ORIGINAL ARTICLE

Evaluation of Copper, Zinc and Copper/Zinc Ratio in the Serum of Pulmonary Tuberculosis Children

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Abstract: Tuberculosis is considered as one of the most important infectious diseases. It results in changes in the serum level of many micronutrients. Copper and Zinc are two important micronutrients in the immune system and metabolism. In this study the serum levels of Copper and zinc are evaluated in the children suffering from pulmonary tuberculosis. Also the effects of anti tuberculosis therapy on the serum levels of these micronutrients have been studied. This two phases (cross-sectional, cohort) study was conducted in National Research Institute of Tuberculosis and Lung Disease (NRITLD) during 2003-2004. A total of 45 children were divided into three groups: 1- Children suffering from pulmonary tuberculosis 2- Children with malnutrition 3- Healthy children, considered as control group. The serum levels of Copper and Zinc were measured before stating treatment was initiated. In children with tuberculosis, serum Copper and Zinc levels were measured one and four months after therapy. Results demonstrated higher serum levels of Copper in children with tuberculosis as compared to the other two groups ($P<0.05$) and similar serum Zinc levels ($P=0.5$). Anti tuberculosis therapy results in a significant decrease in serum Copper levels. The zinc level in children with tuberculosis was significantly decreased and then increased during treatment. This study, in which the levels of Zinc and Copper have been studied in children suffering from pulmonary tuberculosis, is the first of such kind conducted in Iran.

Key words: Pulmonary tuberculosis, Children, Copper, Zinc, Malnutrition.

Introduction: Tuberculosis (TB) is considered as one of the most important infectious diseases in the world and its incidence is on rise. Annually Mycobacterium tuberculosis (MTB) causes diseases in about 1,300,000 children. The mortality rate of TB in children had been estimated about 450,000 per year. [1]

TB results changes in many clinical and paraclinical parameters in affected patients. Observing these changes, could help physicians to reach an accurate diagnosis and also could evaluate the response to the treatment. [2] TB in children is usually associated with malnutrition and loss of weight. Several studies have been shown that malnutrition in children results in decrease in the serum levels of many micronutrients and vitamins. [3] Among micronutrients, Copper (Cu) and Zinc (Zn), as two of the most vital micronutrients, have always under consideration.

Normally, the Copper enters the body orally. It is mainly transported in blood by binding to ceruloplasmin. Concentration of copper is highest in liver, brain, heart and kidney. Muscle contains a low level of copper, but because of its large mass, skeletal muscle contains almost 40% of all copper in the body. [4]

The function of copper and its role in various body tissues are known. It is present in the structure of many vital enzymes and co-enzymes. Similarly its role in internal antioxidants, mitochondrial energy production and melanin synthesis are clearly known. [5].

Similarly the role of zinc in the immune system, structure of enzymes and its effects in improving disease such as TB, pneumonia and diarrhea in children are apparent [7,8,9,10] Because of association of copper and zinc in many enzymes structures, and also the important role of them in the immune system most of the evaluations have considered both copper and zinc together.

Because of the limited data available on the relationship between nutritional status and TB and due to the increasing incidence of TB we decided to compare zinc and copper status in children with active pulmonary TB. The aim of this study was to evaluate and compare serum copper and zinc levels in children suffering from pulmonary TB with those of the healthy and malnourished children. In addition it also evaluated the effect of anti TB therapy on the serum level of these two micronutrients.

Methods and Materials: This two phases study was conducted in Masih daneshvari hospital (NRITLD) during 2003-2004. The understudy children (Aged between 5 month -14 yr. old) were divided into three groups (Phase 1: Cross-sectional design):

A. Children with pulmonary TB: It included 15 children that had smear positive pulmonary TB, in whom MTB was detected in smear and culture of gastric washing sample. Children with TB that had a weight percentile below 5 % and those with drug resistant TB were excluded.
B. Malnourished children: This group included 15 patients with weight percentile below 5%. They were considered as 'TB Free' by examining their gastric washing, chest X-ray, Tuberculin skin test and clinical findings. Nutrition therapy and vitamin supplementation was the choice treatment.
C. Control group: 15 healthy children in whom their clinical and laboratory investigations did not demonstrated TB, malnutrition or any other disease.

All those children with congenital diseases, primary or acquired immune deficiency and malabsorption states were excluded from the study.

Data including: age, sex, nationality, height and weight were recorded. Also 5cc of peripheral blood was collected from each child before the onset of therapy and sent to reference laboratory. Serum copper and zinc levels were measured by atomic absorption spectrophotometry method (Chemtech Analytical CTA 2000 AAS, USA) using a hollow cathode lamp at 214.1 nm. The instrument was calibrated with Chemlab Standard Solution obtained from the National Bureau of Standards (NBS, Washington DC, USA).

Standard anti TB therapy (Including four drugs: Isoniazid, Rifampin, Ethambutol, Pyrazinamide) was started for group A. In this group copper and zinc levels were measured after one and four months of onset of treatment. (Phase 2: cohort design)

Considering the normal distribution of results and number of samples, analysis was performed using ANOVA, Turkey, Wilcoxon and Friedman tests and P value < 0.05 was considered as significant. Analysis was done by SPSS v.11.5 software.

Results: A total of 45 children were evaluated in three groups. In each group there were 8 girls (53.2%) and 7 boys (46.7%). Mean age (All understudies children) was 7.7±3.9 year (Mean ± SD) with mean age in group A being 10.1±3.24 years, in group B being 8±3.5 years and in group C being 5.9±4.24 years. The youngest child was 5 month old and oldest was 14 yr. old. Serum Zinc and copper levels in each group are depicted in

	Group A	Group B	Group C	P value
Serum Zinc (mcg/dl)	71.74 ±11.48	72.50 ±11.48	76.88 ±08.89	0.55
Serumcopper (mcg/dl)	95.25 ±33.99	51.13 ±24.20	58.93 ±13.34	>0.001

Cu/Zn ratio was calculated in all groups. Comparing this ratio in different groups demonstrated a significant higher Cu/Zn ratio in TB patients than the other groups (P=0.002). However Cu/Zn ratio in the malnourished group show no significant difference compared to healthy children (P=0.85)

All patients suffering from pulmonary TB received the standard anti TB treatment. In this group serum copper and zinc levels were measured 1 and 4 months after onset of treatment. (Table No.2)

Table 2- Serum levels of copper and zinc during anti TB therapy (mcg/dl)

	1 month after therapy Mean ±SD	4 months after therapy Mean ±SD	P value
Copper	88.9±30.13	79.68±28.73	0.02
Zinc	52.81±18.13	73.20±17.26	0.01

Based on our results, we observed that both copper and zinc serum levels showed significant changes during anti TB treatment. Zinc decreased during initial month of therapy, increasing afterwards. Meanwhile serum copper level decreased throughout the treatment phase.

Discussion: The study of relation of various micronutrients in the body have been started years ago. Savenkov et al point out to the high level of copper in pleural fluid of TB patients in 1975 [11]. In the present study TB patients had a higher serum copper level than the two other groups. Also Cu/Zn ratio was higher in the first group as compared to the control group. For the same fact this study is in concordance [12,13]. Koyonagi et al. studied the serum levels of copper, zinc and selenium in patients suffering from pulmonary TB in 2004.[14] Their study showed that TB patients had higher serum copper level and Cu/Zn ratio as compared to normal individuals, while serum zinc level was lower as compared to control group.

The high copper concentration have been associated with an increase in the synthesis of the copper-binding protein ceruloplasmin, the enzymatic and oxidative properties of copper-ceruloplasmin complex possibly contributing to host defense [14,15]. The level of serum copper also increases in other infectious diseases. Sobol et al studied on the serum level of copper in neonates suffering from pneumonia in1995 [16]. They demonstrated that pneumonia patients had high serum levels of copper in the infection phase.

Researches have shown that before initiation of therapy in TB patients, their serum zinc levels are lower than normal individuals [17]. The low serum zinc level in these patients is due to several reasons, mainly, Redistribution of zinc to the liver, mediated by increased hepatic synthesis of metalothionein. On the other hand tuberculosis is very closely linked to malnutrition. It has been well known that malnutrition is a predisposing factor to hypozincemia.[18] In our study zinc serum level in TB patient was not significantly lower than the other groups. This difference could be results of our study design. In our study children suffering from both TB and malnutrition were excluded. A study which was conducted in Indonesia, researchers observed low serum zinc levels in only those TB patients that were malnourished [19]. Our results were compatible with the results of the above mentioned study.

Another reason for low serum zinc level in TB patient is usage of Zinc by MTB for its growth and reproducing [20]. Superoxidase desmotase (SOD) is one the major enzymes in human body cells and microorganisms that confronts free radicals [21]. The protective role of this metalloenzyme in many bacteria such as shigella and salmonella is clearly known [22,23]. In order to confront the free radicals released by immune system, MTB uses two types of SODs, Cu-Zn-SOD and Fe-SOD. Cu-Zn-SOD by producing a single peptide is presents on the outer layer of bacillus [24]. Multiple researches have shown the protective role of this type of SOD against the free radicals released by macrophages. However it should be noted that SOD and its level in MTB do not have any role in the pathogenicity of MTB [24,25].

In 1998 Ray et al. studied the changes in the serum levels of copper and zinc in the patients that are under anti TB therapy [26]. This research shows that after anti TB therapy, there is a significant rise in the serum zinc level, while serum copper level decreases with anti TB treatment. According to our research, children with pulmonary tuberculosis have a hypozincemic state which normalizes at 4 month of anti tuberculosis therapy. Our research is similar to the above mentioned study in this region. Many conducted researches also prove this fact. Some studies may not have noted significant changes in zinc levels, probably because they assessed the levels too early during the course of therapy. [27]

Different researches have proved the fact that zinc administration has a positive health impact in TB patients [28,29]. In a study conducted in this center, it has been shown that zinc supplementation increases the rate of negativity of sputum smear in TB patients [30].

This research was conducted with the aim of evaluating serum levels of copper and zinc in TB patients as well as determining the effect of anti TB treatment on these two elements. Therefore measuring the serum levels of copper and zinc along with other examinations such as direct microscopy and culture of MTB

would help the physicians in the diagnosis and treatment monitoring of TB. In addition this study recommends considering micronutrients such as copper and zinc and their roles in immune system, in other infectious diseases.

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E-published: November 2007

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