Full Length Research Article

Non-Enzymatic Antioxidants and Nutritional Profiles in Newly Diagnosed Pulmonary Tuberculosis Patients in Nigeria.

Akiibinu M.O1, Arinola O.G2*, Ogunlewe J.O1, and Onih E.A3

1 Department of Chemical Pathology and Immunology, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Ago-Iwoye, Ogun state.
2 Department of Chemical Pathology, Immunology unit, University of Ibadan, Ibadan.
3 Department of Medical Laboratory Sciences, Faculty of Basic Medical Sciences, College of Medicine, Ambrose Alli University Ekpoma, Edo state.

ABSTRACT

The effects of pulmonary tuberculosis on nutritional and antioxidants status was determined in thirty-one patients attending Chest Clinic, Oyo State Hospital, Jericho, Ibadan Nigeria by assessing the levels of total protein, albumin, globulin, total cholesterol, HDL cholesterol, LDL cholesterol, uric acid, vitamin C, vitamin E and total antioxidants. Thirty-four members of staff of State Hospital Adeoyo Ibadan, Nigeria were selected as controls. The mean levels of total protein (7.13±1.06g/dl), albumin (3.27±0.7g/dl), total cholesterol (100.7±23.6mg/dl), HDL cholesterol (28.6±7.2mg/dl), LDL cholesterol (65.5±15.1mg/dl) and triglycerides (61.6±16.2mg/dl) were significantly low while the level of globulin (3.9±0.84g/dl) was significantly high in pulmonary tuberculosis patients when compared with controls (total protein = 7.6±0.7g/dl; albumin = 4.2±0.48g/dl; total cholesterol = 163.2±40.1mg/dl; HDL cholesterol = 41.4±5.4mg/dl; LDL cholesterol = 107.8±27.5mg/dl; globulin = 3.4±0.6g/dl). Levels of vitamin C (18.5±1.6mg/l), vitamin E (6.9±1.7mg/l) and total antioxidant (0.83±0.34mmol/l) were significantly lower while the level of uric acid (5.2±2.1mg/dl) was significantly higher in patients with pulmonary tuberculosis when compared with the controls (vitamin C = 23.1±2.3mg/l; vitamin E = 12.5±1.6mg/l; total antioxidant = 1.65±0.26mmol/l; uric acid = 4.3±1.2mg/dl respectively). Significantly lower levels of antioxidants might have been caused by endogenous uric acid production.


Key words: - Antioxidants, nutritional profiles, pulmonary, tuberculosis.

*Address for Correspondence: Tel:+234 8023451520; e-mail: arinolaog@doctor.co, drarinolaog64@yahoo.com
INTRODUCTION

Tuberculosis is a highly infectious disease that is widely distributed throughout the world and the developing countries have the highest prevalent rate. The most popular causative agent is *Mycobacterium tuberculosis* although, *Mycobacterium bovis*, which affects cattles, can also be found in man (Bates *et al.*, 1997).

Tuberculosis 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 also affect extra pulmonary regions like lymph nodes, bone and joints, subcutaneous, meninges, eyes, the kidneys and the gastro-intestinal tract (Hardy *et al.*, 1968), where it causes an insidious disease that develops without any striking clinical evidence of disease. Congenital tuberculosis is possible from an infected mother to foetus following ingestion of the amniotic fluid containing *Mycobacterium tuberculosis* from the placental (Cantwell *et al.*, 1994). Tuberculosis 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 *Mycobacteria* activate the invaded macrophages resulting to free radical burst (Wiid *et al* 2004; McGarvey *et al*., 2004). High serum levels of these free radicals and high concentration of lipid peroxidation products are characteristics of by patients with advanced tuberculosis (Kwiatkowska *et al*., 1999). The peroxidation could cause reduced concentration of serum lipids and tissue inflammation (Sasaki *et al*., 1999). Yamanaka *et al* 2001 also reported that serum cholesterol was significantly lower in tuberculosis patients and got worse in homeless patients.

This study hypothesized that tissue inflammation, oxidative stress and continuous production of free radicals in pulmonary tuberculosis patients may cause lower levels of antioxidants.

MATERIALS AND METHODS

Subjects
Thirty-one patients with active tuberculosis and were sputum smear- positive volunteered to participate in this study. The radiological examination also showed pulmonary inflammation. Thirty-four apparently healthy sputum smear-negative individuals selected from staffs of State Hospital Adeyo Ring road, Ibadan, Nigeria served as controls.

Ten millilitres (10ml) of blood was collected from these subjects into a lithium heparin container and spun within one hour. The plasma was separated immediately, out of which 1.5ml for vitamin C estimation was mixed with equal volume of 10% TCA within three hours of collection of the blood sample and stored at 4°C. The remaining plasma sample for total antioxidants, lipid profiles, total protein, albumin, total cholesterol, uric acid and vitamin E estimations were stored at −20°C until ready for analysis.

Methods:

**Determination of plasma total antioxidant:** Total antioxidant was measured using a method of Koracevic *et al* (2001) based on the principle that a standardised solution of Fe-EDTA complex reacts with hydrogen peroxide by a Fenton-type reaction leading to the release of hydroxyl radical. These reactive oxygen specie degraded benzoate, resulting in the release of thiobarbituric acid reactive substance. The rate of inhibition of colour development measured at 532nm is proportional to the concentration of oxidative activity in the plasma.

**Vitamin E estimation:** The level of vitamin E was determined in the plasma by using the method of Baker and Frank(1968), which is based on the principle that vitamin E extracted in xylene was made to react with alpha, alpha dipyridyl. The product produces a reddish colour with ferric chloride, which was read at 520nm.

**Vitamin C estimation:** Vitamin C was measured using the titration method of Harris and Ray (1935). The vitamin C constituent of plasma diluted with 10% TCA was titrated against a specified concentration of 2, 6 Dichlorophenolindophenol. The disappearance of the pink
Copper Status in Hypercholesterolemic Patients

Uric acid estimation: Uric acid was measured using phosphotungstate solution purchased from Dialab Production and Vertrieb vonchemisch-technischen, Wien- Panikengasse. This method is based on the principle that, in the presence of uric acid, the phosphotungstate complex is reduced to phos-photungstic complex. The intensity of the colour read at 540nm is proportional to the concentration of uric acid present in the sample.

Total protein, albumin and globulin estimation: Total protein was determined spectrophotometrically at 540nm using alkaline solution of cupper sulphate as described by Reinhold (1953). The albumin concentration was determined by the brilliant cresol green solution supplied by Dialab Production and Vertrieb vonchemisch-technischen, Wien- Panikengasse at 540nm. The globulin level was calculated by subtracting the value of albumin from that of total protein.

Determination of plasma lipids: Total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were measured spectrophotometrically using commercially prepared reagents purchased from Randox Laboratory ltd, UK. Vitamin C was measured using the titration method of Harris and Ray (1935).

Statistical methods: The data generated were represented as mean ± S.D and the differences between the means were determined using Students (t) test.

RESULTS

As shown in Table 1, there was no significant difference in the age ranges (P>0.5) of patients with tuberculosis and controls selected for this study. The mean levels of total protein (7.13±1.06g/dl), albumin (3.27±0.7g/dl) were significantly low while the level of globulin (3.9±0.84g/dl) was significantly high when tuberculosis patients were compared with controls (total protein = 7.6±0.7g/dl; albumin = 4.2±0.48g/dl; globulin = 3.4±0.6g/dl respectively). In table 2, total cholesterol (100.7±23.6mg/dl), HDL cholesterol (28.6±7.2mg/dl), LDL cholesterol (65.5±15.1mg/dl) and triglycerides (61.6±16.2mg/dl) were significantly low when compared with the controls (total cholesterol=163.2±40.1mg/dl; HDL cholesterol = 41.4±5.4mg/dl; LDL cholesterol = 107.8±27.5mg/dl; triglycerides = 101.5±29.6mg/dl).

Table 1: Age, Total Protein, Albumin, and Globulin In Tuberculosis and Controls

<table>
<thead>
<tr>
<th>N</th>
<th>Age(years)</th>
<th>Total Protein(g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34</td>
<td>33.21 ± 11.4</td>
<td>7.6 ± 0.7</td>
<td>4.2 ± 0.5</td>
</tr>
<tr>
<td>TB</td>
<td>31</td>
<td>31.5 ± 10.0</td>
<td>7.1 ± 1.1</td>
<td>3.3 ± 0.7</td>
</tr>
<tr>
<td>t, p values</td>
<td></td>
<td>0.6, 0.50</td>
<td>2.1, 0.04*</td>
<td>6.4, 0.00*</td>
</tr>
</tbody>
</table>

*Significantly different from the controls

Table 2: Lipid Profiles in Tuberculosis and Controls

<table>
<thead>
<tr>
<th>N</th>
<th>Tot. Chol.(mg/dl)</th>
<th>Triglyceride(mg/dl)</th>
<th>HDLC(mg/dl)</th>
<th>LDLCmg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34</td>
<td>163.2 ± 40.1</td>
<td>101.5 ± 29.6</td>
<td>41.4 ± 5.4</td>
</tr>
<tr>
<td>TB</td>
<td>31</td>
<td>100.7 ± 23.6</td>
<td>61.6 ± 16.2</td>
<td>28.6 ± 7.2</td>
</tr>
<tr>
<td>t, p values</td>
<td></td>
<td>7.6, 0.00*</td>
<td>6.7, 0.00*</td>
<td>8.2, 0.00*</td>
</tr>
</tbody>
</table>

*Significantly different from the controls
Table 3: Levels of Uric Acid, Vitamin C, Vitamin E And Total Antioxidant (Ta) In Tuberculosis And Controls

<table>
<thead>
<tr>
<th>N</th>
<th>Uric acid (mg/dl)</th>
<th>Vitamin C(mg/L)</th>
<th>Vitamin E(mg/L)</th>
<th>Ta(mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34</td>
<td>4.2±1.2</td>
<td>23.1±2.3</td>
<td>12.5±1.6</td>
</tr>
<tr>
<td>TB</td>
<td>31</td>
<td>5.2±2.1</td>
<td>18.5±1.6</td>
<td>6.9±1.7</td>
</tr>
</tbody>
</table>

| t, p values | 2.2, 0.03* | 9.4, 0.00* | 14.1, 0.00* | 10.9, 0.00* |

*Significantly different from the controls

Table 3 shows that levels of vitamin C (18.5±1.6mg/l), vitamin E (6.9±1.7mg/l) and total antioxidants (0.83±0.34mmol/l) were significantly lower while the level of uric acid (5.2±2.1mg/dl) was significantly higher in patients with pulmonary tuberculosis when compared with the controls (vitamin C = 23.1±2.3mg/l; vitamin E = 12.5±1.6mg/l; total antioxidants = 1.65±0.26mmol/l; uric acid = 4.3±1.2mg/dl respectively).

DISCUSSION

The present study shows significantly lower levels of total protein and albumin in pulmonary tuberculosis patients. This agrees with Sasaki et al (1999), which stated that albumin and total protein were significantly low in pulmonary tuberculosis. Aily et al (1999) observed low levels of albumin and haematocrit in tuberculosis. Yamanaka et al (2001) reported that the total protein, albumin, cholinesterase, hemoglobin and lymphocyte were significantly lower in homeless patients when compared with non-homeless tuberculosis patients and healthy men. Low levels of total protein and albumin in this study might have been caused by anorexia, malnutrition and mal-absorption commonly observed in tuberculosis. Albumin is an important component of plasma with antioxidant activity that primarily binds free fatty acids, divalent cations and hydrogen oxochloride (HOCl) (Llesuy et al, 1994). The pro-oxidants (free radicals) attack the cell membrane thereby causing tissue damage and wasting disease in pulmonary tuberculosis patients with resultant high level of uric acid. The low level of albumin may therefore contribute to the complications associated with pulmonary tuberculosis.

Significantly higher level of uric acid was observed in this study. Koumbanion et al (1998) observed higher levels of uric acid in those tuberculosis patients on pyrazinamide therapy, which caused increase in the level of uric acid in children with pulmonary tuberculosis (Sanchez-Albisua et al, 1997). Significantly higher level of uric acid observed in this study could be due to continuous tissue breakdown or a compensatory mechanism of reducing the free radical load in the tuberculosis patients.

Significantly high level of globulin observed in tuberculosis in our study might have arisen from combination of elevation of different globulin fractions previously observed. Arinola and Igbi (1998) reported high levels of IgG and IgM in pulmonary tuberculosis. Nagayama et al (1999) also stated that hyperglobulinaemia in tuberculosis is one of the predictive factors for the development of residual pleural thickening in tuberculous pleurisy. Gatner et al (1980) reported higher levels of alpha-1-antitrypsin in pulmonary tuberculosis patients. The high level of globulin observed in this study support the fact that humoral immune response is less affected and raises the possibility of polyclonal B cell activation in tuberculosis patients.

Low levels of total cholesterol, triglyceride, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol were observed in this study. Kwiatkowska et al (1999) and Reddy et al (2004) reported high level of lipid peroxidation in all categories of pulmonary tuberculosis patients, irrespective of treatment status and this might have caused reduction in the concentration of serum lipids as observed in our study. It was shown that total cholesterol was significantly
lower in tuberculosis patients when compared with pulmonary tuberculosis-free controls (Sasaki et al, 1999; Yamanaka et al, 2001). Triglycerides and LDL cholesterol are the chief constituents of cell membranes while the HDL cholesterol protects the arterial walls of the blood circulatory system (Gordon et al, 1977). The low levels of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides observed in this study could be the result of impaired rate of lipid production and enhanced lipid catabolic rate associated with tuberculosis. Low levels of lipids noticed in these patients could be another factor that predisposes them to cell and tissue damage, cardiovascular problems and low cellular immunity.

Previous workers have reported significantly low levels of vitamin E, vitamin A, total antioxidant, Zn and beta-carotene in pulmonary tuberculosis (Plit et al, 1998; Madebo et al, 2003 and Wiid et al, 2004). In our study, low levels of total antioxidant, vitamins C and E were observed in pulmonary tuberculosis. This might be due to malnutrition or exhaustion in attempt to neutralize heavy load of free radical in these patients.

This present study reported low levels of antioxidant and lipid profiles in newly diagnosed PTB. This may be as a result of heavy load of free radicals from oxidative stress of pulmonary tuberculosis. The effect of this is tissue damage and wasting disease. Therefore, supplementation with antioxidant vitamins may be necessary in the management of pulmonary tuberculosis.

REFERENCES


