

A study of micronutrient status in multidrug resistant tuberculosis patients at a tertiary care center

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Abstract

Introduction: Micronutrients deficiency is supposed to impair the overall immune function and causes increased resistance to infection. Such a relationship exists even in tuberculosis. A major threat is multidrug resistant tuberculosis (MDR-TB). In India, few studies are available to discuss about risk factors and serum zinc and selenium status in MDR-TB.

Material and Methods: A case control study of 25 diagnosed MDR-TB patients from Damian foundation and 25 healthy controls from Narayan Medical College were recruited after laboratory tests, chest X-ray and clinical history. 5 ml of blood collected and serum zinc and selenium levels were assessed for both groups and correlated with demographic factors, duration of treatment, and BMI.

Results: The results indicate that the MDR-TB patients have altered profile of the serum zinc and serum selenium in their sera and this could be more due to the active disease, causing oxidative stress which depletes the micronutrients. In this study, the mean serum zinc levels were $79.08 \pm 14.2 \mu\text{g/dL}$ and $91.1 \pm 15.2 \mu\text{g/dL}$ in case and control groups respectively. Serum selenium levels were $56.2 \pm 11.1 \mu\text{g/dL}$ and $65.2 \pm 4.2 \mu\text{g/dL}$ in case and control groups respectively. In both the groups micronutrient levels showed statistical significant.

Conclusions: The risk factors in our study, such as lower level of serum zinc was one of the concern for the development of MDR-TB and an attempt to minimize them might contribute to control the upward slope of MDR-TB.

Keywords: Immune status; Serum zinc; Serum selenium; TB.

Introduction:

Globally, tuberculosis (TB) is a major public health challenge and now ranks alongside HIV as a leading cause of death worldwide. In recent years, progress in TB control and eradication has been major problem by the emergence of drug resistant strains of *Mycobacterium tuberculosis* (Mtb). India is estimated to have a MDR-TB rate of 2.9% and 14.3% among new and relapse cases and it was ranked highest burden in Asia. The innate immune cells; macrophages, neutrophils and dendritic cells, acts as first responder, activator of adaptive immunity and effector cell in Mtb infection. Mtb bacilli upon entry into the lungs are engulfed by resident macrophages and dendritic cells. This leads to transformation of macrophages from a resting state to an activated state with characteristic feature of increased oxygen uptake, enlargement and increased protein synthesis. Also there is an increased influx of neutrophils to the lungs in human TB. Macrophage and neutrophil apoptosis have also been reported to be a potent mechanism for control of inflammation and removal of infected cells in Mtb infection. These lead to a high rate of cellular turn over during infection and increased demand for essential nutrients by the immune cells. Malnutrition is associated with tuberculosis and plays the role in the development of the disease [1,2]. In Tanzania, study reported that malnutrition was evident before and after TB treatment [3].

In India, limited information is available on the zinc status in MDR-TB cases. Zinc plays a vital role in immunity. Macrophages are also affected by zinc deficiency. In person with Zinc deficiency, there was an increased susceptibility to variety of pathogens including *M. tuberculosis*. The development of T and B cells are also affected by zinc deficiency.

Micronutrient deficiency has been described in pulmonary TB patients and several studies have suggested that the patients with TB are at high risk of deficiency of zinc and selenium. Micronutrient deficiency has been reported to impair resistance to infection, lead to active TB disease and poor outcome of anti-TB chemotherapy, this present study therefore determined the serum level of micronutrients [Zn, Se] in MDR-TB patients at the anti-TB chemotherapy.

Material and Methods:

A case control study carried out at Narayana Medical College in collaboration with Damian foundation, Nellore, Andhra Pradesh to determine the Zn, Se and other risk factors associated with MDR-TB.

Study participants: Twenty-five patients admitted into the MDR-TB center, Damien Foundation, Nellore, Andhra Pradesh, India for anti-TB treatment were recruited for the study after obtaining written informed consent.

Inclusion and exclusion criteria: Only the pulmonary MDR-TB patients from Nellore district registered at Damien Foundation, Nellore were included in the study

as cases. Patients who were unwilling to participate in the study, extra-pulmonary tuberculosis and patients not turning up at the hospital during the study period were excluded.

Patients were previously diagnosed as being infected with Isoniazid and Rifampicin resistant strains of Mtb using clinical history, chest X-ray and line probe assay test. Twenty five age matched healthy participants were kept as controls. Five milliliters of blood was drawn from the anti cubital fossa vein into heparin tubes before the commencement of chemotherapy, and blood samples were centrifuged and plasma obtained was analyzed. The plasma was collected and refrigerated till analysis. In both the groups the micronutrient [serum zinc, serum selenium] levels were measured using Atomic Absorption Spectrophotometer [SHIMDAZU, Japan] and a comparative analysis of the findings was made.

MDR-TB treatment protocol: All bacteriologically confirmed MDR-TB patients received an intensive phase for 6–8 months in the hospital followed by 12 months of continuation phase in the community based on the updated RNTCP guidelines. Standardized treatment regimen was used including five drugs: Kanamycin/Amikacin, Levofloxacin, Prothionamide, Cycloserine, Pyrazinamide (with Pyridoxine). This present study was conducted during the intensive phase of treatment.

Micronutrient analysis:

Serum levels of micronutrient (Zinc and Selenium) were determined by Atomic Absorption

Spectrophotometer [SHIMDAZU, Japan] and a comparative analysis of the findings were made.

Statistical analysis:

Data obtained were analyzed by statistical package for social sciences (SPSS) version 17.0. Independent Student *t*-test was used to compare the mean values of MDR TB patients and controls while paired *t*-test was used to compare the mean values of MDR TB patients before the commencement of chemotherapy. Values were considered significant at $p < 0.05$. The two groups were compared based on various demographic factors, duration of treatment, body mass index.

Results:

Out of 25 MDR-TB cases, highest percentage of MDRTB (40%) were found within the age group 21-30 years, followed by age group 41-50 years (35%) and 31-40 years with 25%. Among 25 MDR-TB patients, 15 (60%) were male and 10 (40%) were female. 25 controls (14 male and 11 female) with a mean age of 35 and age ranging from 22 to 56 years were included.

A total of 15 of MDR-TB cases had a history of drug default and 10 had history of irregular treatment of antitubercular therapy.

The mean $\pm$ SD of serum zinc levels were found to be 79.08 ± 14.2 μ g/dL and 91.1 ± 15.2 μ g/dL in cases and control groups respectively.

The mean ($\pm$ SD) serum selenium levels in cases and control groups were found to be 56.2 ± 11.1 μ g/dL and 65.2 ± 4.2 μ g/dL.

Table 1: Analysis of serum zinc and selenium levels in MDR-TB and controls and their significant association

Zinc (80-120 µg/L)				
BMI	Normal	Undernourished	Total	<i>p</i> value
Normal	6 (54.55)	5 (38.46)	11 (45.83)	<i>p</i> >0.05
Low	5 (45.45)	8 (61.54)	13 (54.17)	
Total	11 (100)	13 (100)	24 (100)	
Selenium (55-65 µg/L)				
Normal	7 (53.84)	4 (46.16)	11 (45.83)	<i>p</i> >0.05
Low	6 (46.15)	7 (53.85)	13 (54.17)	
Grand Total	13 (100)	11 (100)	24 (100)	
Micronutrients level		MDR-TB	Controls	<i>p</i> value
Zinc (80-120 µg/L)		79.29± 10.19	91.1 ± 15.2	0.01
Selenium(55-65 µg/L)		55.71 ± 6.09	65.2 ± 4.2	0.01

Discussion:

Mostly, MDR-TB will develop due to interruption in the course of antibiotics and the insufficiency of the drug to kill 100% of bacteria. WHO estimated that 45,000 people fell ill with MDR-TB in 2012 (WHO, 2013) [4]. Low- and middle-income countries

accounted for 95% of TB deaths (WHO, 2013a) [5]. More people are dying of the disease with the emergence and proliferation of MDR-TB bacteria (MDR-TB; WHO, 2013a) [6]. Malnutrition is a risk factor for the development of pulmonary TB and is responsible for the premature death due to active

disease. In India, studies are limited on the nutritional status in MDRTB patients. Current study aimed to analyse the zinc and selenium nutritional status of MDR-TB patients.

Malnutrition may predispose the individuals in the latent infection to active disease because malnutrition affects the cell-mediated immunity [7]. We analysed serum zinc levels in MDR-TB patients, as we assumed that zinc deficiency may play an important role for developing MDR-TB.

In our study we observed 13/24 (54.17%) of our patients had hypozinaemia, among them 8/13 (61.54%) were undernourished. A study reports says that hypozinaemia is often observed at the time of diagnosis and this difference was statistically significant ($P < 0.05$) [8]. In a study also lower level of serum zinc was observed in MDR-TB than controls [9].

Lower level of serum zinc was reported in some studies in the same condition [10,11]. Serum zinc level in pulmonary TB was 65.53 (9.8) $\mu\text{g/dl}$ and 52.5 (19.5) $\mu\text{g/dl}$ in Ramakrishnan et al., and Ahmad et al., studies respectively and these levels were lower than normal ranges.

Selenium deficient lymphocytes were less able to proliferate in response to mitogen [12]. Current study analysis showed that the mean selenium level is $55.71 \pm 6.09 \mu\text{g/L}$ in the Mtb group and $65.2 \pm 4.2 \mu\text{g/L}$ in the control group.

Selenium is also important for achieving optimal functionality of neutrophils. In a study from Malawi involving 801 TB patients of whom 579 were HIV-positive, selenium deficiency was found in 87% of the participants when cut-off for deficiency was set at $0.89 \mu\text{mol/L}$ [13].

Patients (98%) in our study had previous history of TB. Various studies had shown very significant correlation between past history of TB and MDR-TB [14-17]. Also, drugs that are of poor quality or less in quantity, especially in developing countries contribute to MDR-TB [18]. According to a study, 67% of cases missed medicine at least a few weeks during their previous treatment [19]. Irregularity in taking medicine was shown statistically associated with MDR-TB which was correlated with previous studies [20-22].

In this study, younger age (21-30 years) was found to be statistically associated with MDR-TB. The highest number of MDR-TB patients of the productive age group might be due to the exposure of these age group people to different environment during their work and activities that would make their health more prone to infection by TB organisms [23,24].

Conclusion:

Now a days, MDR-TB is a big challenge to control in the world. Identifying region specific risk factors of MDR-TB infection which are reflective of nutritional status may help to prevent the proliferation of the disease. We recommended further study to compare

serum zinc and selenium level of MDR-TB with pulmonary tuberculosis in a large sample size.

Conflict of interest: None

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