INTRODUCTION

Tuberculosis has re-emerged to become the world’s leading cause of death from a single infectious agent. The precise clinical manifestations of tuberculosis (TB) are likely to result from a complex interaction between the host and the pathogen. Cytokines are primarily involved in host response to disease or infection. TNF-α is a monocyte-activating cytokine, which stimulates antimycobacterial activity and helps to maintain the integrity of the tuberculous granulomas in which M. tuberculosis is contained. TNF-α is believed to play multiple roles in the immune and pathological responses in tuberculosis. Serum TNF-α measurement might play an important role in the evaluation of the inflammatory phenomena in TB.

During pulmonary inflammation, increased amounts of reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) are produced as a consequence of phagocytic respiratory burst. These ROS and RNI induce lipid peroxidation (LP), a general mechanism of tissue damage by free radicals that is known to be responsible for cell damage and may induce many pathological events. Inflammatory cytokines play an important role during the course of the disease and may be responsible for tissue damage by lipid peroxidation.
In the present study, serum levels of Tumor necrosis factor alpha & Malondialdehyde were estimated to assess the effect of antituberculor therapy.

MATERIAL AND METHODS

Subjects
Thirty-five patients (20 male and 15 female) with pulmonary TB and 32 healthy controls (17 male and 15 female) participated in the study. All patients were recruited from the TB & Chest department Shri Aurobindo Institute of Medical sciences & Manorama Raje Tuberculosis hospital, Indore. Their mean age was 31.62 ± 6.37 (range 18-55 years). On entry, all pulmonary tuberculosis patients belonged to CAT I (Two sputum specimens positive for acid-fast bacilli by direct microscopy or one sputum specimen positive for culture) No extrapulmonary involvement was found in any patient. Patients who had diabetes mellitus, pregnancy or immunological or autoimmune diseases other than tuberculosis & subjects with history of smoking, previous antituberculosis medication were excluded from the study. None of the subjects had serological evidence of HIV infection. All patients were administered antituberculosis therapy according to standard antitubercular regimen in which isoniazid, rifampicin, pyrazinamide and streptomycin or ethambutol (2HRZE/4H3R3) was used. A control group of 32 healthy volunteer subjects (17 women and 15 men) with a mean age of 27.74 ± 7.30 (range 18-55 years) was also studied. Consent to participate in the study was obtained from each individual and the study protocol was approved by the Institutional & Human Ethical committees, Shri Aurobindo Institute of Medical sciences, Indore.

Blood collection
Fasting blood samples (5 ml) was collected from antecubital venipuncture in a plain tube, and was left to clot & then centrifuged at 1000 rpm for 10 min. The serum was then aliquoted & stored until used for assay of parameters. Blood samples were collected from all controls and from the PTB patients at baseline, that is, before starting antituberculosis treatment (ATT) and after one month of ATT.

Estimation of serum TNF alpha
Serum TNF alpha was estimated by using a commercially available immunoassay Kit from DIACLONE United Kingdom. The kit was used according to the manufacturer’s instructions. The concentration of the cytokines in the samples was determined using a standard curve and the results were expressed as pg/ml. [Normal range = < 8pg/ml]

Estimation of serum MDA
Estimation of MDA in the serum was done by thiobarbituric acid method of Wilbur K M et al (1949). The thiobarbituric acid reacts with serum malondialdehyde produced by hydrolysis of lipid hydroperoxides to form the pink red color complex that absorbs strongly & can be measured spectrophotometrically at 532 nm. (The results were expressed as nmoles/ml.) The complex is usually quantified against MDA standards generated from 1, 1, 3, 3 tetraethoxypropane under the same reaction conditions.

Statistics
Unpaired t-test and paired t-test were used for statistical assessments with SPSS Version 10 to evaluate mean levels of variables between study groups and controls, and to determine values of pretreatment and after treatment in study group. Values were expressed as Mean ± SD and pd’0.05 was significant.

RESULTS
There was no significant difference between mean ages of control & pulmonary tuberculosis patients (p>0.05). The serum levels of TNF alpha & MDA were highly significant in pulmonary tuberculosis patients when compared with control (p<0.001) (Table no 1)

Serum TNF alpha & MDA levels were found to be significantly decreased (p< 0.05) after antituberculosis therapy. (Table no 2)

DISCUSSION
The present study showed significant increase in serum TNF α & Malondialdehyde levels in pulmonary tuberculosis patients as compared to controls. TNF α also appears crucial for the formation of M. tuberculosis-constraining granulomas, infection control and elimination of mycobacteria. An
Table 1: Comparison of mean age & serum levels of TNF α & MDA in control group & pulmonary tuberculosis patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Tuberculosis patients</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>31.62 ± 6.37</td>
<td>29.37 ± 8.2</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Serum MDA nmol/ml</td>
<td>2.1 ± 0.82</td>
<td>5.04 ± 1.15</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Serum TNF α Pg/ml</td>
<td>14.59 ± 8.88</td>
<td>53.14 ± 19.06</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2: Serum levels of TNF α & MDA before & after antituberculor treatment in pulmonary tuberculosis patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before therapyMean ± SD</th>
<th>After therapyMean ± SD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum MDA nmol/ml</td>
<td>5.04 ± 1.15</td>
<td>4.54 ± 0.90</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Serum TNF α Pg/ml</td>
<td>53.14 ± 19.06</td>
<td>44.54 ± 15.48</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

association between TNF α with pathology in TB accompanied by fever, weight loss, shock, tissue necrosis is reported. Previous studies have shown higher serum levels of TNF α in pulmonary TB patients as compared to control subjects. The serum TNF α level may be a good marker to predict the TB patient’s clinical evolution. Evaluating the serum cytokine levels may be useful in evaluating the clinical effect of antituberculous therapy. An effect of antitubercular therapy in the present study is consistent with the other studies. Although, contradictory results have also been reported suggesting involvement of TNF in delayed-type hypersensitivity responses. TNF- alpha secretion continued to escalate even after treatment with ATT.

Our findings are consistent with the previous reports that a serum MDA concentration, that is a measure of lipid peroxidation and reflecting the degree of oxidative stress, were significantly higher in patients with tuberculosis as compared to healthy controls.

The level of MDA is decreased significantly after antituberculor treatment in present study.

Reddy et al., (2004) & Madhab Lamsal et al., (2007) demonstrated a significantly lower MDA concentration and higher antioxidant levels in patients with clinical improvement after chemotherapy. While Jack et al., (1994) and Plit et al., (1998) did not find any such significance. Anti-TB drugs induce formation of ROS and those patients with poor antioxidant mechanisms are at a greater risk of toxicity. Oxidative stress could play a role in the pathogenesis of antitubercular drug (ATD) induced hepatotoxicity and lower levels of plasma glutathione and higher levels of MDA may be due to oxidative stress resulting from ATD therapy.

The present study showed raised levels of Tumor necrosis factor alpha and Malondialdehyde in pulmonary tuberculosis patients. After antituberculor therapy, these parameters decreased significantly. This is useful for evaluating the asperity of TB disease and monitoring the clinical effects of antitubercular drugs. TNF alpha immune modulation should be evaluated in M.tuberculosis therapy in future studies. Because of oxidative tissue damage, suitable anti-oxidant supplementation for protection from free radical attack is warranted.

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