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Original Research Article

Relevance of copper/zinc ratio and its association with redox status in chronic obstructive pulmonary disease

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ABSTRACT

Background: Chronic obstructive pulmonary disease is a progressive and persistent inflammatory condition associated with significant morbidity and mortality. Increased oxidative stress amongst others, plays an important role in the pathogenesis of this disease. The objective of the present study was to conduct a preliminary yet comprehensive examination of metal exposure specifically copper and zinc levels and their association with overall oxidative stress in COPD.
Methods: A cross sectional study was carried out in a tertiary care hospital in South India. Two groups were included in the study. One group with 20 COPD patients and the other group of 20 healthy controls. Plasma samples were obtained from both the groups and serum levels of copper and zinc were studied by atomic absorption spectrometry. Cu/Zn ratio obtained from the results was further correlated with oxidative stress index calculated from total oxidant status and total antioxidant status in COPD with respect to controls.
Results: The copper levels were significantly higher and the zinc levels lower in the COPD group as compared to the control group. The copper/zinc ratio was higher in COPD as compared to control population. The correlation between Cu/Zn ratio and oxidative stress index showed a positive correlation with a regression coefficient of 0.7.
Conclusions: The study throws light on the trace element imbalance in COPD and how these could induce oxidative stress, contributing to persistent inflammation in COPD. In the clinical perspective, monitoring Cu/Zn ratio in COPD patients may lead to better risk mitigation and thereby better therapeutic management of the disease.

Keywords: Copper/zinc ratio, COPD exacerbation, Occupational exposure, Oxidative stress index

INTRODUCTION

Chronic obstructive pulmonary disease (COPD), as defined by Global Initiative for Obstructive Lung Disease (GOLD) 2023, is a diverse lung condition characterised by chronic respiratory symptoms such as dyspnea, cough, and expectoration caused by pulmonary abnormalities such as bronchitis, bronchiolitis as well as emphysema. This disorder is generally characterised by persistent and progressive airway restriction, the underlying causes of which include recurring inflammation, oxidative stress, and an imbalance in protease/antiprotease activities.

There are diverse risk factors which play a role in the development and progression of chronic obstructive pulmonary disease, including environmental factors such as smoking, occupational exposure to trace elements and air pollution. In low and middle income countries

(LMIC), approximately 60 to 70 % of COPD is brought about by risk factors other than smoking.[1] These include indoor air pollution, caused by the burning of biomass (wood, animal dung and crop residues) in primitive stove setups, posing a significant health concern. This biomass burning has been linked to noxious particulate matter, heavy metals, trace elements and greenhouse gases, all reported to play a role in COPD disease progression. Even regions with moderate air pollution have been reported to have pulmonary impairment in children and declined lung function in adults, when chronically exposed to specific occupational contaminants.[2]

Early detection of Chronic Obstructive Pulmonary Disease (COPD) is essential because patients often experience severe pulmonary function impairment by the time of diagnosis and biomarkers may be a useful tool for early diagnosis and prediction of COPD progression. The present-day studies have been increasingly focusing on the relevance of trace elements as age related bio-markers in COPD. Copper and zinc are two such essential micronutrients showing great promise as COPD markers as Cu/Zn ratio has been reported many times to be increasingly associated with chronic inflammation in COPD progression. A high Cu/Zn score have been correlated with increased risk of heart diseases, heart failure, cancers, all-cause mortality and infectious diseases such as pneumonia.[3] Cu/Zn ratio has also been reported as being linked to inducing inflammation in the body via oxidative stress and it is widely recognized that oxidative stress plays a crucial role in the pathogenic process underlying chronic obstructive pulmonary disease. Hence, the primary objective of this study was to conduct a preliminary yet comprehensive examination of Cu/Zn ratio and their association with overall stress in the body in COPD samples obtained from a regional population in mid Kerala. This study attempts to lay the groundwork for future studies addressing occupational exposure to trace elements and its effect in inducing oxidative stress in COPD. Understanding micronutrient levels and their dysregulation could also help in designing better therapeutic interventions in COPD treatment leading to stress mitigation and better quality of life in COPD patients.

METHODS

This study was a comparative cross-sectional study carried out at a tertiary care hospital, in central Kerala. The study was in compliance with the Institutional Ethics Committee and the Helsinki Declaration of 1975, revised in 2000. Written informed consent was taken from all participants. Blood samples were collected from COPD and healthy control participants that visited the tertiary care hospital during 2020-2022. All the participants were in the age group 40-75years. Mean age of COPD patients was 67 and that of the healthy controls was 60.5. Demographic details of the participants are listed in Table 1.

Table 1: Demographic features of the study participants.

Variable	Healthy control	COPD	P value
Age (years)	60.5±0.9	67±4.3	-
Geography	Mid-Kerala states of Thrissur, Malappuram and Palakkad		-
Male to female ratio	85%		-
CRP	0.3±0.01	3.22±1.22	<0.05

The study consisted of two groups, namely COPD and healthy controls; each group comprising of 20 samples each. COPD patients were diagnosed on the basis of spirometry showing post bronchodilator FEV1/FVC ratio less than 70% with a post bronchodilator reversibility less than 12% or patients who were already on treatment for COPD from a pulmonologist. The samples were also categorized strictly based on GOLD guidelines and biochemical parameters such as CRP levels.

Patients not willing to participate and immunocompromised patients like those with HIV or immunosuppressants were excluded from the study.

About 3 mL of blood was collected by venipuncture in heparinised vacutainer. The samples were centrifuged at 2800 rpm for 10 minutes and the plasma separated. The separated plasma samples were used for further biochemical analysis.

Heavy metal analysis

Plasma sample preparation for analysis of copper, zinc levels were performed by wet mineralization. Each sample was mineralized in concentrated nitric acid and the content of the trace elements in the resulting acid solutions were determined by atomic absorption spectrometry using (AAS) Perkin Elmer PinAAcle 900H atomic absorbtion spectrometer.[4]

Total oxidant status

Total oxidant status (TOS) in COPD versus healthy control blood samples was determined using the ferric-xylenol orange (FOX1) assay, which provides an indirect measure of total peroxide content. The assay calibration used hydrogen peroxide as the standard, measured at 560 nm. The results were expressed in mEq µmol hydrogen peroxide per L.[5]

Total anti-oxidant status

Total antioxidant status (TAS), a measure of the body's ability to fight oxidants, was determined using the Cayman chemical antioxidant assay kit in accordance with the standardised kit procedure. The assay is based on the ability of antioxidants in the sample to block the

oxidation of ABTS to ABTS+ by metmyoglobin, as detected at 405 nm with Trolox as a reference.

Oxidative stress index

The oxidative stress index was calculated as the ratio of TOS to TAS represented in arbitrary units.

The OSI is calculated as TOS (mEq µmol hydrogen peroxide/L) divided by TAS (µmol Trolox equivalent/L).

Statistical analysis

The results were expressed in Mean±SEM. Statistical analysis of the oxidative stress parameters were carried out in SPSS version 25 and the plots were constructed using R software. Analysis of biochemical parameters in COPD with respect to healthy controls were performed by Student's t-test. The Cu/Zn ratio was correlated with oxidative stress index using Pearson's correlation. A p-value < 0.05 was considered as significant.

RESULTS

Plasma levels of copper, zinc were analysed in COPD samples and compared with the healthy controls. The results of the trace elements expressed in mg/L are represented in the Table 2.

Table 2: Trace mineral composition in COPD samples with respect to healthy controls.

Trace minerals	COPD	Healthy controls	P value
Copper	1.894±0.25	1.175±0.24	0.00078
Zinc	0.280±0.007	0.283±0.09	0.9681
Cu/Zn	7.23±2.32	4.55±1.67	0.1352

Plasma copper levels were significantly increased in COPD patients. Zinc (Zn) plays a key role in many processes, such as DNA and RNA synthesis, energy metabolism, and in the regulation of the immune system. Plasma zinc levels shows a decrease in trend significant difference in COPD with respect to controls. Cu/Zn ratio has been implicated with increased risk of severity in chronic obstructive pulmonary disease studies which was corroborated by our findings, wherein a significant increase in Cu/Zn ratio was observed in COPD with respect to healthy controls (Table 2).

Oxidative stress index

OSI is a marker of redox imbalance in the human system. It represents the ratio of TOS to TAS. A higher OSI may indicate a redox imbalance, where pro-oxidant levels exceed antioxidant levels, which is typically observed in pathophysiological circumstances. Table 3 summarises the mean value of the TOS, TAS, and OSI data from the current investigation.

Table 3: Summarises the mean value of the TOS, TAS, and OSI data from the current investigation.

	Control	COPD
TOS (µmol/L)	9.84±2.96	9.70±4.85
TAS (mmol/L)	3.61±0.2711	1.7±0.269
OSI (TOS/TAS)	2.72±0.84	5.67±4.28

The correlation between Cu/Zn ratio and oxidative stress index showed a positive correlation, with a regression coefficient of 0.7 as shown in Figure 1. This indicates that with an increase in copper to zinc ratio indicative of increased severity in COPD, there is an increase in oxidative stress in the system.

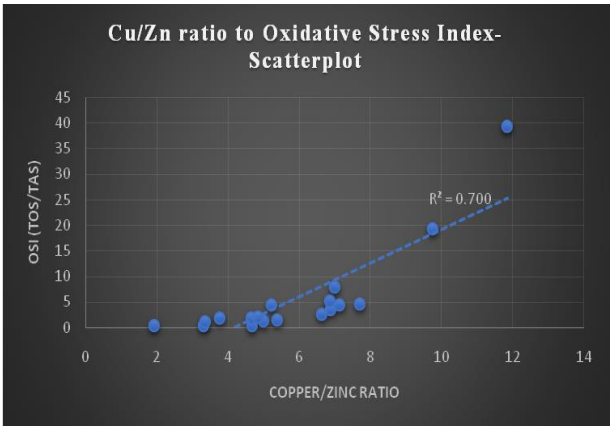

Figure 1: Correlation between Cu/Zn ratio and oxidative stress index.

DISCUSSION

COPD is a multifaceted respiratory condition with systemic manifestations such as oxidative stress. In the present study, we examined Cu/Zn ratio and their association with oxidative stress index in COPD, by keeping healthy controls as a baseline reference.

When comparing COPD to healthy controls, our data demonstrated a significant increase in copper levels. There have been many previous reports of high levels of copper inducing oxidative stress through Fenton type chemistry by producing highly damaging hydroxyl radicals leading to high lipid peroxidation in COPD.[6,7] Elevated copper levels may also increase the risk for COPD by fostering inflammation through its close link to ceruloplasmin, a copper binding acute phase reactant, significantly elevated during inflammation.[8] Previous studies have reported the toxic effects of increased copper levels in COPD, wherein it amplifies oxidative stress and induces lung fibroblast activation leading to pulmonary fibrosis.[9]

Along with copper, we also studied zinc levels which showed a decreasing trend in COPD with respect to healthy controls. This observation corroborates previous findings, as zinc has been previously reported for its

antagonistic effects on copper toxicity and low levels of zinc has been reported in COPD patients with recurrent exacerbations, that showed a marked improvement upon nutritional zinc supplementation. This could be attributed to the anti-inflammatory, anti-oxidant and immunomodulatory properties of zinc.[10-12]

Our study further observed a higher Cu/Zn ratio in COPD which in turn showed a positive correlation to oxidative stress index, that is in line with the findings of previous studies, that have reported higher Cu/Zn ratios to be associated with higher oxidative stress and lower oxygen saturation.[13] A higher Cu/Zn ratio also suggests a dysregulated copper and zinc mobilization and redistribution, which induces pro-inflammatory cytokines such as IL-1 leading to acute phase reaction and chronic inflammation in inflammatory pathophysiologies.[14] There have been previous reports that age-related inflammatory conditions is in general signalled by decreased Zn and/or increased Cu in the plasma which corroborates with our study wherein COPD is influenced both by aging and persistent inflammation.[15]

In a clinical perspective, a number of reports highlight the potential of the Cu/Zn ratio as a prognostic indicator for COPD progression and severity. These studies contribute to a growing body of evidence supporting the utility of the Cu/Zn ratio as a biomarker in the assessment of oxidative stress dynamics in COPD patients.[16,17] Moreover, monitoring the Cu/Zn ratio in COPD patients could help in restoring a balanced Cu/Zn ratio through supplementation leading to better therapeutic management of the disease through stress mitigation. Nevertheless, the present work has its limitations such as a small sample size and short duration of the study. The biological variability due to age, race, sex and genetic selection were also not taken into account. Further research is needed to validate Cu/Zn as a prognostic biomarker and efforts could be taken to better characterise subjects under study.

CONCLUSION

This study conducted in a tertiary care hospital in Kerala holds significance as it showed a positive association between Cu/Zn ratioand oxidative stress index in COPD. The study observed higher levels of copper and lower levels of zinc and thereby a higher Cu/Zn ratio in COPD with respect to healthy controls. The findings of the study supports existing literature proposing the Cu/Zn ratio as a potential prognostic indicator for COPD progression and severity, offering insights into the dynamic nature of oxidative stress in COPD patients.

In the clinical perspective, monitoring the Cu/Zn ratio in COPD patients may guide therapeutic interventions aimed at restoring the micronutrient balance through targeted supplementation, leading to stress mitigation and enhanced therapeutic management of the disease.

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