LIPID PEROXIDATION AND OXIDATIVE STRESS IN PULMONARY TUBERCULOSIS IN EDO STATE, NIGERIA

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Abstract
Aims: The purpose of the study was to investigate the blood lipid peroxidation products and important free radical scavenging enzymes.

Methods: The study comprised of a total of 230 subjects made up of normal human volunteers (n=50) as control group, newly diagnosed PTB patients without anti-tuberculosis therapy, PTB-WATT, (n=60) and a case group of PTB patients on anti-tuberculosis therapy, PTB-ATT, (n=120). TBARS, MDA, SOD and CAT were analyzed by standard methods.

Results: The TBARS levels in PTB, PTB-WATT, PTB-ATT patients were significantly higher (p < 0.05) than the control group. Also the MDA levels in PTB, PTB-WATT, PTB-ATT patients were significantly higher (p < 0.05) than the control group. While the levels of SOD activity in PTB, PTB-WATT, PTB-ATT patients were significantly lower (p < 0.05) than the control group. Equally, the CAT levels in PPTB, PTB-WATT, PTB-ATT patients were significantly lower (p < 0.05) than the control group.

Conclusion: The study showed an increased oxidative stress and high lipid peroxidation in the plasma of PTB patients with or without anti-tuberculosis therapy. Therefore, there is a need to beef-up antioxidant therapy with proper nutritional antioxidant supplementation in such patients.

Key Words: Oxidative stress, Lipid peroxidation, Pulmonary tuberculosis.

INTRODUCTION
Pulmonary tuberculosis, an infectious disease caused by Mycobacterium tuberculosis, is one of the world’s leading causes of death (Paton et al., 2004). The pathogenesis of TB is multifactorial and includes the effects of oxidative stress (Janiszewska-Drobinska et al., 2001; Madebo et al., 2003; Wiid et al.,2004). Reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) are induced by mycobacteria through the activation of phagocytes (May and Spagnuolo, 1987; Kuo et al., 1996; Plit et al., 1998) by respiratory burst mechanism (Kwiatkowska et al., 1999) which is crucial to host defense but may promote tissue injury, inflammation (Jackson et al., 1994; Wiid et al., 2004) and may further contribute to immunosuppression (Hugo 1963). Pulmonary fibrosis and dysfunction in TB are thought to be a consequence of chronic inflammatory events involving pro-inflammatory cytokines, activated macrophages and ROS that stimulate fibroblast proliferation and mononuclear cell DNA damage (Orme et al., 1993; Jack et al., 1994; Ellner et al., 1997). It is spread through air, when the infected individual cough, sneeze, or spit and most infections in humans result in asymptomatic, latent infection, and about one in ten latent infections eventually progress to active disease, which if left untreated, kills more than 50% of its victims (Konstantinos, 2010). Karyadi et al., (2002); Vijayamalini and Manoharan (2004)
MOKOGWU ET AL  PULMONARY TUBERCULOSIS

stated that in developing countries, infection and malnutrition form a vicious cycle, hence TB is often said to be the disease of the malnourished. Lipid peroxidation which is a general mechanism of tissue damage by free radicals is known to be responsible for cell damage and may induce many pathological events (Reddy et al., 2009). And this process (lipid peroxidation) is one of oxidative conversion of polyunsaturated fatty acids to lipid peroxidation products of which malondialdehyde (MDA) is the most widely studied biologically free radical reaction. MDA itself, owing to its high cytotoxicity and inhibitory action on protective enzymes, is suggested to act as a tumor promoter and a co-carcinogenic agent (Hashni et al., 2012). MDA is a well-characterized mutagen that reacts with deoxyguanosine to form a major endogenous adduct found in the DNA of human liver (Bakan et al., 2002). TB patients have been reported to have decreased concentrations of antioxidants, enhanced generation of ROS (Madebo et al., 2003) and increased levels of lipid peroxidation product as a consequence of impaired activity of scavenging enzymes (Srinivasan et al.,2004). Superoxide which is a free radical is produced during cellular metabolic reactions. Superoxide dismutase (SOD), an antioxidant enzyme, changes superoxide anion into hydrogen peroxide and oxygen. Superoxide dismutase is expressed at high levels in mammalian lungs compared with other tissues (Gao et al., 2008). In Nigeria, there is little or no work on the effects of oxidative stress and antioxidants in patients with pulmonary tuberculosis. Hence this study was to investigate the levels of lipid peroxidation products (thiobarbituric acid reducing substances, TBARS; malondialdehyde, MDA), and the intensity of oxidative stress by the measurement of superoxide dismutase (SOD), and catalase (CAT) in normal human volunteers and pulmonary tuberculosis patients.

MATERIALS AND METHODS

The study, a cross-sectional survey was carried out at University of Benin Teaching Hospital (UBTH) Leprosy Tuberculosis Centre located at Ogan Village, near Benin-City, Edo State.

Sample Population
This comprised of a control group, made up of apparently healthy individuals and a case group who were patients of known and established cases of Pulmonary Tuberculosis (PTB).

Sample Size
A total of 230 subjects, age range between 22-57yr were studied. They were made up of 120 cases of pulmonary tuberculosis patients on antituberculosis therapy (PTB-ATT), 60 newly diagnosed pulmonary tuberculosis patients without antituberculosis therapy (PTB-WATT) and 50 apparently healthy subjects as control groups.

Sample Collection
Blood samples were collected from the subjects after an informed consent was sought along side with the administration of a questionnaire. Blood sample (about 5ml) was collected from each of the subjects under aseptic condition using 5ml disposable syringe and needle. Each blood sample was transferred into separate lithium heparin tubes and mixed gently. At the end of each day’s collection, the heparinized blood samples were centrifuged using Mega centrifuge 1.0 Heraus instrument of German made. The instrument was set at 5,000 rpm for 5 minutes, followed by subsequent separation of each of the plasma samples into plain tubes and labeled appropriately. Plasma samples were analyzed on the same day of collection. The control samples were collected from apparently healthy individuals and were treated as the study group.

Sample Analysis
Plasma thiobarbituric acid reducing substances (TBARS) was determined by method of Yagi, 1996. To 1ml of plasma sample in a test tube was added 2ml of TCA-TBA-HCL reagent, while 3ml of the reagent was used as the blank tube. The solutions were heated in a boiling water bath for 15minutes and allowed to cool. The tubes were centrifuged to remove flocculent precipitate at 1000g for 10minutes. Subsequently the absorbance of the supernatant sample was read against the reagent blank at 535nm. Malondialdehyde (MDA) activity was assayed as developed by Nichans and Samualson, 1968. Into test tubes labeled test and blank were added 3ml of glacial acetic acid. This was followed by the addition of 3ml of 1% thiobbituric acid (TBA). Into the test was added 0.6ml of sample while 0.6ml of distilled water served as blank. They were mixed and incubated at 100˚C water bath for 15min. Then they were allowed to cool and subsequently centrifuged at 2000rpm for 5min. The absorbance of the test was read spectrophotometrically at 532nm after zeroing
with the blank. Superoxide dismutase (SOD) activity was assayed by the method of Kakkar et al., 1984. Into test tubes labeled test and blank were added 2.5ml of 0.05M carbonate buffer. 0.2ml of sample was added into the test while 0.2ml of distilled water was used as blank. This was followed by the addition of 0.3ml of 0.03mM of adrenalin. They were then mixed and the absorbance of test read spectrophotometrically at 420nm after zeroing the instrument with the blank. Catalase (CAT) activity was determined by the method of Sinha, 1972. 0.5ml of sample was added into a test tube labeled test while 0.5ml of distilled water was used as blank. Then 5ml of cold 30mM peroxide was added into each of the tubes. The solutions were mixed by inversion and allowed to stand on the bench for 3min. This was followed by the addition of 1ml of 6M H$_2$SO$_4$ each to both the sample and the blank as well as into a standard tube containing 5.5ml of 0.05M phosphate buffer of pH 7.4. The 3 tubes were mixed by inversion followed by the addition of 7ml of 0.01M KMnO$_4$. The absorbance of test and standard were read at 480nm after zeroing with the blank.

**Data Analysis**

All data were analyzed using Epinfo version 3.5.1, 2008. Means, standard deviations and proportions were determined as applicable. Student’s t-test was used, while a probability value (P value) less than 0.05 was taken as statistically significant.

**RESULTS**

Age range of control group was 22-54yr with a mean of 33-20. Age range of PTB group was 20-58yr with a mean of 35-21. There was no significant age difference between the control and the case group. The mean concentration of TBARS obtained in PTB patients (4.67 ± 0.89) was significantly higher (p < 0.05) than the control group (2.98 ± 0.67). Also the value of MDA obtained in PTB patients (7.77 ± 1.22) was significantly higher (p < 0.05) than the control group (3.78 ± 1.66). Equally the mean concentration of SOD obtained in PTB patients (1.08 ± 0.27) was significantly lower (p < 0.05) than the control group (1.86 ± 0.47), while the mean concentration of CAT obtained in PTB patients (4.34 ± 0.63) was significantly lower (p < 0.05) than the control group (5.09 ± 0.62) all as shown in Table 1. Table 2 shows the plasma concentration of TBARS, MDA, SOD, CAT in PTB-WATT and PTB-ATT patients with control group. The mean concentration of TBARS and MDA obtained in PTB-WATT patients (5.09 ± 0.62 and 8.34 ±1.58) were significantly higher (p < 0.05) than the control group (2.98 ± 0.67 and 3.78 ± 1.66). Also the concentration of SOD, CAT in PTB-WATT patients (0.86 ± 0.28, 3.89 ± 0.59) were found to be significantly lower (p < 0.05) than the control group (1.86 ± 0.47, 5.09 ± 0.89) in that order (Table 2). While the mean concentration of TBARS, MDA in PTB-ATT patients (4.24 ± 1.10, 7.19 ± 0.86) were found to be significantly higher (p < 0.05) than the control group (2.98 ± 0.67, 3.78 ± 1.66) and also the SOD, CAT concentrations obtained in PTB-ATT patients (1.30 ±0.26, 4.78 ± 0.67) were found to be significantly lower (p < 0.05) than that of control group (1.86 ± 0.47, 5.09 ± 0.89) in that order (Table 2). Table 3 shows the comparison of TBARS, MDA, SOD, CAT in PTB-WATT and PTB-ATT patients. The levels of TBARS, MDA in PTB-WATT patients (5.09 ± 0.62, 8.34 ±1.58) were found to be significantly higher (p < 0.05) than that of PTB-ATT patients. While the levels of SOD, CAT in PTB-WATT patients (0.86 ± 0.28, 3.89 ± 0.59) were found to be significantly lower (p < 0.05) than that of PTB-ATT patients (1.30 ± 0.26, 4.78 ± 0.67) as shown in Table 3.

### Table 1. Plasma concentration of TBARS, MDA, SOD & CAT in PTB patients and Control Group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>PTB Patients</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol/ml)</td>
<td>2.98 ± 0.67</td>
<td>4.67 ± 0.89</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>3.78 ± 1.66</td>
<td>7.77 ± 1.22</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>SOD (IU/ml)</td>
<td>1.86 ± 0.47</td>
<td>1.08 ± 0.27</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>CAT (IU/ml)</td>
<td>5.09 ± 0.89</td>
<td>4.34 ± 0.63</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

**Key:**

- Values = expressed in x ± STD  
- CAT = Catalase  
- MDA = Malondialdehyde  
- SOD = Superoxide dismutase  
- PTB = Pulmonary tuberculosis  
- TBARS = Thiobarbituric acid reducing substances
Table 2. Plasma concentration of TBARS, MDA, SOD & CAT in PTB-WATT patients, PTB-ATT patients and Control group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>PTB-WATT</th>
<th>PTB-ATT</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol/ml)</td>
<td>2.98 ± 0.67</td>
<td>5.09 ± 0.62</td>
<td>4.24 ± 1.10</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>3.78 ± 1.66</td>
<td>8.34 ± 1.58</td>
<td>7.19 ± 0.86</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>SOD (IU/ml)</td>
<td>1.86 ± 0.47</td>
<td>0.86 ± 0.28</td>
<td>1.30 ± 0.25</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>CAT (IU/ml)</td>
<td>5.09 ± 0.89</td>
<td>3.89 ± 0.59</td>
<td>4.78 ± 0.67</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

Key:
- Values = expressed in x ± STD
- MDA = Malondialdehyde
- TBARS = Thiobarbituric acid reducing substances
- PTB = Pulmonary tuberculosis
- PTB-ATT = Pulmonary tuberculosis on anti-tuberculosis therapy
- CAT = Catalase
- PTB-WATT = Pulmonary tuberculosis without anti-tuberculosis therapy

Table 3. Plasma concentration of TBARS, MDA, SOD, CAT in PTB-WATT & PTB-ATT patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PTB-WATT</th>
<th>PTB-ATT</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol/ml)</td>
<td>5.09 ± 0.62</td>
<td>4.24 ± 1.10</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>8.34 ± 1.58</td>
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<td>p &lt; 0.05</td>
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<td>SOD (IU/ml)</td>
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<td>1.30 ± 0.25</td>
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</tr>
<tr>
<td>CAT (IU/ml)</td>
<td>3.89 ± 0.59</td>
<td>4.78 ± 0.67</td>
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</table>

Key:
- Values = expressed in x ± STD
- TBARS = Thiobarbituric acid reducing substances
- PTB = Pulmonary tuberculosis
- PTB-ATT = Pulmonary tuberculosis on anti-tuberculosis therapy
- CAT = Catalase
- PTB-WATT = Pulmonary tuberculosis without anti-tuberculosis therapy

DISCUSSION

The oxidative stress is measured not only by the production of free radicals and by antioxidant enzymes but also by enhanced lipid peroxidation products (TBARS and MDA) in PTB patients. Our findings of greatly increased levels of TBARS and MDA in pulmonary tuberculosis patients in this study (means of 4.67 ± 0.89 and 7.77 ± 1.22) compared to control levels (2.98 ± 0.67 and 3.78 ± 1.66) is in agreement with the work of Hashni et al.,2012 who observed a 2.5 fold increase in MDA in TB patients. The increased level of MDA is also in agreement with the work of Nwanjo and Eze, 2007 who noted a high level of MDA in PTB patients infected with human immunodeficiency virus. The findings also corroborates with the work of Reddy et al., 2004 who recorded a mean MDA level of 8.64 ± 1.84 in PTB patients. Our findings of significant reduction in enzymatic antioxidants (SOD, CAT) is also in line with the findings of Hashni et al., 2012 and Reddy et al., 2004 who noted a significant reduction in both SOD and CAT. Our results are also in accordance with various other studies (Guzel et al., 2006;Reddy et al., 2009; Suresh et al., 2010) in which different methodologies were used to assess the total antioxidant capacity. Our findings of a significant correlation between high TBARS, MDA concentrations and low concentrations of antioxidant enzymes correlates with the work of Madebo et al., (2003) who suggested increased utilization of ROS as an important contributing factor to the lower concentrations of antioxidants in TB patients. The contribution of malnutrition leading to decreased supplementation of antioxidants and enhanced ROS generated leading to increased utilization of these compounds may represent a pathogenic loop that results to markedly enhanced oxidative stress during tuberculosis. Our results of reduced antioxidant capacity in PTB patients undergoing ATT are not only based on comparison with control group (table 2) but also comparison of the various parameters analyzed in patients without anti tuberculosis therapy and those on tuberculosis therapy (Table 3). The significant reduction (p < 0.05) in TBARS, MDA (4.20 ± 1.10, 7.17 ± 0.87) and significant increase (p < 0.05) in SOD, CAT (1.30 ± 0.26, 4.78 ± 0.67) in pulmonary tuberculosis patients undergoing anti-tuberculosis therapy (PTB-ATT) compared to TBARS, MDA (5.09 ± 0.62, 8.34 ± 1.58) and SOD, CAT (0.86 ± 0.28, 3.89 ± 0.59) in pulmonary tuberculosis patients without anti-
tuberculosis therapy (PTB-WATT) are in accordance with the observation of Reddy et al., 2009 using pleural fluid in PTB patients, who recorded a significant increase in antioxidant levels in patients under treatment in contrast with untreated cases.

Conclusion: Our study showed high lipid peroxidation and increased oxidative stress in plasma of pulmonary tuberculosis patients with or without therapy as compared to normal healthy control subjects. These findings further support a link between oxidative stress and tuberculosis infection. Hence, nutritional support may represent a novel approach for swift recovery.

REFERENCES


