ASSESSMENT OF TOTAL ANTIOXIDANT STATUS OF PULMONARY TUBERCULOSIS PATIENTS IN EKPOMA AND IRRUA, EDO STATE, NIGERIA

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ABSTRACT

Pulmonary tuberculosis (TB) is one of the most common infectious diseases globally. This study was carried out to assess the total antioxidant status of pulmonary tuberculosis patients in Ekpoma and Irrua, Edo State, Nigeria. A total of 140 individuals (males and females), comprising 50 newly diagnosed pulmonary tuberculosis patients yet to be placed on drug, 50 old cases that are on drugs and control group consisting of 40 apparently healthy individuals of the same age range (16-55) with the subjects were investigated. Serum total antioxidant status (TAS) was determined using standard method. The mean ± SD values newly diagnosed patients’ TAS (1.03±0.09), mean ± SD values of old cases’ TAS (1.20±0.13) and the controls’ TAS (1.63±0.10) were compared. The analysis showed a significant difference (p<0.05) in the value of TAS (1.03±0.09) of new cases when compared with both controls (1.63±0.10) and old cases (1.20±0.13). There was a significant difference (p<0.05) between old cases (1.03±0.09) and control individuals (1.63±0.10). The results of this study have shown that total antioxidants status is significantly reduced in pulmonary tuberculosis patients that may be associated with high levels of free radicals and oxidative stress. This study has also shown that total antioxidant can be improved with appropriate therapy.

Key words: Tuberculosis, Pulmonary, Total, Antioxidant, Status

INTRODUCTION

Tuberculosis is an infectious disease caused by the bacterium Mycobacterium tuberculosis (MTB) (WHO, 2015). It is a highly infectious disease that is widely distributed throughout the world. The disease is influenced by economic and nutritional factors; although educational background, immunity and hormonal status have been associated with the prevalence (Cruickshank, 1973; Halliwell, 1992).

The economic and nutritional factors accounts for the highest prevalence in developing countries. The World Health Organization (WHO) reports showed that there were an estimated 9.3 million cases of TB in 2007 (World Health Organization, 2009). The WHO declared TB a global health emergency in 1993, and the “Stop TB” Partnership developed a Global Plan to Stop Tuberculosis that aims to save 14 million lives between 2006 and 2015 (Martin, 2006). In 2004, around 14.6 million people had active TB disease with 9 million new cases. The annual incidence rate varies from 356 per 100,000 in Africa to 41 per 100,000 in the Americas (World Health Organization, 2009). The rise in human immune virus (HIV) infection and the neglect of TB control programs have enabled a resurgence of tuberculosis. The emergence of drug-resistant strains has also contributed to the TB epidemic, with 20% of TB cases from 2000 to 2004 being resistant to standard TB treatments, and 2% resistant to second-line TB drugs (Sobero and Peabody, 2006).

Although Mycobacterium tuberculosis is more common, Mycobacterium bovis which affects cattle can also be found in man (Bates et al., 1997). It is commonly a disease of the lungs (pulmonary tuberculosis) where it forms a localized infection after inhalation (Mohr et al., 1969; Cruickshank, 1973). It can affect extra pulmonary regions like lymph nodes,
bone and joints, subcutaneous, meninges, eyes, the kidneys, and also the gastro-intestinal tract, where it causes an insidious disease that develops without any striking clinical evidence (Hardy et al., 1968). It can also cause congenital tuberculosis transmissible from an infected mother to fetus following ingestion of the amniotic fluid containing Mycobacterium tuberculosis (Cantwell et al., 1994).

The pathogenesis of TB is multifactorial and includes the effects of oxidative stress (Janiszewska-Drobinska et al., 2001; Madebo et al., 2003; Wild 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 (Jack et al., 1994; Wild et al., 2004) and may further contribute to immunosuppression (Beulter et al., 1963; 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, 1997).

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may cause cellular damage. Antioxidants such as thiols or ascorbic acid (vitamin C), terminate these chain reactions (oxidation). Plants and Animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase) produced internally or the dietary antioxidants such as vitamin A, vitamin C and vitamin E (Abner et al., 2011).

Oxidative stress can be considered as either a cause or consequence of some diseases, an area of research stimulating drug development for antioxidant compounds for use as potential therapies. Free radicals are responsible for widespread and indiscriminate oxidation and peroxidation of lipids causing cell death or organ damage. Free radicals oxidative stress has been implicated in the pathogenesis of a variety of human diseases (Ansari, 1993). When a host tissue is challenged by a pathologic insult of either an immunologic or non-immunological nature, an inflammatory reaction may occur, with subsequent clearance of the pathologic stimulus by phagocytic cell. Tissue injury may result from either the direct effects of the pathologic agent or as a consequence of an inflammatory cell influx (Fantone and Ward, 1982). Upon recognition of a pathocytic or soluble stimulus, both neutrophils and macrophages experience a “respiratory burst” which is characterized by an increase in oxygen consumption and increase glucose metabolism via hexose monophosphate shunt.

In conjunction with an increase in oxygen consumption, neutrophils and macrophages secrete both superoxide(O2-) and hydrogen peroxide(H2O2) as a defense mechanism (Fantone and Ward, 1982). The biological effects of these highly reactive compounds are controlled in vivo by a whole spectrum of antioxidative defense mechanisms: vitamin E and C, carotenoids, metabolites such as glutathione and uric acid, and antioxidant enzymes (superoxide dismutase, glutathione peroxidase and catalase). During pulmonary inflammation increased amounts of reactive oxygen species and reactive nitrogen intermediates are produced as a consequence of phagocytic respiratory burst (Ansari, 1993). Though pulmonary tuberculosis is a disease of most common occurrence and widely studied, many questions in this field still remain unanswered. Therefore, in the present study an attempt has been made to define more precisely the total antioxidant status (TAS) in patients with pulmonary tuberculosis.

MATERIALS AND METHODS

Research Design: A total of 140 individuals (males and females) which comprised of 50 newly diagnosed pulmonary tuberculosis patients yet to be placed on drugs and 50 old cases that were on drugs attending Irrua Specialist Teaching Hospital, Irrua and General Hospital Ekpoma, were enrolled for this study. A group of 40 apparently healthy individuals of the same age range (16-55) with the subjects was used as control. Serum total antioxidant status (TAS) was determined using standard method. ANOVA was used to analyze the results and differences was considered significant at P<0.05 level of confidence. All data was expressed as Mean ± Standard deviation (SD).

Geographical Description of the study area: This study was carried out in Ekpoma and Irrua, in Esan land, Edo State. Esan land comprises 5 local government areas of Esan west, Esan central, Esan north-east, Esan south-west and Igbeben in Edo State, Nigeria. Esan land is located on a plateau; we have the top and bottom of sections of the plateau (Segynola, 2015). This area is located between latitude 6° 10 and 6° 45’ north of the equator and between longitudes 6° 10’ and 6° 30’ east of the Greenwich Meridian (Akinbode, 1983). The 2006 national census put the

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population of the study area at 591,534 people (NGSA, 2006). Projected to 2015 at 2.8 percent national growth rate, the 2015 population of the study area is 740,601 people.

**Inclusion Criteria:** Only subject with active pulmonary tuberculosis within the age range 16-55 years were recruited for this study

**Exclusion Criteria:** Pulmonary tuberculosis patients with HIV/AIDS, pregnancy, DM, history of smoking and drinking were excluded from this study.

**Ethical Consideration:** Ethical approval was obtained from the Edo State Hospital Management Board (HMB) and informed consent was sought from the subjects before sample collection.

**Sample Collection:** Five millilitres of venous blood was collected from the subjects/controls using sterile disposable syringes and needles at the anti cubital fossa vein by venin-puncture after sterilization with 70% alcohol with the use of tourniquet into a sodium citrate sample container. The blood samples were centrifuged at 3000rpm for 12 minutes. The plasma was separated into a clean dry plain container and stored frozen at -70°C until analysis was done at room temperature.

**Laboratory Analysis: Total antioxidant status was determined** using the method described by Apak et al., (2006). The reduction potential of the sample/standard effectively converts Cu²⁺ to Cu⁺, thus changing the ion’s absorption characteristics. This form of copper will selectively form a stable 2:1 complex chromogenic reagent with an absorption maximum at 450 nm. A known concentration of trolox is used to create a calibration curve with data been expressed as mM Trolos equivalents or in µM copper reducing equivalents.

**Procedure:** Two hundred microlitre (200µl) of sample and standard was placed to a microcuvette. Blank contained dilution buffer in place of sample/standard. 1ml of assay buffer is added to the cuvette. The cuvette was read at 450nm for a reference measurement. 100µl of chromogen was added and incubated for 5 minutes at room temperature. The cuvette was read the second time at 450nm. Total antioxidant status was extrapolated from the calibration curve plotted with the standard.

**Statistical Analysis:** The data generated from the study (both control and test groups) was subjected to basic statistical measurement using parametric analysis of variance (ANOVA) as well as the comparison of the test with the control using Students’-test using the Statistical Package for Social Sciences (SPSS, version 21.0) windows application at 95% level of confidence. All results were reported as mean ± standard deviation (SD).

**RESULTS**

The results of this study are presented in the tables below. Table 1 shows the Mean ± SD values of total antioxidant status (TAS) of pulmonary tuberculosis subjects and the control subjects. The analysis showed a significant difference (p<0.05) in the values of TAS (1.11±0.14) of pulmonary tuberculosis subjects when compared with control subjects of values 1.63±0.10

Table 2 shows comparison of the Mean ± SD values of total antioxidant status of pulmonary tuberculosis subjects (new cases), pulmonary tuberculosis subjects (old cases) and the control subjects. The analysis showed a significant difference (p<0.05) in the value of TAS (1.03±0.09) of new cases when compared with both controls (1.63±0.10) and old cases (1.20±0.13). There was a significant difference (p<0.05) between old cases (1.03±0.09) and control individuals (1.63±0.10).

Table 3 shows the comparison of Mean ± SD values of total antioxidant status of female pulmonary tuberculosis subjects (new cases), female pulmonary tuberculosis subjects (old cases) and the female control subjects. The analysis showed a significant difference (p<0.05) in the value of TAS (1.01±0.11) of female new cases when compared with both female controls (1.57±0.11) and female old cases (1.17±0.08). There was a significant difference (p<0.05) between female old cases (1.17±0.08) and female control individuals (1.57±0.11).

Table 4 shows the comparison of Mean ± SD values of total antioxidant status of male pulmonary tuberculosis subjects (new cases), male pulmonary tuberculosis subjects (old cases) and the male control subjects. The analysis showed a significant difference (p<0.05) in the value of TAS (1.04±0.08) of male new cases when compared with both male controls (1.66±0.09) and male old cases (1.22±0.15). There was a significant difference (p<0.05) between female old cases (1.22±0.15) and female control individuals (1.66±0.09).
Table 1: Total antioxidant status of pulmonary tuberculosis subjects and Controls

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>CONTROLS Mean±SD</th>
<th>SUBJECTS Mean±SD</th>
<th>T-VALUE</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 40</td>
<td></td>
<td>N = 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAS (mmol/l)</td>
<td>1.63±0.10</td>
<td>1.11±0.14</td>
<td>21.039</td>
<td>0.00 (S)</td>
</tr>
</tbody>
</table>

Keys: TAS = Total Antioxidant Status; (S) = Significant

Table 2: Total antioxidant status of old and new pulmonary tuberculosis subjects and control subjects

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>Control Mean±SD</th>
<th>Subjects Mean±SD</th>
<th>F-VALUE</th>
<th>P-VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 40</td>
<td>(New case) mean±sd</td>
<td>N = 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 50</td>
<td>(Old case) Mean±SD</td>
<td>N = 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAS(mmol/l)</td>
<td>1.63±0.10a</td>
<td>1.03±0.09b</td>
<td>1.20±0.13c</td>
<td>339.988</td>
</tr>
</tbody>
</table>

Keys: Values in a row with a different superscript are significantly different at P<0.05; TAS = TOTAL Antioxidant Status; (S) = Significant; 1 = Control; 2 = New Case; 3 = Old Case

Table 3: Total antioxidant status of females with pulmonary tuberculosis and control subjects

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>Female Control Mean±SD</th>
<th>Female Subjects Mean±SD</th>
<th>F-VALUE</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 12</td>
<td></td>
<td>N = 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 19</td>
<td></td>
<td>N = 19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAS(mmol/l)</td>
<td>1.57±0.11a</td>
<td>1.01±0.11b</td>
<td>1.17±0.08c</td>
<td>115.857</td>
</tr>
</tbody>
</table>

Keys: Values in a row with a different superscript are significantly different at P<0.05; TAS = TOTAL Antioxidant Status; (S) = Significant; 1 = Control; 2 = New Case; 3 = Old Case

DISCUSSION

From this present study, there was a significant decrease (p<0.05) in the total antioxidant status of pulmonary tuberculosis subjects when compared with control. This is in agreement with previous works done by Plit et al., 1998, Reddy et al., 2004, Wild et al., 2004, Guzel et al., 2006, and Parchwani et al., 2011.

The lower levels of total antioxidants in pulmonary tuberculosis patients could be associated with heavy load of free radicals, oxidative stress and lipid peroxidation. Free radicals and peroxydes are clearly involved in physiological phenomenon such as synthesis of prostaglandins, thromoxanes and in the pathogenesis of various diseases (Southorn and Powis, 1988). During pulmonary inflammation increased amounts of reactive oxygen species and reactive oxygen nitrogen intermediates are involved as a consequence of phagocyte respiratory burst (Kwiatkowska et al., 1999). Thus, toxic free radicals are implicated in the development of lung fibrosis, which may be a long term sequel of pulmonary tuberculosis (Wild et al., 2004).

Also from this study, there was a significant increase (p<0.05) in the levels of total antioxidant of pulmonary
tuberculosis subjects on drugs when compared with those that were not on drugs. This agreed with previous studies by Parchwani et al., (2011) and Akiibinu et al., (2008).

In conclusion, the results of this study have shown that total antioxidants status is significantly reduced in pulmonary tuberculosis patients which may be associated with high levels of free radicals and oxidative stress. This study has also shown that total antioxidant can be improved with appropriate therapy.

**RECOMMENDATION**

We therefore recommend that;

i. Total antioxidant status test be routinely monitored in patients with pulmonary tuberculosis.

ii. Drugs for the treatment of tuberculosis be made regularly available and affordable for tuberculosis.

iii. Patients’ compliance with medication should be encouraged.

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**REFERENCES**


AUTHOR’S CONTRIBUTIONS

AIRHOMWANBOR, K.O., Chief researcher, laboratory supervisor and content analyst. DIC-IJIEWERE, O.E.; Resource supervisor and result analyst. SHABBAAH, G. M.; Sampling/Laboratory analyst. IDEHEN, I. C.; Co-researcher. EIDANGBE, A. P.; Co-researcher.