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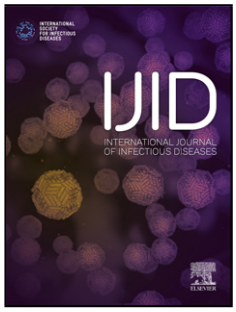

COVID-19: Poor outcomes in patients with Zinc deficiency

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PII: S1201-9712(20)30730-X

DOI: https://doi.org/10.1016/j.ijid.2020.09.014

Reference: IJID 4597

To appear in: *International Journal of Infectious Diseases*

Received Date: 22 July 2020

Revised Date: 24 August 2020

Accepted Date: 7 September 2020

Please cite this article as: Jothimani D, Kailasam E, Danielraj S, Nallathambi B, Ramachandran H, Sekar P, Manoharan S, Ramani V, Narasimhan G, Kaliamoorthy I, Rela M, COVID-19: Poor outcomes in patients with Zinc deficiency, *International Journal of Infectious Diseases* (2020), doi: https://doi.org/10.1016/j.ijid.2020.09.014

TITLE PAGE

Manuscript title: COVID-19: Poor outcomes in patients with Zinc deficiency

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This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest: None

Ethical Approval: Obtained

Highlights

- COVID-19 patients had significantly low Zinc levels in comparison with healthy controls.
- Zinc deficient patients developed more complications (70.4% vs 30.0%, P=0.009).
- Zinc deficient COVID-19 patients had a prolonged hospital stay 7.9 and 5.7 days (P=0.048)
- Invitro studies show reduced Zinc level favours interaction of ACE2 to SARS-CoV-2 spike protein and likewise increased Zinc level inhibits ACE2 expression resulting in reduced viral interaction.

Abstract

Background: Zinc is a trace element with potent immunoregulatory, antiviral properties and is utilized in the treatment of COVID-19. However, we do not know the clinical significance of serum Zinc level in COVID-19 patients. **Aim**: To determine the clinical significance of serum Zinc in COVID-19 patients and to establish a correlation with disease severity. **Methodology:** A prospective study on COVID-19 patients underwent fasting Zinc level at the time of hospitalisation. An initial comparative analysis was carried out between COVID-19 patients and healthy controls. Zinc deficiency COVID-19 patients were compared to those with normal levels. **Results**: COVID-19 patients (n=47) showed significantly low Zinc levels compared to healthy controls (n=45), median 74.5 (IQR 53.4-94.6) vs 105.8 (IQR 95.65120.90) µg/dl, P<0.001. Amongst COVID-19 positive patients, 27 (57.4%) were found Zinc deficient. These patients were found to have higher complications (P=0.009), ARDS (18.5% vs 0%, P=0.06), received corticosteroid therapy (P=0.02), prolonged hospital stay (P=0.05) and increased mortality (18.5% vs 0%, P=0.06). The Odds ratio (OR) of developing complications in Zinc deficient COVID-19 patients was 5.54. **Conclusion**: Our data clearly shows that significant number of COVID-19 patients are Zinc deficient. These Zinc deficient patients developed more complications with prolonged hospital stay and were associated with increased mortality.

Keywords: SARS-CoV-2, Zinc, COVID-19.

Main text

Main text Word count - 2313

Background: COVID-19 pandemic caused by SARS-CoV-2 is a major healthcare problem around the world with significantly higher morbidity and mortality in patients with coexisting conditions such as diabetes mellitus and hypertension[1,2]. Clinical presentation can be heterogenous from being asymptomatic to severe disease which can be associated with cytokine storm. Pathogenesis of COVID-19 is not fully understood but is probably multi-factorial causing systemic hyperinflammatory response and associated thromboembolic complications in severe cases[3,4] .

Zinc is a trace element with potent immunoregulatory and antiviral properties. Zinc is essential for growth, reproductive health, immunity and neurobehavioral development[5]. Recommended daily intake of zinc ranges between 3 – 16 mg. Under physiological conditions, Zinc is essential for cellular growth and maturation of immune cells, particularly in the development and activation of T-lymphocytes[6] . Studies show around 10% of our body proteins utilizes Zinc and is a cofactor in at least two-hundred immunomodulatory and anti-oxidant reactions[7]. Prolonged deficiency is associated with immune system dysfunction, sterility in males, neurosensory disorders and decreased body mass[8]. Studies show increased viral infection in patients with Zinc deficiency[9].

Antiviral property of Zinc has been extensively studied in various viral infections including Coronavirus, Hepatitis C virus and Human immunodeficiency virus[10]. However, the exact role of Zinc in SARS-CoV-2 is not well studied. The proposed mechanisms of antiviral property of Zinc include inhibition of RNA synthesis, topoisomerase and viral replication[11].

Until now, there is no definitive curative therapy for COVID-19. Therefore, the current treatment involves a multimodal approach with corticosteroids, antivirals and anticoagulation therapy. Multivitamin supplements are not uncommon in 'flu' prescriptions. Supplementation with Zinc is increasingly recommended in the management of COVID-19 patients[12,13]. However, it is unclear whether these patients are actually deficient in Zinc.

Aim: To determine the clinical significance of serum Zinc level in COVID-19 patients and to establish a correlation with disease severity.

Methodology: A prospective observational study was conducted from 17th May 2020 to 27th May 2020 to test serum Zinc levels in all consecutive SARS-CoV-2 RT-PCR positive patients referred to our Dr Rela Institute and Medical centre, Chennai, for secondary and tertiary care management of COVID-19 patients. This is a multi-speciality tertiary care institution, currently managing a significant volume of patients with COVID-19. About 5ml blood was collected after an informed consent in a BD gel vacutainer after 6 hours fasting from the time of hospital admission. Biochemical analysis was performed on the serum sample after separation and Zinc level was measured with fully automated Indiko Plus (Thermo Scientific, USA) analyzer using colorimetric method. The reference range used for Zinc concentration was 80-120 µg/dl. To verify the accuracy of the method, two levels of random control (Randox chemistry control: Human Assay control-2 LOT-1369 UN; Human Assay control 3 LOT-1066 UE, Randox Immunoassay control: level -1 LOT-1862, level-2 LOT-1877 and level-

3 LOT 1867) were analyzed. Method performance was monitored by analysis of the same control serum within each of the batch. The obtained result agreed with the certified values.

This study was carried out following an approval from the hospital ethics committee. Only SARS-CoV-2 positive adult patients admitted to hospital during the study period were included. Patients already on Zinc supplements, those who did not require hospital admission or unwilling to participate in the study were excluded from enrolment. Controls were hospital staff members from the out patients department with no underlying comorbidities underwent blood test to estimate zinc levels following an informed consent.

A comparative analysis was carried out between COVID-19 patients and healthy volunteers. COVID-19 patients were further stratified according to their serum Zinc concentration. A Zinc level <80 μg/dl was defined as 'deficient'. Further, COVID-19 positive patients with Zinc deficiency were identified and compared to those with normal levels. Corticosteroid therapy was initiated in patients with 'moderate' disease defined as the presence of any of hypoxia (saturation<92%) measured by pulse oximetry, requiring oxygen therapy, tachycardia or tachypnoea, and in patients with 'severe' disease defined as any of oxygen saturation <90%, hypotension, Acute respiratory distress syndrome (ARDS) or end organ damage. All patients received hydroxychloroquine (HCQ), antibiotics, multivitamins, including vitamin C 500 mg twice a day and Zinc 150 mg once a day (after the test), and patients with moderate and severe disease received additional subcutaneous anticoagulation for the duration of hospital stay as the standard of care. Patients were managed in the intensive care unit in case of

clinical deterioration causing haemodynamic instability, requiring organ support and invasive ventilation.

A descriptive statistical analysis was carried out for all variables using SPSS v21.0 consisting of mean, standard deviation, percentage, median and interquartile range (IQR: 25%, 75%). Proportions/Associations between characteristics of the study groups were compared by Fisher's exact test. Mann-Whitney U-test and t-test was used to compare continuous variables between the study groups. An univariate logistic regression analysis was carried out to identify Odds Ratio and for evaluation of 95% Confidence Intervals. Results were considered statistically significant when the P value was <0.05.

Results: A comparative analysis of COVID-19 patients (n=47) and healthy controls (n=45) showed a median age 34.0 years (18-77 years) vs 32.0 (18-60 years), (P=0.067), sex ratio (M:F), 1.6:1 vs 2.1:1 (P=0.09), respectively. COVID-19 patients had significantly low Zinc levels in comparison with healthy controls, median 74.5 (IQR 53.4-94.6) vs 105.8 (IQR 95.65-120.90) µg/dl, P=0.00 **(Figure 1)**. 5 out of 45 healthy controls had low Zinc levels (71.8-79.6 µg/dl).

COVID-19 Patients: Zinc deficiency vs Normal levels

Amongst COVID-19 (n=47) patients, 27 (57.4%) were found Zinc deficient. A comparative analysis was carried out between COVID-19 patients with Zinc deficiency and normal Zinc levels. Majority of patients presented with fever and cough and there was no statistical

significance between the groups (P=0.481 and P=0.121). Other symptoms included sore throat, myalgia, gastrointestinal symptoms were observed in both groups with no statistical significance. Pre-existing comorbid conditions such as age >60 years (7.4% vs 10%, P=1.0), diabetes mellitus (14.8% vs 15%, P=1.0), hypertension (14.8% vs 25%, P=0.40), coronary artery disease (3.7% vs 20%, P=0.70), pregnancy 7.4% vs 0, P=1.0), hypothyroidism (3.7% vs 0, P=0.5), rheumatoid arthritis (3.7% vs 0, P=1.0), obesity (0 vs 5%, P=0.42) and bronchial asthma (0 vs 5%, P=0.42) did not differ between the Zinc deficient and normal Zinc level in COVID-19 patients. At the time of hospitalization overall, 4 (8.5%) patients required non-invasive oxygen therapy ranging from 2 to 8 litres and 4 (8.5%) required mechanical ventilation. COVID-19 disease severity on admission showed mild, moderate and severe in 21(77.8%) vs 18 (90%), 1 (3.7%) vs 2 (10%) and 5 (18.5%) vs 0 (P=0.09) between patients with Zinc deficiency and Normal zinc levels, respectively **(Table 1)**.

In total, 14 (29.7%) patients received corticosteroids, commenced at day 5 (median, 1-7 days) from the time of admission of whom 12 (85.7%) were in the Zinc deficient patients. Twelve patients received oxygen therapy during the hospital stay, including 6 patients on invasive mechanical ventilation.

Complications: Overall, Zinc deficient patients developed more complications (19 (70.4%) vs 6 (30.0%), P=0.009). A subgroup analysis showed higher number of patients had Acute Respiratory Distress Syndrome (ARDS) (18.5% vs 0%, P=0.063), hypotension (14.8% vs 0%, P=0.126) and elevated Interleukin-6 (IL-6) (33.3% vs 15%, P=0.110) level in the Zinc

deficient group compared to the normal Zinc COVID-19 group. Interestingly, the median peak IL-6 level was 67.8 (IQR 23.8-158.1) vs 10.4 (IQR 3.05-44.03) pg/ml, P=0.029, between Zinc deficient and normal Zinc COVID-19 patients.

Higher number of Zinc deficient COVID-19 patients had a prolonged hospital stay (≥ 7 days) compared to patients with normal Zinc levels (16 vs 6 patients P=0.047), with a mean hospital stay of 7.9 and 5.7 days (t=2.036; df=44.7; P=0.048), respectively. Similarly, more number of patients in the Zinc deficient group received corticosteroids (12 vs 2, P=0.022), required ICU care (7 vs 2, P=0.266) and recorded deaths (5 (18.5%) vs 0 (0%), P=0.06) in comparison to patients with normal Zinc levels. Clinical and treatment characteristics of expired patients is shown in **Table 3**.

The Odds ratio (OR) of developing any complications in Zinc deficient COVID-19 patients was 5.54 (95% CI 1.56-19.6 (P=0.008). On further analysis, the OR for corticosteroid use, hospital stay ≥ 7 days, ICU admission and mortality was 7.2 (CI 1.39-37.35, P=0.02), 3.39 (0.99-11.57, P= 0.076), 3.15 (0.58-17.67, P=0.266) and 5.48 (0.61-49.35, P= 0.129), respectively.

Discussion: To our knowledge this is the first clinical study correlating lower baseline Zinc levels in patients with COVID-19 compared to healthy controls (74.5 vs 105.8 μg/dl, P=0.00) Amongst COVID-19 patients, 57.4% (n=27) were Zinc deficient. However, we do not know whether Zinc deficiency in these patients is a causation or an epiphenomenon. *In vitro* studies showed SARS-CoV-2 viral spike protein interacts with ACE2 (Angiotensin Converting Enzyme2) and serine protease TMPRSS2 (Transmembrane Protease Serine2) in the alveoli

permitting its entry into the cells. Interestingly, ACE2 is a Zinc dependent peptidyl dipeptide hydrolase composed of two subdomains (I and II) out of which N-terminal containing subdomain I and C-terminus containing subdomain II are involved with Zn binding[14]. This process is facilitated and coordinated by amino acids His^{374}, His^{378}, Glu^{402} (HEXXH + E motif) and a molecule of water at subdomain I and by amino acids Arg^{169}, Trp^{477}, and Lys^{481} with a chloride ion at subdomain two[15]. Earlier studies demonstrated that decreased Zn level favours this interactionn of ACE2 to SARS-CoV-2 spike protein and likewise increased Zn level inhibits ACE2 expression resulting in reduced viral interaction[16,17].

Zinc - viral particle interplay has been studied previously with other RNA viruses like Hepatitis C, Coronavirus, HIV and Influenza[18,19,20,21]. Zinc supplements are traditionally prescribed for common cold ailments, usually caused by coronaviruses. Zinc supplements were associated with shortened duration of symptoms, reduced severity of illness and more importantly it was associated with reduced morbidity and mortality in children[22].

Zinc has shown to exhibit antiviral property by inhibition of RNA synthesis, viral replication, DNA polymerase, reverse transcriptase and viral proteases[9,23,24]. However, literature is unclear with SARS-CoV-2 and Zinc. Interestingly, Hydroxychloroquine, a drug used initially in the management of COVID-19, is an ionophore that transports Zinc across the hydrophobic cell membrane[24,25]. Moreover, evidences specifically suggest that Zinc supplemented with antiviral drugs containing Zinc-ionophores precisely targets and binds to SARS-CoV-2 preventing its replication within the infected host cells[26]. Intracellularly, Zinc binds with RNA

dependent RNA polymerase causing elongation inhibition and decreased template binding of the viral mRNA[25,26].

Zinc plays a major role at various levels in the process of immune development and acts as an immunomodulator. Zinc deficiency has been associated with poor development of lymphoid tissue, reduced Natural Killer (NK) cell function leading to poor innate immunity[27]. Zinc deficiency is associated with reduced macrophage activation and cytokine generation. Zinc is involved in T- cell and B-cell function. Thymulin, a zinc dependent thymus hormone binds to T-cell receptor and promotes T-cell maturation and cytotoxicity.[8] In addition, Zinc deficiency is associated with downregulation of Interferon gamma, resulting in severe impairment of cell mediated immunity. Also, it enhances the production of interleukins particularly IL-2 via activation of Nuclear Factor kB (NFkB) [28]. Above *in vitro* studies, indicate that Zinc deficiency is associated with immune dysfunction and risk of infection. Role of Zinc in the immunology SARS-CoV-2 infection definitely warrants further clinical research.

*A d*isintegrin *a*nd *m*etalloproteinase (ADAM) enzymes are zinc dependent cell surface proteins of the Adamalysin protein family known to play a major role in inflammation. ADAM 17 catalysis the activation of proinflammatory cytokine, Tumor Necrosis Factor (TNF) – α and conversion membrane bound (m)IL 6 to soluble (s)IL6. Targeting the inhibition of ADAM 17 at the zinc co factor site inhibits the enzyme, causing downregulation of inflammation by inhibiting these two pathways[29, 30].

These *in vitro* evidences suggest that Zinc may have a pivotal role in COVID-19 **(Figure 2).** Therefore, Zinc deficiency in COVID-19 patients may not be just a mere association. More studies are required to ascertain the relationship between COVID-19 and Zinc.

Our data clearly demonstrates higher complication rates (70.4% vs 30.0%, P=0.009) in Zinc deficient COVID-19 patients, with an OR 5.54. In addition, these patients showed an increased trend towards development of ARDS (5 vs 0, P=0.06), longer hospital stay (Mean, 7.9 and 5.7 days, P=0.048), more likely to have received corticosteroids (12 vs 2, P=0.02) and had increased mortality (5 (18.5%) vs 0 (0%), P=0.06), indicative of severe disease spectrum in these patients. Our study shows an association between baseline zinc level and COVID 19 disease course, such that zinc deficient patients encounter more complications and mortality.

LDH is an intracellular enzyme, present in most cells, catalyses the interconversion of pyruvate and lactate. LDH is a marker of organ injury particularly related to hypoxia[31]. A pooled analysis of 1532 COVID -19 patients showing elevated LDH was associated with 6-fold risk of severe disease and 16-fold risk of death[32]. Elevated LDH in our study is probably indicative of severe disease as a result of Zinc deficiency.

Ours is a single centre study with limited number of patients, requiring hospitalisation for COVID-19. It would be interesting to study Zinc level and its role in the entire spectrum of the disease including asymptomatic patients with no comorbid conditions who are

otherwise managed with home isolation. Moreover, it is unclear, whether low Zinc is a simple association or a causative factor for COVID 19. The literature and the understanding of Zinc in COVID-19 patients is currently limited. Clearly, a multi-centre study is required to throw more light on this specific issue.

Conclusion: Our study clearly demonstrates that COVID-19 patients are Zinc deficient compared to healthy adults. It is convincing that low baseline Zinc levels in these patients were associated with more complications, leading to prolonged hospitalisation and increased mortality. It is not clear whether supplementing Zinc after admission to hospital helps to reduce severity of disease. It is worth exploring the exact role of Zinc in COVID-19 patients and establish the appropriate dosage to improve their survival. With more research Zinc could provide a cost-effective therapy for COVID-19, certainly the need of the hour in this pandemic.

Declaration of interests: None

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest: None

Ethical Approval: Obtained

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Variables	Zinc deficient COVID-19 patients (n=27) (57.4%)		Normal Zinc level COVID-19 patients (n=20)(42.6%)		P Value
	n	%	n	%	
Age (Median, years)	33 (18-75)		35 (27-77)		0.546
Sex Ratio (M: F)	1.7:1		3:1		0.529
Asymptomatic	1	3.7	2	10	0.567
Fever	20	74.1	17	85	0.481
Cough	12	44.4	4	20	0.121
Sore Throat	5	18.5	1	5	0.221
Loose stools	4	14.8	4	20	0.707
Myalgia	6	22.2	4	25	0.5
Nausea	0	0	1	5	0.426
Anosmia	1	3.7	0	0	1.0
Dyspnoea	4	14.8	3	15	1.0
Comorbidities					
Diabetes Mellitus	4	14.8	3	15	1.0
Systemic Hypertension	4	14.8	5	25	0.405

Coronary Artery Disease	1	3.7	4	20	0.707
Pregnancy	2	7.4	0	0	1.0
Hypothyroidism	1	3.7	0	0	0.5
Rheumatoid Arthritis	1	3.7	0	0	1.0
Obesity	0	0	1	5	0.426
Age > 60 years	2	7.4	2	10	1.0
Bronchial Asthma	0	0	1	5	0.426
Laboratory indices Median (IQR)					
Bilirubin (Normal 0.2-1.2) mg/dl	0.48 (0.35-0.48)		0.57 (0.38-0.90)		0.241
AST (Normal 0-45) U/L	28 (18-34)		25 (18-32)		0.639
ALT (Normal 0-47) U/L	18 (11-32)		22 (19-28)		0.517
Creatinine	0.80 (0.69-0.93)		0.96 (0.64-1.05)		0.166

(Normal 0.5-1.3) mg/dl			
LDH (Normal=135-225) (U/L)	264 (206.5-417.5)	200 (169-242)	0.006
Ferritin (Normal=28-397) ng/ml	216.0 (70.5-511.2)	202.3 (98.7-313.4)	0.622
C-Reactive Protein (Normal< 5) mg/L	11.0 (3.5-48.5)	3.6 (1.3-35.8)	0.144
D- Dimer (Normal <250) ng/ml	499.0 (237-603)	158.5 (106.75-487.5)	0.108
Fasting Glucose (Normal 70-100) mg/dl	110 (93-128)	101.5 (92.7-142.5)	0.780
Triglyceride (Normal <150) mg/dl	103 (76-167)	124 (101.2-190.2)	0.165

Vitamin D **(Normal 0-30)** **ng/ml**	13.6 (11.3-25.7)	19.3 (12.9-22.2)	0.533
Disease severity on admission			
Mild	21 (77.8%)	18 (90%)	0.09
Moderate	1 (3.7%)	2 (10%)	
Severe	5 (18.5%)	0	

AST-Aspartate Amino transferase, ALT-Alanine Amino transferase, LDH-Lactate dehydrogenase

Table 1. Comparison of variables in COVID-19 patients on admission: Zinc deficient vs Normal Zinc level.

Complications	Zinc deficient COVID-19 patients (n=27) (57.4%)		Normal Zinc level COVID-19 patients (n=20) (42.6%)		P Value
	n	%	n	%	
Corticosteroids	12	44.48	2	10	0.022
ARDS	5	18.5	0	0	0.063
Hypotension	4	14.8	0	0	0.126
Sepsis	1	3.7	0	0	1.0
IL-6 > 7 pg/ml	9	33.3	3	15	0.110
Others	2	7.4	1	5	1.0
ICU	7	25.9	2	10	0.266
Hospital stay ≥ 7 days	16	59.3	6	30	0.047

Death	5	18.5	0	0	0.06

ICU – Intensive care unit

Others- melaena, hyponatremia and hypokalemia in each patient

Table 2. Complications in COVID-19 patients during hospital stay: Zinc deficient vs Normal Zinc level.

	Case 1	**Case 2**	**Case 3**	**Case 4**	**Case 5**
Age/Sex	40/M	51/F	50/F	72/F	75/F
Comorbidities	CAD	DM HT	DM, HT Hypothyroid	DM HT	Nil
Initial symptoms	Fever Myalgia Cough	Fever Anorexia	Fever Dyspnoea	Dyspnoea	Fever Dyspnoea
Duration of symptoms (Days)	2	5	7	3	2
Complications	ARDS	Sepsis, ARDS	ARDS, MODS	ARDS	ARDS
Treatment	Methylprednisolone Supplements Antibiotics Enoxaparin	Methylprednisolone Supplements Piperacillin-tazobactam Enoxaparin	Methylprednisolone Supplements Piperacillin-tazobactam Enoxaparin	Methylprednisolone Supplements Piperacillin-tazobactam Enoxaparin	Methylprednisolone Supplements Meropenem Enoxaparin
Hospital stay (Days)	3	7	3	8	18
Admission Zinc level (µg/dl)	36.4	47	57	59	81
WCC X 10^9/L	4.6	16.8	13.3	9.07	18.2
Lymphocyte X 10^9/L	0.73	0.67	1.33	1.18	1.27
CRP	32.5	108.3	227	193.3	300.9

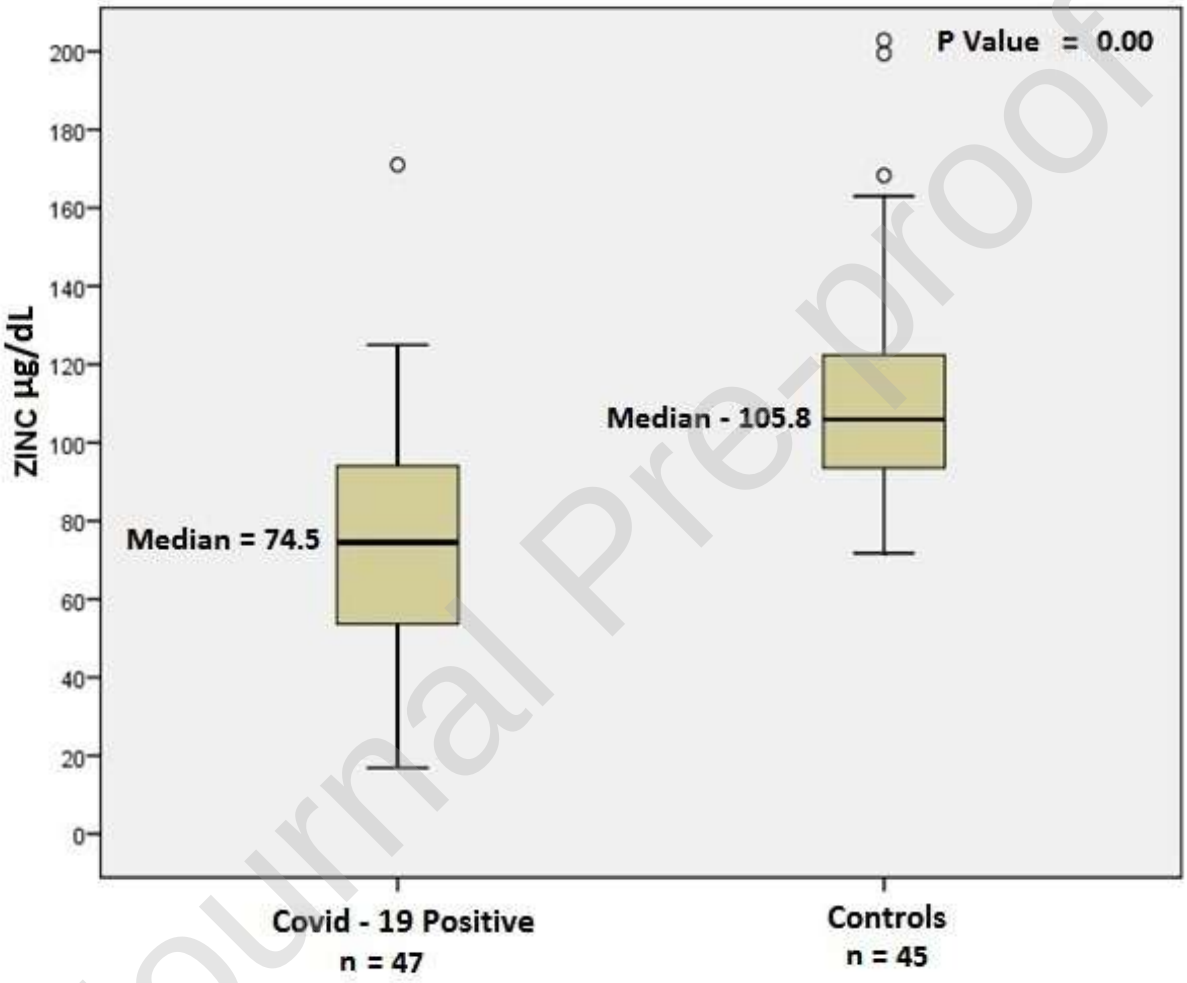
(ng/ml)					
Ferritin (ng/dl)	979.8	203.6	636.5	514.5	1441

CAD- Coronary Artery Disease; HT- Systemic Hypertension; DM-Type 2 Diabetes Mellitus ARDS-Acute Respiratory Distress Syndrome; MODS- Multi-organ Dysfunction syndrome WCC-White Cell Count; CRP- C-reactive Protein; Supplements- Vitamin C 500MG twice a day and Zinc 150mg once a day.

Table 3.Clinical and treatment characteristics of COVID 19 expired patients.

Legend 1: Figure 1. Serum Zinc levels in COVID-19 positive patients and healthy controls

Legend 2: Figure 2. Illustration of antiviral and immunomodulatory properties of Zinc in COVID-19.



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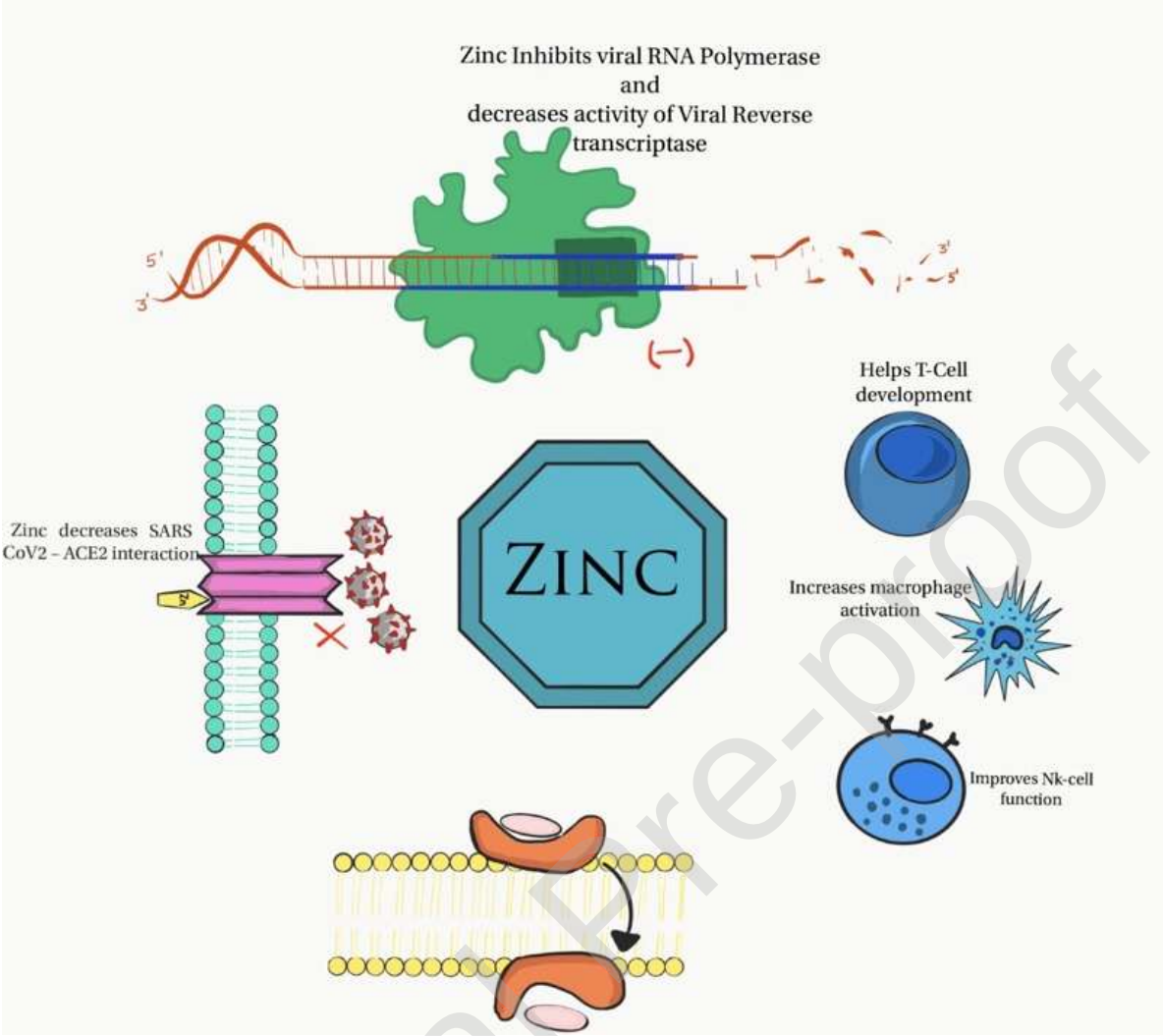

HCQ aides transporting Zinc across the cell membrane

Figure 2. Illustration of antiviral and immunomodulatory properties of Zinc in COVID-19.