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Assessment of Total Serum Immunoglobulin Levels and CD4$^+$ T-Lymphocyte Counts in Pulmonary Tuberculosis Patients Co-Infected With HIV in Uyo, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author AEM designed the study and wrote the protocol. Author DMI wrote the first draft of the manuscript, managed the analyses of the study and performed the statistical analysis and author VGB managed the literature searches and analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: Poor disease diagnosis and monitoring, could aid in the progression of Tuberculosis especially in Tuberculosis/HIV co-infected patients.
Aim: This study aimed to evaluate the levels of cellular (CD4$^+$ T-Cell) and humoral (immunoglobulin classes IgA, IgG, IgM) markers associated with PTB and HIV infection in sputum producing patients in Uyo, Southsouth, Nigeria.
Study Design: This was a cross sectional study of patients suspected with PTB and HIV.
Study Location and Duration: The study was conducted at the Tuberculosis and HIV clinics of University of Uyo Teaching Hospital and St. Luke's Hospital all in Uyo, Akwa Ibom State, Nigeria, from October, 2013 to September, 2014.

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Methodology: Sputum producing adult patients, 105 (male 48, female 57; age range 18 - >60 years), who were referred to the laboratory for investigation, were recruited for the study. On the spot and overnight sputum samples were collected and analysed using Ziehl Neelsen's technique and fluorescence microscopy. Blood samples were collected by veni-puncture and assayed for HIV status using rapid test kits while $CD4^+$ T-cell counts and total serum immunoglobulin levels were determined by flow cytometry and ELISA techniques, respectively. Subjects were classified into PTB, HIV and PTB/HIV-infected groups.

Results: Mean $CD4^+$ T-cell counts (cells/ul) in the order of PTB/HIV (175.12±85.8)<HIV (311.06±228.6)<PTB (576.31±326.7) were significantly lower than apparently healthy control subjects (1294±334.9) ($p<0.05$). Mean IgA values (mg/dl) in the order of HIV (208.85±104.9)>PTB/HIV (206.0±71.7) were significantly higher than the control group (126.81±35.5). Mean IgG values (mg/dl) of PTB/HIV (1498.54±35.5) was significantly higher than other patient and control groups ($p<0.001$). The mean IgM values (mg/dl) of patient groups was significantly higher than those of control (50.00±32.3) ($p<0.001$). There was a significant moderate negative correlation ($r=-0.56$) between categorized $CD4^+$ T-cell counts (≤200 cells/µl and >200 cells/µl) and total serum IgG levels in PTB/HIV group. PTB/HIV cases with severe cellular immune suppression had very high IgG levels.

Conclusion: $CD4^+$ T-cell counts and levels of immunoglobulin classes, particularly IgG, were significantly affected in PTB/HIV co-infection. Therefore, these immune markers could be useful in predicting and monitoring disease progression in PTB/HIV coinfected patients.

Keywords: Tuberculosis; HIV; immunoglobulin levels; CD4+ T-cell counts.

1. INTRODUCTION

Pulmonary tuberculosis (PTB) is an infectious disease caused by *Mycobacterium tuberculosis*. It continues to remain a major public health problem in most of the developing countries of the world and sub-Saharan Africa in particular [1]. Some reports over the past several years have indicated that TB problem was on the wane in most developed countries suggesting a decline in TB burden with increase in developing countries where intricate factors such as malnutrition, poverty, homelessness and overcrowding favour TB incidence and prevalence [2,3]. Human immunodeficiency virus (HIV) and *Mycobacterium tuberculosis* co-infection is a fast growing problem in the AIDS pandemic in Africa and Asia [4]. Pulmonary tuberculosis associated with HIV infection, usually leads to death in many cases that are not properly treated [2] and tuberculosis is the most common opportunistic infection among HIV sero-positive patients [5]. A Report from India, indicates that about one- third of all patients with tuberculosis also have HIV infection [1]. In some countries of sub-Saharan Africa, two-third of HIV-infected subjects is co-infected with TB [6]. In the Niger Delta region of Nigeria, recent TB/HIV co-infection prevalence rate is reported to be 5.9% [7].

Despite Nigeria's rising TB detection rates and programme coverage, many TB cases are still undetected because of poor and insensitive diagnostic technique coupled with high cost of assessing sensitive and advanced molecular techniques. The high rates of TB/HIV co-infection in Nigeria has resulted in a significant health challenge in the HIV/AIDS response. Early diagnosis and disease monitoring through the use of serological markers of immune activation associated with HIV and tuberculosis infections could help in early diagnosis, check disease progression as well as provide information about disease activity [8].

Nigeria is a country with high incidence of tuberculosis with or without HIV co-infection [9]. It has a generalised HIV epidemic. However, prevalence varies across states, rural and urban areas [10]. Akwa Ibom State, Nigeria has the second highest HIV prevalence in Nigeria and at least 300,000 persons have tested positive for the dreaded HIV/AIDS [11]. This study was aimed to assess cellular and humoral immunological markers associated with TB/HIV co-infection as a predictor of early disease and monitoring of disease progression in Uyo, Akwa Ibom State of Nigeria.

2. MATERIALS AND METHODS

2.1 Study Location

This study was carried out at the University of Uyo Teaching Hospital (UUTH) and St. Luke's Hospital, Anua, both in Uyo-Nigeria, from October, 2013 to September, 2014. The

hospitals are tertiary and secondary health facilities, respectively and UUTH serve patients with various illnesses not limited to referrals. Uyo is the capital of Akwa Ibom State and the state is located in the coastal Southsouth region of Nigeria, West Africa.

2.2 Study Population

The study involved consecutive sampling of 105 sputum producing adult male and female patients suspected with tuberculosis at the tuberculosis clinic of University of Uyo Teaching Hospital (UUTH) and St. Luke's Hospital in Uyo, and referred to the laboratory for investigation. Control samples were obtained from thirty-one (31) age-and sex matched apparently healthy blood donors whose consent were sought and obtained for this research.

2.3 Study Design

This was a cross sectional study where eligible subjects were divided into three study groups and a control group. The first group had subjects with PTB infection only, the second group comprised persons with HIV infection only, and the third group had subjects with PTB/HIV co-infections. The control group comprised apparently healthy blood donors.

2.4 Inclusion Criteria

Subjects sampled included individuals who were suspected by clinicians to have suffered from pulmonary tuberculosis, having one or more symptoms compatible with tuberculosis (cough, weight loss, night fever, chest pain, haemoptysis, fatigue, loss of appetite, shortness of breath) for greater than two weeks. Subjects who were HIV sero-positive, having cough for more than two weeks were also recruited. Both male and female adults, 18 years and above, who gave informed consent were eligible for inclusion. The blood donors recruited into the study were apparently healthy individuals without any clinical symptoms. They were screened for HIV, HBsAg, HCV including physical & clinical examinations in line with the National Blood Transfusion guidelines criteria and were tested negative for these diseases.

2.5 Exclusion Criteria

Subjects excluded were children (0-17 years), pregnant women and non-sputum producing patients. Patients on antiretroviral therapy and anti-Kochs treatment were also excluded.

2.6 Ethical Considerations

All subjects had given written informed consent prior to enrolment in this study. Written application for ethical approval of study protocol was done and approval obtained from the Institutional Review Board of University of Uyo Teaching Hospital, Uyo where the study was conducted.

2.7 Samples Collection

This study used whole blood and sputum specimen collected from eligible subjects. Blood sample collection was done by a trained Phlebotomist. Appropriate personal protective equipment were worn and all aseptic techniques followed.

Seven milliliter of venous blood sample was collected from both test and control subjects and serum extracted and freeze stored at -20℃ until assay, while samples in EDTA bottle were sent to the laboratory for $CD4^+$ T-cell analysis within six hours of collection. Test subjects were given two sputum containers for on the spot deep-coughed out sputum, and over-night sample for laboratory processing.

2.8 Laboratory Analysis

Two on the spot and one early morning sputum samples were collected from each patient and tested by Ziehl-Neelsen and fluorescence microscopy techniques following standard procedures. HIV rapid test was done according to the National serial algorithm, using 3 types of commercial kits: Determine, Uni-Gold and Stat-Pak (tie-breaker) and testing was conducted according to manufacturer's manual. $CD4^+$ T-Lymphocyte count was done using Cytflow counter kit (Partec Cytflow Counter, Germany). The quantification of serum immunoglobulin IgA, IgG and IgM levels were performed using Total Human IgA, IgG and IgM test kits, respectively (Immunology Consultants Laboratory, Incorporated, USA) which employed a highly sensitive two-site Enzyme Linked Immunoassay (ELISA) technique. Optical Density of tests were read at dual wave length of 450nm and 620nm using a spectrophotometer.

2.9 Data Analysis

Descriptive statistics (Mean and Standard deviation) were generated for normally distributed quantitative variables. Inferential statistics (Student t-test and Analysis of Variance) were used to compare the means of $CD4^+$ T-Cell

count and total serum IgA, IgG, IgM levels in the respective group of patients and control. All statistical significant difference were determined at $p<0.05$.

3. RESULTS

3.1 Age and Sex Distribution of PTB, HIV and PTB/HIV Co-infected Patients in Uyo

Out of the 105 sputum producing patients examined, those with PTB only were 13(12.4%), HIV only, 35(33.3%) and PTB/HIV co-infected, 17(16.2%). Of the 13 PTB and 35 HIV infected patients, those in the age bracket of 21-30 years were the majority (n=5 and n=16, respectively). Of the 17 PTB/HIV co-infected patients, those mostly infected were in the age bracket of 31-40 years (n=8), closely followed by 21-30 years (n=6). None was infected with HIV and PTB/HIV within the age bracket of >60 years. Among those in the PTB and HIV group, the number of males and females infected were almost equal (7 versus 6 and 8 versus 9, respectively) but in the PTB/HIV category, more females (n=19) than males (n=16) were infected (Table 1).

3.2 Mean Absolute $CD4^+$ T-cell Count in the Various Groups

The mean $CD4^+$ T-cell counts of the various test and control groups are presented in Table 2. The mean $CD4^+$ T-cell count of PTB (576.31±326.7 cells/µl), HIV (311.06±228.6 cells/µl) and PTB/HIV co-infected patients (175.12±85.8 cells/µl) were low compared with the control subjects (1282.9±203.5 cells/µl). Comparison of the mean $CD4^+$ T-cell counts in the categorical groups using ANOVA showed that a significant difference exist between the PTB and HIV groups, and those with PTB/HIV co-infection ($p<0.05$). The post hoc analysis showed that the mean $CD4^+$ T-cell counts in the patient groups were significantly lower than the control subjects ($p<0.05$).

3.3 Mean Total Serum Immunoglobulin Levels in the Various Groups

The mean total serum IgA, IgG and IgM values of the various patient groups and control are presented in Table 3. The mean IgA values of the PTB group (190.91±94.8 mg/dl) and control group (126.81±35.5 mg/dl) were not significantly different ($p>0.05$). The mean IgA levels in HIV infected (208.85±104.9 mg/dl) and PTB/HIV co-infected (206.07±71.7 mg/dl) groups were significantly higher than the control group (126.81 ± 35.5 mg/dl) ($p<0.05$). The mean IgG level of the PTB/HIV co-infected group (1498.54±903.9 mg/dl) was 4-fold higher than the control values (379.23±174.9 mg/dl) and 2-fold higher than PTB group ($p<0.05$). However, the difference between the mean IgG levels in PTB and HIV infected groups was not statistically significant ($P>0.05$). The mean IgM values of each of the test groups; PTB (317.75±146.11 mg/dl), HIV (283.73±256.4 mg/dl) and PTB/HIV co-infected (299.70±118.42 mg/dl) was higher than the control group (50.00±32.3 mg/dl) ($p<0.05$).

3.4 Chart of Mean Values of Total Serum Immunoglobulin Classes and $CD4^+$ T-cell Counts

The mean comparison values of respective total serum immunoglobulin classes and $CD4^+$ T-cell counts of PTB, HIV, PTB/HIV co-infected and control groups are presented in Fig. 1. The PTB/HIVco-infected group had the highest serum IgG level followed by the PTB group while at the cellular immune level, $CD4^+$ T-Cell count was the lowest in the PTB/HIV group, followed by the HIV group, with their respective standard deviation error bars.

Table 1. Age and sex-specific distribution of PTB, HIV and PTB/HIV co-infection among sputum producing patients in Uyo

Age group (Years)/ Gender	No. tested (N=105)	PTB (n=13) No. positive	HIV (n=35) No. positive	PTB/HIV (n=17) No. positive
18-20	18	2	2	1
21-30	30	5	16	6
31-40	23	2	9	8
41-50	16	1	7	1
51-60	11	2	1	1
>60	7	1	0	0
Male	48	7	16	8
Female	57	6	19	9

Table 2. Mean $CD4^+$ 5T-cell counts of the categorical patients and apparently healthy persons in Uyo

Subject Group	No. tested	$CD4^+$ (Mean±SD) (cell/µl)	F-value	P-value
PTB	13	576.31±326.7[a]		
HIV	35	311.06±228.6[a]		
PTB/HIV	17	175.12±85.8[b]		
Control	31	1282.9±203.5[c]	131.6	<0.05

a,b,c: Values with different superscript are significantly different (p<0.05)

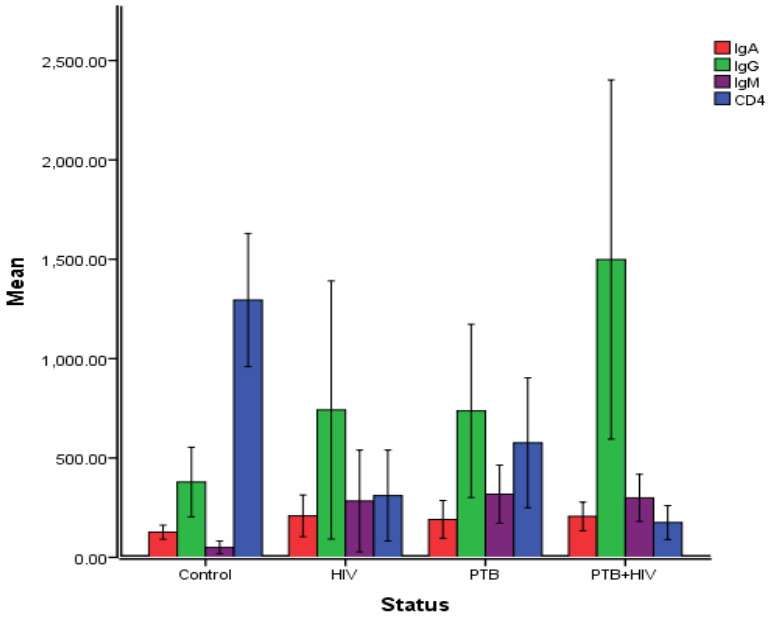

Fig. 1. Mean $CD4^+$ T-cell count, IgA, IgG and IgM levels in patients and control groups

Table 3. Mean total Serum IgA, IgG and IgM levels in patient and control groups

Subject group	No. Tested	IgA (Mean±SD) (mg/dl)	IgG (Mean±SD) (mg/dl)	IgM (Mean±SD) (mg/dl)
PTB	13	190.91±94.8[ab]	737.29±435.8[a]	317.75±146.11[a]
HIV	35	208.85±104.9[a]	741.06±649.6[a]	283.73±256.4[a]
PTB/HIV	17	206.07±71.7[a]	1498.54±903.9[b]	299.70±118.42[a]
Control	31	126.81±35.5[b]	379.23±174.9[c]	50.00±32.3[b]

a,b,c: Values with different superscript are significantly different (p<0.05)

3.5 Mean Serum Immunoglobulin (IgG, IgA, IgM) Levels among the Categorical Patient Groups with $CD4^+$ T-Cell Counts Less or Greater than 200 cells/µl

The mean serum immunoglobulin (IgG, IgA, IgM) levels among patients with low (≤200 cells/µl) and raised (>200 cells/µl) CD4 cell counts in the categorical groups are shown in Tables 4 – 6, respectively. There was no statistical significant difference between the estimated levels of immunoglobulin G, A and M and $CD4^+$ T-cell count (≤ 200 or > 200 cell/µl) in PTB (Table 4) and HIV infected patients (Table 5). However, mean serum IgG levels were significantly higher in PTB/HIV co-infected patients with $CD4^+$ T-cell count ≤ 200 cell/µl than those with $CD4^+$ T-cell counts > 200 cell/µl (Table 6). Hence, a negative correlation (r= -0.56) was observed between categorical $CD4^+$ T-cell count and IgG levels in PTB/HIV co-infected patients. Linear regression showed that 31.6% of the variance in the $CD4^+$ T-cell count of PTB/HIV co-infection may be accounted for by the serum IgG level.

Table 4. Comparison of mean serum immunoglobulin (IgG, IgA, IgM) levels in PTB patients with $CD4^+$ T-cell count of less or greater than 200 cells/µl

Immunoglobulin (mg/dl)	$CD4^+$ T Cell Count		T-test value	p-value
	(≤200)cells/µl	(>200)cells/µl		
IgG	740.40±289.07	736.73±408.6	0.10	0.99
IgA	209.91±18.12	187.46±103.27	2.96	0.77
IgM	450.53±68.87	291.19±144.45	1.48	1.69

Table 5. Comparison of mean immunoglobulin (IgG, IgA, IgM) levels in HIV infected patients with $CD4^+$ T cell count of less or greater than 200 cells/µl

Immunoglobulin (mg/dl)	$CD4^+$ T cell count		T-test value	p-value
	(≤200)cells/µl	(>200)cells/µl		
IgG	639.00±375.00	631.00±366.00	0.12	0.91
IgA	216.15±125.85	204.62±94.17	0.29	0.78
IgM	289.84±284.89	279.95±243.84	0.11	0.91

Table 6. Comparison of mean immunoglobulin (IgG, IgA, IgM) levels in PTB/HIV co-infected patients with $CD4^+$ T cell count of less or greater than 200 cells/µl

Immunoglobulin (mg/dl)	$CD4^+$ T cell count		T-test value	p-value
	(≤200)cells/µl	(>200)cells/µl		
IgG	1814.48±976.06	1047.20±587.88	2.02	0.04
IgA	186.74±78.56	260.94±57.65	1.25	0.23
IgM	284.28±91.41	330.55±168.80	0.70	0.50

4. DISCUSSION

Monitoring of laboratory indices such as levels of cellular and humoral immunological markers using $CD4^+$ T-lymphocyte counts and levels of total serum immunoglobulin classes (IgA, IgG, IgM), could help physicians predict or monitor TB disease progression especially in TB/HIV coinfection.

In this study, sputum producing patients with PTB only were 12.4% while those with PTB/HIV co-infection were 16.2%. These rates are twice lower than that reported elsewhere [12,13] possibly due to the use of cultural methods as against the less sensitive smear microscopy employed in this study. Majority of those with PTB and PTB/HIV coinfection were in the age groups of 21-30 yr. and 31-40 yr., respectively and this is in agreement with previous report by WHO indicating that individuals in their economically productive years in Africa, are seen to be most infected with TB [14]. In Nigeria, tuberculosis still poses a serious public health challenge especially in persons co-infected with HIV. Nigeria was one of the 22 high burden countries of the world that could not meet the mandatory 50% reduction in TB prevalence rate by 2015 compared with the 1990 prevalence and HIV prevalence accounts for 5-19% among new TB cases [15].

Findings in this study revealed that the mean $CD4^+$ T-lymphocyte counts in patients with PTB, HIV and PTB/HIV co-infection were significantly reduced compared with apparently healthy persons, and this finding agrees with previous report of studies carried out within and outside Nigeria [16-19]. A recent study in an Indian population also reported a significant decrease in $CD4^+$ T-cell count among PTB patients [13]. This could be due to the suppression of cellular immune response by HIV infection and other clinical conditions that promote immunosuppression [3].

In this study, the mean total serum levels of IgA, IgG, IgM in PTB, HIV and PTB/HIV co-infected patients were elevated variably. This finding is in agreement with previous reports in southeastern Nigeria [16]; Ibadan, Southwestern Nigeria [20] as well as reports from other African countries such as Gambia, West Africa [21] and Dares Salaam, East Africa [22]. The increase in IgA levels observed in all patient groups in this study buttress the fact that IgA is the most important immunoglobulin in mucosal immunity. Its increase is imperative in mucosal infection as tuberculosis affects the respiratory system. The

level of IgA concentration is dependent upon severity of infection. The presence of low bacillary burden in less severe PTB cases could elicit low IgA concentration unlike in the more severe cases, especially among those co-infected with HIV [3,23]. The mean serum IgG levels in PTB, HIV and PTB/HIV co-infected patients in this study were significantly elevated ($p<0.05$), most especially in the PTB/HIV coinfected group where a 4-fold increase compared with the control and 2-fold increase compared with the PTB and HIV groups were recorded. Gomez and co-workers in Gambia [21] reported a 4-fold increase in the mean serum IgG levels in PTB patients compared to latently infected controls. They also observed a 2-fold increase in IgG levels following successful TB treatment compared with pretreated cases and latently infected controls. The prime function of IgG in opsonization, complement activation and antibody dependent cell-mediated cytotoxicity during infections accounts for the rise in the total serum levels as observed in this study [3]. Also, bacillary burden and bacterial accessibility through its antigenic parts determines the levels of humoral immune response [21]. Determining the total serum IgG levels in the management of PTB/HIV coinfection could nonetheless aid in monitoring and predicting disease progression [21]. The mean serum IgM levels in PTB, HIV and PTB/HIV coinfected patients in this study were significantly elevated in agreement with the report of Amilo et al. [16]. Although the difference between the raised IgM levels in the respective patient groups were not significant, their serum concentrations were high compared to the control values. The reason for the consistent rise in serum IgM levels may be unconnected with its role during acute and active infections before class switching to IgG occurs [24].

In this study, patients infected with PTB, HIV and PTB/HIV co-infection, were further stratified into two categories based on their levels of $CD4^+$ T-cell counts (≤200 cells/µl and >200 cells/µl). This was done to compare the categorical $CD4^+$ T-cell counts in the respective patient groups and immunoglobulin levels and to correlate disease severity in the respective patient groups (owing to depressed cellular immunity) with total serum immunoglobulin levels. It was observed that no correlation existed between serum immunoglobulin levels of PTB and HIV groups, whether with low or raised cellular immunity whereas a negative correlation ($r= -0.56$) was observed in patients co-infected with PTB/HIV. Linear regression analysis indicated that 31.6% of the variance observed between the categorical $CD4^+$ T-cell count in PTB/HIV co-infection may be accounted for by IgG. It was further observed that the difference between the mean serum IgG levels and the categorical $CD4^+$ T-cell count in PTB and HIV groups were not significant unlike in the PTB/HIV group where a high significant difference was observed as previously documented [16,22,25]. This could be attributed to the fact that the presence of TB bacilli in HIV infected person increases viral replication/ viral load, leading to suppressed cellular immunity and severe disease state [26,27]. A study in Ibadan, Nigeria, conducted among HIV infected patients, equally reported no significant reduction in the concentration of plasma IgG levels in HIV patients receiving Highly Active Anti-Retroviral Therapy (HAART), whose $CD4^+$ T-Cell Counts were either below or greater than 200 cells/µl [18]. Going by these reports, the estimation of plasma concentration of immunoglobulin classes alone in cases of HIV infection might not be very useful in predicting severity of HIV disease. However, in cases of PTB/HIV co-infection which is crucial in developing and resource poor countries, predicting disease progression and monitoring treatment failures could be achieved by estimating serum IgG concentration and $CD4^+$ T-cell counts of the patients concurrently.

The appreciable increase in levels of all the immunoglobulin classes (IgA, IgG, and IgM) observed in this study is a clear evidence of polyclonal B-cell activation in patients with advancing TB and HIV diseases. As earlier reported by other workers, the HIV viral envelope proteins especially glycoprotein (Gp) 41 which induces polyclonal B-cell activation usually result in excess and abnormal serum levels of immunoglobulins production [20,23]. However, in spite of this observed polyclonal B-cell activation and abundance of immunoglobulins, HV disease progression to AIDS still occurs [28,29]. This therefore implies that estimating serum IgA, IgG and IgM levels in patients with TB and HIV diseases, particularly in PTB/HIV co-infection, along with $CD4^+$ T-cell count may provide useful information on disease progression, and may probably signal some kind of intervention to be made that may reduce further immune suppression, and delay progression to AIDS.

5. CONCLUSION

The levels of $CD4^+$T-cell count and immunoglobulin classes (IgA, IgG and IgM), were

significantly affected in patients with PTB, HIV and PTB/HIV co-infection. All patient groups had reduced $CD4^+$ T-cell counts, especially those with PTB/HIV co-infection. Similarly, serum IgG levels were highly raised in PTB/HIV co-infected patients followed by those infected with either HIV or PTB. Hence, the use of $CD4^+$ T-cell count and total serum IgG levels in PTB/HIV co-infection along with other known systemic markers and parameters could aid in predicting disease progression and monitoring treatment failure. This approach could complement current efforts in TB management, particularly in patients with TB/HIV co-infection. Moreover, there still exist some challenges in low resource settings such as poor case detection rate (50% in Nigeria) using the conventional acid-fast technique to diagnose cases of HIV infected patients, long duration of culture where facilities are available, smear negative tuberculosis cases and expensive PCR based methods. An expanded population study is advocated to validate the findings in this study.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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