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Evaluation of Serum Levels of Essential Trace Elements in Patients with Pulmonary Tuberculosis Before and After Treatment by Age and Gender

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Abstract: The purpose of this study was to evaluate the levels of Zinc, Copper, Iron and Copper/Zinc ratio in the serum of adult patients with pulmonary tuberculosis in Iran. Serum levels of Zinc and Copper were determined by flame atomic absorption spectrophotometer and serum iron concentration was measured by using an Auto Analyzer. The study group consisted of 50 pulmonary tuberculosis patients before treatment and after 6 months of anti-tubercular therapy. Levels of serum Zn ($p<0.001$) and Fe ($p<0.001$) in TB patients were significantly increased after 6 months of anti-tubercular therapy. However, serum Cu concentration ($p<0.01$) and Cu/Zn ratio ($p<0.05$) were decreased after 6 months of anti-tubercular therapy. Some studies indicated a strong association of Zn, Cu, Fe and the Cu/Zn ratio with TB. In this study, we found remarkable change in Cu/Zn ratio. Some researchers mentioned that serum Cu/Zn ratio could be used as an important laboratory marker for diagnosis and treatment of tuberculosis. They also mentioned that trace element levels must be closely monitored during the process of disease.

Key words: Pulmonary tuberculosis, Zn, Cu, Fe, Cu/Zn ratio

INTRODUCTION

Pulmonary Tuberculosis (TB) is caused by the bacteria *Mycobacterium tuberculosis*. One can get TB by breathing in air droplets from a cough or sneeze of an infected person. This disease is known as an ancient disease of our planet and still is one of the most serious health problems in the world (Abbasi *et al*., 2006).

The Centers for Disease Control and Prevention (CDC) predicts that between 2002 and 2020, approximately one billion people will be newly infected, over 150 million will get sick and 36 million will die of TB if proper control measures are not instituted (Grange and Zumla, 2002). Tuberculosis prevalence in Iran is about 39 cases in 100,000 (World TB Day, 2003).

The biological role of trace elements, especially Copper, Zinc and Iron in different pathologic conditions has been extensively investigated in many diseases (Naser *et al*., 2009).

Zinc is a component of more than 200 different enzyme system functions, that included in most events such as immunity and cell division (Tudor *et al*., 2005). Zinc is a cofactor for the antioxidant enzyme Superoxide Dismutase (SOD) and also involved in carbohydrate and protein metabolism (Al-Numair, 2006). Zinc deficiency affects host defence in a variety of ways. It results in decreased phagocytes and leads to a reduced numbers of circulating T-cells, therefore reduced tuberculin reactivity, at least in animals (Karyadi *et al*., 2000).

Cu is an essential trace element involved in the metabolism of several key enzymes including cytochrome oxidase of the mitochondrial electron transport and cytosolic superoxide dismutase (Adewumi *et al*., 2007).

Moreover, 60% of Copper in the blood is tightly bounded to a Copper-Zinc dependent enzyme known as superoxide dismutase (Cu/Zn/SOD) which is a powerful anti-oxidant (Maybauer *et al*., 2006).

Iron also is the most important essential trace elements in transporting of oxition via hemoglobin in the red blood cells. Iron also intervenes in the constitution of enzymatic systems such an catalase, peroxidase and cytochromes that play an essential role in cellular respiratory mechanisms, especially in the mitochondrial respiratory channel (Inocent *et al*., 2008).

As there is limited data available about the relationship of the trace elements in adult patients with pulmonary tuberculosis in Iran and due to the increasing incidence of TB, we decided to investigate the association

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of essential trace elements such as Zinc (Zn), Copper (Cu) and Iron (Fe) levels in serum of patients with pulmonary tuberculosis before and after treatment.

MATERIALS AND METHODS

The subjects of this study were collected from the Central Hospital of Qom, a province of Iran during an 18-month period from April 2008 to September 2009. The study group consisted of 50 proven pulmonary tuberculosis patients (26 F, 24 M; mean of ages = 41.1±19.8 years). All individuals gave a written consent that they accepted the terms and conditions of the entire experiment and this study was reviewed and approved by the Human Subjects Ethical Community of the Pasteur Institute of Iran. They were interviewed with structured questionnaire requesting information related to various criteria. Conventional laboratory methods for the diagnosis of *Mycobacterium tuberculosis* were performed on all patients using Ziehl-Neelsen staining method for Acid-fast Microscopy (AFM) (Talat *et al*., 2002) and culture for growth of the organism on Lowenstein-Jensen (LJ) medium (Fatolahzadeh *et al*., 2007). All studied cases were also tested for radiographic abnormalities.

Five milliliter of venous blood was drawn after overnight fasting from all proven patients before and after treatment by Isoniazid, Rifampin, Ethambutol, and Pyrazinamide. The blood samples were allowed to clot at room temperature for about one hour. For separating sera, samples were centrifuged at 3000 rpm for 15 min at room temperature. Hemolytic sera were discarded. Sera were aliquot into the eppendorf tubes and stored at -20°C until they were tested in the Biochemistry Department of Pasteur Institute of Iran.

Analysis of Copper and Zinc were measured by using flame atomic absorption spectrometry (Thermo Jarrel Ash, Germany). According to the method of Kirgbright (1980), serum samples were diluted by deionized water. Different concentrations of trace elements were prepared for calibration of standard graphs. The Absorbances were read at 324.7 and 213.9 nm for Copper and Zinc, respectively. For accuracy, the standard solutions were run for every 10-test sample. Serum samples were run in triplicate and individual values were averaged (Narain *et al*., 1992).

Serum Iron concentration was measured by using an Auto Analyzer (Technicon, RA1000, USA) with commercial kit (Shim Enzym, Iran).

The SPSS software package (Windows version 14, SPSS, Chicago, Ill., USA) was used for all statistical analyses. All values are shown as standard deviation (SD). Statistical analyses were performed by paired t-test. The p-value of less than 5%, was considered significant.

RESULTS AND DISCUSSION

Concentration of serum Zn, Fe, Cu and Cu/Zn ratio in patients with pulmonary tuberculosis were compared before treatment and after 6 months of anti-tubercular therapy. Levels of serum Zn ($p<0.001$) and Fe ($p<0.001$) in TB patients were significantly increased after 6 months of anti-tubercular therapy (Table 1). However, serum Cu concentration ($p<0.05$) and Cu/Zn ratio ($p<0.01$) were decreased after 6 months of anti-tubercular therapy (Table 1).

For better evaluation, the patients have been divided into two groups on the basis of age (higher and lower than 30 years) and gender, each parameter was studied separately upon these basis .It was observed that serum Zinc levels significantly increased after treatment, especially in the >30 age group ($p<0.001$) with no difference in gender, both males and females showing significant increased in Zn levels after treatment ($p<0.01$) (Table 2). Interestingly serum Cu levels were significant only in ≤30 years age groups ($p<0.01$) and between the genders, it was significant in females but not in males ($p<0.05$) (Table 3).

Table 1: Comparison of serum trace elements concentrations in patients with pulmonary tuberculosis before and after treatment

	Treatment		
Trace elements	Patient group before	Patient group after	p-value*
Serum Zn (μg dL^{-1})	93.70±10.38	109.31±26.62	<0.001*
Serum Cu (μg dL^{-1})	132.99±19.76	122.58±21.87	<0.05*
Serum Fe (μg dL^{-1})	65.82±40.25	105.52±72.13	<0.001*
Cu/Zn ratio	1.43±0.23	1.18±0.36	<0.001*

*The mean difference is significant at p-value<0.05. Values are as Mean±SD

Table 2: Serum Zn concentration μg dL^{-1} for age and gender of patients

		Treatment		
Parameter	(No. of pts.)	Before	After	p-value
Age	≤30 years (16)	95.15±12.80	108.41±19.65	<0.05*
	≥30 years (34)	93.01±9.17	111.62±25.21	<0.001*
Sex	Female (26)	96.87±11.18	112.61±27.29	<0.01*
	Male (24)	90.26±8.37	105.74±25.98	<0.01*

*The mean difference is significant at p<0.05. Values are as Mean±SD

Table 3: Serum Cu concentration μg dL^{-1} for age and gender of patients

Parameter	(No. of pts.)	Before treatment	After treatment	p-value
Age	≤30 years (16)	143.27±23.34	117.30±23.72	<0.01*
	>30 years (34)	128.15±16.02	125.07±20.84	>0.05
Sex	Female (26)	136.11±20.15	122.13±21.49	<0.05*
	Male (24)	129.00±19.17	123.06±22.72	>0.05

*The mean difference is significant at p<0.05. Values are as Mean±SD

Table 4: Serum Fe concentration μg dL^{-1} for age and gender of patients

Parameter	(No. of pts.)	Before treatment	After treatment	p-value
Age	≤30 years (16)	61.88±36.55	107.75±70.17	<0.05*
	>30 years (34)	67.68±42.28	104.47±74.06	<0.05*
Sex	Female (26)	65.62±35.91	105.27±80.60	<0.05*
	Male (24)	66.04±45.28	105.79±63.44	<0.05*

*The mean difference is significant at $p<0.05$. Values are as Mean±SD

Table 5: Serum Cu/Zn ratio concentration μg dL^{-1} for age and gender of patients

Parameter	(No. of pts.)	Before treatment	After treatment	p-value
Age	≤30 years (16)	1.51±0.23	1.20±0.40	<0.01*
	>30 years (34)	1.39±0.22	1.17±0.05	<0.01*
Sex	Female (26)	1.41±0.24	1.15±0.38	<0.01*
	Male (24)	1.44±0.21	1.22±0.34	<0.01*

*The mean difference is significant at $p<0.05$. Values are as Mean±SD

Table 4 shows that serum Fe levels were significant in all the groups, irrespective of age and gender ($p<0.05$). Serum Cu/Zn ratio level were also seen to be significant ($p<0.01$) in both groups (Table 5).

Tuberculosis is considered as one of the most important infectious diseases and still is one of a prevalent disease in Iran and many other countries (Sharma and Mohan, 2003).

The global incidence of tuberculosis is expected to increase, from 8.8 million cases from 1995 to 11.9 million by 2005, also 3 million deaths due to tuberculosis occurred in 1995 (Reichman, 1996).

Trace elements are associated with many diseases; the determination of them in various diseases has been carried out for many years (Naser *et al*., 2009).

We have measured the Zn and Cu levels by flame atomic absorption spectrophotometer and serum iron concentration was measured by using an auto analyzer, however, with the advent of new technology, like mass spectrometry and 1 HNMR newer methods can be used for diagnosis (Massire *et al*., 2010) monitoring the drug response, immunity (Zamani *et al*., 2011) and hematological parameters to tuberculosis (Arjmand *et al*., 2010).

In this study we have found low concentration of Zinc in our patients but after treatment its level was significantly increased (Table 1).

The same findings were reported in the study by Sharda and Bhandari (1997). They reported a fall of serum Zinc in patients suffering from pulmonary tuberculosis, which reversed after the anti-tubercular treatment. The results of this study confirmed the findings of the study conducted by Ray *et al*. (1998) and Ghulam *et al*. (2009) in India. Researchers mainly believe that decreasing the levels of serum Zn in patients is because of the redistribution of Zinc in their liver. Metallothionein (MT) binds 7 g atoms of Zn per mol and serves to draw Zn away from free-circulating pools which are induced by IL-1 *in vivo* (Rofe *et al*., 1996). Canneva *et al*. (2005) described another reason for decreasing serum Zn levels in TB patients. They explained that MTB used Zinc for its growth and reproduction. The protective role of this metalloenzyme is known in many bacteria such as Shigella and Salmonella (Melov, 2002).

In this study we also observed that in TB patients, serum Cu levels and Cu/Zn ratio were significantly higher than healthy subjects but after the anti-tubercular treatment both Cu levels and Cu/Zn ratio were significantly decreased (Table 1). Abdolnasser *et al*. (1982) reported that after 10 days treatment by Ethambutol, Cu levels in patients with pulmonary tuberculosis were significantly decreased. They also mentioned that this result supports the hypothesis that Ethambutol may change serum Cu concentration in human. They explained that Ethambutol along with Copper may make chelating complex in the body and it causes the change in the Cu concentration.

On the other hand, Ciftci *et al*. (2003) described that serum Zinc, Copper levels of 18 healthy subjects and 22 TB patients before treatment and after 4 weeks of anti-tubercular therapy. After 2 months of therapy, they reported no change in the serum Cu levels.

We also found significant difference between serum concentrations of Cu in male patients and in patients older than 30 years before and after treatment (Table 3). It is in contrast with the result of study by Nazari *et al*. (2009). Increased serum Cu is associated with an increase in the synthesis of the Copper binding protein, ceruloplasmin (Cousins, 1985) the level of serum Copper also increases in other infectious disease such as Pneumonia, Cancer (Sobol and Pyda, 1995) and Leishmaniasis (Pourfallah *et al*., 2009).

We found that serum Iron concentration ($p<0.01$) in patients with TB were significantly lower than healthy group but there was a significant increase in serum Iron levels ($p<0.01$) when the patients were treated (Table 1). Anemia (iron deficiency) has been reported in patients with pulmonary TB, as indicated by low concentrations of blood hemoglobin , Iron and total iron binding capacity of serum but how iron deficiency affects host defense against it unclear (Deveci and Ilhan, 2003). Ratledge (2004) contended that while extracellular pathogens have direct access to host extracellular iron chelates, bacteria that replicate within macrophage phagosomes such as *M. tuberculosis* faces a greater challenge to obtain adequate iron to meet their metabolic needs.

The findings of these studies indicate a strong association of Zn, Cu and Fe and the Cu/Zn ratio with TB before and after treatment. Since the determination of serum Zn, Cu and Fe is simple, some researchers

suggested that they could be used as a valuable laboratory tool (Karyadi *et al*., 2002). Bogden *et al*. (1977) claimed that lowering of Zn plasma has been considered to be more sensitive index of tuberculosis infection than raising the levels of plasma Copper. Low plasma Zn levels with normal plasma Cu may mean that infection is in early stages (Khanna *et al*., 1982).

In conclusion, the results of the present study show that serum Cu levels and Cu/Zn ratio were significantly increased during the disease, whereas Fe and Zn levels had fallen significantly But after treatment reverses the picture with an increase in Fe and Zn levels and a significant decrease in Cu levels (only in male patients and patients older than 30 years) and Cu/Zn ratio.

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