

Comparative study of serum copper, zinc, Cu/Zn ratio in pulmonary tuberculosis patients with normal subjects

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Abstract

Introduction: Tuberculosis (TB) is an important global health problem. It has more impact on developing and underdeveloped countries. The impact of tuberculosis can be seen by the fact that in 1993, the world Health Organization (WHO) declared tuberculosis a Global Emergency. WHO estimated that tuberculosis infects about one-third of world's population. Every year, 8 million people contract tuberculosis, of which 95% are in the developing world.¹

Aims and Objectives: To estimate and compare serum Copper, Zinc levels, and Cu/Zn ratio in newly detected pulmonary tuberculosis patients with healthy volunteers. To estimate Total Protein and Albumin levels to rule out hypoproteinemia.

Materials and Methods: The study included 40 newly detected sputum positive pulmonary tuberculosis patients and 40 healthy volunteers. Serum copper and zinc was estimated by colorimetric method using RA-50 semi autoanalyser. Total protein was estimated by biuret method and Albumin, by bromocresol green method using automated analyser.

Results and Conclusions: There was a significant increase in serum copper levels in pulmonary tuberculosis patients when compared to control group (P<0.0001). The mean Copper levels in pulmonary tuberculosis patients was 221.93 + 64.92 µg/dl and in controls, it was 137.83 + 26.71 µg/dl. There was a statistically significant decrease in the mean Zinc values in tuberculosis patients when compared to the control group (P<0.0001). The mean Zinc levels in tuberculosis patients was 170.97 + 34.99 µg/dl and in the controls it was 207.71 + 38.37 µg/dl. The Cu/Zn ratio in tuberculosis patients is 1.35 + 0.46 and in the controls it was 0.69 + 0.19. There was a statistically significant (P<0.0001). From the results of present study it can be concluded that estimation of serum copper, zinc, Cu/Zn ratio can have an auxillary value in the early diagnosis and in monitoring the prognosis of subjects with pulmonary tuberculosis, but further studies with larger group of patients is necessary.

Keywords: Pulmonary Tuberculosis, Copper, Zinc, Cu/Zn Ratio, Total Protein, Albumin.

Introduction

Tuberculosis (TB) is an important global health problem. It has more impact on developing and under developing countries. The Impact of tuberculosis can be seen by the fact that in 1993, The World Health Organization (WHO) declared tuberculosis is a global emergency. WHO estimated that tuberculosis infects about 1/3rd of world's population. Every year, 8 million people contact tuberculosis of which 95% are in the developing world.¹

It is estimated that between 2002 and 2020, tuberculosis will be affect nearly 1 million people and 35 million will be die of tuberculosis, if it is not controlled. Therefore control of Tuberculosis and better chemotherapy is still a challenge in the management of tuberculosis infection in the 21st century. The basic problem arises in early diagnosis of the disease and by the time of the patient comes to the treatment, the disease is already fully established.²

Many elements present in minute quantities in man are essential nutrition. Their presence was long overlooked and it has been only in recent years that analytical techniques capable of measuring such trace levels were developed. These trace elements perform functions in dispensable to the maintenance and growth of tissues.

The trace metals have been examined critically as a potential key factor in various diseases. The two important micro nutrients, namely Zinc and Copper play distinctive role in the pathophysiological process of Tuberculosis.³⁻⁵

Several studies have been done to study the influence of these individual risk factors on the pathophysiology of tuberculosis. However, very few studies have been done to study the correlation between these risk factors in pulmonary tuberculosis. This study for serum copper, zinc levels in patients of tuberculosis is being undertaken to see if changes of these parameters might be helpful in the early diagnosis of the disease leading to decreased morbidity and mortality by early treatment.

Materials and Methods

The study included 40 untreated newly detected sputum positive adult pulmonary tuberculosis patients. Patients were selected from the outpatient and inpatient departments of pulmonology and Medicine of JSS Medical College Hospital, Mysore. Clinical Diagnosis was based on history and Sputum Microscopy for AFB (Acid Fast Bacilli). The age of patients varied from 20-40 years. The cases were selected on the basis of simple

random sampling method. The study protocol was approved by the Institutional Ethical Committee and Informed consent was obtained from the subjects under study. Patients with metabolic diseases altering the levels of Zinc and Copper and other preexisting illness likely to affect the levels of Copper and Zinc metabolism are excluded. The results were compared with 40 randomly selected normal healthy individuals after obtaining due consent. The controls were age and sex matched with cases.

Under all aseptic precautions, about 3ml of venous blood was collected in gel tubes, allowed to clot and it was centrifuged at 4000 rpm for 5 minutes. The serum separated was then used for the estimation of parameters. The Investigations included fasting blood sugar, serum Copper, Zinc, total protein and albumin. The same was estimated in controls also.

Serum copper and zinc was estimated by colorimetric method using RA-50 semi auto analyzer. At pH 4.7, copper which is bound to ceruloplasmin, release by reducing agent. It can react with a specific color reagents 3,5-Di-Br-PAESA-4 (3, 5-dibromo-2 pyridylazo-) N-Ethyl-N-(3-Sulphopropyl) aniline to form a stable, colored chelate. The intensity of the color is directly proportional to the amount of copper in the sample.⁹

Zinc present in the sample is chelated by 5-Br-PAPS (2-(5-Bromo-2-pyridylazo) -5-(N-propyl-N-sulpho propyl amino) – phenol in the reagent. The formation of this complex is measured at the wavelength of 560 nm.¹⁰

T Protein and Albumin was estimated using the Randox, Daytona auto analyzer. T protein was estimated by Biuret method, end point using Erba Mannheim kit FBCER0057.¹¹ Albumin was estimated by Bromocresol green method using Randox kit AB380012. Statistical analysis was done using SPSS software version 16, student t test (two tailed, independent) to find the significance between cases and controls.

Results

The present study was conducted on 40 untreated adult pulmonary tuberculosis patients and 40 healthy controls. The cases and controls were age and sex matched. The age group was between 20-40 years. The mean age in tuberculosis patients was 34.4 ± 5.85 years and in controls, it was 34 ± 5.48 . The total protein, albumin A/G ratio in patients was 7.27 ± 0.92 g/dl, 3.61 ± 0.17 g/dl and 1.04 ± 0.26 g/dl respectively. In controls, it was 8.47 ± 0.43 g/dl, 4.43 ± 0.42 g/dl and 1.12 ± 0.21 g/dl respectively and were within normal limits in both group.

There was significant increase in serum copper levels in pulmonary tuberculosis patients when compared to the control group (221.93 ± 64.92 µg/dl and 137.83 ± 26.71 µg/dl respectively). There was significant

decrease in Zn in pulmonary tuberculosis patients when compared to controls, (170.97 ± 34.99 µg/dl and 207.7 ± 38.37 µg/dl respectively). The cu/Zn ratio was significantly higher in patients than control (1.35 ± 0.46 and 0.69 ± 0.19 respectively).

Table 1: Mean and SD of Serum Cu, Zn and Cu/Zn Ratio in the study group

	Mean + SD		p-value
	Tuberculosis	Controls	
Copper	221.93± 64.92	137.83± 26.71	<0.0001
Zinc	170.97± 34.99	207.71± 38.37	<0.0001
Cu/Zn	1.35 ± 0.46	0.69± 0.19	<0.0001

*p value < 0.05 (Statistically significant)

Discussion

The present study was conducted on 40 pulmonary tuberculosis patients and 40 healthy controls. Total protein, albumin was done to rule out hypoproteinemia. Both in patients and controls, the total protein level and albumin levels was within normal range and the criteria to rule out hypoproteinemia was met.

In the present study there was significant increase in copper levels in pulmonary tuberculosis (TB) patients than in controls. The mean copper level in PTB was 221.93 ± 64.92 µg/dl and in the controls it was 137.83 ± 26.71 µg/dl ($P < 0.0001$). Our findings on copper levels were comparable with previous study done by the Ciftici T.U. et al., who have found that there is significant increase in serum, copper levels in tuberculosis than in normal subjects.¹³

Reza B.M et al., have also shown in their study that serum copper levels were significantly higher in patients with active pulmonary tuberculosis than in healthy control.¹² Another study reveals that rise in serum copper level during infection or inflammatory stress conditions was due to the release of IL-1 which stimulate production of ceruloplasmin and increases copper level.^{14,19}

In the present study, there was significant decrease in Serum Zinc levels in the patients than in controls groups. The mean serum zinc levels in patients and controls was 170.97 ± 34.99 and 207.71 ± 38.37 respectively ($p < 0.0001$). Oluboya P O et al., in their study have pointed that pulmonary TB affects metabolism of trace elements like any other infection diseases. In their study, there was fall in plasma zinc level in the cases and was found to be statistically significant.¹⁴ Muthuraj M et al., in their study found significantly decrease in serum zinc in patients with PTB, irrespective of their HIV status. The possible for the low serum Zinc were considered to be nutritional factors enteropathy and acute phase reactant proteins.⁶

Low Zinc status in patients with inflammatory disease has been attributed to less of Zinc form

catabolized tissue and increased urinary excretion of Zn subsequent to its mobilization by IL-1. Low zinc are likely the result of re distribution of Zinc from plasma to other or reduction of hepatic production of the zinc carrier protein α -2 macro globulin and of a rise in the production of metallothionein.¹⁵

In our study, copper/Zinc (Cu/Zn) ratio was calculated and we found that there was significant increase in Cu/Zn ratio in tuberculosis patients than in the controls. Then mean Cu/Zn ratio in PTB patients and controls was 1.35 ± 0.46 0.69 ± 0.19 respectively, ($p < 0.0001$).

Our findings of Cu/Zn ratio were comparable with study done by the Ciftci T.U et al., who have found an initial increase in Cu/Zn ratio in patients prior to treatment and on follow up of same group after 2 months with treatment there was a significant decrease in Cu/Zn ratio and thus concluded saying that serum Zn level and Cu/Zn ratio could serve as a diagnostic tool to assess the beneficial effects of antituberculosis therapy.¹⁶

Mohan G et al., observed an initial significant increase in Cu/Zn ratio prior to treatment and there was significant decrease after anti tubercular therapy and have hypothesized that Cu/Zn ratio can be used as an important laboratory marker for the diagnosis of tuberculosis.¹⁷ Reza B.M., et al., in their study on children with PTB found a higher Cu/Zn ratio in patients than in controls, which was in agreement with our study.¹⁸

Our study showed increase in Cu levels and Cu/Zn ratio reflecting the ongoing inflammatory process. Decreased level of Zn could be due to pre-existing malnutrition and increased usage of Zn by the tuberculosis bacteria itself. Thus these parameters directly reflect the pathophysiological state of the disease process.

Conclusion

Based on the results of present study and data available from literature, it can be implicated that dietary deficiency of trace elements like copper and zinc have been associated with defective function of immune mechanisms in humans and hence this imbalance in these elements may be associated in the pathophysiological of infectious diseases like PTB. From the results of present study, it can be concluded that estimation of serum copper, Zinc, Cu/Zn ratio can have an auxiliary values in the early diagnosis and in monitoring the prognosis of subjects with pulmonary tuberculosis. On the other hand we can only speculate as to whether the serum copper Zinc and Cu/Zn ratio could serve as a diagnostic tool and to assess the beneficial effects of anti-tuberculosis therapy.

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