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

[1]Ibokette, D. M., [1]Umo, A. N., *[2]Akinjogunla, O. J., [1]Moses, A. E

[1]Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, University of Uyo, Nigeria
[2]Department of Microbiology, Faculty of Science, University of Uyo, P.M.B. 1017, Uyo, Nigeria

*Corresponding authors' email: **papajyde2000@yahoo.com** Phone: 08064069404

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 Ishaleku, 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 (Umo *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; Umo *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 (Umo *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 -70ºC) 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 Cytoflow counter (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. tuberculosis,* while from the 27 samples collected from St Luke's Hospital, only 2 samples were positive for *M. tuberculosis.*

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 ±

18.12mg/dL, while in PTB patients with CD4$^+$ T-lymphocyte counts of > 200cells / µL the mean IgA level was 187.46 ± 103.27mg/dL. The mean IgM level in PTB patients with CD4$^+$ T-lymphocyte counts of ≤ 200cells / µL was higher than that of PTB patients with CD4$^+$ T-lymphocyte counts of > 200cells / µL. The mean IgG level in PTB patients with CD4$^+$ T-lymphocyte counts of ≤ 200cells / µL was 740.40 ± 289.07mg/dL, while PTB patients with CD4$^+$ T-lymphocyte counts (> 200cells / µL) had the mean IgG level of 736.73 ± 408.61mg/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±94.8mg/dL) was significantly higher than that of apparently healthy subjects (126.81±35.5mg/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±32.2mg/dL. The mean IgM value of PTB patients and that of the apparently healthy subjects was 317.75±146.11 and 50.00±32.3 mg/dL, respectively. There was statistically significant difference between the immunoglobulin levels of PTB patients and the apparently healthy subjectsat 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

Hospital	No of Samples Collected	No of Samples Positive for *M. tuberculosis*	Prevalence (%)	t-value	p-value	χ^2
UUTH	78	11	14.1	0.98	0.47	1.29
St Luke's	27	2	8.3	0.24	0.36	0.87
Total	105	13	12.4	0.24	0.36	1.13

Key: UUTH: University of Uyo Teaching Hospital

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

Age Group (yrs)	No (%) of Samples Collected		No (%) of Samples Positive for *M. tuberculosis*		t-value	p-value	χ^2
	Male	Female	Male	Female			
>18-20	7(38.9)	11(61.1)	2(28.6)	0(0.0)	0.99	0.38	0.71
21-30	10(33.3)	20(66.7)	3(33.3)	2(10.0)	1.01	0.36	0.43
31-40	13(56.5)	10(43.5)	1(7.7)	1(10.0)	0.33	0.53	0.25
41-50	10(62.5)	6(37.5)	0(0.0)	1(16.7)	0.24	0.36	0.43
51-60	6(54.5)	5(45.5)	2(33.3)	0(0.0)	1.12	0.49	0.68
> 60	2(28.6)	5(71.4)	0(0.0)	1(20.0)	0.29	0.65	0.20
Total	48(45.7)	57(54.3)	8(16.7)	5(8.8)	1.46	0.57	0.31

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

Immunoglobulin Types	CD4$^+$ T-Lymphocyte Counts (cells / µL)	mm±SD Immunoglobulin Level (mg/dL)	t-value	p-value	χ^2
IgA	≤ 200	209.91 ± 18.12	2.96	0.77	1.45
	> 200	187.46 ± 103.27			
IgG	≤ 200	740.40 ± 289.07	0.10	0.99	1.14
	> 200	736.73 ± 408.61			
IgM	≤ 200	450.53 ± 68.87	1.48	1.69	2.98
	> 200	291.19 ± 144.45			

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

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

Immunoglobulin Types	Subjects	No. Tested	mm±SD Immunoglobulin Level (mg/dL)	t-value	p-value	χ^2
IgA	PTB	13	190.91±94.8			
	AHS	15	126.81±35.5	3.33	< 0.05	4.45
IgG	PTB	13	737.29±435.8			
	AHS	15	23.81±32.2	33.06	< 0.001	10.21
IgM	PTB	13	317.75±146.11			
	AHS	15	50.00±32.3	6.76	< 0.001	5.69

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).It this study, the mean serum levels of IgA, IgG and IgM in PTB patients were significantly high and this agrees with studies in 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 ($\leq$200 cells/μL and >200cells/μL). Our results showed that there was no significant relationship ($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 whoseCD4^{+} T-lymphocyte counts were either below or greater than200 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 enable 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.

REFERENCES

Abdul KA, Andrew HL. (2009). Basic Immunology: Functions and Disorders of the Immune System. *Saunders Elsevier*. pp 212-222.

Akinjogunla, O. J., Ekuma, E.A., Etukudo I. U., Oshosanya, G.O. and Inyang, D. E. (2020). CD4+ T- lymphocyte values, bloodstream bacterial isolates and their antibiotic susceptibility profiles among human immunodeficiency virus infected patients in Uyo, Nigeria. *Tropical Journal of Natural Product Research*, 4 (9): 612-620.

Amilo GI, Meludu SC, Onyenekwe C, Ekejindu OC, Ifeanyichukwu O. (2012). Evaluation of CD4+ and CD8+ T- lymphocyte counts and serum levels of immunoglobulins in pulmonary tuberculosis (PTB) patients with or without HIV coinfection in South Eastern Nigeria. *African Journal of Pharmacy and Pharmacology*. 6(23):1639-1643.

Arinola OG, Igbi J. (1998). Serum immunoglobulin and circulating immune complexes in Nigeria with human immunodeficiency virus and pulmonary tuberculosis infection. *Tropical Journal of Medical Research.* 2(2):41-48.

Cheesbrough M. (2006). *District Laboratory Practice in Tropical countries.* Low Price editions, UK: Cambridge University Press, Pp, 39-78, 209.

Federal Ministry of Health - Nigeria. (2012) Report first national TB prevalence survey 2012, Nigeria. Abuja: Federal Ministry of Health.

Gomez MP, Donkor S, Adetifa IM, Ota MOC, Sutherland JS. (2012). Analysis of LAM and 38 kDa Antibody levels for diagnosis of TB in a case-control study in West Africa. *International Scholarly Research Network ISRN Immunology,* 23:1-6.

Goodridge A, Zhang T, Miyata T, Sangwei LU, Riley LW. (2014). Antiphospholipid IgM antibody response in acute and chronic *Mycobacterium tuberculosis* mouse infection model. *Clin Respir J*., 8: 137-144.

Itah AY, Udofia SM. (2005). Epidemiology and Endemically of Pulmonary Tuberculosis (PTB) in South-Eastern Nigeria. *South Eastern Asian Tropical Medicine Public Health*, 36(2): 317-323.

Jain VK, Bishnoi HS, Beniwal OP, Misra S N. (1984). Immunoglobulin profile in pulmonary tuberculosis. *Journal of Postgraduate Medicine*, 30(80): 1-5.

Kent PT, Kubica GP. (1985). *Public Health Mycobacteriology: A Guide for Level III Laboratory*. Atlanta: Center for Disease Control. pp 10-154.

Kochak HE, Seyed-Alinaghi S, Zarghom O, Hekmat S, Jam S, Abdi Z. (2010). Evaluation of serological tests using A60 antigen for diagnosis of tuberculosis. *Acta Med Iran*, 48: 21-26.

Lawn SD, Acheampong JW. (2009).Pulmonary tuberculosis in adults: factors associated with mortality at a Ghanaian teaching hospital. West Afr J Med. 18(4):270-274.

Lyamuya EF, Maselle SY, Matre R. (1999). Serum Immunoglobulin Profile in Asymptomatic HIV-1 Seropositive Adults and in Patients with AIDS in Dares Salaam Tanzania. *East African Medical Journal.* 76 (7): 370-375.

Nwachukwu E, Peter GA. (2010). Prevalence of Mycobacterium tuberculosis and human immuno-deficiency virus (HIV) infections in Umuahia, Abia state, Nigeria. African Journal of Microbiology Research, 4 (14):1486-1490.

Nzou C, Kambarami RA, Onyango FE, Ndhlovu CE, Chikwasha V. (2010). Clinical predictors of low CD4 count among HIV-infected pulmonary tuberculosis clients: A health facility- based survey. *S. Afri Med J.* 100:602–605.

Olaniyi JA, Arinola GO. (2011).Humoral immunological factoral and nitrioxide levels in HIV Patients with low CD4$^+$ T-lymphocytes counts. *International Journal of Health Research.* 4(2): 69-74.

Schneider BE, Korbel D, Hagens K, Koch M, Raupach B, Enders J. (2010). A role for IL-18 in protective immunity against *Mycobacterium tuberculosis.* *Eur. J. Immunol.* 40: 396–405.

Selma WB, Harizi H, Boukadida J. (2011).Immunochromatographic IgG/IgM test for rapid diagnosis of active tuberculosis, *Clin Vaccine Immunol.*, 18(12): 2090–2094.

Tripathy S, Meron P, Joshi DR, Patil U, Gadkari DA, Paranjape RS. (2000). CD4 and CD8 Lymphocytes counts in *Mycobacterium tuberculosis* in pure India*. Indian Journal of Medical Research*, 111: 195-198.

Umeh EU, Ishaleku D, Iheukwumere CC. (2007). HIV/Tuberculosis co-infection referred chest clinic in Nasarawa State, Nigeria. *Journal of Applied Science,* 7: 1-9.

Umo AN, Akinjogunla OJ, Umoh NO, Uzono GE. (2020). Diagnosis and risk factors of latent tuberculosis infection among healthcare workers using whole blood human interferon-gamma release assay and tuberculin skin testing. *Asian Journal of Research in Infectious Diseases,* 3 (3):15-21.

Verma S, Mahajan V. (2008). HIV- Tuberculosis Co-infection. *The Internet Journal of Pulmonary Medicine.* 10(1): 1531-2984.

Wilson D, Badri M, Maartens G. (2011). Performance of serum C-reactive protein as a screening test for smear-negative tuberculosis in an ambulatory high HIV prevalence population. PLoS One. 6(1):e15248.

World Health Organization (2017). Global tuberculosis report 2017. Geneva: World Health Organization.

World Health Organization (WHO) (2009). A guide to monitoring and evaluation for collaborative TB/HIV activities, World Health Organization.