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Evaluation of Relationship Between Iron Status and Pulmonary and Extra Pulmonary Tuberculosis: A Case-control Study

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Abstract: Several line of evidences have been suggested that iron is critical for mycobacterium tuberculosis growth in macrophage and plays some role in the susceptibility to and outcome of tuberculosis. In view of the increasing burden of tuberculosis and lack of known risk factors for activation of tuberculosis in some cases, the researchers decided to study this relationship using iron indicating serum markers. In a case control study, 52 patients with pulmonary and extra pulmonary TB and without any known risk factor for tuberculosis activation compared with healthy subjects for serum iron, TIBC and ferritin level and transferrin saturation rate. A total of 52 TB patients 24 with pulmonary and 28 with extrapulmonary TB and 52 healthy individuals as control group were enrolled in the study. Mean serum ferritin level ±SD for case and control groups were 215.95±204.37 μg dL^{-1} and 61.67±45.8 μg dL^{-1}, respectively. Mean±SD for serum iron level in case group was 62.88±30.41 μg dL^{-1} and for control was 86.75±27.56. Mean±SD for TIBC in case and control groups were 270.21±62.98 μg dL^{-1} and 231.71±48.57 μg dL^{-1}, respectively. Mean±SD for transferrin saturation in case group was 23.63±10.87% and in control was 27.15±8.99%. The levels of iron, TIBC and ferritin showed significant difference in 2 groups but there was no significant difference about levels of transferrin saturation rate.

Key words: Iron, ferritin, transferrin saturation, tuberculosis, TB, Iran

INTRODUCTION

Tuberculosis (TB) caused by Mycobacterium Tuberculosis (MTB) is a worldwide health problem, especially in developing countries with 8 million active new cases and 2-3 million death annually (Ratledge, 2004). The registered number of new tuberculosis cases in Iran was over 10000 in 2008, from those over 70% was pulmonary tuberculosis. MTB is an intra-cellular pathogen which grows within phagosomes of macrophages (Basaraba *et al*., 2008).

Iron is an essential micronutrient for all living organisms and plays a pivotal role in modulating the battle for survival between mammalian hosts and their pathogens. Its role is well known in metabolic processes such as cell respiration, growth and DNA synthesis (Gangaidzo *et al*., 2001). Iron has a crucial role in TB infection. It has been shown that iron overload promotes free radical induced tissue damage and organ failure, decreases in immune protection and increases in pathogen invasion (Sahiratmadja *et al*., 2007). *In vitro* and *in vivo* iron loading of macrophages results in impaired defense because of decrease in synthesis of Tumor Necrosis Factor α (TNFα) and Nitric oxide (No) (Crichton *et al*., 2002). In the past decade, several studies indicated that excess iron in body lead to decrease in resistance to mycobacterium tuberculosis (Boelaert *et al*., 2007; Crichton *et al*., 2002; Cronje and Bornman, 2005).

On the other hand, iron deficiency reduces the ability of neutrophils to kill bacteria, reduces lymphocyte response and Impairs Natural Killer (NK) cells activity (Finch and Huebers, 1982). According to various effects of iron, it seems that an optimal level of iron is needed for natural immunity against MTB growth. Several preliminary studies conducted *in vitro*, in experimental animals and human indicated that iron status may have some effects on the occurrence and outcome of tuberculosis but the exact influence of iron on this disease remained unclear (Gangaidzo *et al*., 2001; Lill *et al*., 2006; Lounis *et al*., 2001).

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Gangaidzo *et al.* (2001) showed that iron overload can increase the occurrence and severity of pulmonary TB (Finch and Huebers, 1982; Lounis *et al*., 2001; McDermid and Prentice, 2006). In another study by Carine and colleagues, iron chelation indicated as a potential additive therapy for TB (Crichton *et al*., 2002). Both of these studies conducted in Africa with high prevalence of iron overload in that area.

One study by Boelaert *et al.* (2003) showed that macrophage iron loading contribute to an increased predisposition toward TB in HIV infected patients (Boelaert *et al*., 2003).

The researchers studied the potential relationship between iron status and pulmonary and extra pulmonary tuberculosis by a case-control study in Iran, a country with high prevalence of TB.

MATERIALS AND METHODS

This case-control study was conducted between May, 2008 and January, 2011 at Taleghani Hospital of Urmia, Iran after Institutional Review Board and Ethics Committee approval was obtained. Case group selected from the patients with diagnosis of pulmonary and extra pulmonary TB based on the clinical presentation, Chest X-Ray (CXR) and confirmed by two consecutive acid-fast bacilli-positive sputa, culture results and histopathologic examination and response to anti-TB drugs. The patients had no history of drug use (including vitamins, iron or antibiotics) at the time of assessment. They also had no history of blood transfusion in the last 6 months and no recent history of blood loss or other condition that may disturb iron status. Additional excluding criteria in females were pregnancy, lactating and having menses problem. Patients with HIV seropositivity, metabolic problems including diabetic mellitus, chronic renal failure or chronic liver disease, receiving immune suppressive drugs, silicosis, gastrectomy, malabsorption syndromes and underweight and other conditions that is known as risk factor for activation of TB were excluded from the study. In the same period, healthy individuals who matched by age, sex and living condition and socioeconomic class were enrolled as control group. After obtaining informed consent from all subjects, blood samples were obtained by venipuncture. Fe status indicators including serum Fe, serum Total Iron Binding Capacity (TIBC), transferrin saturation and serum ferritin were measured. Serum iron was measured imetrically (Direct method) using a Ferene assay kit (Biolabo Reagents, France). TIBC was measured using aREF 92308 assay kit (Biolabo Reagents, France). Transferrin saturation percentage was calculated according to the following:

$$\text{Transferrin saturation (\%)} = \text{Serum Iron concentration} \times 100/\text{TIBC}$$

Finally, serum ferritin was measured, using the ELFA technique (Enzyme Linked Fluorescent Assay, VIDAS) instrument and a Ferritin kit (both fromBio Merieux, France). The data were analyzed using SPSS(R) software Version 16 then were expressed as mean±Standard Deviation (SD). Statistical comparisons were performed using the student's t-test. The $p<0.05$ was considered to be statistically significant.

RESULTS

A total of 52 patients (28 male and 24 female) including 24 pulmonary, 11 lymph node TB, 8 pleural TB, 6 milliary and 3 meningeal TB in case group were compared to 52 healthy subjects (28 male and 24 female) in control group. The mean±SD (age) in case group was 48.4±20.19 year ranging from 15-86 and in control group was 50±18.83 from 19-82.

The mean±SD serum ferritin level in case group (215.95±204.37 μg dL^{-1}) was significantly higher than control group (61.67±45.8 μg dL^{-1}) ($p<0.001$).

The mean±SD serum iron level in the case group (62.88±30.41 μg dL^{-1})was significantly lower than control group (86.75±27.56 μg dL^{-1}) ($p<0.001$).

The mean±SD TIBC in case group (270.21±62.98 μg dL^{-1}) was significantly higher than control group (231.71±48.57 μg dL^{-1}) ($p<0.001$). The mean±SD transferrin saturation rate in the case group was 23.63±10.87% and in the control group was 27.15±8.99% (Table 1, Fig. 1). The difference was not statistically significant ($p<0.075$).

Table 1: Compared variables in case and control groups

Variables	Mean±SD	p-values
Age		0.67
Case	48.40±20.190	
Control	50.00±18.830	
Ferritin (μg dL^{-1})		0.001
Case	215.95±204.37	
Control	61.67 ± 45.80	
Iron (μg dL^{-1})		0.001
Case	62.88± 30.41	
Control	86.75± 27.56	
TIBC (μg dL^{-1})		0.001
Case	270.21±62.98	
Control	231.71±48.57	
Tsat (%)		0.075
Case	23.63±10.87	
Control	27.15±8.990	

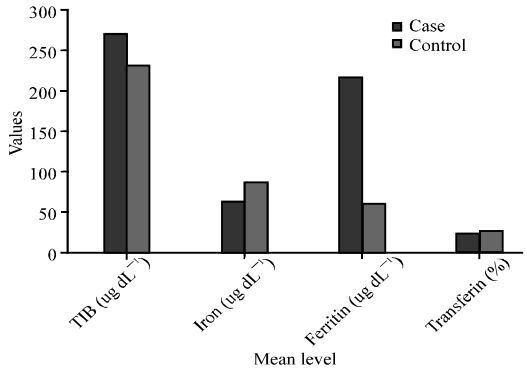

Fig. 1: Mean level of analyzed parameters

DISCUSSION

The findings showed that there were statistically significant differences in serum iron, serum ferritin and TIBC levels of patients who had tuberculosis compared to healthy subjects whom matched by age and sex with case group. Serum ferritin and TIBC levels in TB group were higher and serum iron was lower than the control group, also transferrin saturation level in patients with TB was lower than healthy subjects but the difference was not significant.

Serum ferritin is a valuable factor in measurement of iron reserve of the body but because of being an acute phase reactive protein, it could be increased in pulmonary tuberculosis due to inflammatory state (Gangaidzo *et al*., 2001), this factor is not reliable in judgment about iron status in patients with TB, therefore the researchers measured serum Fe, TIBC level and transferrine saturation rate. Transferrin saturation rate did not show any relationship between iron status and TB disease and has not significant difference with control group.

One study showed that iron overload has a relationship with occurrence and severity of pulmonary tuberculosis (Finch and Huebers, 1982) or in another study, iron chelation indicated as a potential additive therapy for TB (Cronje and Bornman, 2005). Both of these studies conducted in Africa with high prevalence of iron overload in that area that may have some influence on their results, however the researchers did not have any case of iron overload in this study.

Another study showed that the management of dietary iron can be influential in aiding the outcome of TB (McDermid and Prentice, 2006) and it is of interest that Para Amino Salicylate (PAS) anti-TB effect is related to inhibition of iron assimilation and metabolism (McDermid and Prentice, 2006).

One of the limitations in present study was that iron status was estimated by measurement of several serum indices, not by bone marrow aspiration or liver biopsy to determine true iron status of the host and another limitation was that we cannot determine any relationship between disease severity and iron status.

According to the findings, iron status has not any relationship with TB disease but further studies with larger sample size and by using other methods to estimate true iron status of patients are suggested.

CONCLUSION

This study did not show that iron overload or deficiency is a risk factor for tuberculosis so it seems more studies is needed to judge if iron plays any role in activation tuberculosis or not.

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