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Micronutrients Imbalance in Tuberculosis: A Comparative Study of Zinc and Copper Levels in Multidrug Resistant and Drug-Sensitive Pulmonary Tuberculosis

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ABSTRACT

OBJECTIVE: To compare serum copper and zinc levels, along with and Cu/Zn ratio, between individuals who have multi-drug resistant pulmonary tuberculosis (TB-MDR) and those with drug-sensitive Tuberculosis (TB-DS).

METHODOLOGY: Confirmed cases of TB- DS and TB-MDR were compared in a cross-sectional study. Venous blood samples were collected under aseptic conditions, copper & zinc levels were measured in serum by atomic absorption spectrophotometry. The data was analyzed using SPSS version 22. Normality of the data was checked using Kolmogorov-Smirnov test. Serum Cu and Zn levels in the TB-DS and TB-MDR comparison was done by using independent sample t-tests.

RESULTS: A statistically significant decrease ($p < 0.001$) was observed in serum zinc levels in Group B (TB-MDR, 65 ± 28 µg/dL) compared to Group A (TB-DS, 89 ± 30 µg/dL). The Cu/Zn ratio was increased in group B (1.09) compared to group A (0.71). There was no difference between copper levels among the two groups(p=0.08,71±23µg/dL vs 63±23µg/dL).

CONCLUSION: Patients with TB- MDR exhibited significantly lower zinc levels and a higher Cu/Zn ratio than drug-sensitive TB patients, suggesting impaired antioxidant status and increased inflammatory burden.

Keywords: Copper, Zinc, Pulmonary Tuberculosis, multidrug resistant Tuberculosis, Drug-Sensitive Tuberculosis, Trace Elements

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INTRODUCTION

Tuberculosis (TB) has surpassed HIV/AIDS as the leading cause of death from a single infectious disease worldwide and is ranked among the top ten causes of death globally.[1] An estimated 10.6 million people suffered tuberculosis in 2021, compared with 10.1 million in 2020, and 1.6 million people died from tuberculosis in 2021 (including 187000 individuals with HIV), compared with 1.5 million in 2020 (including 214000 individuals with HIV). In addition, the incidence rate of tuberculosis increased by 3.6% in 2021 relative to 2020, suggesting a reversal from the trend of nearly 2% decrease per year during the past two decades.[2] Over 10 million new cases of TB were recorded worldwide by the World Health Organization (WHO) in 2023, and almost 500,000 of those cases were resistant to rifampicin, the most potent first line anti-TB medication, making them TB-MDR.[3]

Pakistan is among the top high burden countries globally, ranking 5th for TB incidence, with an estimated hundreds of thousands of cases annually and a substantial proportion attributed to TB- MDR. Recent regional data indicate that TB-MDR[4] prevalence remains alarmingly high, reaching up to 18.8% in Pakistani population, especially among previously treated patients.[5] Across Asia and other low and middle-income regions, inadequate treatment adherence, delayed diagnosis, and inappropriate antibiotic use continue to drive the emergence of drug-resistant strains.[6] The nutritional state of the host has a significant impact on the course and outcome of TB. The control of immunological function depends on micronutrients, especially trace elements like copper (Cu) and zinc (Zn). Copper plays an important role in the control of oxidative stress and the action of various enzymes essential to immune defense, whereas zinc is essential for the growth and operation of T-lymphocytes as well as the preservation of epithelial barriers.[7,8]

Research shows that the levels of copper & zinc are altered in TB, likely deficiency of zinc has been shown to altered immunity causing high risk of disease.[9] Nevertheless, increased copper

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levels were seen in certain infective conditions, which shows their role in acute phase reactions.[10] Moreover, the Cu/Zn ratio is progressively documented as an indicator of inflammation and oxidative stress.[11] It is an important marker for evaluation , progression and prognosis of oxidative stress in infectious diseases.[12] Patients having TB-MDR often have prolonged sickness, different drugs therapies, and leading to severe metabolic irregularities, in which the micronutrient status is being disturbed.[13]

Although several studies have reported the burden and risk factors of TB-MDR, there is a relative paucity of data directly comparing biochemical and nutritional biomarkers like trace elements between TB-MDR and TB- DS patients within regional populations. This represents a critical knowledge gap, particularly given that host nutritional and micronutrient status may influence immune response, disease progression, and treatment outcomes. Furthermore, global and regional analyses highlight that TB-MDR disproportionately affects populations in low and middle-socioeconomic settings, including South Asia, yet context specific biological differences between TB-MDR and TB- DS patients remain underexplored.[14] Keeping in view all these inconsistencies and the therapeutic implications, this present research compared the serum Cu & Zn levels of persons having TB-MDR and TB-DS.

METHODOLOGY

This cross sectional study was conducted in the Hayatabad Medical Complex Peshawar (HMC) and Programmatic Management of Drug-Resistant Tuberculosis (PMDT) unit LRH, for infectious diseases. The ethical approval was granted Khyber Medical University Ethical Committee (Approval No. DIR/KMU/EB-CZ000684). A total of 110 patients with proven pulmonary tuberculosis between 18 to 55 years of age were enrolled. The sample size was calculated with the help of Open Epi software®. Assuming confidence interval of 80%, a total of 55 participants were required to conduct this study.[15]

Study Population: Two groups were formed from the cohort: i) TB-DS had drug-sensitive pulmonary TB. ii) TB-MDR: Individuals with molecular line probe assay and drug susceptibility testing (DST) confirmed multi-drug-resistant TB. Sputum smear microscopy and GeneXpert tests were used to confirm the diagnosis.

Inclusion Criteria: (1) Confirmed pulmonary TB (either DS or MDR). (2) Newly diagnosed with no prior anti-TB therapy in the past 6 months. (3) Willingness to provide informed consent. (4) Either gender.

Exclusion Criteria: (1) Patients with HIV, liver or renal dysfunction. (2) Pregnant or lactating women. (3) History of copper or zinc supplementation in the past 3 months.

Data collection: Self-administered questionnaires were provided to gather demographic data. Anthropometric indices were also taken and based upon this BMI was calculated through standard protocol.

Sample Collection and Processing: Five milliliters of venous blood were drawn using trace element free vacutainer in an aseptic setting. After centrifugation for 10 minutes at 3000 rpm, the serum was kept at -20°C. The samples were prepared at Khyber Medical University Peshawar's Institute of Basic Medical Sciences.

Biochemical Analysis: Serum Cu & Zn concentrations were determined using chemistry analyzer following standardized protocols using colorimetric method with Dibrom-PAESA for copper and 5-B-PAPS for zinc. All assays were performed in duplicate and internal quality control samples were run concurrently. The data was analyzed using SPSS version 22. Normality of the data was checked using Kolmogorov-Smirnov test. Serum Cu and Zn levels in the TB-DS and TB-MDR comparison was done by using independent sample t-tests.

RESULTS

A statistically significant difference was observed in mean age, BMI and height among the study groups. BMI was significantly lower in TB-MDR compared to TB-DS (16.6 ± 3.1 kg/m2 vs 18.9 ± 3.6kg/m2) as shown in table 1. Figure 1 shows the gender distribution among TB-DS and TB-MDR.

Participant's Characteristics at baseline:

Table I: Baseline demographic and anthropometric characteristics of TB-DS and TB-MDR patients

Parameters	(TB-DS) Mean ± SD (n=55)	(TB-MDR) Mean± SD (n=55)	p-value
Height (ft. inches) either inches or feet or cm or m	5.5± 0.4	5.3 ± 0.3	**<0.01
Weight (kg)	46.9 ± 8	48.8±12.4	0.19
BMI (kg/m2)	18.9 ± 3.6	16.6 ± 3.1	**<0.01
Age	26 ± 10	32 ±13	*0.008

Significantly different (*P<0.05, **P<0.01)

In drug sensitive tuberculosis group, 18 patients were males and 37 were females whereas, in multi-drug-resistant tuberculosis group 27 were males and 28 were females.

Table 2 shows the clinical characteristics of the participants. Majority of the participants had a previous history of TB (64.5%). Line probe assay for TB was done among 50% of the

pa **Figure I: Gender wise distribution of the participants** tie

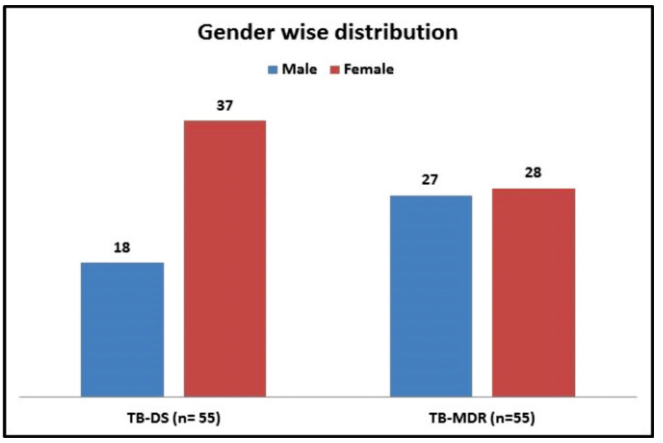

Table 2: Clinical findings in DS and MDR groups

TB history n=110(100%)	
No history of TB	39(35.5%)
History of TB	71(64.5%)
Sputum smear	
Negative	----
Positive	110 (100%)
Culture	
No	55(50%)
Yes	55(50%)
Line probe assay done	
No	54 (49%)
Yes	56(50%)
Gene Xpert done	
No	-----
Yes	110(100%)
Co-morbidity	
None	93(84%)
Diabetes	9(8%)
Blood pressure	6(5%)
Hepatitis	1(.9%)

Table 3: Levels of Copper, Zinc & Cu/Zn ratio comparison among the groups

Parameters	Group A (TB-DS)	Group B (TB-MDR)	P value	Cu/Zn ratio
Zinc(µg/dL)	89 ± 30	65 ± 28	< 0.001	
Copper(µg/dL)	63 ± 23	71 ± 23	0.068	
Cu/Zn ratio	0.71	1.09	-----	↑ in TB

significance (*P<0.05, **P<0.001)

A significant decrease in serum zinc levels was observed in TB-MDR as compared to TB-DS (p. < 0.001, 65 ± 28 µg/dL vs 89 ± 30 µg/dL) respectively. There was no significant difference between Cu levels among the two groups (p=0.08, 71±23 µg/dL vs 63±23 µg/dL). The Cu/Zn ratio was higher in the TB-MDR group (1.09) compared to the TB-DS group (0.71). This suggests a relative Zn deficiency and Cu excess in TB-MDR patients, possibly linked to inflammation or altered micronutrient status.

DISCUSSION

This study highlighted significant differences in serum trace element levels between TB-DS and TB-MDR patients. Serum zinc levels were markedly reduced in both groups, with a more pronounced deficiency observed in TB-MDR patients. This is consistent with earlier reports indicating zinc depletion in chronic infectious diseases due to increased metabolic demand, poor intake, and redistribution during inflammation.[16,17] Zinc levels in MDR patients showed comparable findings in one study conducted at the Institute Pasteur of Côte d'Ivoire (IPCI) in Abidjan.[18] A study from Bangladesh (2016),[19] also found reduced zinc level in fifty TB-MDR patients compared to non-resistant patients.

In the present study, copper levels showed no statistically significant difference between the two groups. This finding is consistent with previous research that has reported similar patterns of unchanged or non-significant variations in serum copper concentrations among patients with different forms of tuberculosis. For instance, research by Edem et al. and Bahi et al. found that although serum copper levels tended to be elevated in TB patients due to the acute-phase response, the differences were not always statistically significant when comparing drug-sensitive and drug-resistant cases, possibly due to the wide individual variation and compensatory mechanisms regulating copper homeostasis during infection and inflammation.[18,20] The normal copper levels in our study might be because of use of drugs and supplements used for TB treatment & also diet variations by the participants compared to other geographical areas. Although some previous studies reported higher serum copper and lower zinc concentrations in TB patients.[21] They observed elevation in copper levels reflecting an intensified acute phase response and hepatic synthesis of ceruloplasmin, a copper-carrying protein often up regulated during infection.[22]

The Cu/Zn ratio was remarkably higher in the MDR-TB (1.09) compared with TB-DS (0.71), indicating a relative zinc deficiency or copper overload in patients with TB-MDR. This altered ratio is proposed as a potential biomarker for disease severity, oxidative stress, and immune dysfunction in TB. According to recent research, a greater Cu/Zn ratio in patients with MDR-TB may be a reflection of both chronic inflammation and poor nutritional condition. According to Escobedo-Monge et al. and Malavolta et al., low Cu/Zn ratio is associated with decreased immunity causing inflammation and infections, like tuberculosis.[11,12] These results propose that the Cu/Zn ratio may

play a dependable indicator for nutrient imbalances than their individual concentrations. The greater Cu/Zn ratio in TB-MDR group as compared to TB-DS group may show widespread inflammation and the degree of the disease progress. Studies have shown that an increased Cu/Zn ratio is linked with adverse clinical outcomes for various chronic diseases and cancers.[11] The present study revealed that the ratios of trace element, not individual concentrations should be taken into consideration when assessing micronutrient levels in the setting of infections.

Lower BMI observed in the TB- MDR group is an important consideration, as nutritional status is a well-established determinant of micronutrient homeostasis, including serum zinc levels. Zinc deficiency is strongly associated with protein-energy malnutrition and impaired immune function, both of which are common in tuberculosis patients. Therefore, the reduced zinc levels identified in TB- MDR patients may not solely reflect disease severity or drug resistance but could also be influenced by their poorer nutritional status. This highlights BMI as a potential confounder in the relationship between TB type and serum zinc levels. Previous studies have similarly reported that under-nutrition is highly prevalent among TB patients and is linked with altered trace element profiles, particularly reduced zinc concentrations and elevated Cu/Zn ratios.[23,24]

The fundamental cause in this scenario can be changed immune response and oxidative stress coping mechanism. The person's ability to combat Mycobacterium TB may be hindered by zinc deficiency, changing macrophage & T cell function. Meanwhile, if antioxidant systems are not balanced properly, then increase in the copper levels will deteriorate tissues by oxidative stress. These findings focus on the importance of using trace elements concentrations, especially copper and zinc, in TB patients as part of a more comprehensive nutritional and immunological evaluation from a clinical standpoint. Addressing the micronutrient deficiencies with supplements or dietary therapy may improve immunological response and improve treatment outcomes, especially in TB-MDR patients, although more research and interventional studies are required. Implementation of nutritional strategies in addition to conventional treatment regimens may help in boosting the immune response and lead to fast recovery of the patients. There were certain limitations of this study such as; nutrient consumption and eating habits were not controlled; a single time-point measurement restricted the interpretation of causality. Secondly, the cross-sectional design precluded casual inference. Thirdly, the sample size in this study was relatively small; larger multi-center studies are needed.

CONCLUSION

This study showed that MDR-TB was associated with alterations in trace element status compared to TB-DS, while no difference was observed in copper levels. However, TB-MDR patients have higher Cu/Zn ratio compared to TB-DS patients, indicating an association with impaired antioxidant status and increased inflammation. Further longitudinal studies are required to determine whether correction of micronutrient deficiencies improves treatment outcomes in TB-MDR patients.

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CONFLICT OF INTEREST

Author declared no conflict of interest

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AUTHORS CONTRIBUTIONS

HZ: Conceptualization, study design, data collection, manuscript drafting, critical revision of the manuscript, final approval of the version to be published, and accountability for all aspects of the work.

NS: Conceptualization, study design, data acquisition, data analysis, manuscript drafting, critical revision of the manuscript, final approval of the version to be published, and accountability for all aspects of the work.

MZ: Conceptualization, study design, data interpretation, manuscript drafting, critical revision of the manuscript, final approval of the version to be published, and accountability for all aspects of the work.

BI: Data collection, manuscript drafting, critical revision of the manuscript, final approval of the version to be published, and accountability for all aspects of the work.

KT: Supervision of the study, Conceptualization, study design, data collection, data analysis, manuscript drafting, supervision of the study, critical revision of the manuscript, final approval of the version to be published, and accountability for all aspects of the work.

DATA SHARING POLICY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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