concentrations of the trace elements between the different groups. The Spearman correlation test was used to assess the correlation between the analyzed elements. For calculations, results below the limit of detection (LOD) were considered as LOD/2. Differences were considered statistically significant at $p < 0.05$. Results are shown as mean \pm standard deviation (SD).

3. Results and discussion

3.1. Levels of essential and toxic elements

The demography and clinical characteristics of the individuals under study classified according to the severity of the COVID-19 (asymptomatic, mild, severe and control) are shown in Table 1. The infected

Table 1
Characteristics of the participants in the study.

	COVID-19 +	COVID-19 +	COVID-19 +	COVID-19-/control
COVID-19	Asymptomatic n = 23	Mild n = 79	Severe n = 22	n = 86
Age	79.0	71	67.5	62.0
Median (IQR)	(63.0–84.0)	(57.5–83.0)	(60.0–71.0)	(50.0–69.0)
Sex				
Female	16 (69.6%)	34 (43.0%)	7 (31.8%)	60 (69.8%)
Male	7 (30.4%)	45 (57.0%)	15 (68.2%)	26 (30.2%)
Smoking				
Yes	1 (4.3%)	3 (3.8%)	0	13 (15.1%)
No	21 (91.3%)	60 (75.9%)	17 (77.3%)	54 (62.8%)
Ex-smoker	1 (4.3%)	16 (20.3%)	5 (22.7%)	19 (22.1%)
Alcohol consumption	1 (4.3%)	4 (4.9%)	3 (13.6%)	7 (7.9%)
Comorbidities				
Diabetes Mellitus	6 (26.1%)	18 (22.2%)	6 (27.3%)	10 (11.4%)
Cardiovascular disease	12 (52.2%)	42 (51.9%)	14 (63.6%)	30 (34.1%)
Chronic liver disease	0	0	1 (4.5%)	
Chronic lung disease	2 (8.7%)	14 (17.3%)	2 (9.1%)	
Chronic kidney disease	4 (17.4%)	14 (17.3%)	4 (18.2%)	8 (9.1%)
Chronic neurological disease	3 (13.0%)	22 (27.2%)	4 (18.2%)	
Previous infectious disease	0	1 (1.2%)	0	
Cancer	7 (30.4%)	5 (6.2%)	4 (18.2%)	4 (4.5%)
Medication				
Anticoagulants	20 (86.9%)	72 (88.9%)	20 (90.9%)	28 (31.8%)
Corticoids	7 (30.4%)	39 (48.1%)	9 (40.9%)	
Insulin	7 (30.4%)	17 (20.9%)	4 (18.2%)	4 (4.5%)
Statins	4 (17.4%)	32 (39.5%)	8 (36.4%)	28 (31.8%)
Clinical outcomes				
Fever	-	63 (77.8%)	17 (77.3%)	-
Cough	-	44 (54.3%)	15 (68.2%)	-
Pneumonia	-	52 (64.2%)	17 (77.3%)	-
Odynophagia	-	2 (2.47%)	2 (9.1%)	-
Chills	-	22 (27.2%)	5 (22.7%)	-
Respiratory distress	-	54 (66.7%)	17 (77.3%)	-
Vomits	-	6 (7.4%)	2 (9.1%)	-
Diarrhea	-	21 (25.9%)	5 (22.7%)	-
Acute respiratory distress syndrome	-	3 (3.7%)	3 (13.6%)	-
Acute kidney failure	-	9 (11.1%)	6 (27.3%)	-
Other symptoms	-	3 (3.7%)	1 (4.5%)	-
Length of stay (IQR). Days	28 (19–47)	10 (7–19)	27 (19–43)	
Recovered	20 (87.0%)	63 (77.8%)	14 (63.6%)	-
Deceased	3 (13.0%)	18 (22.2%)	8 (36.4%)	-

participants were mainly men (54.8%), non-smokers, and non-alcohol consumers. The predominant comorbidity was cardiovascular disease (CVD), whose incidence was slightly higher in the severe COVID-19 group than in the rest of groups (asymptomatic, mild and control). Consequently, anticoagulant treatment before SARS-CoV-2 infection was the most frequent among all individuals, regardless of the COVID-19 course. There was only one patient that had suffered a previous infectious disease and another subject with chronic liver disease, who underwent mild and severe COVID-19, respectively. Other comorbidities such as chronic lung disease and chronic neurological disease, were more frequent in the mild group. Most individuals with cancer underwent asymptomatic COVID-19, while there were no differences in the rate of diabetes and chronic kidney disease among groups. Other chronic

treatments included corticoids, insulin and statins, which were more frequent among the patients having mild COVID-19. As expected, the incidence of most clinical outcomes (cough, pneumonia, odynophagia, respiratory distress, vomits, acute respiratory distress syndrome, acute kidney failure, and various other symptoms) was higher in patients suffering severe symptoms than in those with mild COVID-19. Hence, their stay at the hospital was longer, while the recovering and mortality rates from COVID-19 were lower and higher, respectively.

The concentrations of Cd, Cr, Cu, Fe, Mg, Mn, Pb, Se, V and Zn in asymptomatic, mild COVID-19, severe COVID-19 and control individuals are depicted in Fig. 1. Additionally, individual concentrations are shown in Supplementary Material (Table S1). Serum levels of Mg, V, Cr, Cu, Cd, and Pb were higher in COVID-19 positive patients than those in the control group. Although no significant differences were observed between the different groups of patients, the concentrations of Cd, Pb, V and Zn showed a tendency to be higher in individuals with severe COVID-19 than in those showing mild symptoms or being asymptomatic. In turn, Fig. 2 shows the correlations between the concentrations of the different metals/metalloids. Almost all elements hereby assessed showed significant positive correlations, with the exception of Se-Cd which was negative, although very weak (-0.169). The highest correlations were found among Pb, Cd, Mn and Zn, showing correlation coefficients of 0.909 for Pb-Cd, 0.705 for Pb-Mn, 0.662 for Mn-Cd, and 0.630 for Mn-Zn.

Because inflammatory response is associated with COVID-19 severity [34], we also evaluated the correlations among essential/toxic elements along with biochemical blood parameters (Table 2). These included C-reactive protein (CRP), glucose, creatinine, lipid profile, the hepatic enzymes aspartate and alanine aminotransferases (AST and ALT), hemoglobin and blood cell counts (erythrocytes, leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils and platelets). The highest positive correlations were found between Pb, Mn and Cd and CRP, with correlation coefficients of 0.450, 0.434, 0.422, respectively. Hence, these elements might be related to the inflammation response of the infected individual, as CRP blood levels rise in response to inflammation. On the other hand, Mg was positively correlated with leukocytes (0.298) and neutrophils (0.347), while Cu was correlated with leukocytes (0.232), neutrophils (0.278) and platelets (0.239). Fe and Se showed positive correlations with hemoglobin (0.208 and 0.339), and Se also with erythrocytes (0.298). It is important to note that the correlation coefficient between Fe and hemoglobin is lower than that previously reported by Zeng et al. [35]. This might be related to the fact that the levels of toxic/essential elements - including Fe - were not analyzed in whole blood. Interestingly, Cd and Pb correlated positively with AST (0.331 and 0.300). These two toxic elements have been already associated with liver injury [36,37].

In the scientific literature, there is a very limited information on the potential role of toxic/essential trace elements on COVID-19 outcomes [38–40]. To date, most investigations have been focused on the important role of the nutritional status and essential trace elements supplementation on COVID-19 immunity and adverse health outcomes, without studying the potential significant role of toxic elements. The current results show that the occurrence of neither essential minerals, nor toxic elements, play a potential role on the severity degree (asymptomatic, mild or severe) along the SARS-CoV-2 infection. However, the levels of certain toxic elements, which included Cd, Pb and V, are higher in infected individuals than in non-infected subjects. Regarding this, it is known that Cd, Pb and V, may cause adverse effects on the human respiratory system. These elements might have a joint inflammatory and immunotoxic effect [39, 41–43]. Therefore, those individuals exposed to Cd, Pb and V, could have a weakened immune system, and consequently, leading to an increased risk of infection with SARS-CoV-2 [38].

In spite of the tremendous amount of information published on various aspects of the COVID-19, data on the body levels of trace elements in this disease is rather scarce [26–28,35,40,44,45]. Our results

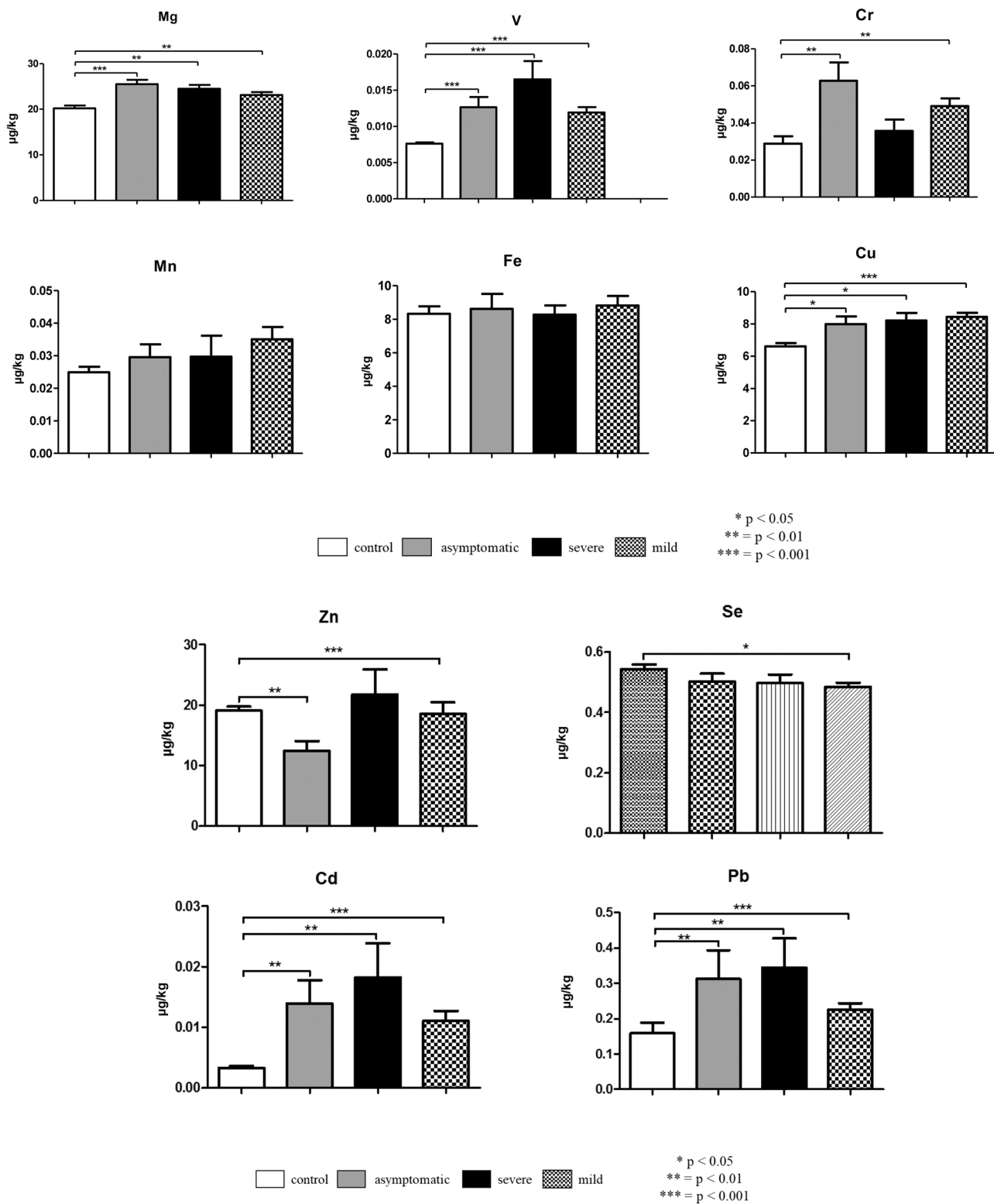


Fig. 1. Median concentrations (IQR) of toxic and essential trace elements in COVID-19 (asymptomatic, severe and mild) and control individuals. Fig. 1 (continued). Median concentrations (IQR) of toxic and essential trace elements in COVID-19 (asymptomatic, severe and mild) and control individuals.

disagree with some results of Zeng et al. [35], who determined the levels of a number of trace elements in whole blood samples of COVID-19 patients. The differences concern the lower correlation between Fe and hemoglobin hereby found (0.208 vs 0.75), the lower detection rates of certain elements (i.e., As and Hg), as well as the lack of significant differences between COVID-19 severity groups (asymptomatic, mild and

severe). Anyway, the different type of sample analyzed (serum vs. whole blood) makes the comparison between both studies rather difficult. In turn, PVSN et al. [46] also reported decreased levels of Mg in controls. Albeit this finding agrees with our results, statistical significance ($p < 0.01$) between controls and severity groups was only found in our study. In turn, PVSN et al. [46] reported significantly decreased levels of

	Mg	V	Cr	Mn	Fe	Cu	Zn	Se	Cd
Mg	--								
V	0.268**	--							
Cr	0.127	0.231**	--						
Mn	0.347**	0.419**	0.279**	--					
Fe	0.195*	0.293**	0.268**	0.256**	--				
Cu	0.351**	0.154	0.207*	0.235**	-0.041	--			
Zn	0.283**	0.234**	-0.069	0.630**	0.171*	0.285**	--		
Se	0.110	-0.022	0.111	-0.127	0.120	0.250**	0.005	--	
Cd	0.294**	0.263**	0.158	0.662**	0.145	0.101	0.427**	-0.169*	--
Pb	0.240**	0.302**	0.148	0.705**	0.161	0.114	0.456**	-0.138	0.909**

* $p < 0.05$; ** $p < 0.01$

Fig. 2. Correlation factors between essential and toxic elements in serum of SARS-CoV-2 infected individuals.

Table 2

Correlation factors between essential and toxic elements in serum of infected individuals along with biochemical blood parameters (C-reactive protein (CRP), glucose, creatinine, cholesterol, triglycerides, High-density lipoprotein (HDL), Low-density lipoprotein (LDL) and Very low-density lipoprotein (VLDL), Aspartate transaminase (AST) and Alanine transaminase (ALT), hemoglobin and blood cell counts (erythrocytes, leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils and platelets).

	CRP	Glucose	Creatinine	Cholesterol	HDL	LDL	VLDL	Triglycerides	AST	ALT	Hemoglobin
Mg	0.136	0.166	0.016	-0.118	-0.223 **	-0.128	0.171 *	0.154	0.186 *	0.039	-0.056
V	0.085	0.013	0.124	-0.148	-0.098	-0.117	-0.067	-0.076	0.071	-0.032	-0.291 **
Cr	0.045	-0.057	0.015	-0.134	-0.024	-0.133	0.018	-0.004	0.103	0.063	-0.019
Mn	0.413 **	0.123	0.055	-0.251 **	-0.302 **	-0.222 *	0.034	0.027	0.242 **	0.130	-0.300 **
Fe	-0.165	-0.099	-0.200 *	0.100	0.038	0.093	-0.063	-0.056	0.100	0.084	0.111
Cu	0.193 *	0.041	-0.003	0.035	-0.058	-0.030	0.200 *	0.196 *	0.023	-0.020	-0.179 *
Zn	0.212 *	-0.070	-0.155	-0.095	-0.100	-0.093	-0.116	-0.060	0.110	0.086	-0.180 *
Se	-0.260 **	-0.017	-0.125	0.265 **	0.269 **	0.183 *	-0.025	-0.024	-0.129	0.044	0.290 **
Cd	0.355 **	0.180 *	0.121	-0.218 *	-0.279 **	-0.180 *	-0.088	-0.063	0.352 **	0.148	-0.163
Pb	0.350 **	0.160	0.106	-0.211 *	-0.301 **	-0.186 *	-0.020	0.010	0.320 **	0.151	-0.199 *
	Erythrocyte	Leukocyte	Neutrophil	Lymphocytes	Monocytes	Eosinophils	Basophils	Platelets			
Mg	0.054	0.164	0.218 *	-0.200 *	-0.031	-0.145	-0.069	-0.033			
V	-0.237 **	-0.088	-0.036	-0.133	-0.152	0.048	-0.019	-0.078			
Cr	0.072	0.064	0.096	-0.139	-0.011	-0.067	-0.084	-0.101			
Mn	-0.185 *	0.125	0.158	-0.247 **	0.030	-0.005	0.015	0.004			
Fe	0.122	-0.002	-0.033	0.030	0.020	-0.060	0.000	0.017			
Cu	-0.040	0.134	0.163	-0.062	0.049	0.069	0.074	0.155			
Zn	-0.086	0.018	0.038	-0.129	-0.047	-0.044	-0.144	0.008			
Se	0.322 **	0.087	0.098	0.105	0.096	0.057	-0.055	0.085			
Cd	-0.096	0.134	0.151	-0.258 **	0.034	-0.022	0.051	-0.031			
Pb	-0.121	0.149	0.156	-0.270 **	0.037	0.046	0.086	0.017			

* $p < 0.05$; ** $p < 0.01$

Cu and Zn in COVID-19 patients when compared to those in controls, disagreeing with our results, which showed higher levels of Cu in COVID-19 patients than in controls, as well as higher levels of Zn in patients undergoing a severe SARS-CoV-2 infection, than patients with an asymptomatic or mild infection, along with controls. Additionally, our results disagree with Yadav et al. [47], who found a significantly decreased levels of Fe in COVID-19 patients. Although Fe was also determined in serum, the different analytical strategies were applied (direct colorimetric method vs. ICP-MS) might had led to different results.

4. Conclusions

In the present study, a comprehensive analysis of a number of essential/toxic elements in serum samples of COVID-19 patients was carried out. Arsenic and Hg were rarely detected in serum of the participants in the study, regardless if they were infected by SARS-CoV-2, or not. However, special attention must be paid at the exposure to Cd, Pb and V. Chronic exposure to certain levels of these elements might cause immunotoxicity, meaning a worsening the immune system of the exposed individuals, and therefore, leading to be more prone to become infected by SARS-CoV-2. Although a protective role of essential elements

was not hereby found, Mg and Cu concentrations were higher in severe COVID-19 patients than in non-infected individuals. Anyhow, further research is needed to cover the above-mentioned limitations. It should properly fill the knowledge gap on this potentially important field, not only for patients with COVID-19, but also for those affected by other respiratory viruses and potential further pandemics with coronaviruses.

Declaration of Competing Interest

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jtemb.2023.127160](https://doi.org/10.1016/j.jtemb.2023.127160).

References

[1] F. Wu, S. Zhao, B. Yu, Y.M. Chen, W. Wang, Z.G. Song, Y. Hu, Z.W. Tao, J.H. Tian, Y.Y. Pei, M.L. Yuan, Y.L. Zhang, F.H. Dai, Y. Liu, Q.M. Wang, J.J. Zheng, L. Xu, E.

- C. Holmes, Y.Z. Zhang, A new coronavirus associated with human respiratory disease in China, *Nature* 579 (2020) 265–269.
- [2] D. Cucinotta, M. Vanelli, WHO declares COVID-19 a pandemic, *Acta Biomed.* 91 (2020) 157–160.
- [3] J.L. Domingo, What we know and what we need to know about the origin of SARS-CoV-2, *Environ. Res.* 200 (2021), 111785.
- [4] J.L. Domingo, An updated review of the scientific literature on the origin of SARS-CoV-2, *Environ. Res.* (2022).
- [5] C. Aragón-Benedí, P. Oliver-Forniés, F. Galluccio, E. Yamak Altinpulluk, T. Ergonenc, A. El Sayed Allam, C. Salazar, M. Fajardo-Pérez, Is the heart rate variability monitoring using the analgesia nociception index a predictor of illness severity and mortality in critically ill patients with COVID-19? A pilot study, *PLoS One* 16 (2021), e0249128.
- [6] Tricarico G., Travagli V. Patient variability in severity of COVID-19 disease. Main suspect: vascular endothelium. *J. Allergy Clin. Immunol. Pract.* 2023;9:2540–2541.
- [7] R.M. Viner, J.L. Ward, L.D. Hudson, M. Ashe, S.V. Patel, D. Hargreaves, E. Whittaker, Systematic review of reviews of symptoms and signs of COVID-19 in children and adolescents, *Arch. Dis. Child.* 106 (2021) 802–807.
- [8] P. Sarzi-Puttini, V. Giorgi, S. Sirotti, D. Marotto, S. Ardizzone, G. Rizzardini, S. Antinori, M. Galli, COVID-19, cytokines and immunosuppression: what can we learn from severe acute respiratory syndrome? *Clin. Exp. Rheuma* 38 (2020) 337–342.
- [9] A. Aslan, C. Aslan, N.M. Zolbanin, R. Jafari, Acute respiratory distress syndrome in COVID-19: possible mechanisms and therapeutic management, *Pneumonia* 13 (2021) 14.
- [10] P.G. Gibson, L. Qin, S.H. Puah, COVID-19 acute respiratory distress syndrome (ARDS): clinical features and differences from typical pre-COVID-19 ARDS, *Med. J. Aust.* 213 (2020) 54–56, e1.
- [11] S.B. Brosnahan, A.H. Jonkman, M.C. Kugler, J.S. Munger, D.A. Kaufman, COVID-19 and respiratory system disorders: current knowledge, future clinical and translational research questions, *Arterioscler. Thromb. Vasc. Biol.* 40 (2020) 2586–2597.
- [12] H. Ejaz, A. Alsrhani, A. Zafar, H. Javed, K. Junaid, A.E. Abdalla, K.O.A. Abosalif, Z. Ahmed, S. Younas, COVID-19 and comorbidities: deleterious impact on infected patients, *J. Infect. Public Health* 13 (2020) 1833–1839.
- [13] A. Wingert, J. Pillay, M. Gates, S. Guitard, S. Rahman, A. Beck, B. Vandermeer, L. Hartling, Risk factors for severity of COVID-19: a rapid review to inform vaccine prioritisation in Canada, *BMJ Open* 11 (2021), e044684.
- [14] T. Zhang, W.S. Huang, W. Guan, Z. Hong, J. Gao, G. Gao, G. Wu, Y.Y. Qin, Risk factors and predictors associated with the severity of COVID-19 in China: a systematic review, meta-analysis, and meta-regression, *J. Thorac. Dis.* 12 (2020) 7429–7441.
- [15] Y.D. Gao, M. Ding, X. Dong, J.J. Zhang, A. Kursat Azkur, D. Azkur, H. Gan, Y. L. Sun, W. Fu, W. Li, H.L. Liang, Y.Y. Cao, Q. Yan, C. Cao, H.Y. Gao, M.C. Brügggen, W. van de Veen, M. Sokolowska, M. Akdis, C.A. Akdis, Risk factors for severe and critically ill COVID-19 patients: a review, *Allergy* 76 (2021) 428–455.
- [16] Y. Zhou, J. Chi, W. Lv, Y. Wang, Obesity and diabetes as high-risk factors for severe coronavirus disease 2019 (Covid-19), *Diabetes Metab. Res. Rev.* 37 (2021), e3377.
- [17] A. Tsatsakis, D. Petrakis, T.K. Nikolouzakis, A.O. Docea, D. Calina, M. Vinceti, M. Goumenou, R.N. Kostoff, C. Mamoulakis, M. Aschner, A.F. Hernández, COVID-19, an opportunity to reevaluate the correlation between long-term effects of anthropogenic pollutants on viral epidemic/pandemic events and prevalence, *Food Chem. Toxicol.* 141 (2020), 111418.
- [18] J.L. Domingo, J. Rovira, Effects of air pollutants on the transmission and severity of respiratory viral infections, *Environ. Res.* 187 (2020), 109650.
- [19] M. Marquès, J. Rovira, M. Nadal, J.L. Domingo, Effects of air pollution on the potential transmission and mortality of COVID-19: a preliminary case-study in Tarragona Province (Catalonia, Spain), *Environ. Res.* 192 (2021), 110315.
- [20] M. Marquès, J.L. Domingo, Positive association between outdoor air pollution and the incidence and severity of COVID-19. A review of the recent scientific evidences, *Environ. Res.* 203 (2022), 111930.
- [21] M. Marquès, E. Correig, D. Ibarretxe, E. Anoro, J. Antonio Arroyo, C. Jericó, R. M. Borrallo, M. Miret, S. Náf, A. Pardo, V. Perea, R. Pérez-Bernalte, R. Ramírez-Montesinos, M. Royuela, C. Soler, M. Urquiza-Padilla, A. Zamora, J. Pedro-Botet, STACOV-XULA research group, L. Masana, J.L. Domingo, Long-term exposure to PM₁₀ above WHO guidelines exacerbates COVID-19 severity and mortality, *Environ. Int* 158 (2022), 106930.
- [22] S. Maggini, A. Pierre, P.C. Calder, Immune function and micronutrient requirements change over the life course, *Nutrients* 10 (2018) 1531.
- [23] P.C. Calder, A.C. Carr, A.F. Gombart, M. Eggersdorfer, Optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections, *Nutrients* 12 (2020) 1181.
- [24] F. Pecora, F. Persico, A. Argentiero, C. Neglia, S. Esposito, The role of micronutrients in support of the immune response against viral infections, *Nutrients* 12 (2020) 3198.
- [25] S. Taheri, S. Asadi, M. Nilashi, R. Ali Abumalloh, N.M.A. Ghabban, S.Y. Mohd Yusuf, E. Supriyanto, S. Samad, A literature review on beneficial role of vitamins and trace elements: evidence from published clinical studies, *J. Trace Elem. Med. Biol.* 67 (2021), 126789.
- [26] A.B. Engin, E.D. Engin, A. Engin, Can iron, zinc, copper and selenium status be a prognostic determinant in COVID-19 patients? *Environ. Toxicol. Pharmacol.* 95 (2022), 103937.
- [27] K. Dharmalingam, A. Birdi, S. Tomo, K. Sreenivasulu, J. Charan, D. Yadav, P. Purohit, P. Sharma, Trace elements as immunoregulators in SARS-CoV-2 and other viral infections, *Indian J. Clin. Biochem* 36 (2021) 416–426.
- [28] I. Al-Saleh, N. Alrushud, H. Alnuwaysir, R. Elkhatib, M. Shoukri, F. Aldayel, R. Bakheet, M. Almozaeni, Essential metals, vitamins and antioxidant enzyme activities in COVID-19 patients and their potential associations with the disease severity, *Biomaterials* 35 (2022) 125–145.
- [29] M.K. Fath, M. Naderi, H. Hamzavi, M. Ganji, S. Shabani, F.N. Ghahroodi, B. Khalesi, N. Pourzardosht, Z.S. Hashemi, S. Khalili, Molecular mechanisms and therapeutic effects of different vitamins and minerals in COVID-19 patients, *J. Trace Elem. Med. Biol.* 73 (2022), 127044.
- [30] M. Nordberg, G.F. Nordberg, Trace element research-historical and future aspects, *J. Trace Elem. Med. Biol.* 38 (2016) 46–52.
- [31] T. Syversen, P. Kaur, The toxicology of mercury and its compounds, *J. Trace Elem. Med. Biol.* 26 (2012) 215–226.
- [32] M. Peana, A. Pelucelli, S. Medici, R. Cappai, V.M. Nurchi, M.A. Zoroddu, Metal toxicity and speciation: a review, *Curr. Med. Chem.* 28 (2021) 7190–7208.
- [33] WHO, World Health Organization, 2020. Clinical management of COVID-19: interim guidance, 27 May 2020. Geneva, Switzerland, available at: <https://apps.who.int/iris/handle/10665/332196>
- [34] C. Signorini, P. Pignatti, T. Coccini, How do inflammatory mediators, immune response and air pollution contribute to COVID-19 disease severity? A Lesson Learn. *Life* 11 (2021) 182.
- [35] H.L. Zeng, Q. Yang, P. Yuan, X. Wang, L. Cheng, Associations of essential and toxic metals/metalloids in whole blood with both disease severity and mortality in patients with COVID-19, *FASEB J.* 35 (2021), e21392.
- [36] T. Syversen, P. Kaur, The toxicology of mercury and its compounds, *J. Trace Elem. Med. Biol.* 26 (2012) 215–226.
- [37] D.W. Kim, J. Ock, K.W. Moon, C.H. Park, Association between Pb, Cd, and Hg exposure and liver injury among Korean adults, *Int. J. Environ. Res. Public Health* 18 (2021) 6783.
- [38] J.L. Domingo, M. Marquès, The effects of some essential and toxic metals/metalloids in COVID-19: a review, *Food Chem. Toxicol.* 152 (2021), 112161.
- [39] A.V. Skalny, T.R.R. Lima, T. Ke, J.C. Zhou, J. Bornhorst, S.I. Alekseenko, J. Aaseth, O. Anesti, D.A. Sarigiannis, A. Tsatsakis, M. Aschner, A.A. Tinkov, Toxic metal exposure as a possible risk factor for COVID-19 and other respiratory infectious diseases, *Food Chem. Toxicol.* 146 (2020), 111809.
- [40] H.L. Zeng, B. Zhang, X. Wang, Q. Yang, L. Cheng, Urinary trace elements in association with disease severity and outcome in patients with COVID-19, *Environ. Res.* 194 (2021), 110670.
- [41] R.R. Dietert, J.E. Lee, I. Hussain, M. Piepenbrink, Developmental immunotoxicology of lead, *Toxicol. Appl. Pharmacol.* 198 (2004) 86–94.
- [42] I. Mirkov, A. Popov Aleksandrov, M. Ninkov, D. Tucovic, J. Kulas, M. Zeljkovic, D. Popovic, M. Kataranovski, Immunotoxicology of cadmium: cells of the immune system as targets and effectors of cadmium toxicity, *Food Chem. Toxicol.* 149 (2021), 112026.
- [43] I. Zwolak, Vanadium carcinogenic, immunotoxic and neurotoxic effects: a review of in vitro studies, *Toxicol. Mech. Methods* 24 (2014) 1–12.
- [44] G.Q. Chanihoon, H.I. Afridi, A. Unar, F.N. Talpur, H.B. Kalochi, R. Nassani, N. Laghari, N. Uddin, A. Ghulam, A.U.R. Chandio, Selenium and mercury concentrations in biological samples from patients with COVID-19, *J. Trace Elem. Med. Biol.* 73 (2022), 127038.
- [45] A. Šcibior, E. Wnuk, Elements and COVID-19: a comprehensive overview of studies on their blood/urinary levels and supplementation with an update on clinical trials, *Biology* 11 (2022) 215.
- [46] K.K. Pvsan, S. Tomo, P. Purohit, S. Sankanagoudar, J. Charan, A. Purohit, V. Nag, P. Bhatia, K. Singh, N. Dutt, M.K. Garg, P. Sharma, S. Misra, D. Yadav, Comparative analysis of serum zinc, copper and magnesium level and their relations in association with severity and mortality in SARS-CoV-2 patients, *Biol. Trace Elem. Res.* 201 (1) (2023) 23–30.
- [47] D. Yadav, K.K. Pvsan, S. Tomo, S. Sankanagoudar, J. Charan, A. Purohit, V. Nag, P. Bhatia, K. Singh, N. Dutt, M.K. Garg, P. Sharma, S. Misra, P. Purohit, Association of iron-related biomarkers with severity and mortality in COVID-19 patients, *J. Trace Elem. Med. Biol.* 74 (2022), 127075.