The Relationship Between Zinc Levels, Length of Hospital Stay, and Mortality in Intensive Care Unit of COVID-19 Patients

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Original Article

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ABSTRACT

Aim: The aim of this study is to evaluate the relationship between disease severity and serum Zinc(Zn) levels in COVID-19 patients.

Materials and Methods: The study included 153 COVID-19 patients confirmed by RT-PCR test, were divided into two groups according to the severity of the disease: hospitalized COVID-19 patients (n=81) and intensive care unit patients (n=72). Additionally, 78 healthy controls were included. Serum levels of various biomarkers, including WBC, Neutrophil, Lymphocyte, Monocyte, Platelet, PT, APTT, INR, D-Dimer, CRP, PCT, Ferritin, and Zinc, were measured for all participants. Demographic data and length of hospitalization were also recorded.

Results: The study found significant differences between the groups in age, hospitalizations, chronic disease, and length of hospital stay ($p \leq 0.001$). COVID-19 patients had lower median zinc levels (89µg/dl) compared to healthy individuals (99.50µg/dl), and this difference was statistically significant (p=0.023). As zinc levels increased, the CORADS score decreased (r=-0.248, p=0.031). There was an inverse relationship between zinc level and intensive care unit admission, indicating that lower zinc levels were associated with a higher probability of intensive care hospitalization (r=-0.260, p=0.023).

Conclusion: This study showed that COVID-19 patients had significantly lower zinc levels than healthy individuals. The difference between the average zinc level of COVID-19 patients and the average zinc level of healthy individuals were found to be statistically significant. Additionally, a significant inverse relationship were found between zinc level and intensive care unit hospitalization; As the zinc level decreases, the likelihood of being hospitalized in intensive care increases.

Key Words: CORADS score, COVID-19, intensive care, RT-PCR, zinc (Zn).

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INTRODUCTION

COVID-19 is a multisystem disease caused by SARS-CoV-2, which emerged in 2019 and has spread globally. The severe course of the disease is primarily driven by an excessive release of pro-inflammatory cytokines, such as IL-6 and TNF-α, leading to a cytokine storm. These cytokines are secreted by innate immune cells, causing vascular hyperpermeability, systemic inflammation, and multiple organ failure.These features of COVID-19 have become particularly important in the treatment and management of the disease. Research shows that treatments that suppress the activity of these pro-inflammatory cytokines can positively impact the course of the disease[1].

Malnutrition and mineral deficiencies can weaken the immune system, increase susceptibility to infectious diseases, and exacerbate their symptoms[2]. Copper, zinc and selenium minerals provide protection against viral infections due to their enzymatic antioxidant roles, and since they have many different effects on the immune system, their adequate intake is very important in defending against diseases[3]. Zinc (Zn) is the second most abundant trace element after iron in the human body. In addition to its immune response strengthening effect, this trace element has also been reported to play a role in defense against viruses[4]. Zinc is an essential mineral that plays a crucial role in various cellular processes, including enzyme catalysis, immune function, protein synthesis, wound healing, DNA replication, and taste

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perception. It is vital for the production of white blood cells and antibodies, making it indispensable for the proper functioning of the immune system. In a condition of zinc deficiency, the levels of pro-inflammatory signaling molecules (IL-1, IL-6, and TNF alpha) rise[5]. Supplementing with zinc can enhance the infection-fighting capacity of polymorphonuclear cells[6]. Impaired zinc homeostasis is believed to increase susceptibility to COVID-19 by impairing immune functions. Correcting low zinc levels in high-risk groups may play a crucial role in preventing and managing COVID-19[7]. The COVID-19 virus uses the angiotensin-converting enzyme2 (ACE2)to gain entry into target cells. Research has indicated that zinc gluconate can counteract the impact of the virus's spike protein on ACE2, potentially offering a protective effect. Consequently, zinc gluconate may provide protection by decreasing the activity of ACE2, which is established as the receptor for the COVID-19 virus[8]. Zinc has direct antiviral activity against some RNA viruses and plays a role in immune functions. Zinc cations, particularly when combined with the zinc ionophore pyrithione, inhibit the SARS-coronavirus RNA polymerase enzyme, suggesting that intracellular zinc concentration is crucial in combating coronaviruses[9]. Zn2+ may have antiviral properties by increasing interferon production, improving respiratory function, and modulating immune responses that can limit cytokine storms[10].

AIM OF THE STUDY

This study aimed to examine whether there is a relationship between disease severity and Zn levels in COVID-19 patients, depending on their prognosis and whether they are admitted to the service or intensive care unit.

MATERIALS AND METHODS

Establishing Working Groups

This cross-sectional study included 153 COVID-19 cases (patient group) who applied to the COVID-19Clinic of our hospital between 15 April 2021 & 15 July 2021, 78 healthy individuals (control group) who did not have a known chronic disease such as heart disease, diabetes, hypertension, active/chronic infection or rheumatological disease, and who had not used Zn-containing drugs in the last year were included. Patients under the age of 18 were not included in the study. COVID-19 patients were selected from two separate patient groups, namely services or intensive care patients, according to their prognosis. Participants in the study, including COVID-19 patients and the control group, provided written consent to take part. The demographic and laboratory information for the study groups was gathered from the hospital's data management system.

Collection of Blood Samples/ Specimens

Venous bloods were collected from COVID-19 patients and healthy controls into a yellow-capped gel biochemistry tube to obtain serum. Serums of patients followed in the service and intensive care unit were stored at -20°C. Demographic data, hospital stay and CORAS Score of the patients were statistically analyzed with the SPSS statistical program. Zinc levels were measured in the serum samples of COVID-19 patients who were followed in the service and intensive care unit and came to our laboratory for other tests. 5 ml venous blood samples taken with suspicion of COVID-19 were collected in a BD gel vacuum cleaner and stored at -20°C from patients followed in SBU Samsun Training and Research Hospital COVID-19 services between 15.03.2020 and 15.06.2020. The Zn level was measured by a colorimetric method using the Automatic Indiko Plus analyzer (Thermo Scientific, USA) from stored serum samples. The comparison range used to assess Zn levels was established as 70-110 micrograms per deciliter(µg/dl). To validate the correctness of the measurement technique, two sets of random control samples were analyzed. These control samples were provided by Randox, a manufacturer of clinical diagnostic products, and included the Human Test Control-2 (LOT-1369UN) and Human Control-3(LOT-1066UE) for chemistry analyses, as well as the COVID-19 patient control for immunoassay testing. Additionally, the COVID-19 patients were categorized based on their serum zinc concentrations. Individuals with zinc levels below 70 µg/dl were considered to have a 'deficient' zinc status. The researchers then identified COVID-19 patients who exhibited zinc deficiency and compared them to those with normal zinc levels. This comprehensive analysis of zinc levels in COVID-19patients, using a standardized reference range and validated control samples, allowed the researchers to better understand the potential role of zinc status in the clinical course and outcomes of individuals affected by the COVID-19 virus.

PCR Analysis

The study included individuals who had been diagnosed with COVID-19 based on the results of reverse transcription-polymerase chain reaction(RT-PCR) tests conducted on samples obtained from their nasopharynx, oropharynx, or sputum. The RT-PCR test used to confirm the presence of SARS-CoV-2, the virus that causes COVID-19, was the

Montania4896 Real-Time PCR Instrument, manufactured by Anatolia Diagnostics Inc. in Istanbul, Turkey. The test was performed in accordance with the instructions provided by the manufacturer, Bioeksen R&D Tech. Ltd., also based in Istanbul, Turkey. One of the RT-PCR kits is the Bio-Speddy® (Bioeksen R&D Technologies Inc. COVID-19 RT-qPCR Detection Kit v2.0, Istanbul-Turkey) determined valuable by the "Turkish Ministry of Health" and used throughout COVID-19 pandemic. Each RT-PCR kit production followed CDC's and WHO's detection guidelines[11-13]. However, each kits are included in the WHO Emergency Use Listing for SARS-CoV-2 in vitro diagnostic products (https://www.who.int/diagnostics_laboratory/EUL/en/).

Statistical analysis

In this study, sample size calculations were conducted to test the difference between two independent groups consisting of 153 patients and 75 control subjects. The calculations were performed using a specified statistical power and significance level. The statistical significance level was set at a 95% confidence interval (Z = 1.96), with a power of 80% (Z = 0.84). A medium effect size (Cohen's d = 0.5) and a population standard deviation (σ = 1) were assumed. The sample size calculation indicated that approximately 63 participants are required for each group. Therefore, the current sample sizes of 153 patients and 75 controls are adequate for the study.

All statistical analyses were performed using SPSS software program, version 23.0(IBMCorporation,Armonk,NY,USA). Continuous variables are expressed as mean±standard deviation(SD), while categorical data are presented as numbers and percentages. To compare categorical data, the researchers used the Chi-square test or Fisher's exact test, depending on the appropriate conditions. The conformity of the variables to normal distribution was assessed using both visual (histogram) and analytical (Kolmogorov-Smirnov test and Shapiro-Wilk test) methods. Kruskal-Wallis test was applied for comparison of more than two independent groups. The Mann-Whitney U test with Bonferroni correction (p<0.05/5) was used as a post-hoc test to perform pairwise comparisons of groups that differed in the Kruskal-Wallis test. Spearman correlation analysis was used to examine correlations between study parameters. Throughout the statistical analyses, a *p-value* less than 0.05 was considered statistically significant.

RESULTS

Table 1. Demographic and clinical characteristics of the study groups.

	Intensive care/ ICU (n=72)	Service (n=81)	Healthy (n=78)	p
Age (year) †	67.54 ± 13.322	50.67 ±12.048	50.15±16.024	<0.11*
Sex ‡				
Female	42 (58.3)	60 (%74,1)	48(%61.5)	0.454**
Male	30 (41.7)	21(%25,9)	30(%38.5)	
Survey‡				
Living	15(%20.8)	75(%92,6)	78(%100)	<0.001**
Exitus	57(%79.2)	6(%7,4)	0(%0)	
Presence of chronic disease‡	32 (%44.4)	6 (%7.4)		<0.001**
Length of stay in hospital†	14.3 ±10.290	7.22 ± 3.916		0.007***
Zinc level	89.00±19.470	90.00±16.464	99.50±12.084	0.063*

†: mean ± standard deviation, ‡: n (%).
*. Kruskal Wallis Test
**. Pearson Chi-Square, Fisher's Exact test
*** Independent samples t-test

The study divided participants into three groups: intensive care patients, service patients, and healthy controls. There were significant differences in age, survey responses, chronic disease prevalence, and hospital stay duration between the groups. COVID-19patients (n=153) had significantly lower zinc levels(median 89 µg/dl) compared to healthy individuals(n=78, median 99.50 µg/dl, p=0.023).

Table 2: Comparison of COVID-19 patients and Healthy Group.

	COVİD-19patient (*n*=153)	Healthy Group (*n*=78)	*p*
Age (year) †	59.00 ± 15.149	54.00 ±16.024	<0.61*
Sex ‡			
Female	102 (66.7)	16(%61.5)	0.847**
Male	51 (33.3)	10(%38.5)	
Survey‡			
Living	90(%58.8)	26(%100)	<0.001**
Exitus	63(%41.2)	0(%0)	
Zinc level	89.00±17.968	99.50±12.084	0.023*

†: mean ± standard deviation, ‡: n (%).
*. Mann-Whitney U
**. Pearson Chi-Square, Fisher's Exact test

Table 3: Correlation between numerical parameters and the Zinc levels.

Parameters			Spearman's rho	*p*
Zinc levels	-	Age	-0.085	0.465
	-	Length of stay in hospital	-0.141	0.223

Spearman's Rho correlation coefficient.

There was an inverse relationship between zinc level and CORADS in the Spearman correlation analysis. It was determined that CORADS decreased as the zinc level increased (r=-0.248 *p*=0.031). No significant relationship was found between zinc levels and variables such as age and hospitalization day (Table 3).

There was a significant inverse relationship between zinc level and ICU stay. As the zinc level decreased, the probability of being hospitalized in intensive care increased (r=-0.260 *p*=0.023).

Table 4: Laboratory Findings.

Laboratory Findings (X±SD)	Intensive care/ ICU (1)	Service (2)	Control (3)	*p* (1-2)	*p* (1-3)	*p* (2-3)
WBC (*109/l)	15.03±9.13	4.12±1.06	6.49±2.60	<0.001	<0.001	<0.001
Neutrophil (*109/l)	11.86±7.17	3.18±1.99	4.32±2.28	<0.001	<0.001	>0.05
Lymphocyte (*109/l)	0.92±0.78	1.10±0.44	1.43±0.45	>0.05	0.005	0.008
Monocyte (*109/l)	0.51±0.27	0.39±0.19	0.55±0.30	>0.05	>0.05	0.015
Platelet (*109/l)	176.09±105.01	217.93±81.88	283.59±92.62	>0.05	<0.001	0.007
PT	15.56±5.68	12.21±1.06	11.29±0.62	0.04	<0.001	<0.001
APTT	27.40±10.62	24.49±3.68	24.79±3.75	>0.05	>0.05	>0.05
INR	1.38±0.53	1.06±2.30	0.98±0.06	0.003	<0.001	<0.001
D-DIMER	5.03±7.60	0.42±0.37	0.21±0.12	0.002	0.002	0.008
CRP (mg/l)	130.65±88.90	23.54±28.77	4.70±1.90	<0.001	<0.001	0.001
PCT	1.82±3.55	0.11±0.12	0.06±0.06	0.011	0.016	0.027
Ferritin	895.72±88.90	250.13±295.50	138.59±99.71	<0.001	<0.001	>0.05

Critically ill patients admitted to the intensive care unit(ICU) had significantly elevated levels of several biomarkers compared to the other two groups. In particular, white blood cell(WBC) count, neutrophil count, prothrombin time(PT), international normalized ratio(INR), D-dimer, C-reactive protein(CRP), procalcitonin(PCT) and ferritin levels were all significantly higher than the corresponding values in the other groups (as shown in Table 4). In contrast, lymphocyte and platelet counts were lower in ICU patients compared to the control group. Similarly, individuals hospitalized in the general ward also exhibited higher PT, INR, D-dimer, CRP and PCT levels compared to the control group (Table 4). Furthermore, patients in this ward had lower WBC, lymphocyte, monocyte and platelet counts compared to the control group.

DISCUSSION

Severe acute respiratory syndrome viral pathogens such as coronavirus(SARS-CoV) still pose serious health problems worldwide. Respiratory tract infections of viral origin are directly associated with other pathophysiological

processes such as cytokine production, inflammation, cell death and redox imbalance or oxidative stress. Immune cells respond when SARS-CoV-2 enters respiratory epithelial cells to prevent viral replication from the beginning of this pathophysiological process. The novel coronavirus has the ability to disrupt and hinder the body's production of type I interferons, a crucial class of signaling proteins that play a vital role in the immune system's initial antiviral response. At the same time, the virus triggers the release of inflammatory cytokines and stimulates an oxidative burst, which collectively contribute to creating a pro-inflammatory state within the infected host. This multifaceted immune evasion strategy employed by the virus serves to undermine the body's first line of defense, leaving it vulnerable to the virus's continued proliferation and pathogenic effects. By simultaneously suppressing the interferons that would normally mount a rapid antiviral response, while inducing a heightened inflammatory milieu, the virus is able to establish a foothold and avoid immediate clearance by the immune system[14]. Insufficient counter-regulatory immune responses can exacerbate hyperinflammatory conditions and "cytokine storms" through activation of the Th1/Th17 helper T cell phenotypes[14-16]. Previous studies have shown that seriously ill patients tend to have high concentrations of interleukin-6 (IL-6), tumor necrosis factor (TNF-α), and various inflammatory cytokines. Excessive production of these pro-inflammatory cytokines, which causes an increased systemic inflammatory response with excessive vascular permeability and multiple organ failure. This is defined as cytokine storm and may contribute to high mortality rates[17,18]. However, the healthy immune system neutralizes the virus, resolves the infection and produces virus-specific antibodies[16,19].

Our bodies are normally well protected against attacks by a complex and integrated immune system. However, an important exogenous factor supporting immune functions is nutrition. Lack of diversity in nutrition and nutritional deficiencies can have negative effects on the immune system. Especially the oxidant-antioxidant balance is vital for the immune system. Viral threats can exacerbate these negative effects[20]. The synergy of endogenous and exogenous antioxidants plays an important role in protecting against inflammation in the respiratory system resulting from exacarbations of asthma and acute respiratory infections[21]. Vitamins A,C,D,E,B6,B9,B12, and trace elements like zinc, iron, copper, selenium, and magnesium are potential protective and therapeutic antiviral nutrients that support the immune response against viruses[22,23]. Decreases in vitamins and minerals and biochemical blood tests are important markers in determining the prognosis of the disease[24,25].

Zinc, which was the focus of this investigation, is a micronutrient with potent immune-regulating and antiviral characteristics. The human body incorporates zinc into approximately 10% of its proteins and it serves as a cofactor in at least 200 processes that modulate the immune system and provide antioxidant protection[26]. It is present as a cofactor in metalloenzymes and thus maintains the integrity of immune barriers[20]. Effective communication between the endoplasmic reticulum and the golgi is crucial for a properly functioning secretory pathway. The regulation of zinc levels in our body is tightly controlled by zinc transporters. Despite this, zinc deficiency is prevalent, affecting approximately 17% of the global population. The impact of this deficiency on public health is quite substantial[27], and it disrupts the balance between oxidants and antioxidants within the organism. SARS-CoV-2 infection may trigger autoimmunity and may be associated with the development of autoantibodies and inflammation[28,29].

Zinc, an antioxidant, offers protection against reactive oxygen species(ROS) and reactive nitrogen species(RNS). This essential mineral is crucial for the development, differentiation, and activation of T lymphocytes[30,31]. It promotes the development of regulatory T (Treg) cells, which are vital for maintaining immune tolerance[32,33], and it suppresses the development of proinflammatory Th17 and Th9 cells[34-36]. Zinc influences the formation of cytokines, such as IL-2, IL-6, andTNF-α, and induces the proliferation of cytotoxic T cells[32,33]. Additionally, it plays a role in the differentiation, development, and activation of T cells, cytokine production of Th1 cells, and the development of regulatory T cells[37]. Zinc is also important for the production of antibodies, particularly Immunoglobulin G (IgG)[38,39].

The anti-viral properties of zinc include the inhibition ofRNA synthesis, topoisomerase, and viral replication. Additionally, zinc has been found to exhibit direct antiviral activity against various RNA viruses[7]. In vitro evidence suggests that zinc may also have a crucial role in COVID-19[8]. It also has beneficial immunomodulatory effects against respiratory infections that improve the immune response, including the response against SARS-CoV[40]. It has been proven that zinc effectively inhibits SARS-CoV replication in cell culture and that intracellular zinc has an important role in inhibiting virus replication[7]. The zinc finger domain, present in various CoV proteins, plays a crucial role in viral replication and transcription. Mutations within this domain can reduce the antiviral response, while increasing intracellular zinc levels can disrupt CoV replication effectively[41]. It has also been shown that the zinc binding domain may begin to open during the initial passage of SARS-CoV, leading to a decrease in pathogen virulence[42]. In a study, serum zinc levels were significantly lower in COVID-19 patients compared to healthy individuals[43]. In another study, the

average zinc level in COVID-19patients was found to be 71.7±24.6 micrograms per deciliter. However, this level was significantly higher, measuring 97.5±29.4 micrograms per deciliter, in healthy individuals[44]. In a recent study, researchers examined serum zinc levels in healthy individuals and patients with mild, moderate, and severe COVID-19 cases. The findings indicate that compared to the control group, zinc levels were notably lower in the moderate and severe COVID-19 patient groups[45]. Numerous reports have indicated that elevating the zinc concentration within cells can significantly disrupt the replication of coronaviruses. Furthermore, the combination of zinc and the compound pyrithione has been shown to inhibit the replication of SARS-CoV specifically[9]. Furthermore, zinc significantly inhibited infections with SARS-CoV[46], H5N1/H1N1 virus[47]. Numerous medical studies have indicated that the addition of zinc supplements can decrease the length of time that symptoms persist, lower the number of affected individuals, enhance the turnover of lymphocytes and phagocytosis, and improve the effectiveness of immunotherapy in treating various viral illnesses[48].

While serum Zn levels were observed to be significantly lower in intubated COVID-19patients compared to the mild-moderate patient group[49]. The study in Spain found that COVID-19patients who died had significantly lower plasma zinc levels (43 µg/dl)compared to those who survived(63.1 µg/dl). Each unit increase in plasma zinc reduced the risk of in-hospital death by 7%. Patients with plasma zinc levels below 50µg/dl had a two- to three-fold higher risk of in-hospital death. Lower serum zinc levels(less than 50 µg/dl)were associated with worse clinical outcomes, longer time to stability, and higher mortality[50]. The study found that individuals hospitalized with COVID-19 had significantly lower zinc levels compared to a healthy control group. Additionally, patients with zinc deficiency experienced more complications and longer hospital stays[20]. The study found that COVID-19 patients who took zinc salt lozenges orally experienced symptom improvement[51].

CONCLUSION

In this study, significantly lower zinc levels were detected in COVID-19patients compared to healthy individuals. The difference between the average zinc level of COVID-19 patients and the average zinc level of healthy people were found to be statistically significant. There was a significant inverse relationship between zinc level and intensive care unit admission. As the zinc level decreases, the likelihood of being hospitalized in intensive care increases.

AUTHORS' CONTRIBUTIONS

HK: Conceptualization, Project administration, Resources, Validation, Writing – original draft.

RA: Conceptualization, Formal Analysis, Resources, Software, Visualization, Writing – review and editing.

AK: Data curation, Formal Analysis, Resources.

UD: Data curation, Formal Analysis, Resources.

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ETHICS COMMITTEE APPROVAL

The study design and the consent form were approved by the clinical research ethics committee of Samsun University under protocols (2021/4/8).

INFORMED CONSENT

Written informed consent was obtained from all the participants in the study.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

REFERENCES

1. **Doğan S, Bal T, Çabalak M, Dikmen N, Yaqoobi H, Ozcan O.** Oxidative stress index can be a new marker related to disease severity in COVID-19. Turkish J Biochem. 2021;46:349-57. doi.org/10.1515/tjb-2021-0013.

2. **Maggini S, Pierre A, Calder PC.** Immune function and micronutrient requirements change over the life course. Nutrients. 2018;10(10):1531. doi.org/10.3390/nu10101531.

3. **Junaid K, Ejaz H, Abdalla AE, Abosalif KOA, Ullah MI, Yasmeen H, *et al.*** Effective immune functions of micronutrients against SARS-CoV-2. Nutrients. 2020;12(10):2992. doi.org/10.3390/nu12102992.

4. **Pal A, Squitti R, Picozza M, Pawar A, Rongioletti M, Dutta AK, *et al.***: Zinc and COVID-19: basis of current clinical trials. Biol Trace Elem Res. 2021;199:2882-2892. doi.org/10.1007/s12011-020-02437-9.

5. **Güven G, Köseoğlu P, Lohmann E, Samancı B, Şahin E, Bilgiç B, Hanagası HA, Gürvit H, Erginel-Ünaltuna N.** Peripheral Expression of IL-6, TNF-α and TGF-β1 in Alzheimer's Disease Patients. Turk J Immunol. 2024;12(1):28-34. DOI:10.4274/tji.galenos.2024.76598.

6. **Vallee BL, Falchuk KH.** The biochemical basis of zinc physiology. Physiological reviews, 1993. 73;1:79-118. doi.org/10.1152/physrev.1993.73.1.79.

7. **Mossink JP.** Zinc as nutritional intervention and prevention measure for COVID–19 disease. BMJ Nutr Prev Heal. 2020;3(1):111-117. 10.1136/bmjnph-2020-000095.

8. **Abdel-Mottaleb MS, Abdel-Mottaleb Y.** In search for effective and safe drugs against SARS-CoV-2: Part II] The role of selected salts and organometallics of copper, zinc, selenium, and iodine food supplements. ChemRxiv. 2020;1. doi:10.26434/chemrxiv.12234743.v1.

9. **Te Velthuis AJW, Van Den Worm SHE, Sims AC, Baric RS, Snijder EJ, *et al.*** Zn2+ inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. PLoS Pathog. 2010;6(11):e1001176. doi.org/10.1371/journal.ppat.1001176.

10. **Skalny AV, Rink L, Ajsuvakova OP, Aschner M, Gritsenko VA, Alekseenko SI, *et al.*** Zinc and respiratory tract infections: Perspectives for COVID-19. Int J Mol Med. 2020;46(1):17-26. doi.org/10.3892/ijmm.2020.4575.

11. **World Health Organization.** Global surveillance for COVID-19 caused by human infection with COVID-19 virus: interim guidance, 20 March 2020. No. WHO/2019-nCoV/SurveillanceGuidance/2020.6. World Health Organization, 2020.

12. **Centers for Disease Control and Prevention.** "Division of Viral Diseases. 2020. 2019-novel coronavirus (2019-nCoV) real-time rRT-PCR panel primers and probes. Centers for Disease Control and Prevention."

13. **World Health Organization.** "Coronavirus Disease (COVID-19) Pandemic-Emergency Use Listing Procedure (EUL)." Status of COVID-19 Vaccines within WHO EUL/PQ evaluation process. Available online: extranet. who. int (accessed on 29 October 2024).

14. **Prompetchara E, Ketloy C, Palaga T.** Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic. Asian Pac J Allergy Immunol. 2020;38:1-9. 10.12932/ap-200220-0772.

15. **Mehta P, McAuley DF, Brown M, Sanchez E. Tattersall RS, Manson JJ, *et al.*** COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033-1034. 10.1016/S0140-6736(20)30628-0.

16. **Shi Y, Wang Y, Shao C, Huang J, Gan J, Huang X, *et al.*** COVID-19 infection: the perspectives on immune responses, Cell Death Differ. 2020;27:1451-1454. 10.1038/s41418-020-0530-3.

17. **Zhang C, Wu Z, Li JW, Zhao H, Wang GQ.** Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality. Int J Antimicrob Agents. 2020;55(5):105954. doi.org/10.1016/j.ijantimicag.2020.105954.

18. **Ponti G, Maccaferri M, Ruini C, Tomasi A, Ozben T.** Biomarkers associated with COVID-19 disease progression. Crit Rev Clin Lab Sci 2020;57:389-99. doi.org/10.1080/10408363.2020.1770685.

19. **Shanmugaraj B, Siriwattananon K, Wangkanont K, Phoolcharoen W.** Perspectives on monoclonal antibody therapy as potential therapeutic intervention for Coronavirus disease-19 (COVID-19). Asian Pac J Allergy Immunol. 2020;38:10-18. 10.12932/ap-200220-0773.

20. **Haryanto B, Suksmasari T, Wintergerst E, Maggini S.** Multivitamin supplementation supports immune function and ameliorates conditions triggered by reduced air quality. Vitam Min. 2015;4(2):1-15. 10.4172/2376-1318.1000128.

21. **Majkowska-Wojciechowska B,. Kowalski ML.** Allergens, air pollutants and immune system function in the era of global warming. Air pollution–monitoring, modelling, health and control. Rijeka/Croatia. InTech. 2012;221-254. ISBN 978-953-51-0381-3.

22. **Gasmi A, Tippairote T, Mujawdiya PK, Peana M, Menzel A, Dadar M, *et al.*** Micronutrients as immunomodulatory tools for COVID-19 management. Clinical Immunology. 2020;220;108545. doi. org/10.1016/j.clim.2020.108545.

23. **Yigit S, Tural S, Aci R, Sezer O.** Vascular endothelial growth factor gene insertion/deletion polymorphism is associated with Vitamin D level in Turkish patients with coronavirus disease 2019. Rev. Assoc. Med. Bras. 2023;69(7):e20221713.

24. **Aci R, Keskin A.** The impact of COVID-19 on some aspects of laboratory activities. Riv Ital Med Lab. 2022;18(2):85-90. DOI: 10.23736/S1825-859X.22.00143-8.

25. **Keskin A, Aci R.** The Relationship Between Some Laboratory Findings and the Prognostic Significance of Phosphorus and 25-(OH) Vitamin D3 Values in SARS-CoV-2 Cases. Avicenna J Med Biochem. 2022;10(2):108-113. doi: 10.34172/ajmb.2022.2387.

26. **Jothimani D, Kailasam E, Danielraj S, Nallathambi B, Ramachandran H, Sekar P, *et al.*** COVID-19: Poor outcomes in patients with zinc deficiency. Int J Infect Dis. 2020;100:343-349. doi.org/10.1016/j.ijid.2020.09.014.

27. **Bailey RL, West Jr KP, Black RE.** The epidemiology of global micronutrient deficiencies. Ann Nutr Metab. 2015;66(2):22-33. doi.org/10.1159/000371618.

28. **Aci R, Erdem M, Üstün GÜ, Duran U, Keskin A, Bilgin M.** The Relationship Between Inflammatory Indicators and the Severity of the Disease in Coronavirus Disease. Meandros Med. Dental J. 2022;23(2):208-213. doi:10.4274/meandros.galenos.2021.65477

29. **Bilgin M, Basbulut E, Baklacioglu HS, Keskin A, Aci R.** Could SARS-CoV-2 Trigger the Formation of Antinuclear Antibodies?. Turk J Immunol. 2022;10(3):155-161. DOI: 10.4274/tji.galenos.2022.25238

30. **Maggini S, Beveridge S, Sorbara JP, Senatore G.** Feeding the immune system: The role of micronutrients in restoring resistance to infections. CAB Rev. 2008;3:1–21. doi: 10.1079/PAVSNNR20083098.

31. **Wintergerst ES, Maggini S, Hornig, DH.** Immune-enhancing role of vitamin C and zinc and effect on clinical conditions. Ann Nutr Metab. 2006;50(2):85-94. doi.org/10.1159/000090495.

32. **Rosenkranz E, Maywald M, Hilgers RD, Brieger A, Clarner T, Kipp M, *et al.*** Induction of regulatory T cells in Th1-/Th17-driven experimental autoimmune encephalomyelitis by zinc administration. J Nutr Biochem. 2016;29:116–123. doi.org/10.1016/j.jnutbio.2015.11.010.

33. **Rosenkranz E, Metz CHD, Maywald M, Hilgers RD, Weßels I, Senff T, *et al.*** Zinc supplementation induces regulatory T cells by inhibition of Sirt-1 deacetylase in mixed lymphocyte cultures. Mol Nutr Food Res. 2016;60(3):661-671. doi.org/10.1002/mnfr.201500524.

34. **Wu D, Lewis ED, Pae M, Meydani SN.** Nutritional modulation of immune function: Analysis of evidence, mechanisms, and clinical relevance. Front Immunol. 2019;9:3160. doi: 10.3389/fimmu.2018.03160.

35. **Kitabayashi C, Fukada T, Kanamoto M, Ohashi W, Hojyo S, Atsumi T, *et al.*** Zinc suppresses Th17 development via inhibition of STAT3 activation. Int Immunol. 2010;22(5):375-386. doi.org/10.1093/intimm/dxq017.

36. **Maywald M, Wang F, Rink L.** Zinc supplementation plays a crucial role in T helper 9 differentiation in allogeneic immune reactions and non-activated T cells. J Trace Elem Med Biol. 2018;50:482-488. doi.org/10.1016/j.jtemb.2018.02.004.

37. **Wintergerst ES, Maggini S, Hornig DH.** Contribution of selected vitamins and trace elements to immune function. Ann Nutr Metab. 2007;51(4):301-323. 10.1159/000107673.

38. **Shankar AH, Prasad AS.** Zinc and immune function: The biological basis of altered resistance to infection. Am J Clin Nutr. 1998;68(2):447-463. doi.org/10.1093/ajcn/68.2.447S.

39. **Ibs KH, Rink L.** Zinc-Altered Immune function. J Nutr. 2003;133(5):1452-1456. doi.org/10.1093/jn/133.5.1452S.

40. **Quiles JL, Rivas-Garcia L, Varela-López A, Llopis J, Battino M, Sanchez-Gonzalez C.** Do nutrients and other bioactive molecules from foods have anything to say in the treatment against COVID-19?. Environ Res. 2020;191:110053. doi.org/10.1016/j.envres.2020.110053.

41. **Gorji A, Ghadiri MK.** Potential roles of micronutrient deficiency and immune system dysfunction in the coronavirus disease 2019 (COVID-19) pandemic. Nutrition. 2021;82:111047. doi.org/10.1016/j.nut.2020.111047.

42. **Chou YW, Cheng SC, Lai HY, Chou CY.** Differential domain structure stability of the severe acute respiratory syndrome coronavirus papain-like protease. Arch Biochem Biophys. 2012;520(2):74-80. doi.org/10.1016/j.abb.2012.02.015.

43. **Elham AS, Azam K, Azam J, Mostafa L, Nasrin B, Marzieh N.** Serum vitamin D, calcium, and zinc levels in patients with COVID-19. Clin Nutr ESPEN. 2021;43:276-82. doi.org/10.1016/j.clnesp.2021.03.040.

44. **Heller RA, Sun Q, Hackler J, Seelig J, Seibert L, Cherkezov A, *et al.*** Prediction of survival odds in COVID-19 by zinc, age and selenoprotein P as composite biomarker. Redox Biol. 2021;38:101764. doi.org/10.1016/j.redox.2020.101764.

45. **Skalny AV, Timashev PS, Aschner M, Aaseth J, Chernova LN, Belyaev VE, *et al.*** Serum zinc, copper, and other biometals are associated with COVID-19 severity markers. Metabolites. 2021;11(4):244. 10.3390/metabo11040244.

46. **Warnes SL, Little ZR, Keevil CW.** Human coronavirus 229E remains infectious on common touch surface materials. mBio. 2015;6(6): e01697-15. doi:10.1128/mBio.01697-15.

47. **Barnard DL, Wong MH, Bailey K, Day CW, Sidwell RW, Hickok SS, *et al.*** Effect of oral gavage treatment with ZnAL42 and other metallo-ion formulations on influenza A H5N1 and H1N1 virus infections in mice. Antivir Chem Chemother. 2007;18:125-132. doi.org/10.1177/095632020701800302.

48. **Gammoh NZ, Rink L.** Zinc in infection and inflammation. Nutrients. 2017;9(6):624. doi.org/10.3390/nu9060624

49. **Yasui Y, Yasui H, Suzuki K, Saitou T, Yamamoto Y, Ishizaka T, *et al.*** Analysis of the predictive factors for a critical illness of COVID-19 during treatment-relationship between serum zinc level and critical illness of COVID-19. Int J Infect Dis. 2020;100:230-236. doi.org/10.1016/j.ijid.2020.09.008.

50. **Vogel-González M, Talló-Parra M, Herrera-Fernández V, Pérez-Vilaró G, Chillón M, Nogués X, *et al.*** Low zinc levels at admission associates with poor clinical outcomes in SARS-CoV-2 infection. Nutrients. 2021;13(2):562. doi.org/10.3390/nu13020562.

51. **Finzi E.** Treatment of SARS-CoV-2 with high dose oral zinc salts: A report on four patients. Int J Infect Dis. 2020;99:307-309. doi.org/10.1016/j.ijid.2020.06.006.