Chapter -5

Serum Zinc and Albumin levels in pulmonary tuberculosis patients with and without HIV
Malnutrition is observed frequently in patients with pulmonary tuberculosis (TB), but their nutritional status, especially of micronutrients (zinc), is still poorly documented. Low concentrations of these nutrients may affect the host’s defence mechanisms.

Objective

In this chapter following things are focused V. To study serum zinc and albumin status in adult pulmonary tuberculosis (PTB) with and without HIV co infection and compare with healthy control.

Materials

A cross sectional study was designed to study serum zinc and albumin levels in newly detected, adult untreated active PTB cases attending to Government Rajaji Hospital, Madurai. Selection of cases was based on positive microscopy for AFB atleast on two sputum samples. Exclusion criteria for cases were as follows: previous Anti Retro viral Treatment (ART), pregnancy, lactation, other active inflammatory conditions, moderate-to-severe injury or surgery during the last month, acute or chronic liver, renal or cardiac diseases, diabetes mellitus (as measured by elevated fasting blood glucose levels) and neoplasm (as determined by clinical examination) and use of corticosteroids or supplements containing zinc during the previous month. All those cases included for the study purpose were free from alcoholism. Healthy adult family members of the cases matched for age with no history of pulmonary TB, or any overt illnesses were kept as control.
Data collection

Potential cases and control were interviewed using structured questionnaire requesting information related to the inclusion and exclusion criteria. Those apparently eligible were then screened clinically including a chest X-ray. Cases were offered HIV testing after counseling and were screened for HIV antibodies using ELISA test established by two different kits i.e., Innotest™ HIV 1/HIV 2 by Belgium and Lab systems Finland.

Standard procedures were used to determine height (cm) and weight (kg). Body Mass Index (BMI) was calculated as body weight divided by height in meter square (kg/m\(^2\)). Cases and control were regarded as being malnourished (W H O, 1995) if BMI was below 18.5.

Blood samples were collected in two vacutainers from fasting subjects via venepuncture for estimation of serum albumin by the bromo cresol green method and serum zinc by atomic absorption spectrometer in the Department of Biotechnology, Lady Doak College, Madurai with values of a quality control analyzed with each set of determinations within 3% of certified values.

(A) **Serum total protein & albumin** using by Automated Chemical Analyzer

(Cobas mira),

(B) **Serum zinc** by Atomic absorption spectrophotometer.

All laboratory procedures adhered to good laboratory practice followed established standard operational procedures (SOPs). The biochemical parameters underwent additional internal and external quality assessment.
**Estimation of serum albumin**

Serum albumin was analysed by the bromo cresol green method (Doumas et al., 1971) at Department of Biobiochemistry, Government Rajaji Hospital, and Madurai. To clean sterile test tube 1.0 ml of BCG (bromo cresol green) reagent was added, to which 10 μ of freshly collected serum sample was also added. After the sample was read with in 10 minutes at 628 nm absorbance was against distilled water.

**Estimation of serum zinc**

Serum zinc was analyzed by Atomic absorption spectrophotometer.

**Principle**

Atomic absorption spectrophotometry utilizes the phenomenon that atoms absorb radiation of particular wavelength. By atomic absorption spectrophotometer, the metals in water (organic) sample can be analyzed.

**Procedure**

The sample was obtained of which 0.5 ml was delivered into a 16 mm plastic test-tube and then 2 ml of demonized water was added. Then the solutions were mixed thoroughly and gas flow settings were established to optimize signal and minimize background noise. Glycerol diluents were aspirated into the flame and then, setting was changed into baseline to read zero absorbance.

Baseline fluctuations, were again corrected, glycerol diluents were aspirated before and after each aspiration of calibrates and specimen was reset the baseline to zero again. Then, the zinc working calibrators were aspirated sequentially from the most dilute to the most concentrated, aspiration was done, until the reading was stable (± 0.004A). The sample concentration was read directly from the display unit of the instrument and
then finally the resulting values were used to establish the calibration curve by use of a least squares regression fit.

**Reference range**

The accepted reference interval for zinc in plasma is 70 to 120 μg/dl.

**Statistical analysis**

A one-sample Kolmogorov-Smirnov test was used to check whether data were normally distributed. Mean and standard deviation (SD) were calculated for zinc and albumin. (for reporting normally distributed data). An independent sample ‘t’ test was used to assess the differences between cases and control for normally distributed parameters. The SPSS software package (Windows version 14, SPSS, Chicago, IL) was used for all statistical analyses and a P-value of < 0.05 was considered significant.

**Results**

There were 40 healthy controls, and 100 each of HIV positive and HIV negative cases with active PTB. Age of the patients ranged from 29 years to 52 years. The mean ± SD for age among HIV positive patients were 34.5 ± 6.5, HIV negative patients were 35.3 ± 11.3 and control were 34.5 ± 8.8 years respectively. There was no statistical difference among them.

**Body Mass Index**

Body Mass Index was below 18.5 in 4 of 40 healthy control, where as it was 15 and 6 among PTB with and with out HIV infection respectively. The range of body mass index among healthy control varied from 18 to 20.1, and the median19.2 and mean ± SD 19.6 ± 0.6 respectively, where as range of body mass index in those with HIV positive PTB patients 17.6 to 18.9, median 18.2, and mean ± SD 18 ± 0.4; and HIV
negative PTB patients. Body Mass Index were 17.8 to 18.9, median 18.4, and mean ± SD 18.5 ± 0.6 respectively (Table 9).

The Body Mass Index status was significantly low in PTB patients with HIV coinfection when compared to control (p<0.004) and with HIV negative PTB patients (p<0.005). A positive correlation was observed between body mass index and albumin as well as body mass index and zinc. Micronutrient zinc deficiency was more in those with low body mass index. Moreover serum zinc and albumin were positively correlated (Pearson correlation, r = 0.583, p<0.0001).

**Serum zinc and albumin levels**

Serum zinc and albumin levels of control and cases are given in (Table 9). Compared with the control group, the concentrations of zinc and albumin were significantly lower ($P < 0.001$) in PTB cases. Among the cases, serum zinc and albumin levels were significantly lower in those with HIV co infection than without. The $P$ value for zinc and albumin were $P < 0.001$ and $P<0.002$ respectively.
<table>
<thead>
<tr>
<th>Category</th>
<th>BMI (Kg/m²)</th>
<th>Serum Zinc ($) in μg/dL</th>
<th>Serum Albumin (¥) in g/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control (n=40)</strong></td>
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<tr>
<td>Range</td>
<td>18-20.1</td>
<td>79-120</td>
<td>3.2</td>
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<tr>
<td>Median</td>
<td>19.2</td>
<td>116</td>
<td>4.0</td>
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<tr>
<td>Mean ±SD</td>
<td>19.6 ± 0.6</td>
<td>117.13 ± 4.2</td>
<td>4.1 ± 0.6</td>
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<tr>
<td><strong>PTB with HIV (n=100)</strong></td>
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<tr>
<td>Range</td>
<td>17.6-18.9</td>
<td>49-56</td>
<td>2.1-3</td>
</tr>
<tr>
<td>Median</td>
<td>18.2</td>
<td>52</td>
<td>2.8</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>18 ± 0.4 *</td>
<td>53.9 ± 8</td>
<td>2.9 ± 0.4</td>
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<tr>
<td><strong>PTB without HIV (n=100)</strong></td>
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<tr>
<td>Range</td>
<td>17.8-18.9</td>
<td>60-77</td>
<td>3.0-3.9</td>
</tr>
<tr>
<td>Median</td>
<td>18.4</td>
<td>64</td>
<td>3.6</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>18.5 ± 0.6 **</td>
<td>65.53 ± 9.8</td>
<td>3.6 ± 0.7</td>
</tr>
<tr>
<td><strong>Significance</strong></td>
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<tr>
<td>$</td>
<td>* P&lt;0.004</td>
<td>$ P&lt;0.001</td>
<td>¥ P&lt;0.002</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>**P&lt;0.005</td>
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**TABLE: 9. BMI, Zinc and Albumin in pulmonary tuberculosis with and without HIV**

BMI- Body mass index  
HIV-Human immunodeficiency virus  
PTB-Pulmonary tuberculosis  
SD-Standard deviation.
Discussion

Infection induces reduction in serum zinc and albumin level in human beings as well as experimental animals. Serum zinc and albumin zinc levels were significantly reduced in patients with pulmonary tuberculosis irrespective of their HIV status than healthy control (Ramakrishnan et al., 2008). The possible causes for low serum zinc and albumin in PTB patients were attributable to nutritional factors, enteropathy and acute phase reactant proteins. (Karyadi et al., 2000, & World Health Organization Expert Committee, 1995). The hepatic synthesis of acute phase reactant proteins are induced by cytokines such as interleukin-6 and tumor necrosis factor- (Xing et al., 1998 and Gabay et al., 1999) which inhibit the production of serum albumin and cause dramatic shifts in plasma concentration of certain essential micronutrients and albumin.

Reduced level of serum zinc observed among TB patients was significantly lower than that of control and is in agreement with a study at Indonesia (Karyadi et al., 2000). Also this was likely due to redistribution of zinc from plasma to other tissues or reduction of the hepatic production of the zinc-carrier protein macroglobulin and to a rise in the production of metallothionin, a protein that transports zinc to the liver (Gabay et al., 1999).

Body Mass Index was significantly lower in patients with active pulmonary TB compared with healthy control which is in concurrence with a recent report from Southern India (Swaminathan et al., 2008). The low body mass index among patients with pulmonary TB may be due to poor dietary intake, anorexia, impaired absorption of nutrients or increased catabolism (Hopewell, 1994). The present published observations (Madurai) are in concurrence with the study done in Indonesia (Karyadi et al., 2000)
and Malawi (van Lettow et al., 2004). Low body mass index is a known risk factor for mortality (van Lettow et al., 2003).

Since, HIV-infected adults with pulmonary TB had significantly low zinc, albumin and Body mass index, this subgroup may potentially benefit from nutritional interventions. Several studies (Jon et al., 2006, and Karyadi et al., 2000) revealed that micronutrient supplementation to patients with active TB and HIV improve their health by way of increase in CD4+ count (Jon et al., 2006), weight gain (Range et al., 2006), efficacy of drugs (Range et al., 2006) as well as decrease in opportunistic infections (Karyadi et al., 2000). Interestingly, intake of zinc beyond certain amount was associated with increased relative risk for disease progression (Baum et al., 2000) where as it was contradicted by different workers (Neera et al., 2002).

**Conclusion**

This chapter concludes that nutritional status of patients with active pulmonary TB is poor when compared with healthy controls. Low concentrations of plasma albumin and zinc as well as wasting were significant in PT patients with HIV infection when compared to non HIV subjects. Also the study indicates need for therapeutic supplementation of zinc and proteins to all patients with active TB with and without HIV.

**Strength**

The strength of the present total study was rigid criteria adopted for selection of all cases and standard methods followed for sputum collection as well as processing the samples by senior microbiologist.