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Original Article

Alteration In The Levels Of Serum Micronutrients In Tuberculosis Patients

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ABSTRACT

Background: Tuberculosis, a severe bacterial infection caused by Mycobacterium tuberculosis, compromises the immune system. Inadequate intake of micronutrients alters the immune response of the host predisposing to infection. The aim of the present study was to assess the influence of vitamins and trace elements on immunity status in patients with tuberculosis. Methods: In this study, forty patients diagnosed with tuberculosis on the basis of history, clinical examination, chest radiography, sputum examination and related laboratory parameters were recruited from The Institute of Thoracic Medicine and were compared with age and sex matched healthy volunteers (n=35). Zinc, selenium, iron, vitamins A, E, haptoglobin, ceruloplasmin levels in serum were determined. Results: Levels of vitamins A, E, trace elements, zinc, selenium and iron were significantly low ($p<0.001$) in tuberculosis patients. Haptoglobin and ceruloplasmin levels were found to be increased significantly ($p<0.001$) when compared with normal healthy volunteers. Conclusion: The results indicate that TB patients have altered profile of micronutrients in their sera and this could be more due to the active disease rather than underlying deficiencies. However, the authors suggest additional research with randomized controlled trials to validate or refute the presence of underlying micronutrient deficiencies in tuberculosis.

1. Introduction

Tuberculosis (TB), a severe bacterial infection is caused by Mycobacterium tuberculosis, and continues to be one of the main causes of morbidity and mortality (1). TB is known to be associated with weight loss and protein and calorie malnutrition (2-5) and these are believed to be predictive factors of adverse events including treatment failure and early death (6-8). However, there are very few studies to report the nutritional status with respect to micronutrients in tuberculosis patients. Recent reviews have shown that deficiency in the micronutrients and trace elements may have adverse effects on the immune status (9, 10) and micronutrient supplementation can lead to boosting of the immune system, which may help improve the response to TB treatment (11). A great deal of research supports the fact that the impairment of the immune system due to trace element deficiency can be sufficient to increase the risk of mortality and morbidity due to infections. It is also stated that the supplementation of trace elements restores immune competence (12).

The biological role of trace elements like Cu, Fe, Zn has been extensively studied in many pathological conditions (13).Fe mediates enzymatic reactions like catalase, peroxidase and cytochromes (14). Cu and Zn are also linked in cytosolic defense against reactive oxygen and nitrogen species (Cu/Zn/SOD a powerful antioxidant) (15). The role of Fe is very significant in myeloperoxidase-dependent generation of hypochlorous acid, the microbicidal factor.

Several studies have proven low concentrations levels of vitamin A and zinc in pulmonary tuberculosis patients. Randomized trial reports have shown that vitamin A and micronutrient supplementation resulted in improved prognosis in

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pulmonary tuberculosis patients (16). Decreased vitamin E and reduced glutathione levels show the potential of oxidative damage to erythrocytes and erythrocyte membranes of pulmonary tuberculosis patients (17). Since there are a lot of studies reporting malnutrition and beneficial effects of micronutrient supplementation but very few studies on the deficiency status of these micronutrients per se in tuberculosis patients, the present study was carried out to assess the levels of micronutrients in the serum of patients diagnosed with tuberculosis.

2. Subjects and Methods

The subjects of this study were recruited from The Institute of Thoracic Medicine, Chennai, India. The study group consisted of 40 pulmonary tuberculosis patients (25–75 yrs). The diagnosis of tuberculosis was performed using Ziehl-Neelsen staining method for Acid-fast Microscopy (AFM) (18) and culture for growth of the organism on Lowenstein-Jensen (LJ) medium (19). The patients were also tested for radiographic abnormalities and various other laboratory parameters. Age and sex matched healthy volunteers (n=40) were also included in the study for comparison of results.

Ten milliliter of venous blood was drawn and transferred to a centrifuged tube without any anticoagulant, after overnight fasting from all proven patients. A portion of the blood sample was allowed to clot for about one hour and subjected to centrifugation at 3000 × g for 15 min at room temperature. The separated serum was then collected and stored at -20°C until analysis. All the experiments were performed within 8 hrs of sample collection. Remaining portion of the blood was used for isolation of lymphocytes. About 6ml of peripheral blood was added with 0.5 ml of 3.8% sodium citrate followed by equal volume of 3% dextran in phosphate buffer-saline. The mixture was drawn into two syringes and placed on a ring stand with tip facing up for 30 minutes at room temperature to allow sedimentation of red blood cells. The leukocyte rich plasma was collected and layered with 5 ml of 1.08 Ficoll-Hypaque followed by 5 ml of 1.105 Ficoll-Hypaque. The contents were centrifuged at 1000 × g for 30 minutes at room temperature. The cells in the bottom layer were harvested which contained mostly neutrophils (11). After Ficoll-Hypaque density gradient centrifugation, cell pellets were mixed with 6% dextran solution and allowed to stand for 1 hr at 37°C for PMNL isolation. The PMNL supernatant was then collected and centrifuged at 400 × g for 10 minutes.

Serum levels of zinc and selenium was measured using atomic absorption spectrophotometry (AAS). Serum Iron concentration was measured by using an Auto Analyzer using a commercial kit.

Serum haptoglobin was assayed based on the peroxidase activity after mixing serum with excess of free hemoglobin, the so called Hb binding capacity by the method described by Tarukoshi (20). Ceruloplasmin was measured by the method of paraphenylenediamine (21). Vitamins A and E levels were assessed by the method of Kamangar et al (22) and Martinek (23) respectively.

Statistical Analysis

The SPSS software package (Version 16) was used for all statistical analysis. All values were represented as mean ± SD. Statistical analysis was performed by student's t-test. A p-value of <0.05 was considered as statistically significant

Conflicts of Interest

There were no conflicts of interest regarding the publication of this article. The study protocol was approved by the Institutional ethics committee and was carried out in accordance with the principle of Declaration of Helsinki. Informed consent was obtained from all the subjects.

3. Results

The clinical characteristics and results of micronutrient assessment in tuberculosis patients and in normal healthy volunteers are presented in Table 1. As shown in table 1, a majority of the patients were severely malnourished and showed the expected clinical symptoms in tuberculosis. The levels of ceruloplasmin and haptoglobin ($p<0.001$) were significantly higher in TB patients than in healthy volunteers. Likewise, as shown in table 2, serum levels of trace elements and vitamins were significantly decreased in TB patients when compared to healthy volunteers.

As shown in table 3, serum ceruloplasmin and haptoglobin ($p<0.001$) were significantly higher in TB patients than in healthy volunteers.

Table 1.Clinical characteristics of patients.

Clinical characteristics	No. of subjects
Total Number	40
Age	25 ± 75 years
Sex	Male - 35; Female - 5
Basal Metabolic Rate	
Severe Malnutrition (< 15.9 kg/m^2)	30
Moderately (16 – 16.9 kg/m^2)	7
Mild17 – 18.4 kg/m^2)	2
Normal>18.5 kg/m^2)	1
Clinical Signs	
Cough	40
Fever	25 – 30
Weight loss	40
Night sweats	25 – 30

Table 2. Levels of serum zinc, selenium, iron, vitamins A, E, haptoglobin and ceruloplasmin in normal volunteers and tuberculosis patients.

Parameters	Normal volunteers (n = 40)	Tuberculosis Patients (n = 40)
Iron (μM)	25.1 ± 2.9	11.3 ± 1.5
Zinc (μM)	28.2 ± 2.3	10.8 ± 2.9
Selenium (μM)	23.9 ± 3.5	9.5 ± 0.8
Vitamin A (mg/dl)	48.2 ± 5.2	17.34 ± 2.4
Vitamin E (mg/dl)	2.5 ± 0.5	0.76 ± 0.05
Haptogloblin (mg/dl)	210.3 ± 16.9	258.7 ± 21.4
Ceruloplasmin (mg/dl)	33.7 ± 30.1	38.9 ± 27.8
Values are expressed as mean ±SD		

4. Discussion

A great number of studies report malnutrition to be a risk factor in progression to active TB after pulmonary infection (24-26). Also, malnutrition is frequently observed in tuberculosis patients but little is known about their nutritional status with respect to the micronutrients. In the present study, serum levels of all the measured micronutrients were significantly decreased in tuberculosis patients when compared with healthy volunteers. These findings corroborate those reported by Karyadi E et al (27) who have reported a significant reduction in serum levels of micronutrients like retinol, α-tochopherol and zinc in malnourished tuberculosis patients when compared with well-nourished healthy controls, malnourished healthy controls and well-nourished tuberculosis patients. The results of the present study also match with those reported by Taneja (28) who has reported a decrease in plasma zinc concentrations in tuberculosis patients although contrary findings have been reported with respect to zinc by Pourfullah F et al (29). The contrary findings could be due to variations in the stages of the disease in the patients studied.

The decreased concentrations of these micronutrients could be due to a number of factors. From the findings and discussions of previous studies, it seems more likely that these deficiencies are due to the active disease and not due to an underlying deficiency. As discussed by Karyadi et al (27) and Visser ME et al (30), low concentrations of vitamins A in tuberculosis could be due to anorexia-induced low intake or due to low absorption of fat. It could also be due to infection- induced increase in the excretion of these nutrients. These explanations can be extrapolated to the decrease in serum levels of the other fat soluble vitamin, vitamin E, found in the present study. In fact, anorexia-induced low intake of food and infection-induced increase in excretion can be attributed as causative factors in the observed decrease of all studied micronutrients in the present research. An acute phase response could also result in decreased production of retinol binding protein or increased utilization of the vitamins by tissues

The decrease in serum zinc concentrations in this study could be due to an acute phase response-induced increase in the production of metalothionein, a protein that transports zinc to liver Visser ME et al (30), redistribution of zinc from plasma to other tissues (31) or reduction of the hepatic production of α2-macroglobulin, a zinc carrier protein (32).

The decrease in serum iron could be due to the body's defense mechanism to create an iron-scarce environment for the bacteria because organisms tightly sequester and regulate their iron supplies to limit the toxicity of the ferrous ion and to deal with the insolubility of the ferric ion (33) and in the mammalian host, these mechanisms create an iron-scarce environment for bacterial pathogens (34). Also, an acute phase response-induced increase in ceruloplasmin and haptoglobin, seen in our study, could have resulted in increased binding of iron in the host, thus resulting in decreased serum levels, in a bid to decrease iron availability for the bacteria. The role of the acute phase protein, hepcidin, which stimulates the internalization of ferroportin, preventing release of iron bound by ferritin, may also be considered, although the present study has not researched on this parameter.

The decrease in serum selenium in the present study corroborates with the findings of van Lettow M et al (35) who have reported decreased plasma selenium in pulmonary tuberculosis patients with or without HIV. Again, an acute phase response, involving decreased hepatic production of serum albumin and other plasma proteins need to be considered here, since 90% of selenium in serum is protein bound and only 10% is not (36-38).

5. Conclusion

The results indicate that TB patients have altered profile of micronutrients in their sera and this is more due to the active disease rather than underlying deficiencies, as gathered from results of previous studies. However, the authors suggest additional research with randomized controlled trials to validate or refute the presence of underlying micronutrient deficiencies in tuberculosis. Also, in depth research involving newer acute phase proteins like hepcidin and others, may be carried out to elucidate the molecular mechanisms involved in altered micronutrient metabolism so that strategies for trace elements supplementation can be planned.

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