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Significance of serum copper zinc ratio in smear negative pulmonary tuberculosis cases in Rohilkhand region

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ABSTRACT

Introduction: Tuberculosis being a highly contagious chronic pulmonary and systemic disease is explicit cause of morbidity and mortality worldwide. There is a direct relationship between zinc and copper in maturation of immunity, Cu/Zn ratio in serum serves to assess levels of oxidative stress in infectious diseases and thus could be used as a diagnostic tool in early diagnosis of smear negative tuberculosis.
Aim: To study and evaluate significance of serum Cu and Zn concentration and Cu/Zn ratio in smear negative pulmonary tuberculosis patients in comparison to apparently healthy controls.
Materials and Methods: Observational case control study was carried out on patients attending IPD and OPD, Department of Pulmonary Medicine, RMCH. Fifty cases of smear negative PTB patients(group A) was taken and compared with fifty age and sex matched apparently healthy controls(group B). Serum Cu and Zn were estimated in both groups by colorimetric method using EM Chem5Plus V2 Semi autoanalyzer.
Statistical analysis: Data is presented as mean ± SD. Comparison of serum levels of the parameters between the two groups was performed by Student's't' test. The p value <0.05 was considered as statistically significant.
Result: We found serum Zinc to be significantly lower in group I than group II(p<0.00), serum Copper & Cu/Zn ratio to be significantly higher in group I than II(p<0.00).
Conclusion: The results suggests that early estimation of serum Cu, Zn & Cu/Zn ratio serves as useful diagnostic marker for smear negative pulmonary TB cases though more extensive multicentric study will provide more insights.

1. Introduction

Tuberculosis is a highly contagious chronic pulmonary and systemic disease caused by *Mycobacterium tuberculosis.* It is an important cause of morbidity and mortality worldwide. According to WHO, it has resulted in 1.8 million deaths in 2016 alone.[1] India is the country with the highest burden of tuberculosis. The incidence rate for India is 2.79 million cases in which Uttar Pradesh accounts to 217,800,000 cases in 2016.[2]

There is a direct relationship between micronutrients and immune system which has been reported in various studies. Among these micronutrients, Zinc and Copper have crucial role in the proliferation and differentiation of cells of innate and acquired immunity.[3]

Zinc is an essential trace element with diverse functions such as maintaining immunological integrity, cellular immunity and anti-oxidant activity. Its deficiency affects

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host defences in variety of ways primarily because of decreased phagocytosis due to reduction in number of circulating T-cells and reduced tuberculin reactivity.

Various studies conducted on active pulmonary tuberculosis patients had shown significant decrease in plasma zinc levels in comparison to healthy controls irrespective of their nutritional status. Thus plasma zinc status of an individual can be used as a marker for monitoring the severity of disease and response to ATT therapy.

Copper is also an important trace element involved in the metabolism of several key enzymes involved in oxidative and reductive reactions. It is important micronutrients for *Mycobacterium* and in optimum concentration it is require for *M.tuberculosis* viability by protecting it from activated macrophages that generates oxidative bursts.

Cu/Zn ratio serves to be an important indicator to assess the level of oxidative stress in case of infectious disease[4,5] and was found to be significantly higher in the serum of tuberculosis patients in several studies.[6–10] Though various studies has been conducted worldwide regarding the role of serum Cu/Zn ration in active pulmonary tuberculosis but very less studies were done from north Indian population and till date no studies was reported on smear negative pulmonary tuberculosis patients.[11–19]

Objective of this study is to find diagnostic efficacy of serum Cu/Zn ratio in smear negative pulmonary tuberculosis patients.

2. Materials and Methods

This observational case control study was conducted in the Dept. Of Biochemistry from February to April 2019 in collaboration with Dept. Of Pulmonary Medicine, Rohilkhand Medical College and Hospital, Bareilly, Uttar Pradesh. Prior approval was obtained from Institutional Ethical Committee with vide reference no. **I**EC/9/19/JAN.

50 cases of diagnosed smear negative pulmonary tuberculosis (Group A) were taken and compared with 50 age and gender matched apparently healthy controls. Sample size was calculated by using Power formula for quantitative case control study.

Formal consent was collected from all the patients included in the study in the form of written consent form after explaining aims, objectives, significance and privacy policy for the study in local language (Hindi).

2.1. Standardised diagnostic criteria for smear negative tuberculosis were

1. Detection by QuantiFERON-TB-Gold-In Tube Test.
2. PCR amplification of *Mycobacterium tuberculosis* DNA.
3. Compatible clinical history and radiological examination, in patients with a lymphocytic exudates and serum Adenosine deaminase > 24.0 IU/L.

2.2. The patients excluded from our study settings which were as follows

1. Other cases of Lung Pathologies.
2. COPD cases.
3. All subjects with previous anti-TB treatment, pregnant and lactating women.
4. Subjects using immunosuppressive drugs.
5. Patients with metabolic disease altering the levels of Copper and Zinc.
6. Subjects using micronutrients therapeutically including herbal preparations.
7. Chronic diseases like diabetes mellitus, hypertension etc.
8. Any liver, renal and muscular disorders.
9. Known HIV positive cases.

The following serum parameters were evaluated i.e. serum zinc, serum copper and serum Cu/Zn ratio. Quantizations of these parameters were done by EM chem5plus V2 semi auto analyzer. Serum zinc was assayed by using colorimetric end point Nitro-PAPS method, serum copper was assayed by using colorimetric end point Di-Br-PAESA method and Serum Cu/Zn ratio was calculated mathematically.

4 mL of blood was collected in serum separation tube (SST) after 10-12hrs of fasting by venipuncture under aseptic condition and employing tourniquet for as short as possible. Modified OSHA (Occupational Safety and Health Administration, 1991)[20] guidelines were implemented in all steps of sample collection, processing and handling of bio-medical waste. Serum was separated after allowing the blood sample to stand for 30 min at 37^0C, then centrifuged at 2000 rpm for 10 min. The sample was processed for analysis immediately or was stored at -20^0C and was processed within 7 days. All the sample processing and analysis were performed in Central Biochemistry laboratory of our college. All the assessment protocols were standardized and calibrated prior to quantization of copper and zinc as per NCCLS (National Committee for Clinical Laboratory Standards)[21] guidelines.

2.3. Statistical analysis

Data was presented as mean ± standard deviation. Comparison of serum levels of the parameters between group A and B was performed by unpaired Student's 't' test. P value < 0.05 considered as statistically significant. ROC analysis was done for each parameter to obtain cut off value with maximum sensitivity and specificity to find out diagnostic efficacy of each parameters. Appropriate box plots showing mean ± SD, median were done by using SPSS version 20 software.

3. Results

The demographic distribution of our study population for the smear negative pulmonary tuberculosis group (Group A) and control group (Group B) is shown in Table 1.

The mean ± SD of serum zinc in Group A (73.798±18.57μg/dL) was found to be significantly lower than that of Group B (90.512±15.670μg/dL) with 'p' value <0.00 [Table 2]. Box plot demonstrating upper quartile, median, lower quartile along with maximum and minimum values are represented in Figure 1. ROC curve analysis shows best cut off value at 83.35μg/dL with 70.0% sensitivity and 62.0% specificity represented in Figure 2.

The mean±SD of serum copper in Group A(403.838±73.285μg/dL) was found to be significantly higher than Group B(116.218±21.629μg/dL) with 'p' value<0.00 [Table 3]. Box plot demonstrating upper quartile, median, lower quartile along with maximum and minimum values are represented in Figure-3. ROC curve analysis shows best cut off value at 228.75μg/dL with 100.0% sensitivity and 100.0% specificity represented in Figure 4.

The mean±SD of serum Copper/Zinc ratio in Group A(5.721±1.398) was found to be significantly higher than Group B(1.303±0.263) with 'p' value<0.00 [Table 4]. Box plot demonstrating upper quartile, median, lower quartile along with maximum and minimum values are represented in Figure 5. ROC curve analysis shows best cut off value at 2.83 with 100.0% sensitivity and 100.0% specificity represented in Figure 6.

Table 1: Demographic characteristic of study population

Parameters	Tuberculosis (Group A) (mean±SD)	Controls (Group B) (mean±SD)
Age(Yrs)	44.020 ± 12.266	44.000 ± 12.358
Mean age of Males	45.500 ± 12.580	45.640 ± 12.419
Mean age of Females	42.136 ± 11.873	42.045 ±12.132

Table 2: Serum zinc concentration in smear negative tuberculosis and control group

Tuberculosis Group (Group A)		Control Group (Group B)	
Mean ± SD	Range	Mean ± SD	Range
73.798 ± 18.572 μg/dL *Vs Control 'p' <0.00	40.2 – 105.5 μg/Dl	90.512 ± 15.670 μg/dL	65.5– 123.0 μg/dL

Figure 1 shows Box Plot demonstrating distribution of upper quartile, median and lower quartile of serum Zinc (μg/dL) in smear negative pulmonary tuberculosis and control group. Mean ± SD of smear negative pulmonary tuberculosis group was 73.798 ± 18.572 μg/dL median was 72.6μg/dL, mean ± SD of control group was 90.512 ± 15.670μg/dL, median for control group was 89. 5μg/dL.

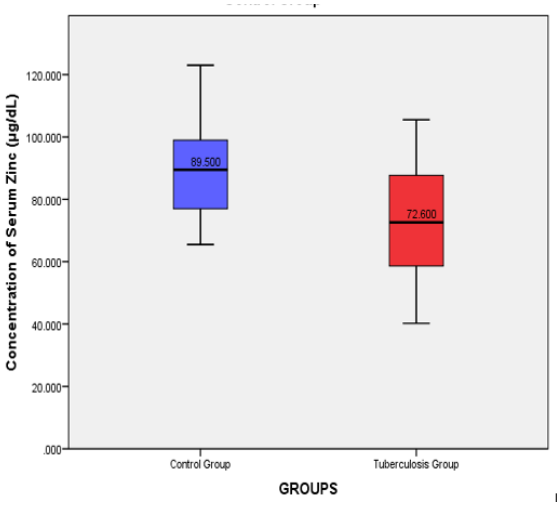

Fig. 1: Boxplot demonstrating upper quartile, median and lower quartile along with max and min values obtained for serum zinc concentration (μg/dL) in TB and control group

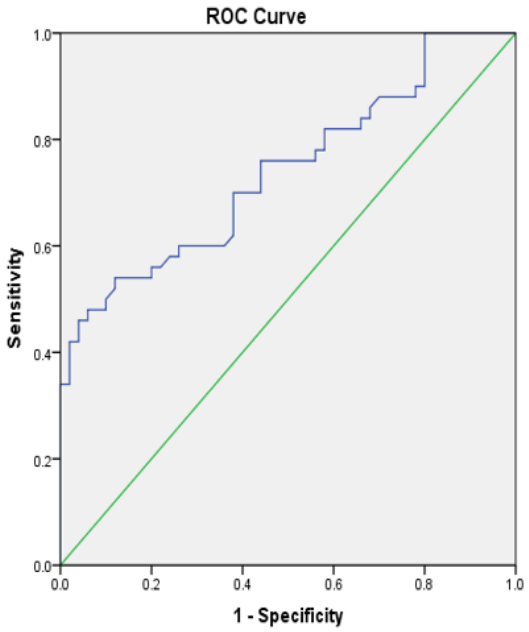

Fig. 2: ROC curve analysis of serum zinc concentration (μg/dL) in smear negative tuberculosis and control group

Figure 2 shows ROC curve analysis of Serum Zinc in smear negative pulmonary tuberculosis and control group. Area under curve was 0.740; standard error as per Hanley & McNeil was 0.049; 'p' value <0.00; Sensitivity was 70.0%, Specificity was 62.0% with best cut off value 83.35 μg/dL.

Table 3: Serum copper concentration in smear negative tuberculosis and control group

Tuberculosis Group (Group A)		Control Group (Group B)	
Mean ± SD	**Range**	**Mean ± SD**	**Range**
403.838 ± 73.285 μg/dL *Vs Control 'p' <0.00	298.5 – 542.5 μg/Dl	116.218 ± 21.629 μg/dL	79.5– 159.0 μg/dL

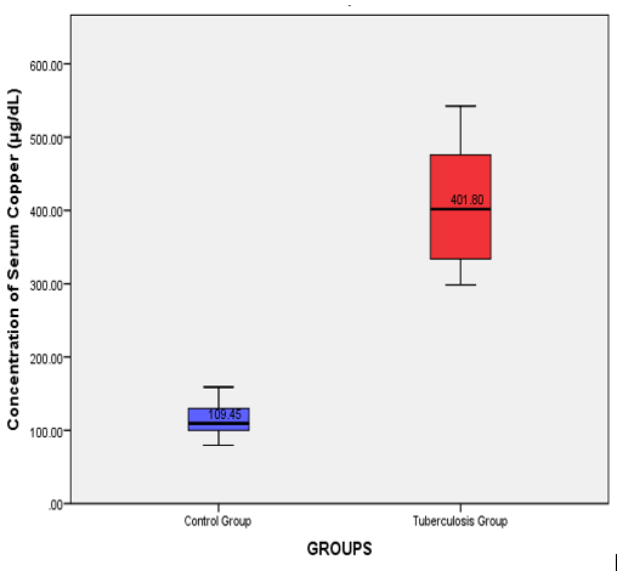

Fig. 3: Boxplot demonstrating upper, quartile, median and lower quartile along with max and min values obtained for serum copper concentration (μg/dL) in TB and control group.

Fig-3 shows Box Plot demonstrating distribution of upper quartile, median and lower quartile of serum Copper (μg/dL) in smear negative pulmonary tuberculosis and control group. Mean ± SD of smear negative pulmonary tuberculosis group was 403.838 ± 73.285 μg/dL, median was 401.80 μg/dL, mean ± SD of control group was 116.218 ± 21.629 μg/dL, median for control group was 109.45 μg/dL.

Figure 4 shows ROC curve analysis of Serum Copper in smear negative pulmonary tuberculosis and control group. Area under curve was 1.00; standard error as per Hanley & McNeil was 0.00; 'p' value <0.00; Sensitivity was 100.0%, Specificity was 100.0% with best cut off value 228.75 μg/dL.

Figure 5 shows Box Plot demonstrating distribution of upper quartile, median and lower quartile of serum Copper/Zinc ratio in smear negative pulmonary tuberculosis and control group. Mean ± SD of smear negative pulmonary tuberculosis group was 5.721 ± 1.398, median was 5.46, mean ± SD of control group was 1.303 ± 0.263, median for control group was 1.26.

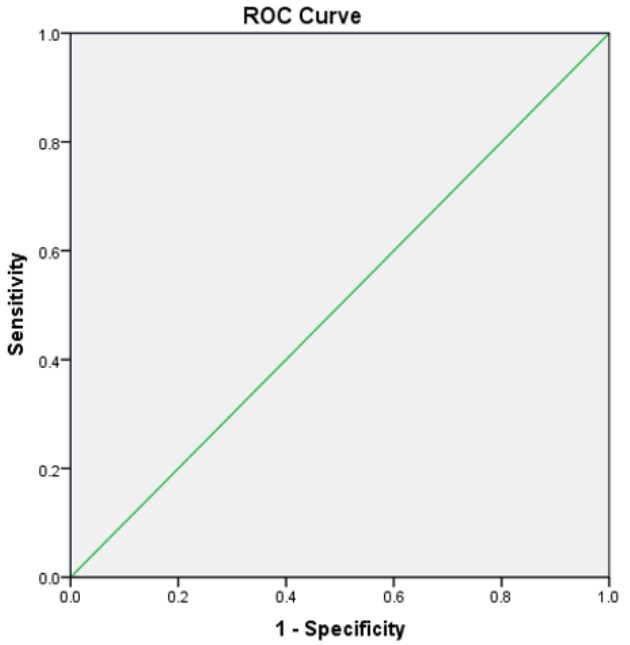

Fig. 4: ROC curve analysis of serum copper contrition (μg/dL) in smear negative tuberculosis and control group.

Table 4: Serum copper/zinc ratio in smear negative tuberculosis and control group

Tuberculosis Group		Control Group	
Mean ± SD	**Range**	**Mean ± SD**	**Range**
5.721 ± 1.398 *Vs Control 'p' <0.00	3.644 – 8.9	1.303± 0.263	0.998– 2.021

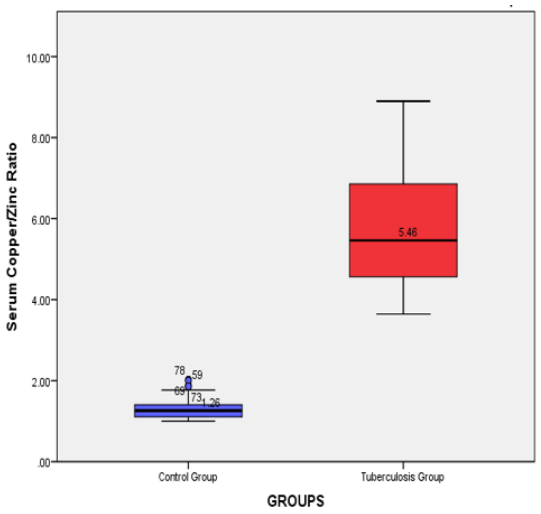

Fig. 5: Boxplot demonstrating upper quartile, median and lower quartile along with max and min values obtained for serum CulZn ratio in TB and control group

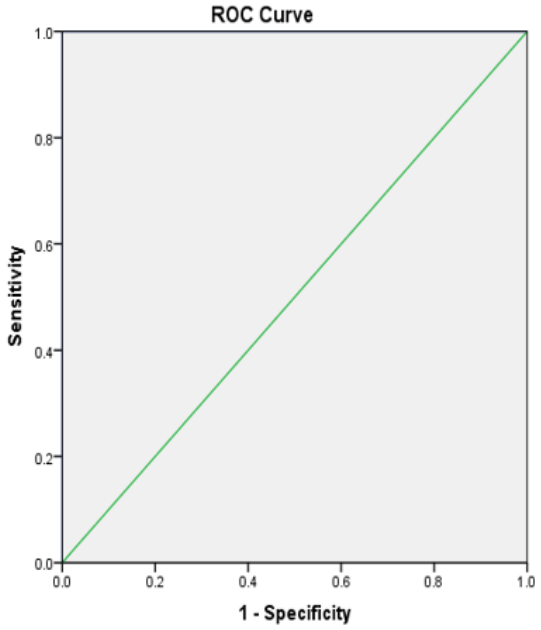

Fig. 6: ROC curve analysis of serum copper/zinc ratio in smear negative tuberculosis and control group

Figure 6 shows ROC curve analysis of Serum Copper/Zinc ratio in smear negative pulmonary tuberculosis and control group. Area under curve was 1.00; standard error as per Hanley & McNeil was 0.00; 'p' value <0.00; Sensitivity was 100.0%, Specificity was 100.0% with best cut off value 2.83.

4. Discussion

The present study was conducted on 50 smear negative pulmonary tuberculosis patients and 50 healthy controls.

In this study, the mean ± standard deviation of serum Zn levels in smear negative pulmonary tuberculosis patients was significantly lower i.e. 73.798 ± 18.572 μg/dl in comparison to control group whose value was 90.512 ± 15.670 μg/dl with 'p' value < 0.00 when compared by student's 't' test.

Similar study was conducted by Zahra Sepehri et al[7] in pulmonary tuberculosis patients. They reported that mean serum Zn concentration in pulmonary tuberculosis patients was significantly lower in contrast to control group. Karthik et al[8] also conducted a study on serum concentration of Zn in tuberculosis infected patients. Similarly they also observed a low Zn concentration in serum of pulmonary tuberculosis patients. Low serum Zn concentrations in tuberculosis patients could be due to redistribution of Zn from plasma to other tissues or reduction of hepatic production of Zn carrier protein alpha-2 macro globulin and increase in the production of metallothionein, a protein that transports Zn to liver.[22]

In this study, the mean ± standard deviation of serum Cu in smear negative pulmonary tuberculosis patients was significantly higher i.e. 403.838 ± 73.285 μg/dl in comparison to control group whose value was 116.218 ± 21.629 μg/dl with 'p' value < 0.00 when compared by student's 't' test.

Karthik G et al[8] and Mohammad Alizadehet et al[10] also reported the similar findings in their study.

Another study reveals that rise in serum Cu level during infection or in inflammatory stress condition was due to release of IL-1 which stimulates the production of ceruloplasmin and increases Cu level.[21] Another explanation for association of high Cu concentration in tuberculosis patients is that decrease in Zn level which occurs in tuberculosis patients, prevents entrance of Cu in tissues and this leads to elevation of serum level of Cu.[23,24]

In our study, Cu/Zn ratio was calculated, and we found that the mean ± SD of Cu/Zn ratio in smear negative pulmonary tb patients was significantly higher i.e. 5.721 ± 1.398 in comparison to control group whose value was 1.303 ± 0.263 with 'p' value < 0.00 when compared by students 't' test.

Our findings of Cu/Zn ratio were comparable with study of Karthik et al[8] who also found increase in Cu/Zn ratio in pulmonary tb patients. Mohammad Alizadeh et al[10] also observed initial significant increase in the ratio prior to treatment which was in agreement with our study.

We have also done ROC analysis of serum Cu, Zn and serum Cu/Zn ratio in our study which was not done in previous studies.

5. Conclusion

This study shows that serum level of Cu, Zn and Cu/Zn ratio in smear negative pulmonary tuberculosis patients were different when compared to healthy group. We observed low serum Zn concentration in contrast to high serum concentration of Cu and elevated serum Cu/Zn ratio in smear negative pulmonary tuberculosis patients.

Based on this result, it can be stated that imbalance of these trace elements have been associated with defective function of immune mechanism in humans and thus plays a role in patho-physiology of tuberculosis.

Thus it can be concluded that estimation of serum Cu, Zn, Cu/Zn ratio can serve as a diagnostic tool for tuberculosis in as early stage as smear negative pulmonary tuberculosis. Also these values act as a prognostic marker for monitoring the response to anti-tubercular therapy (ATT).

One of the limitations of our study is that, this study was conducted in a single region and sample size was less. So, much larger multi centric study could provide better insights.

6. Conflict of Interest

None.

7. Source of Funding

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