Oxidative Stress and Lipid Profile Status in Pulmonary Tuberculosis Patients in South Western Nigeria

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ABSTRACT

Malnutrition and increased free radicals generation are common findings with tuberculosis patients and these can impair their antioxidant capacity. The present study was carried out to estimate the levels of non-enzymatic antioxidants: albumin, vitamin C and E, selenium and lipid fractions in sixty-five (M = 29; F = 36) newly diagnosed (untreated) adult active tuberculosis (TB) patients and to compare them with the levels in fifty (M = 25; F = 25) apparently healthy tuberculosis free individuals of the same age group and location. Lipid fractions, MDA, albumin, vitamins C and E were estimated by standard spectrophotometric methods while selenium concentration was determined by atomic absorption spectrophotometric method. There was a significantly increased concentration of MDA (P < 0.001) in TB patients when compared with the control subjects and significantly reduced concentrations of albumin, vitamin C and E, selenium, total–cholesterol, LDL-cholesterol (P < 0.001 in each case) and triglyceride (P < 0.01) were observed when the TB patients were compared with non TB infected individuals. The study showed that TB patients are predisposed to oxidative stress and reduced concentrations of lipid fractions. Highly nutritious diet fortified with antioxidants supplements is advocated for TB patients alongside chemotherapeutic management and their lipid fractions status should be monitored while managing the patients.

Keywords: Tuberculosis, oxidative stress, lipid profile, non-enzymatic antioxidants, developing country, vitamin C, vitamin E.

INTRODUCTION

Mycobacterium tuberculosis is considered as an etiologic agent of tuberculosis (TB) with the identifying feature of the organism been an acid –fast property (Nwanjo and Oze, 2007). Tuberculosis is a leading health problem worldwide and remains one of the leading causes of death from infectious disease. It is a highly infectious disease that is widely distributed throughout the globe (Gopi et al., 2008). Almost one third of the world’s population is infected with Mycobacterium tuberculosis and the majority of these individuals live in less developed countries (Swaminathan et al., 2008). It is commonly a disease of the lung where it forms a localized infection after inhalation. Worldwide, TB is responsible for more than 1.5 million deaths every year, (Sultan et al., 2012) with an estimated rate of 13.7 million prevalent cases of TB in 2007 (206 per 100,000 populations). (WHO, 2009). Therefore, despite recent progress, TB remains an important global public health problem (WHO, 2009).

Pulmonary disease is characterized by general symptoms such as unexplained cough, dehydration/vomiting, unexplained tiredness, loss of weight, high remittent or intermittent pyrexia and loss of
appetite at the predromal period and specific respiratory symptoms like haemoptasis, pleural pain and others depending on the site of involved (Folaranmi and Adesiyan, 2004). It can affect extra pulmonary regions like lymph nodes, bones and joints, skin, meninges, eyes, the kidneys and also the gastro-intestinal tracts, where it causes an insidious disease that sometimes develops without any striking clinical evidence (Hardy and Schumidek, 1968).

Recent research suggest that in pulmonary Tuberculosis there is increase in several circulating markers of free radical activity, indicating ongoing oxidative stress and decrease in the antioxidant activity which may contribute to development of lung function abnormalities (Ragunath and Madhavi, 2006). Although these are important part of the host defense against the organism; enhanced reactive oxygen species (ROS) generation may promote tissue injury and inflammation. This further contributes to immune suppression (Jack et al., 1994). Moreover, the malnutrition that is commonly associated with patients with TB may further contribute to the impaired antioxidant capacity in these patients which may result into severe oxidative stress that has been reported in TB patients due to malnutrition and poor immunity (Reddy et al., 2004). To maintain normal lung function, there must be a protective antioxidants balance between toxic ROS and antioxidants which protects the body from the damaging effects of ROS. Antioxidants are physiologic substances that are derived both from endogenous and exogenous sources and acts to quench ROS. Example includes catalase, superoxide dismutase, glutathione peroxidase, vitamin C, vitamin E, albumin and uric acid.

Sasaki et al. (1994), reported that lipid peroxidation could cause reduced concentration of serum lipids. Yamanaka et al. (2001), also reported significantly lower concentration of cholesterol in TB patients. There is paucity of local reports on the status of lipid peroxidation, antioxidants, oxidative stress and lipid fractions in our country. The present study was aimed at investigating the status of lipid peroxidation products (malondialdehyde), non-enzymatic antioxidants, triglyceride, total cholesterol, HDL-cholesterol and LDL-cholesterol in TB patients so as to provide information that will enhance success rate in treatment and management of patients with TB in our country.

MATERIALS AND METHODS

Sixty-five HIV seronegative pulmonary tuberculosis patients with the active disease (M = 29; F = 36) aged 20-60 years from low socio-economic status diagnosed by Ziehl-Nelson staining/demonstration of mycobacteria in sputum participated in the study. They were screened for HIV using genie II HIV 1/2 a dual recognition enzyme immunoassay 92430 Marnesia Cogutte-France. Their chest x-ray revealed pulmonary changes; they are taken as the test subjects. The TB patients were recruited from the patients attending the chest clinic, Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Osun state, Nigeria. The control subjects were 50 (M = 25; F = 25) apparently healthy, age-matched individuals who tested free of Mycobacterium tuberculosis without any previous or present symptom of TB or any pulmonary disease. They were recruited from members of staff and students of the same hospital. They had no history or symptom of any form of pulmonary disease and their chest x-ray indicated no TB or any other pulmonary changes.

Patients suffering from other pulmonary diseases apart from TB, those with complications such as diabetes mellitus, hepatic disease, renal failure and endocrine disorder, pregnancy, and history of smoking were also excluded from the study. Only new patients yet to commence treatment for TB were recruited for the study. The study conformed to the Helsinki declaration. All the subjects gave informed consent to participate in the study.

Sample Collection

Ten milliliters of venous blood was collected from all the subjects into heparin tubes after an overnight fasting period of 14 hours. The plasma was extracted by centrifuging the whole blood in a wisperfuge (model 684) centrifuge at 3000 g for 5 minutes. The plasma was used for the estimation of albumin, triglyceride, total cholesterol, HDL-cholesterol and LDL-cholesterol vitamin E, vitamin C, malondialdehyde and selenium.

Analytical methods

Albumin concentration was determined by the Bromocresol-green method, while triglyceride, total cholesterol and HDL-cholesterol were measured spectrophotometrically using commercially prepared reagents produced by Randox laboratory, UK while LDL-cholesterol was calculated using Friedel Wald formula. Malondialdehyde was estimated by the thiobarbituric acid reactive substance (TBARS) method as described by Nichan and Samuelson.
Selenium level was determined by atomic absorption spectrophotometry. Vitamin C was measured by method of Omaye et al. (1979). The method is based on the reaction of ascorbic acid with DNPH reagent (2% DNPH) and 4% thiourea in 9N sulphuric acid. The red color formed was measured at 520 nm. Vitamin E was estimated by the method of Hansen and Warwick (1969). The method was based on the extraction of Vitamin E into hexane and then quantitated by measuring relative fluorescence with ultraviolet spectrophotometer at 248 nm wavelength.

The results were expressed as mean ± S.D. Comparison was made using student’s t–test while P < 0.05 was regarded as significant and were presented in tabular form as shown below.

RESULTS

Table 1: Biochemical parameters in tuberculosis patients and control subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Subject (M=29, F=36)</th>
<th>TB Patients (M=25, F=25)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmol/L)</td>
<td>0.43±0.23</td>
<td>1.2±0.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Albumin(g/L)</td>
<td>42±2.5</td>
<td>29±0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Vitamin C(mg/100ml)</td>
<td>25.4±3.0</td>
<td>15.7±4.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Vitamin E(mg/100ml)</td>
<td>17.0±1.8</td>
<td>10.56±4.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Selenium(mmol/L)</td>
<td>1.3±0.10</td>
<td>0.63±0.14</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Results expressed as mean ± S.D

Table 2: Comparison of Lipid Profile in tuberculosis patients and control subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control subjects (M=25,F=25)</th>
<th>TB Patients (M=29,F=36)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride(mmol/L)</td>
<td>0.84±0.2</td>
<td>0.55±0.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Cholesterol(mmol/L)</td>
<td>6.6±0.5</td>
<td>4.8±1.0</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.32±0.4</td>
<td>1.02±0.27</td>
<td>0.25</td>
</tr>
<tr>
<td>LDL-cholesterol(mmol/L)</td>
<td>4.1±0.9</td>
<td>2.7±0.9</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Results expressed as mean ± S.D

The lipid peroxidation products (malondialdehyde level) estimated in TB patients was significantly higher (P < 0.001) than that found in control subjects. The concentrations of albumin, vitamin C and E in the tuberculosis patients were all observed to be significantly lower (P < 0.001 in each case) than that in the control subjects. The concentrations of triglyceride and selenium in the control subjects was significantly higher (P < 0.01 and 0.001, respectively) when compared with that of the test subjects. The concentrations of total-cholesterol and LDL-cholesterol in the test subjects were significantly lower (P < 0.01) than that in the control subjects.

There was no significant difference (P > 0.05) in the HDL–cholesterol levels when the test and control subjects were compared together.

DISCUSSION

The present study is on evaluation of lipid peroxidation product (malondialdehyde- MDA), non-enzymatic antioxidants (albumin, vitamins C and E) and lipid profile in tuberculosis (TB) patients without history of smoking or HIV disease. The results showed higher lipid peroxidation products and significantly lower antioxidants potential in TB patients. Additionally, this study supports a role for oxidative stress in the pathogenesis of TB and suggests lower antioxidant capacity and higher free radical levels in the TB patients than in control subjects. Significantly increased MDA in TB
patients than in non-TB individuals has been previously reported by some authors (Reddy et al., 2004; Tesfaye et al., 2003; Reddy et al., 2009). The finding in this study correlates with these previous findings. Increased production of reactive oxygen species / reactive nitrogen intermediates(RNI) secondary to phagocyte respiratory burst occur in pulmonary TB. Evidence suggest that increased circulating levels of free radical activity are found in pathogenesis of active pulmonary TB and hence play a role in resultant fibrosis (Rameh et al., 2011). Free radicals have been implicated in the development of lung fibrosis which may be a long term sequel of pulmonary tuberculosis (Jack, 1994). Free radicals attack the cell membrane causing tissue damage and wasting disease in pulmonary TB patients. Mycobacterium can induce reactive oxygen species production by activating phagocytes (Reddy et al., 2004). Previous studies locally have established significant weight loss and reduced BMI in TB patients when compared with non-TB infected control which improves with chemotherapeutic management (Adebisi et al., 2003; Olusoji and Regina, 2011; Dodor, 2008).

The significantly lower concentration of albumin (P < 0.001) observed in this present study corroborates the findings of previous study among TB patients in other study location (Ramakrishnan et al., 2008). Low levels of albumin in this study might have been predicated by anorexia, malnutrition and mal-absorption which are common findings in TB infection. Albumin is an important component of plasma with antioxidant activity that binds free fatty acids, divalent cations and hydrogen oxochloride. The low level of albumin may therefore contribute to the complications associated with pulmonary TB. Furthermore, albumin is a negative acute phase protein whose value decreases during infection, injury or stress possibly as a result of increased metabolic need for tissue repair and free radical neutralization (Liesuy and Tomato, 1994).

The concentrations of antioxidant water soluble vitamins C and fat soluble vitamin E were significantly lower (P < 0.001 respectively) in TB patients when compared with that of the control subjects. This finding correlates with that of earlier findings (Wiid et al., 2004; Reddy et al., 2004; Madebo et al., 2003). Several factors such as low food intake, nutrient malabsorption, inadequate nutrient release from the liver, acute phase response to infection, inadequate availability of carrier protein may influence circulating antioxidants concentrations (Macallan, 1999; Akiibinu et al., 2008). The increased concentration of lipid peroxidation products associated with TB infection may be a contributing factor to the significantly low level of ascorbic acid and α–tocopherol in the test subjects in comparism with the control subjects.

Triglyceride and LDL–cholesterol are major constituents of cell membrane while HDL–cholesterol protects arterial walls of the blood circulatory system (Liesuy and Tomato, 1994). The significantly low concentrations of triglyceride, total cholesterol and LDL–cholesterol observed among tuberculosis patients in this study correlates with the findings of previous studies (Kwiatkowska et al., 1999). Reddy et al., (2009), have reported increased lipid peroxidation in all categories of TB patients. This might have caused reduction in the concentration of serum lipid as noted in this study. Reduced concentrations of these lipid fractions could have resulted from tissue and cells damage and consequently lead to wasting and weight loss which are often observed in TB patients. The lower levels of the lipid fractions observed in TB patients when compared with the control subjects could also be as a result of impaired rate of lipid production and enhanced rate of lipid catabolic rate associated with pulmonary TB infection.

In conclusion, the present study showed oxidative stress due to increased lipid peroxidation and reduced concentrations of non-enzymatic antioxidants (albumin, vitamins C and E), selenium and reduced concentrations of lipid fractions. Improved nutrition and antioxidant supplementation should be encouraged as a major addition with treatment and management of pulmonary TB patients. However, the status of antioxidant enzymes and other essential trace elements should be examined in future prospective studies on pulmonary TB patients.

REFERENCES


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