EVALUATION OF IMMUNOLOGICAL MARKERS IN PATIENTS WITH PULMONARY TUBERCULOSIS IN SOME HOSPITALS IN UYO, AKWA IBOM STATE, NIGERIA

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ABSTRACT

Pulmonary tuberculosis (PTB) remains a major public health problem especially in the developing countries. Literature evidence opines that serological markers associated with PTB infections enhance early diagnosis. This study employed CD4⁺ T-lymphocyte counts and total serum immunoglobulin to establish early diagnosis in PTB patients in Uyo. Sputum samples of 105 patients were screened for Acid Fast Bacilli, while blood (7ml) was collected for CD4⁺ T-lymphocyte counts and quantification of total serum using cytoflow counter and Enzyme linked Immunoassay, respectively. A prevalence of 12.4% was established for PTB infection among the patients. The mean differences between immunoglobulins levels in relation to CD4⁺ T-lymphocyte counts was not significant at p > 0.05. The mean IgG value of PTB patients was 737.29 ± 435.8 mg/dL, while the apparently healthy subjects (AHS) had the mean IgG value of 23.15 ± 32.2 mg/dL. The mean IgA level in PTB patients (190.91 ± 94.8 mg/dL) was higher than that of AHS (126.81 ± 35.5 mg/dL). The mean IgM value of PTB patients and that of the AHS was 317.75 ± 146.11 and 50.00 ± 32.3 mg/dL, respectively. There was significant difference between the immunoglobulin levels of PTB patients and AHS at p > 0.05 with respect to IgA and at p > 0.001 with respect to IgG and IgM. This study has indicated that biomarkers of humoral immunity were significantly affected when compared with AHS, thus, this study would enhance knowledge of the health practitioners and enable them to grasp the role of biomarkers in disease diagnosis.

Keywords: Biomarkers, Immunoglobulins, Patients, T-lymphocytes, Uyo

INTRODUCTION

Tuberculosis (TB) has continued as one of the major public health problems in developing countries of the world and sub-Saharan Africa in particular (Verma and Mahajan, 2008). Despite the rising incidence in Nigeria, tuberculosis detection rates and programme coverage are still low and/or even undetected owing to very poor and insensitive diagnostic methods techniques and, high cost of assessing more sensitive and advanced techniques such as polymerase chain reaction. Early diagnosis and disease monitoring through the use of serological markers of immune activation associated with tuberculosis infections could help in early diagnosis, check disease progression as well as provide information about disease activity (Umeh and Ishakelu, 2007). Several studies indicating polyclonal raised serum immunoglobulin are common with many infective and inflammatory conditions such as Tuberculosis and HIV (Arinola and Igbi, 1998; Schneider et al., 2010; Wilson et al., 2011). Tuberculosis infection is inflammatory in nature as indicated by the presence of high concentration of certain acute phase proteins in infected individuals (Schneider et al., 2010; Wilson et al., 2011). Pulmonary tuberculosis is diagnosed based upon the clinical, radiological and bacteriological evidence; however, serological diagnosis is considered more significant (Umoe et al., 2020). Studies have shown that a high proportion of patients with tuberculosis have significantly increased levels of antibody to Mycobacterium tuberculosis by using enzyme-linked immunosorbent assay. The immunology of tuberculosis and the significance of delayed hypersensitivity as protective immunity have since been extensively studied in the light of modern sophisticated immunological techniques (Jain et al., 1984). The clinical usefulness of detection of serum immunoglobulin IgG and IgM antibodies have been reported in tuberculosis and other pulmonary diseases (Selma et al., 2011). An immunoprofiling of antigen specific responses is vital for the TB diagnosis and therapeutic monitoring (Goodridge et al., 2014; Umoe et al., 2020). Currently, gold standard methods for TB diagnosis and monitor treatment response include sputum smear microscopy and culture conversion after 2 months of TB treatment (Umoe et al., 2020).

Nonetheless, for patients whose sputum samples are not available, alternative serological tests are needed. Some results showed that combined use of different antibody isotypes allow an increased accuracy in the diagnosis of tuberculosis (Kochak et al., 2010), and the levels of antibody against some antigens decreases together with treatment (Goodridge et al., 2014). Nigeria has been reputed as one of the countries with a high burden of tuberculosis (TB) worldwide (WHO, 2017). Hence, there is a need for studies on the immunological markers associated with TB in Nigeria as these may be relevant in early disease diagnosis and monitoring in our TB programmes. This study therefore estimated the CD4⁺ T-lymphocyte counts and levels of total serum IgA, IgG and IgM in pulmonary tuberculosis patients in Uyo, Akwa Ibom State, Nigeria.

MATERIALS AND METHODS

Study Population

This cross sectional study employed consecutive sampling technique to collect samples from symptomatic and apparently healthy individuals in University of Uyo Teaching Hospital and St Luke’s Hospital, Uyo, Akwa Ibom State. A total of 120 samples were collected; 105 from symptomatic individuals and 15 from apparently healthy individuals making it 7:1 for symptomatic against the control.
Inclusion Criteria
Persons aged 18 year and above presenting with symptoms of tuberculosis and attending the tuberculosis clinic of University of Uyo Teaching Hospital and St Luke’s Hospital were included for the study. Blood donors in the two hospitals were included as control subjects.

Exclusion Criteria
Patients on antiretroviral therapy, anti-Koch treatment and individuals who did not meet the inclusion criteria were excluded.

Study Design
Eligible participants were those presenting the symptoms of pulmonary tuberculosis infection (PTB) only and a control group. The control group were subjects that were apparently healthy blood donors (without PTB infection) in the two hospitals.

Ethical Considerations
Ethical approvals for this study were obtained from the University of Uyo Teaching Hospital and St Luke’s Hospital Ethical committees.

Collection of Blood Samples
Seven millilitres (7ml) of venous blood sample (VBS) was aseptically collected from both the case (n=105) and control group (n=15). The 7 mL VBS collected was shared into 2 sterile plain bottles (first bottle contained 3 mL VBS without additives, while the second bottle contained 4 mL VBS with EDTA anticoagulant). The 3 mL VBS without additives in the first bottle was centrifuged, and the supernatant fluid extracted and freeze-stored (at -70°C) until when required for immunoglobulin assays, while the 4 mL VBS with EDTA anticoagulant in the second bottle was stored at 25°C before being sent for CD4+ T-lymphocyte analysis within six hours interval.

Collection of Sputum Samples
Each of the subjects was given two sputum containers for on-the-spot deeply-coughed-out sputum and over-night sample.

Processing of Samples
Prior to analysis, all sputum samples were processed following the standard N-acetyl-cysteine and sodium hydroxide (NALC-NaOH) method for digestion, decontamination and concentration (Kent and Kubica, 1985).

Detection of Acid Fast Bacilli (AFB)
Detection of Acid Fast M. tuberculosis in the decontaminated samples was done using Ziehl Neelsen’s method (Cheesbrough, 2006)

Determination of Absolute CD4+ T-Lymphocyte Count
Absolute CD4+ T-lymphocyte count of he confirmed PTB Patients (n=13) was determined according to the specification of Centre for Integrated Health Programme (Nzou et al., 2010; Akinjogunla et al., 2020). Briefly, 20 µL of CD4+PE monoclonal antibody and 20 µL of well homogenized EDTA whole blood sample in each tube were mixed and incubated at 25°C in the dark for 15 min. Thereafter, 800 µL CD4 buffer solutions was added into the tube and mixed appropriately. The CD4+ T-Lymphocytes was analysed using Cytocount (Partec Cytoflow Counter, Germany).

Quantitative Assay of Total Serum Immunoglobulin Classes (IgA, IgG and IgM)
The quantification of serum immunoglobulins IgA, IgG and IgM of the confirmed PTB Patients (n=13) and Apparently Healthy Subjects (n=15) were performed independently with slight variations in the procedures using Total Human IgA, IgG and IgM kits, respectively (Immunology Consultants Laboratory, Incorporated, USA). To each of the quantification assays, all reagents and samples were brought to room temperature (25°C), 100 µL of prepared standards and appropriately diluted samples were transferred into appropriate wells using sterile pipettes and followed by incubation (30 ± 2 min for IgA; 60 ± 2 min for IgG and IgM) and aspirating the contents of each well. The wells were filled with wash solution, aspirated thrice and followed by addition of 100 µL appropriately diluted Enzyme-antibody conjugate before incubation at 20 ± 2 min for IgG; 30 ± 2 min for IgA and IgM in the dark and at room temperature (25°C). Thereafter, each of the wells was washed, blotted and 100 µL Chromogen Substrate Solution was added before incubation for 10 mins at room temperature (25°C). Finally, 100 µL stop solution (0.3 M Sulfuric acid) was added to each well, absorbance (450 nm) of the contents of each well was determined and the plate reader was calibrated according to the Manufacturer’s specifications (Stat Fax 2100 plate reader, USA). Calculations of cut-off values from the absorbance values were done as described by the manufacturer’s manual, using the respective dilution factors and were finally converted to a standard unit of mg/dL before recording.

Data Analysis
Data obtained were analysed using Statistical Package for Social Sciences (IBM SPSS, Window software Version 22.0. Armonk, NY: IBM Corp.). Analysis of Variance (ANOVA) were used to compare the mean of CD4+ T-lymphocyte counts and total serum immunoglobulin IgA, IgG, IgM levels in the respective group of patients and control. All statistical significant relationships were determined at p<0.05.

RESULTS
The prevalence of PTB infection among the patients in relation to Hospitals is presented in Table 2. Of the 78 samples collected from the UUTH, 11 samples were positive for M. tuberculosys, while from the 27 samples collected from St Luke’s Hospital, only 2 samples were positive for M. tuberculosys. A total of 105 patients pooled from University of Uyo Teaching Hospital (UUTH) and St Luke’s Hospital, Anua, Uyo, Akwa Ibom State were recruited for this study of which males were 48(45.7%) and females 57(54.3%). Age range of patients was between >18-20 and > 60yrs (mean age of 34.51±35 yrs). The highest number of patients was in the age group 21-30 yrs, while age group > 60 yrs had the least number of patients (Table 2). The prevalence of PTB infection among the patients is presented in Table 2. The results showed that 12.4% (n=13) of 105 patients were infected with PTB. The patients with age range of 21-30 yrs were mostly infected with prevalence of 16.7%, while the lowest prevalence of PTB was obtained among patients with age range of 41-50 yrs and > 60 yrs with 6.3% and 14.3%, respectively. There was no statistically significance difference between the prevalence of PTB among the subjects with respect to sex and age ranges (p > 0.05).

The mean (mm±SD) immunoglobulin levels of PTB patients based on their CD4+ T-lymphocyte counts are presented in Table 3. The mean IgA level in PTB patients with CD4+ T-lymphocyte counts of ≤ 200cells / µL was 209.91 ± 113.96 µg/dL while in the control group it was 82.89 ± 29.53 µg/dL. The mean IgG level in PTB patients with CD4+ T-lymphocyte counts of ≤ 200cells / µL was 418.72 ± 101.17 mg/dL while in the control group it was 237.12 ± 93.21 mg/dL. The mean IgM level in PTB patients with CD4+ T-lymphocyte counts of ≤ 200cells / µL was 109.57 ± 42.94 mg/dL while in the control group it was 64.81 ± 27.13 mg/dL.
18.12 mg/dL, while in PTB patients with CD4+ T-lymphocyte counts of > 200 cells/µL the mean IgA level was 187.46 ± 103.27 mg/dL. The mean IgM level in PTB patients with CD4+ T-lymphocyte counts of ≤ 200 cells/µL was higher than that of PTB patients with CD4+ T-lymphocyte counts of > 200 cells/µL. The mean IgG level in PTB patients with CD4+ T-lymphocyte counts of ≤ 200 cells/µL was 740.40 ± 289.07 mg/dL, while PTB patients with CD4+ T-lymphocyte counts (> 200 cells/µL) had the mean IgG level of 736.73 ± 408.61 mg/dL. There was no statistically significant differences in the immunoglobulin levels of PTB patients based on their CD4+ T-lymphocyte counts at p > 0.05 (Table 3).

The mean (mm±SD) immunoglobulin levels of PTB patients and the apparently healthy subjects (control group) are presented in Table 4. The results showed that the mean IgA level in PTB patients (190.91±94.8 mg/dL) was significantly higher than that of apparently healthy subjects (126.81±35.5 mg/dL). The mean IgG value of PTB patients was 737.29±435.8 mg/dL, while the apparently healthy subjects had the mean IgG value of 23.15±2.2 mg/dL. The mean IgM value of PTB patients and that of the apparently healthy subjects was 317.75±416.11 and 50.00±32.3 mg/dL, respectively. There was statistically significant difference between the immunoglobulin levels of PTB patients and the apparently healthy subject at p > 0.05 with respect to IgA and at p > 0.001 with respect to IgG and IgM.

### Table 1: Occurrence of Pulmonary Tuberculosis among the Subjects (N=105) in relation to Hospitals

<table>
<thead>
<tr>
<th>Hospital</th>
<th>No of Samples Collected</th>
<th>No of Samples Positive for <em>M. tuberculosis</em></th>
<th>Prevalence (%)</th>
<th>t-value</th>
<th>p-value</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>UUTH</td>
<td>78</td>
<td>11</td>
<td>14.1</td>
<td>0.98</td>
<td>0.47</td>
<td>1.29</td>
</tr>
<tr>
<td>St Luke’s</td>
<td>27</td>
<td>2</td>
<td>8.3</td>
<td>0.24</td>
<td>0.36</td>
<td>0.87</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td>13</td>
<td>12.4</td>
<td>0.24</td>
<td>0.36</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Key: UUTH: University of Uyo Teaching Hospital

### Table 2: Occurrence of Pulmonary Tuberculosis in relation to Age and Sex of the Subjects (N=105)

<table>
<thead>
<tr>
<th>Age Group (Yrs)</th>
<th>No (%) of Samples Collected</th>
<th>No (%) of Samples Positive for <em>M. tuberculosis</em></th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-20</td>
<td>Male 7(38.9) Female 11(61.1)</td>
<td>11(28.6) Male 0(0.0) Female 0(0.0)</td>
<td>0.99</td>
</tr>
<tr>
<td>21-30</td>
<td>Male 10(33.3) Female 20(66.7)</td>
<td>3(33.3) Male 2(10.0) Female 1(10.0)</td>
<td>1.01</td>
</tr>
<tr>
<td>31-40</td>
<td>Male 13(56.5) Female 10(43.5)</td>
<td>1(7.7) Male 1(10.0) Female 0(0.0)</td>
<td>0.33</td>
</tr>
<tr>
<td>41-50</td>
<td>Male 10(62.5) Female 6(37.5)</td>
<td>0(0.0) Male 1(16.7) Female 0(0.0)</td>
<td>0.24</td>
</tr>
<tr>
<td>51-60</td>
<td>Male 6(54.5) Female 5(45.5)</td>
<td>2(33.3) Male 0(0.0) Female 0(0.0)</td>
<td>1.12</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>Male 2(28.6) Female 7(71.4)</td>
<td>0(0.0) Male 1(20.0) Female 0(0.0)</td>
<td>0.29</td>
</tr>
<tr>
<td>Total</td>
<td>Male 48(45.7) Female 57(54.3)</td>
<td>8(16.7) Male 5(8.8) Female 14(28.8)</td>
<td>1.46</td>
</tr>
</tbody>
</table>

### Table 3: Mean Immunoglobulin Level of Confirmed PTB Patients in relation to CD4+ T- Lymphocyte Counts

<table>
<thead>
<tr>
<th>Immunoglobulin Types</th>
<th>CD4+ T-Lymphocyte Counts (cells/µL)</th>
<th>Immunoglobulin Level (mg/dL)</th>
<th>t-value</th>
<th>p-value</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>≤ 200</td>
<td>209.91±18.12 mg/dL</td>
<td>2.96</td>
<td>0.77</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td>&gt; 200</td>
<td>187.46±103.27 mg/dL</td>
<td>0.99</td>
<td>0.38</td>
<td>0.71</td>
</tr>
<tr>
<td>IgG</td>
<td>≤ 200</td>
<td>740.40±289.07 mg/dL</td>
<td>0.10</td>
<td>0.99</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>&gt; 200</td>
<td>736.73±408.61 mg/dL</td>
<td>0.33</td>
<td>0.25</td>
<td>0.43</td>
</tr>
<tr>
<td>IgM</td>
<td>≤ 200</td>
<td>450.53±68.87 mg/dL</td>
<td>0.99</td>
<td>0.36</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>&gt; 200</td>
<td>291.19±144.45 mg/dL</td>
<td>0.71</td>
<td>0.43</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Keys: mm: mean; SD: Standard Deviation; PTB: Pulmonary Tuberculosis

### Table 4: Comparative Mean Immunoglobulin Level of Confirmed PTB Patients and Apparently Healthy Subjects

<table>
<thead>
<tr>
<th>Immunoglobulin Types</th>
<th>Subjects</th>
<th>No. Tested</th>
<th>Immunoglobulin Level (mg/dL)</th>
<th>t-value</th>
<th>p-value</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>PTB</td>
<td>13</td>
<td>190.91±94.8 mg/dL</td>
<td>&lt; 0.05</td>
<td>4.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AHS</td>
<td>15</td>
<td>126.81±35.5 mg/dL</td>
<td>3.33</td>
<td>&lt; 0.05</td>
<td>4.45</td>
</tr>
<tr>
<td>IgG</td>
<td>PTB</td>
<td>13</td>
<td>737.29±435.8 mg/dL</td>
<td>&lt; 0.001</td>
<td>10.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AHS</td>
<td>15</td>
<td>23.81±32.2 mg/dL</td>
<td>33.06</td>
<td>&lt; 0.001</td>
<td>10.21</td>
</tr>
<tr>
<td>IgM</td>
<td>PTB</td>
<td>13</td>
<td>317.75±416.11 mg/dL</td>
<td>&lt; 0.001</td>
<td>5.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AHS</td>
<td>15</td>
<td>50.00±32.3 mg/dL</td>
<td>6.76</td>
<td>&lt; 0.001</td>
<td>5.69</td>
</tr>
</tbody>
</table>

Keys: mm: mean; SD: Standard Deviation; PTB: Pulmonary Tuberculosis (Confirmed); AHS: Apparently Healthy Subjects

**DISCUSSION**

Poor disease diagnosis and monitoring could facilitate progression of latent tuberculosis to active tuberculosis. However, monitoring of laboratory indices such as levels of cellular and humoral immunological markers like CD4+ T-lymphocyte counts and immunoglobulin classes (IgA, IgG, IgM) respectively could help physicians detect early PTB disease as well as monitor disease progression. In this study, of 105 suspected patients recruited, 12.4% had PTB and this prevalence was lower than 31.7% earlier
reported in Uyo and some parts of Nigeria (Itah and Udofia, 2005) on epidemiology and endemicity of pulmonary tuberculosis (PTB) in South-Eastern Nigeria. The possible rationale for reduction in PTB prevalence in Uyo might be attributed to the current intense PTB disease surveillance and treatment by the governments and their collaborating partners. However, the prevalent rate in this study was in conformity with 12.0 % prevalent rate of PTB reported in Kano (FMOH, 2012). The highest rate of PTB was observed among the age group 21-30 yrs in our study and this corroborates a report by WHO, that adolescents and young adults are the most affected population in Africa (WHO, 2009) and this also substantiates the findings on the highest prevalence of PTB young adults aged 21-30 yrs in Ghana (Lawn and Acheampong, 2009). With regard to sex and age of the PTB patients, there was no significant difference the prevalence rate of PTB and this agrees with the reports that there was no significant difference between the sex and M. tuberculosis infection among patients in Umuahia, Abia state, Nigeria (Nwachukwu and Peter, 2010).

Our findings on the state of cellular immunity of PTB patients revealed that the mean CD4+ T-lymphocyte counts in patients with PTB were low and this concurs with other reports on studies on CD4+ T-lymphocyte counts among PTB patients in some parts of Nigeria (Olaniyi and Arinola, 2011; Amilo et al., 2012). A significant decrease in CD4+T-lymphocyte counts among PTB patients has also been reported in India (Tripathy et al., 2009). The low CD4+ T-lymphocyte counts in patients with PTB could be attributed to the suppression of cellular immune response by other clinical conditions that promote immunosuppression (Abdul and Andrew, 2009). In this study, the mean serum levels of IgA, IgG and IgM in PTB patients were significantly high and this agrees with studies on Ibadan, Oyo State and South Eastern Nigeria, respectively (Arinola and Igbi, 1998; Amilo et al., 2012). Other reports have showed high serum levels of IgA, IgG and IgM in PTB patients in Gambia, West Africa (Lyamuya et al., 1999) and in Dares Salaam, East Africa (Gomez et al., 2012).

In order to compare the CD4+ T-lymphocyte counts with serum immunoglobulin levels of PTB patients and also to determine the possibility of significant relationship between the low and high CD4+ T-lymphocyte counts and mean serum immunoglobulin levels, the PTB patients were grouped into two based on their CD4+ T-lymphocyte counts (≤200 cells/μL and >200 cells/μL). Our results showed that there was no significant relationship (p > 0.05) between the immunoglobulin levels of PTB patients and CD4+ T-lymphocyte counts. Our findings substantiated the reports in Ibadan, Nigeria and the findings in Dares Salaam, Tanzania (25), on an insignificant relationship between high serum immunoglobulin levels and CD4+ T-lymphocyte counts in PTB patients. Similarly, in Ibadan, Nigeria, Olaniyi and Arinola (2011), reported that there was no significant reduction in IgG levels of PTB patients whose CD4+ T-lymphocyte counts were either below or greater than 200 cells/μL. Thus, suggesting that estimation of plasma concentration of immunoglobulin classes might not be useful in differentiating severity of infection.

CONCLUSION

This study has indicated that, the prevalence rate of PTB in Uyo was 12.4%; and biomarkers of cellular (CD4+ T-lymphocyte counts) and humoral (total serum IgA, IgG and IgM) immunity were significantly affected when compared with apparently healthy control subjects and this study would enhance knowledge of the health practitioners and enabled them to grasp the role of biomarkers as an alternative tool for disease diagnosis. Therefore, a prospective further study to monitor the impact of treatment on PTB patients using CD4+ T-lymphocyte, IgA, IgG and IgM as biomarkers at intervals of 3, 6 and 18 month so as to obtain a more comprehensive data is recommended.

CONFLICT OF INTEREST

The authors have no conflicts of interests with respect to the research, authorship and/or the publication of the manuscript.

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