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Analysis of trace elements (Zn and Cu) levels in COVID-19 patients with ICU and Non-ICU hospitalization

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Abstract

Background: The persistence of the COVID-19 pandemic besides its current resurgence and continuously increasing fatalities indicates a vital need for severity assessment at its early stages. Recent studies have already ascribed mortality to chronic inflammation. But the part of trace elements, especially zinc and copper that have been known to possess antiviral roles for a long time is least studied in COVID-19.

Methods: The study comprised 122 COVID-19-positive participants admitted to the tertiary care hospital. Among them, eighty-one (~66%) were admitted to ICU under high severity. Levels of Zn and Cu along with CRP were analyzed and compared among ICU and non-ICU admitted patients. Using ROC analysis, the potential and precise levels for defining severity were determined.

Results: We found a significant reduction in Zn levels ($p=0.001$) in ICU-admitted patients compared to the non-ICU group which was more pronounced in females and patients aged above 50 years. Reduction in the levels of Zn is accompanied by elevated CRP levels ($p<0.001$) in ICU patients with no effect on Cu levels. Upon ROC analysis, Zn and CRP were found to have significant AUC ($p<0.0001$). Further, CRP to Zn ratio displayed improved AUC with 90% sensitivity indicating their applicability to predict ICU requirements.

Conclusions: The present study was primarily aimed to predict the status of zinc and copper in COVID-19 patients and their utility as a prognostic tool for deciding the severity. Our findings indicate that CRP to Zn ratio might feasibly be used to predict the progression of COVID-19 toward severity. Keywords: COVID-19, Severity, Zinc, Copper, CRP

1. Introduction

Coronavirus disease (COVID-19) is currently the most dangerous global health concern. Identified by WHO on 31st December 2019, after the emergence of a cluster of ‘viral pneumonia’ cases in Wuhan, China, the pandemic has severely affected the world population both in terms of health and finances. As of 26 December 2022 nearly 650 million were affected with 6.5 million deaths worldwide. [WHO COVID-19 Dashboard] Being caused by a novel coronavirus, several aspects of pathogenesis and clinical outcomes of the disease are still unclear. Although some clinical data has been produced since the onset of the pandemic indicating the associations of trace elements with COVID-19,[1] their role in the progression of the disease to severity is still poorly understood. From acting as enzyme cofactors to potent immuno-modulators, trace elements are involved in various physiological processes. Of the several elements, the roles of Zinc (Zn) and Copper (Cu) have already been associated with various pathological conditions including, type 2 diabetes mellitus[2], ischemic heart disease[3], psoriasis[4], preeclampsia[5] as well as viral infections owing to their antiviral roles.[6]

Zinc is a micronutrient indispensable to various cellular processes. Studies have identified the potential antiviral role of Zn mediated through different mechanisms including their role in the suppression of viral RNA replication through the inhibition of RNA-dependent RNA polymerase in the SARS-CoV virus.[7]

Involvement in promoting anti-inflammatory effects, modulation of T cell functions, and increased interferon-gamma (IFN-α) production indicate its crucial role in immune functioning.[8] Zn deficiency is often associated with respiratory infections and lung inflammations.[9] Deficiency of Zn in critically ill COVID-19 patients is often speculated as these patients display symptoms similar to hypozincemia i.e., loss of appetite, diarrhea, hair loss, and impaired immune functions. Chronic deficiency may lead to increased production of pro-inflammatory cytokines,[10] leading to a 'cytokine storm', the principal cause of COVID-19-related mortalities. Moreover, these pro-inflammatory cytokines lead to the hepatic secretion of inflammatory markers as indicated in COVID-19, where aggravation of the disease has been associated with increased serum levels of C-reactive protein (CRP).[11] The antiviral role of Zn has already been established in previous studies as its supplementation has displayed significant effects from reducing the duration of the common cold[12] to suppression of pneumonia symptoms.[13]

Copper is an essential trace element, whose role otherwise is not studied in COVID-19 but known to possess antiviral roles against various viruses for a long time.[14, 15] Owing to this property, SARS-CoV-2 shows the least survival on copper surfaces.[16] The deficiency of Cu has been associated with suppressed secretion of pro-inflammatory cytokines and reduced microbicidal and respiratory burst activity of phagocytes.[17]

Although there are studies depicting the associations between Zn, Cu, and CRP individually with COVID-19 incidence and severity but the precise levels of these parameters for determining COVID-19 severity are still unknown. The present study is aimed to evaluate the status of Zn and Cu in intensive care unit (ICU) and non-ICU admitted COVID-19-positive patients and their correlation with CRP to determine the precise levels for predicting the progression of disease towards severity at early stages.

2. Methods

2.1. Study Participants

This is a cross-sectional prospective study conducted on blood samples of 122 patients with RT-PCR confirmed COVID-19 positive status admitted to the tertiary care unit of the hospital between July and November 2020. These blood samples were received in the biochemistry laboratory for routine patient management from ICU and non-ICU wards of the hospital. Patients aged below 18 years were excluded from the study. The study has been approved by Institutional Ethics Review Board (Reference No: NK/6384/Study/231). The requirement of written consent from the patients had been waived by the Institutional Ethics Committee.

2.2. Sample Collection

Blood samples from ICU and non-ICU admitted patients were collected by the phlebotomists and sent to the biochemistry laboratory. Venous blood (5ml) was centrifuged using standard operating procedures. The serum thus obtained was used for the estimation of CRP, Zn, and Cu levels.

2.3. Sample Analysis

For the analysis of Zn and Cu levels, the serum samples were diluted in a 1:10 ratio with a diluent containing 2% HNO_3 (≥ 69%, Fluka Analytical, TraceSELECT®) and 0.01% Triton X-100. Analysis was done in Agilent 240FS AA atomic absorption spectrometer (Malaysia), equipped with deuterium background correction, through flame method using air-acetylene atomization mode. The optimized conditions for estimation are summarized in Table 1. Calibration curves (2.5 to 50 g/dL) were prepared through stepwise dilution of certified standards (1000 mg/dL, Agilent Technologies). The correlation coefficients (R^2) for calibration curves were 0.9988 and 0.9919 for Zn and Cu respectively. LOB, LOD, and LOQ were estimated as 0.05, 0.67, and 1.25 g/dL for Zn and 0.21, 2.1, and 2.5 g/dL for Cu by following the method of Armbruster *et al*, 2008.[18] Quality assurance of the analytical procedures was done through certified reference material, Hum Asy Control, Acusera (Randox, UK).

Table 1

Optimised conditions for the estimation of Zn and Cu

	Analytical wavelength	Slit (nm)	Lamp current (mA)	Airflow (L/min)	Acetylene flow(L/min)
Zinc	213.9	1.0	5.0	10	2
Copper	324.8	0.5	4.0	10	2

CRP levels were determined in Cobas 8000 Auto Analyser (Roche Diagnostic International Ltd, Switzerland), with certified quality controls, PreciControl ClinChem (Roche, Germany). The reference range considered for Zn, Cu and CRP was 66–110 g/dL, 70–145 g/dL, and 0–5 mg/L respectively. A comparative analysis was carried out between the levels of these parameters in different groups.

2.4. Statistical Analysis

Prism 8.0 and MedCalc 19.6.1 were used for statistical analysis. The normality of the data was analyzed through the Kolmogorov-Smirnov test. Comparison between the levels of Cu, Zn, and CRP in different groups was made through the Mann-Whitney test. Receiver operator characteristics (ROC) curve analysis was used to ascertain the cut-off value, sensitivity, and specificity using MedCalc software.

3. Results

3.1. Analysis of Zn, Cu, and CRP levels in the total study population

Out of the 122 patients recruited in the study, 81 (66.39%) were ICU admitted. The mean age for ICU and non-ICU admitted patients was 50.63 and 40.38 years, respectively. Table 2 depicts the higher persistence of comorbidities in ICU-admitted patients.

Table 2: Patient characteristics in COVID-19 non-ICU and ICU groups

	Non-ICU	*ICU*
Mean Age	40.38±14.90	50.63±16.99
	Gender-wise distribution of patients	
Male	26	52
Female	15	29
Patients with comorbidities (%)		
T2DM	16.66	35
Hypertension	23.80	41.25
CAD	2.38	8.75
CKD	11.90	8.75
Obesity	2.38	3.75
Patients with complications (%)		
ARDS	11.90	46.25
Mortality	2.38	30

Upon analysis of Zn, Cu, and CRP levels in these groups, we found significantly reduced Zn levels with a median value of 53 g/dL in ICU patients, whereas levels were within the normal limits, i.e., median 85.20 ug/dL in the non-ICU group (p=0.001, Figure 1). However, no significant difference was observed in the levels of Cu in ICU (median 90.75 ug/dL) and non-ICU patients (median 88.70 ug/dL) which correspond to the normal range of this parameter. Also, ICU admission was found to be accompanied with thirty-one times elevated CRP levels with a median value of 81.30 mg/L as compared to 2.62 mg/L in non-ICU patients (p<0.0001, Figure 1).

Zn and CRP levels were found to be negatively correlated with spearman coefficient (r) -0.476 (Figure 2A, p<0.0001), while no significant correlation was found between Cu and CRP (r=-0.025, p=0.791) as well as between Zn and Cu (r=-0.173, p=0.058) in the total study population (Figure 2B, 2C).

3.2. Age and gender-related variations in Zn, Cu, and CRP levels in COVID-19 patients

A decrease in Zn levels was more pronounced in females where 39% had levels below LLN (lower limit of normal) in comparison to 27% in males. The percentage of females with deranged levels of Cu (below LLN) and CRP (above ULN) was found to be 23% and 73% in comparison to 31% and 79% in males

respectively (Figure 3A). Below 50 years of age, 25% of the COVID-19 patients had Zn levels below LLN and 62% had CRP above ULN, whereas, in patients aged above 50 years, deranged levels of Zn and CRP were found in 39% and 73% of patients respectively. There was no substantial effect on the levels of Cu among these two groups (Figure 3B)

3.3. ROC Analysis

Receiver operating characteristics (ROC) analysis was done for individual parameters. The area under the curve (AUC) was found to be 0.770, 0.503, and 0.922 for Zn, Cu, and CRP with the Youden index 0.539, 0.112, and 0.767 respectively (Table 3). Since, among these parameters, Zn and CRP had the maximum significant AUC and Youden index, their ratio was analyzed. ROC analysis of the CRP/Zn ratio displayed an improved AUC of 0.930 with a Youden index of 0.772 with a significant p-value ($p<0.0001$, Table 3). The cut-off value of the CRP/Zn ratio (CRP and Zn expressed as mg/L and g/dL respectively) for determining the severity of COVID-19 patients was found to be 0.184. Moreover, the ratio displayed increased sensitivity (90%) in comparison to the individual parameters, i.e. 63.75% and 79.75% for Zn and CRP, respectively (Table 3, Figure 4) indicating the greater potential for the identification of 'true positives' and for determining ICU requirement by COVID-19 patients at early stages.

Table 3: Table showing the summary of ROC analysis for various parameters

Parameters	AUC	95% CI	Youden Index	Specificity	Sensitivity
Zinc	0.770	0.684-0.855	0.539	90.24	63.75
Copper	0.503	0.396-0.610	0.112	00.00	88.75
CRP	0.922	0.866-0.979	0.767	96.97	79.75
CRP/Zinc	0.930	0.882-0.977	0.772	87.18	90.00

3.4. Levels of parameters with respect to the survival and mortality status

Out of the 122 patients recruited in the study, 25 died during hospitalization with 24 causalities reported from the ICU ward. The parameters were compared among the patients who survived and recovered with those who died later during their hospital stay due to COVID-19 complications (Figure 5). No significant difference was found between the median values of Zn in these two groups, i.e. (71.45 and 51.80 ug/dL, respectively). However, levels of CRP were significantly higher in the patients who died (97.47 mg/L), in comparison to the ones who survived (27.47 mg/L). Also, no significant difference was found in the ratio of CRP/Zn between the two groups.

4. Discussion

The COVID-19 pandemic has severely affected the world resulting in more than 6 million deaths to date. Continuously increasing fatalities worldwide indicate an urgent need for a prognostic marker for COVID-

19 severity assessment and treatment. In our study, we found significantly reduced levels of Zn in the ICU-admitted COVID-19 patients with approximately 1.6 times reduction in the median values as compared to the non-ICU group. Reduction in Zn levels was non-significant in non-severe COVID-19 patients admitted in the non-ICU wards in comparison to the normal limits, indicating an exclusive association between Zn levels and severity. These findings are in accordance with the recent studies that reported diminished levels of Zn in COVID-19 patients with higher levels of complications and prolonged hospital stay in the Asian population [19] as well as in the European population.[20] Some studies have also studied the severity of COVID-19 in accordance with other minerals such as Pal et al have found that hypocalcaemia was associated with low severity of the disease.[21] Although the definite role of Zn deficiency in the progression of COVID-19 is still unknown, various studies indicate the potential immuno-modulatory and antiviral roles of Zn.[7, 8, 22] The deficiency of Zn might be responsible for facilitating the entry of viral particles into the cell, as the activity of ACE(Angiotensin-converting enzyme) has been reported to be reduced under Zn deficient conditions[23] that may increase the availability of ACE2 receptors for the viral particles. Excessive Zn supplementation, however, has been shown to reduce the activity of ACE-2 receptors[24] which possibly explains the increased COVID-19 severity in Zn deficiency through hyperactivation of ACE2 receptors. A decrease in the serum levels of Zn in severe COVID-19 patients can also be correlated with the elevated levels of IL-6 that induces the expression of Zn binding metallothionein proteins thus reducing its availability.[25]

Levels of Zn have been reported to be associated with the inflammatory status of the body, with a 10% decrease when CRP level approaches 15 mg/L in mild diseases and up to 40% decrease when CRP level approaches near 100 mg/L in severe cases.[26] Comparable findings were observed in our study with a 20% decrease in Zn levels in ICU patients with median CRP levels corresponding to 81.30 mg/L. ICU-admitted patients displayed 31 times rise in the levels of CRP as compared to non-ICU patients. Numerous studies have correlated CRP levels with COVID-19 severity and mortality.[25–27] CRP is a non-specific inflammatory marker of hepatic origin, whose secretion is enhanced by the increased levels of pro-inflammatory cytokines precisely IL-6, resulting in cytokine storm and pulmonary dysfunctions, one of the major reasons for COVID-19 casualties.[29] Correlation has been reported between the size of lung lesions and CRP levels in severe COVID-19 patients,[30] resulting in the augmentation of oxygen support and mechanical ventilation in patients with raised levels of CRP.[31] The decrease in Zn levels was more pronounced in the female group (39%) of our study population in comparison to males (27%) without having any significant gender-specific difference in the levels of CRP, i.e. 73% and 79% in females and males respectively, which might be supported by a study showing the Zn deficient status in 41% of the females in the north region of our country which is even more pronounced in pregnant women.[32]

Zn has been known to have antiviral effects for a long time. From reducing the symptom severity to minimizing the outbreak recurrence against various viruses like Herpes Simplex,[33] Rhinovirus[34] and Torque teno virus,[35] Zn has also been proven effective against respiratory viruses, like respiratory syncytial virus and severe acute respiratory syndrome (SARS) coronavirus, through the inhibition of RNA dependent RNA polymerases.[36] Zn leads to the up-regulation of antiviral genes through the induction of Type 1 Interferons (IFN-1). However, delayed IFN-1 response has been shown to induce acute respiratory

distress syndrome (ARDS) in SARS-CoV-infected mice.[37] Zn is also involved in the regulation of T cell functions and inhibits NF-kB signalling to prevent cytokine storms.[38]

As of 27 December 2022, 78 studies were found with the keywords Zinc and COVID-19 in the clinical trials website, and 38 of them were completed [Home - ClinicalTrials.gov]. A recent study has reported the ineffectiveness of Zn as a treatment for COVID-19 patients as no significant difference was observed after supplementation with 50 mg Zn gluconate alone or in combination with vitamin C for 10 days.[39] However, Zn supplementation might abate certain symptoms of COVID-19 including loss of appetite and disturbances in smell and taste that are attributed to the lower levels of Zn.[40]

Adverse outcomes of Zn overdose include its toxicity along with Cu deficiency as the two trace elements exhibit competition for intestinal absorption.[41] No significant decrease was found in the levels of Cu in ICU patients compared to non-ICU in our study population which might be due to the absence of Zn supplementation in the patients during hospitalization. This might have had a favourable impact on these patients as Cu too exhibits antiviral roles through disrupting the viral membrane,[42] production of reactive oxygen species[15], and inhibition of RNA polymerase.[43] Also, no significant difference was found between males and females with deranged Cu levels. Though the levels of Cu in females are usually high due to contraceptives, no female subject in this study was on contraceptives, neither oral nor IUCDs.

Although numerous studies have reported the individual roles of Zn and CRP in the context of COVID-19, our study shows the combined effects of these crucial factors. We performed Receiver operating characteristics (ROC) analysis for individual parameters as well as for CRP to Zn ratio. A high Youden index with maximum sensitivity (90%) demonstrates a greater likelihood of considering the CRP/Zn ratio as a predictive marker for deciding the severity of COVID-19. However, this ratio was not significantly different for patients with dead and survival outcomes, indicating this as a predictor for progression towards severity but not for mortality.

Our findings should be interpreted in view of certain limitations. Although the study displayed a significant effect on Zn and CRP levels in COVID-19 patients that worsens with disease progression, one cannot rule out the effects of other trace elements and inflammatory markers. Secondly, we could not include information regarding the Zn status and history of Zn supplementation by the patients before their COVID-positive status. Thirdly, the biochemical analysis including Zn and Cu levels was done in samples received in the biochemistry laboratory irrespective of the information provided to us about the day of hospitalization of the patients in ICU and non-ICU wards. This study is merely a preliminary study to elucidate the levels of trace elements and CRP in COVID-19 patients and to present a cut-off value that might be a deciding factor for ICU requirements in COVID-19 patients during their future course of management. However, the precise mechanism including other potential parameters needs to be studied in the future.

Further, it can be concluded from the findings of the study that the analysis of Zn levels may be included as a part of the routine management of COVID-19 patients particularly in the patients deteriorating and

progressing to ICU during their treatment. Moreover, supplementation of zinc as an adjunct in the standard therapy may be helpful to those found deficient.

Abbreviations

IFN-α, Interferon gamma; CRP, C-reactive protein; LOB, Limit of blank; LOD, Limit of detection; LOQ, Limit of Quantification; ANOVA, Analysis of variance; LLN, Lower limit of normal; ULN, Upper unit of normal; ROC, Receiver operating characteristics; AUC, Area under curve; ARDS, Acute respiratory distress syndrome; T2DM, Type 2 Diabetes Mellitus; CAD, Coronary artery diseases; CKD; Chronic kidney disease

Declarations

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Author contributions:

Sant Ram: Conceptualization, methodology, supervision, re-writing, and finalization of the manuscript.

Neha Saini: Writing- original draft

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Sandeep Kaur, Piyush Pathak: Draft formation and editing

Mohana Kumari Chidananda: Sample collection

Ravjit Jassal: Sample analysis

Deepy Zohmangaihi, Writing- review, and editing

Shiv Lal Soni and Vikas Suri: Provided patients related information

Data availability: The datasets generated during and/or analysed during the current study are not publicly available to maintain patient privacy but are available from the corresponding author upon reasonable request.

Ethical approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by Institutional Ethics Committee (Reference No: NK/6384/Study/231).

Participant's consent: The requirement of written consent from the patients had been waived by the Institutional ethics committee.

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Figures

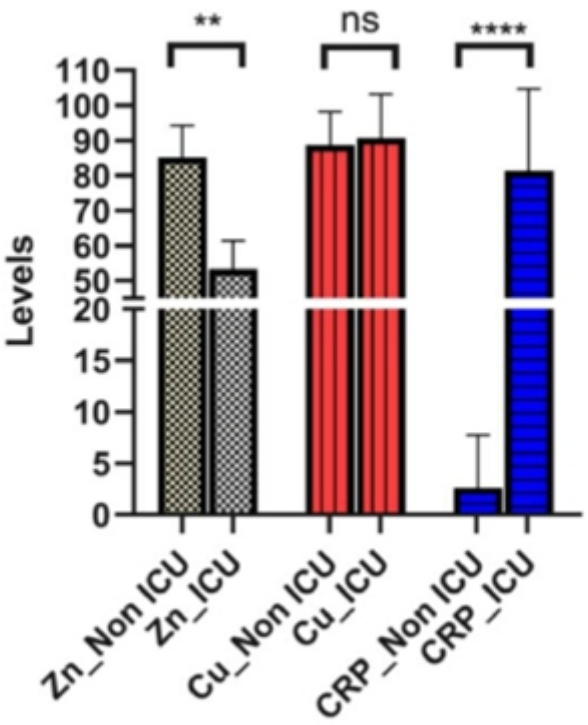

Figure 1

Comparison between Zn, Cu, and CRP levels in ICU and non-ICU admitted COVID-19 patients (unit for Zn and Cu is ug/dL and for CRP, mg/L on the y-axis, error bars represent 95% CI).

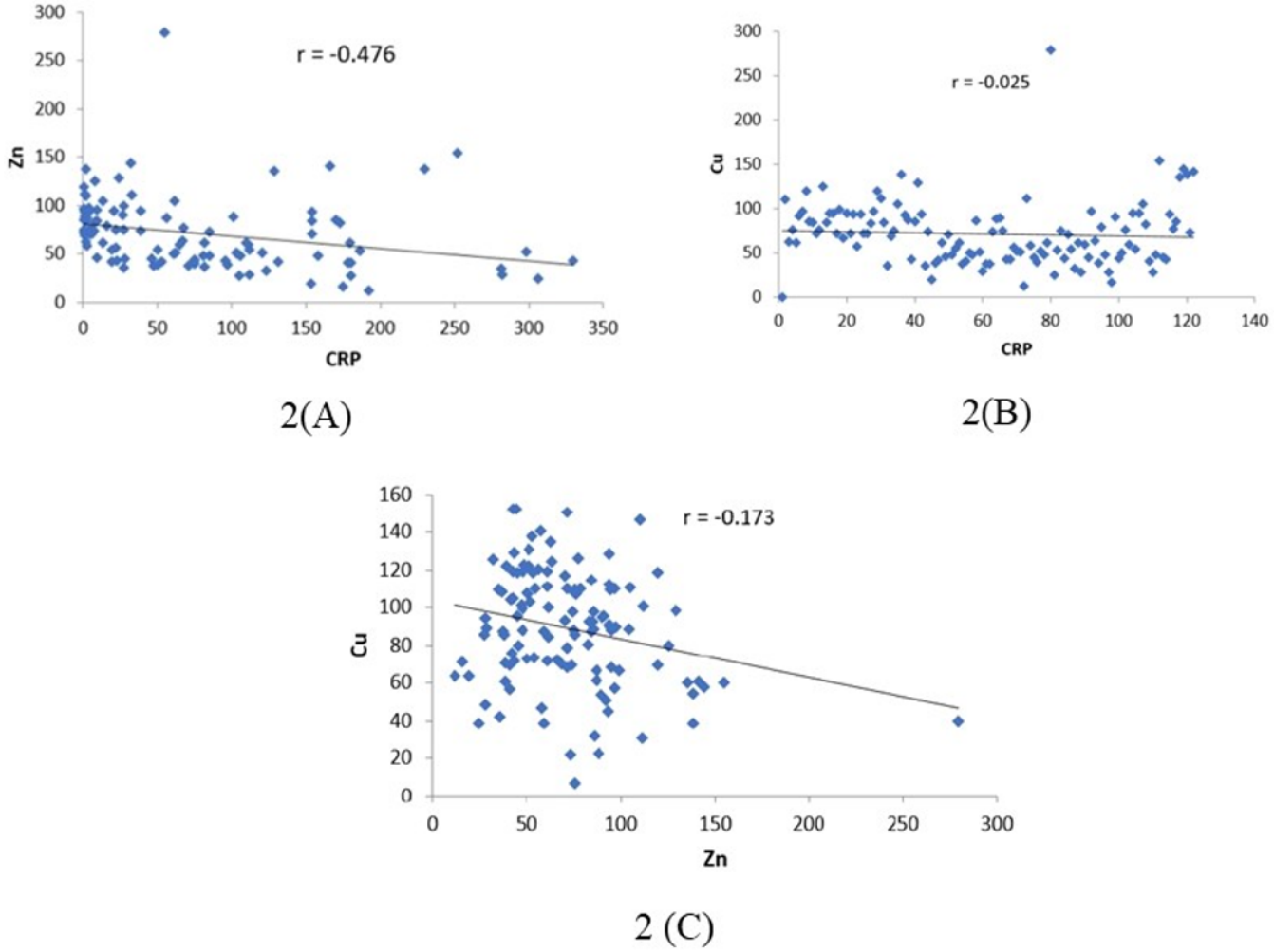

Figure 2

Correlation between Zn and CRP (A), Cu and CRP (B), and Zn and Cu (C) in the total study population.

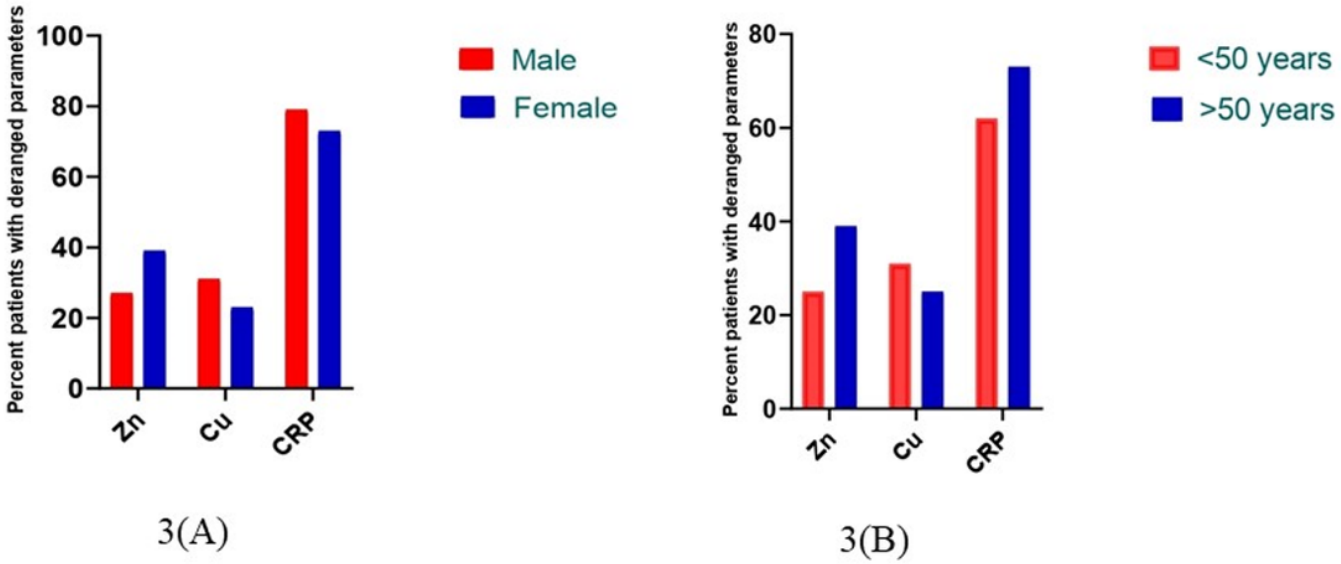

Figure 3

Figure showing the percentage of COVID-19 patients having deranged levels of Zn, Cu (below LLN), and CRP (above ULN) in terms of gender (A) and age (B).

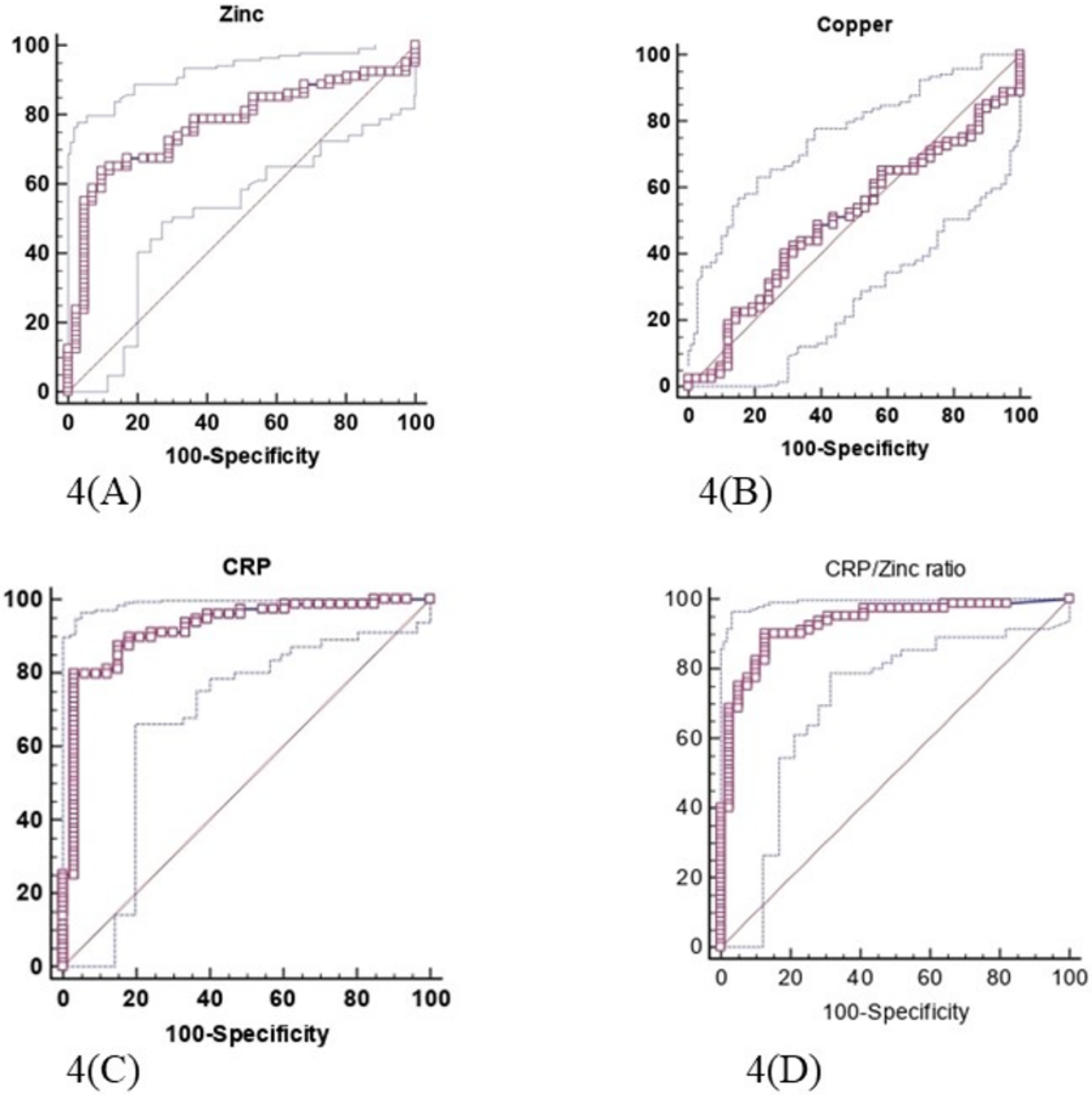

Figure 4

ROC curve analysis for Zn (A), Cu (B), CRP(C), and CRP/Zn ratio(D). p value <0.0001.

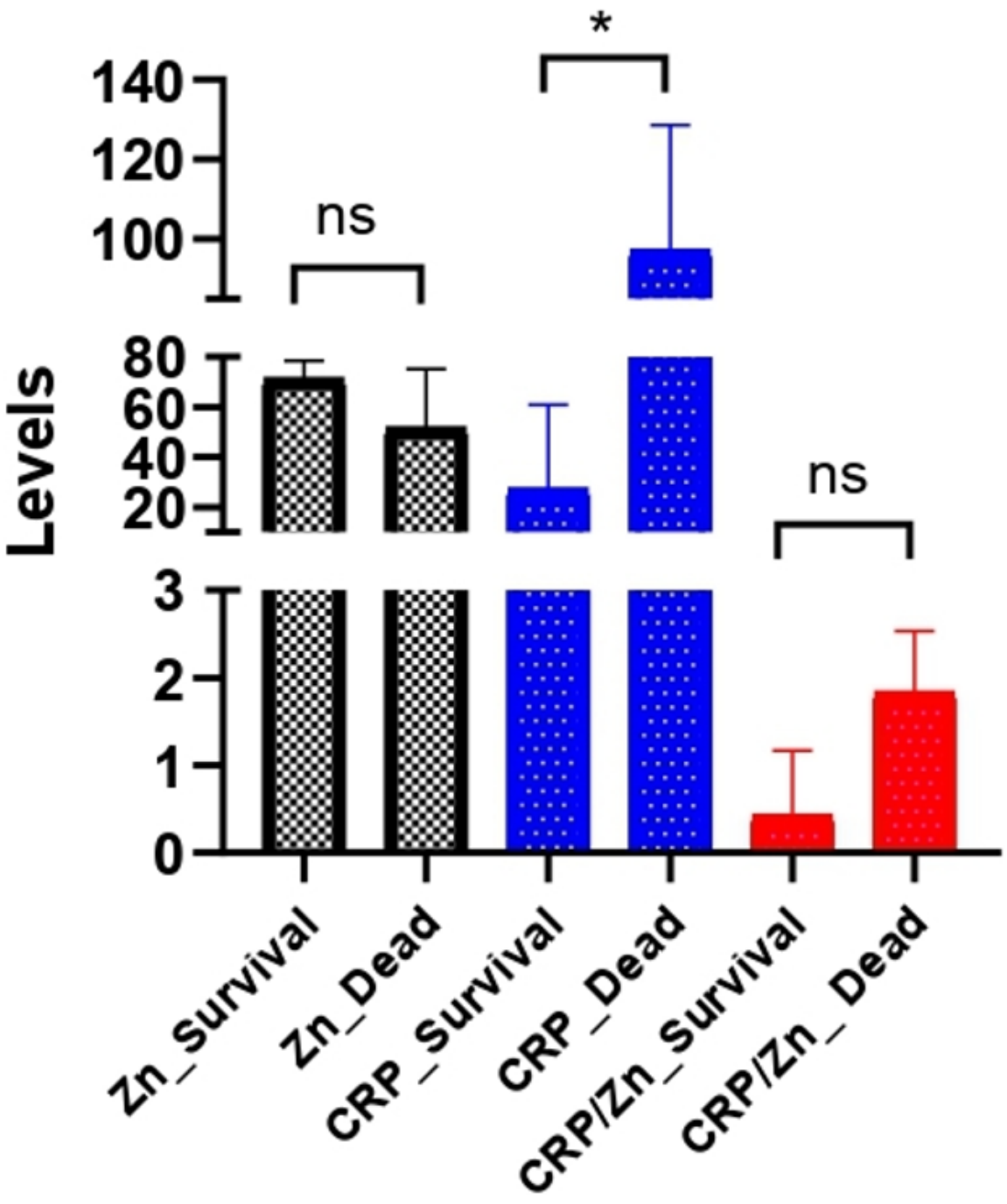

Figure 5

Comparison between Zn, CRP and CRP/Zn ratio among the patients with survival and death outcomes (unit for Zn and Cu is ug/dL and for CRP, mg/L on the y-axis, error bars represent 95% CI).