IJMAAS International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 Volume 4 issue 3 October, 2025 Research Article

Comparative Assessment of *Mycobacterium tuberculosis* Infection In Advanced HIV Disease Patients in Imo State, Nigeria

Achilike, K.M.[1], Amadi[1], L. O*., Ugboma[1], C. J., Peekate, L. P[1].
[11] Department of Microbiology, Faculty of Science, Rivers State University, Nkpolu-Oroworokwu, Port Harcourt, Rivers State, Nigeria.
***Corresponding Author:** *lawrence.amadi1@ust.edu.ng*

ABSTRACT

Tuberculosis (TB) is the leading cause of mortality among Advanced HIV Disease (AHD) patients. Most cases of TB in Nigeria are closely linked to HIV infections. In this study, the prevalence of *Mycobacterium tuberculosis* (*M.tb*) in AHD patients was investigated in some LGAs of Imo State. Imo state was divided into three Districts through cluster sampling technique and purposive sampling approach was used to select three LGAs from each District. Three health facilities chosen from each of the Nine LGAs. Patients' Socio-demographics were collected through questionnaire. The 400 presumptive TB patients were tested for HIV, TB infection and CD4+ counts of the HIV seropositive patients estimated. TB Lateral Flow Lipoarabinomannan (TB LF-LAM) and Genexpert were used to diagnose *M. tb* in AHD patients' samples. Among the 400 patients, the prevalence of HIV infection was 41.5%, *M. tb* was 21.8% and 34.9% had CD4 counts ≤ 200 cells/µl (AHD Patients). The overall Prevalence of *M. tb* in AHD was 30.7%. Based on age, 31-45 years' had *M. tb* in AHD Prevalence of 35.2%. By Gender, Males patients had *M. tb* in AHD prevalence of 33.9% while Females had 29.1%. Occupationally, Civil servants had the least prevalence of 7.7%, Sexual business group had 31.8%. Based on marital status, the Married had 36.8% prevalence of *M. tb* in AHD, the Widows had 35.3%, Singles had 30.4% while the Divorced had 25.0% prevalence of *M. tb* in AHD. At 95% confidence interval, there was no significant difference between the prevalence of *M. tb* among AHD patients across the three senatorial districts of Imo state. This study has become a leeway in the study area for a healthy state and our Nation at large. Further studies on this topic should focused on other opportunistic infections associated with AHD in the entire state. Protective materials should be provided for the Key populations (KPs) of HIV to stop the spread.

Keywords: HIV, *M. tb*, TB, Lipoarabinomannan, TB LF-LAM, AHD, CD4, KPs

Introduction

According to World Health Organization (WHO), Advanced HIV disease (AHD) in adults, adolescents, and children older than five years is defined as CD4+ cell count <200 cells/mm3 or WHO stage 3 or 4 events (WHO, 2007). All Children leaving with HIV (CLHIV), younger than five years of age are considered to have AHD however, those who have been receiving ART for more than one year and who are clinically stable should not be considered to have advanced disease (FMH, 2024). The appearance of opportunistic infections (OIs) is directly related to the extent of immune deficiency; the lower the CD4+ cell count, the higher the likelihood of the appearance of OIs like *Mycobacterium tuberculosis*, *Cryptococcal meningitis*, Toxoplasmosis, *Pneumocystis pneumonia*, *Histoplasmosis* and Severe bacterial infections (FMH, 2024).

Tuberculosis (TB) is the world's deadliest infectious disease that claims more than a million lives each year (WHO, 2020). Human Immunodeficiency Virus (HIV) is characterized by the depletion of CD4 + T-Lymphocytes system, leaving the victim vulnerable to TB by reactivating latent TB to active TB disease (Bigwan *et al.*, 2014; Egah *et al*; 2004 and Pennap *et al.*, 2011). The risk of developing TB is estimated to be between 17 to 27 times greater in PLHIV than those without HIV infection (WHO, 2020). TB progresses faster in people living with HIV and HIV increases TB incidence and mortality.

Globally, the number of people presenting with AHD has constantly remained unchanged, estimates have it that about 30–40% of PLHIV starting ART have a CD4+ cell count of <200 cells/mm^3, and 20% have a CD4+ cell count of <100 c ells/mm^3 (WHO, 2007).

Citation: Achilike *et al*. (2025). Comparative Assessment of *Mycobacterium tuberculosis* Infection in Advanced HIV Disease Patients in Imo State, Nigeria. *International Journal of Microbiology and Applied Sciences*. *4(3)*: 61 – 66.

In some settings, up to 50% of people present to care with AHD. In Nigeria, 21% of newly enrolled clients with baseline CD4 tests in 2023 had with CD4+ cell Count <200cells/mm^3. Previous study has shown that in severely immunosuppressed patients with HIV-TB, early initiation of High Active Anti-Retroviral Therapy (HAART) was associated with reduced mortality and disease progression (Mathew *et al*., 2002).

The diagnostic criteria for AHD include any of the following: WHO clinical stage 3 or 4, CD4+ cell count <200 cell/mm3 and CLHIV <5years. In this study, attention was focused on the prevalence of *Mycobacterium tuberculosis* associated with AHD and management of the diseases in the study area of Imo state to contribute to the field of knowledge regarding *M. tb* among AHD patients.

Materials and Methods

Study area

Imo state is one of the 36 states of the Federal Republic of Nigeria with an administrative structure of 27 Local Government Areas (LGAs). It is located in the Southeastern region of Nigeria within latitudes 5^o 45^{1}N and 7^{o}15^{1}N and Longitude 6 ^{o}50^{1}E and 7^{o}25^{1}E. It has a total land mass of 5530.49 square kilometer (NPC, 2006).

Sample size determination

Sample size for the study shall be determined using established formula (Niang *et al.,* 2006).

$$N = \frac{\left[Z^2(PQ)\right]}{d^2}$$

Where N = Desired sample size

Z = Normal standard distribution that corresponds to 95% at confidence interval as 1.96

P = Prevalence of TB/HIV Co-infection in a previous study, 38.5% for Ibadan (Salami *et al*., 2006; Awoyemi *et al.,* 2002). The estimated sample size was 364 patients with 10% of it to take care of study participants that may be non-responses (Niang *et al.,* 2006), thereby providing a total sample size of 400.

Study population

The study population included 400 TB presumptive patients within the age range of 2 to 65 years attending Directly Observed Treatment Short-Course (DOTS) and Antiretroviral Therapy (ART) clinics in selected Local Government Areas of Imo State, within the period of January to July 2024.

Sampling technique

Imo state was divided into three Districts through cluster sampling technique. Purposive sampling approach was used to avoid random selection of LGAs/health facilities with no level 2 - 3 Biosafety equipment. Another reason is to avoid selection of a heath facility that does not offer DOTS/ART services. One health facility was chosen for the study from each of the Nine LGAs.

Data collection tool

Well-structured questionnaire was used to collect the following information from the patient. A: Age, B: Gender, C; Marital status, D: Occupation, E: Settlement (Urban or Rural), F: Risk assessment of the disease.

Sample collection

TB Sample collection

Sputum specimen collections were done in an open ventilated space in the health facilities while the urine for LF-LAM and stool from children for genexpert whose cough were not productive was in closed space. The patients were given the EndTB sterile wide open mouth screwed-bottles for the samples.

HIV Sample collection

The blood samples were collected with the assistance of well-trained DOTS and Advanced HIV Diseases (AHD) team officers in the selected Comprehensive DOTS/ART facilities of Imo state.

The procedure for Laboratory tests

DETERMINE HIV-1/2

The protective foil cover was removed from each test kit. For whole blood samples, 50µl of sample (precision pipette) was added to the sample pad (marked by the arrow symbol). After one (1) minute, then one drop of chase buffer was applied to the sample pad. Thereafter, the result read after a minimum of 15 minutes. If the control bar does not turn red by assay completion, the test result is invalid.

UNI-GOLD ™

The protective foil cover was removed from each test kit. Two (2) drops (60µl) of serum, plasma or whole blood was added to the sample port using a precision pipette. Thereafter, two (2) drops of Wash Solution (unigold buffer) was applied to the sample port and allowed to react. The result was read after a minimum of 10 minutes. The presence of a double pink/red lines showed a positive test result.

Citation: Achilike *et al*. (2025). Comparative Assessment of *Mycobacterium tuberculosis* Infection in Advanced HIV Disease Patients in Imo State, Nigeria. *International Journal of Microbiology and Applied Sciences*. *4(3)*: 61 – 66.

STAT-PAK HIV 1/2 assay

The protective foil cover was removed from each test kit. A loop full (5µl) of serum, plasma or whole blood was added to the sample port using a precision loop. Thereafter, two (3) drops of Wash Solution (Stat pak buffer) was applied to the sample port and allowed to react. The result was read after a minimum of 10 minutes. The presence of a double pink/red lines showed a positive test result.

VISITECT CD 4 Advanced HIV disease test

The plunger button of the pipette was pressed to the first stop. The disposable tip immersed vertically into the EDTA tube. Plunger button smoothly released by drawing the blood into the disposable tip. Touched was the centre of well A lightly and the bulb squeezed of the blood sample device. The pipette plunger gently depressed to ensure the full 30µl of blood specimen was released into well A. The sampling device/disposable tip was discarded into a sharps/biohazard bin. After 3 minutes, the buffer bottle was held vertically 1cm above well A, 1 drop of buffer added to Well A where the blood has been added. After 17 minutes, the buffer bottle was held vertically 1cm above well B. Carefully 3 drops of buffer were added to well B allowing each drop to absorb into the well before adding the next drop. After 20 minutes, the test was completed and the results interpreted within 5 minutes.

TB Lateral flow-Lipoarabinomannan (TB LF - LAM) as described by FMH (2021). The test units were removed from the cards by bending and tearing at the perforation. The protective foil cover was removed from each test. A precision pipette was used to apply 60 µl of the patient urine sample to the sample pad (White pad marked by the arrow symbol). After 25 minutes the results were read, by visualizing the strip under standard indoor lighting conditions.

GeneXpert MTB/RIF Assay

Genexpert Machine was used to detect and identify Drug Susceptible *M. tb* (DSTB) as described by FMH (2021). Xpert sample reagent (SR) was added in the ratio 1:3 to 0.5ml of the test sample for decontaminated sample and 1:2 for direct sample and shaken vigorously twice during 15 minutes' incubation at room temperature. Two (2) ml of the mixture was transferred to Xpert test cartridge and the cartridge was then loaded into Xpert device. After 90 minutes, the results were interpreted by the GeneXpert DX system from measured fluorescent signals and displayed automatically on the screen.

Statistical analysis

Data obtained from the study were analyzed using Statistical Package for the Social Sciences (SPSS) version 25.0. The results were presented as tables and Figures below. Pearson chi-square test was used to find the relationship between the socio-demographic characteristics of the patients and p - value of <0.05 was statistical significance.

Ethical consideration

Ethical approval for the study was sought for and obtained from the Imo State Ministry of Health prior to the commencement of sample collection.

Results

Out of the 166 PLHIV, 58 (34.9%) had their CD4+ count ≤ 200 cells/µl (AHD) while 108 (65.1%) were ≥ 200 cells/ µl as shown in the Table 1 below. Prevalence of *M.tb* in AHD based on gender showed that Males had 33.9% while Females had 29.1%. Based on occupation, the Sexual business group had 31.8% prevalence of *M. tb* in AHD while Civil servants had the least prevalence of 7.7% *M. tb* in AHD. Comparing by marital status, the Married had 36.8% prevalence of *M. tb* in AHD, followed by the Widows 35.3%, the Singles had 30.4% while the Divorced had 25.0% prevalence of *M. tb* in AHD.

Citation: Achilike *et al*. (2025). Comparative Assessment of *Mycobacterium tuberculosis* Infection in Advanced HIV Disease Patients in Imo State, Nigeria. *International Journal of Microbiology and Applied Sciences*. *4(3)*: 61 – 66.

Table 1: CD4+ Count of HIV Patients across the selected LGAs of Imo State

Senatorial District (LGA)	HIV Positive	$CD4^+$ Count ≥ 200 cells/µl	$CD4^+$ Count ≤ 200 cells/µl
Owerri Senatorial District	56	38	18
Orlu Senatorial District	61	38	23
Okigwe Senatorial District	49	32	17
Total	**166**	**108**	**58**

Table 2: Prevalence of *Mycobacterium tuberculosis* in AHD Among People Living with HIV In Relation to their Socio-Demographic Characteristics

Socio-Demographic Variable	Category (Years)	Population at Risk (PAR)	M. tb in AHD	Prevalence (%)	P-value
Age (Years)	≤ 15	6	3	50.0	
	16 – 30	33	10	30.3	
	31 – 45	71	25	35.2	≥ 0.05
	46 – 65	56	13	47.7	
	Total	**166**	**51**	**30.7**	
Gender	Male	56	19	33.9	
	Female	110	33	29.1	≤ 0.05
	Total	**166**	**51**	**30.7**	
Occupation	Studying	47	10	21.3	
	Sexual Business	66	21	31.8	≥ 0.05
	Civil Service	13	1	7.7	
	Farming	12	11	91.7	
	Artisans	18	4	22.2	
	House Helps	3	2	66.7	≥ 0.05
	House Wives	7	2	28.6	
	Total	**166**	**51**	**30.7**	
Marital Status	Married	57	21	36.8	
	Single	56	17	30.4	
	Divorced	28	7	25.0	≥ 0.05
	Widower	7	0	0.0	
	Widow	17	6	35.3	
	Celibate	1	0	0.0	
	Total	**166**	**51**	**30.7**	

Key: PAR = Population at Risk; *M. tb* = *Mycobacterium tuberculosis;* AHD = Advanced HIV Disease

Citation: Achilike *et al*. (2025). Comparative Assessment of *Mycobacterium tuberculosis* Infection in Advanced HIV Disease Patients in Imo State, Nigeria. *International Journal of Microbiology and Applied Sciences*. *4(3)*: 61 – 66.

Discussion

The study was conducted to determine the prevalence of *M.tb* in AHD among PLHIV attending DOTS/ART health facilities in Imo State. *Mycobacterium tuberculosis* was determined by evaluation of patients samples in genexpert machine and use of TB LF-LAM antigen rapid test kits. Out of the 166 specimens from PLHIV, fifty-one were positive for *M. tb* and also 34.9% had their CD4 count ≤ 200 cells/µl, thereby giving an overall *M.tb* in AHD prevalence of 30.7%. Our findings therefore, were in agreement with report of previous researchers that; HIV depletes the CD4 + T-Lymphocytes system, leaving the victim vulnerable to TB by reactivating latent TB to active TB disease (Bigwan *et al.* 2014; Egah *et al*., 2004 and Pennap *et al.* 2011).

The overall prevalence of 30.7% *M. tb* in AHD is comparable to 33.9% reported by Effiong & Nwakaego, (2015), in a similar study conducted at the University of Benin Teaching Hospital, Edo State. In the same vein, our 30.7% was also comparable to the prevalence of 34.4% reported by Gyar *et al*. (2014) in a study conducted at the Dalhatu Araf Specialist Hospital Lafia, Nassarawa State. Our overall prevalence of 30.7% is also higher than 6.4% recorded among HIV patients attending Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State and 9.6% reported by Agbaji *et al.* (2013) in a study conducted among patients attending HIV clinic at Jos University Teaching Hospital, Jos, Plateau State.

By age, the prevalence of 50% *M. tb* in AHD among children under 1 – 15, was due to the small number of CLHIV. Our report of 30.3% among the age of 16 – 30 years is contrary to Olaniran *et al.* (2011) who had a low figure of 5.8% among the age group of 21-30 years in Ogun State which was lower than ours though with different lower limits. The prevalence of 35.5% *M. tb* in AHD in our study within the age of 31 – 45, is in agreement with the findings of Gyar *et al*. (2014) who reported in a similar study that the highest prevalence of TB in HIV was in the age group 31-40 years in Nasarawa state, Nigeria.

By Gender across the Senatorial districts, the Male had a Prevalence of 33.9% *M. tb* in AHD when compared to their female counterparts with a Prevalence of 29.1%. Our findings were in agreement with similar work reported by the following authors: (Olaniran *et al.*2011; Okonkwo *et al.*2015; & Musa *et al.* 2015).

Contrarily, studies that recorded higher prevalence of among females have been reported by (Effiong and Nwakaego, 2015; Okonkwo *et al.* 2015).

Regarding occupation-related prevalence, patients who engaged in Sexual Businesses had a prevalence of 31.8%. Our reports were agreement with the work done by Oladeinde, *et al.* (2014), in a similar study conducted in Edo State with a prevalence of 41.1% among traders. The higher figure among farmers could be as a result of the sample size of farmers. The public health implications of the high prevalence among the farmers, is that the condition will affect their output, thereby leading to food insecurity. The civil servants in our study, had the lowest prevalence of *M. tb*/AHD with 7.7%. This was similar to the work done by Oladeinde *et al.* (2014), whereby no TB case was recorded among civil servant living with HIV. This could be as result of good health habits, good nutrition and knowledge of TB and HIV among civil servants.

Based on marital status, the Married had a higher *M. tb* in AHD prevalence of 36.8% while the Singles had a prevalence of 30.4% the Widows had 35.3%, while the Divorced had 25.0% prevalence of *M. tb* in AHD. Our results therefore were in agreement with previously documented reports with more *M. tb* in HIV co-infection among the Singles than the married as reported by Oladeinde *et al.* (2014) in Edo state, Nigeria with a prevalence of 32.8%. The result was also in agreement with the findings of Musa *et al.* (2015) in a similar work at Kano state, Nigeria.

Conclusion

The present study has established the link and added to knowledge between *M. tb* and AHD in the study area. *Mycobacterium tuberculosis* remains the most common opportunistic infection (OI) among Advanced HIV Disease patients. We therefore recommend continuous and rapid TB, HIV screening and testing to avoid AHD condition.

References

Awoyemi, O.B, Ige, O.M, & Onadeko, B.O. (2002). Prevalence of active pulmonary tuberculosis in human immunodeficiency Virus seropositive adult patients in University, College Hospital, Ibadan, Nigeria. *African Journal of Medical Science, 31*,329-32

Citation: Achilike *et al*. (2025). Comparative Assessment of *Mycobacterium tuberculosis* Infection in Advanced HIV Disease Patients in Imo State, Nigeria. *International Journal of Microbiology and Applied Sciences*. *4(3)*: 61 – 66.

Bigwan, E.I; Ohaen, M.C; & Okonkwo, H.I. (2014). Prevalence of Acid – Alcohol – Fast Bacilli among patients with suspected cases of pulmonary tuberculosis in Jos, Nigeria. *African Journal of Clinical and Experimental Microbiology*, *15*, 103-108.

Effiong, J.O.& Nwakaego, I.F. (2015). Gender differences among clients attending tuberculosis unit of a teaching hospital in southern Nigeria. *Scholars Journal of Applied Medical Sciences, 3*, 228-233.

Egah, D.Z, Banwat, E.B & Alanana, J.A. (2004). Tuberculosis in Jos, Nigeria: A 9-year review of laboratory report at the Jos University, teaching hospital. *Nigerian Medical Practitioner, 46*, 33-35.

Federal Ministry of Health (2021). *National Tuberculosis Leprosy and Buruli ulcer management and control guidelines*. Seventh edition, 28.

Federal Ministry of Health (2024). National AIDS, STIs and Viral Hepatitis Control Programme. In *National Guidelines for HIV Prevention Treatment and Care, 25* -125

Gyar, S.D, Dauda, E. & Reuben,C.R. (2014). Prevalence of tuberculosis in HIV/AIDS patients in Lafia, Central Nigeria. *International Journal Curr. Microbiol. App. Sci*, 3, 831838.

Matthews, N.J., Fox, G.E.F., & Navaratne, L. (2002). Treatment of tuberculosis in HIV infected persons in the era of highly active antiretroviral therapy. *AIDS, 16,* 75-83.

Musa, B.M, Musa, B. & Muhammed, H. (2015). Incidence of tuberculosis and immunological profile of TB/HIV coinfected patients in Nigeria. *Annals of Thoracic Medicine, 10*, 185.

National Population Commission of Nigeria (2006). *Population and Housing Census*, Available online: http://catalog.ihsn.org/index.php/catalog/3340.

Niang, L., Than, W. & Rusil, N. (2006). Practical issues in calculating the sample size for prevalence studies. *Archives of Orofacial Sciences, 1*, 9 – 14.

Oladeinde, B.H., Olley, M. & Imade, O.S., *et al*. (2014). Prevalence of HIV infection among patients with pulmonary tuberculosis in a rural tertiary hospital in Nigeria. Nigerian *Journal of Experimental and Clinical Biosciences, 2*, 90.

Olaniran, O., Hassan-Olajokun, R.E.& Oyovwevotu, M.A, *et al*. (2011). Prevalence of tuberculosis among HIV/AIDS patients in Obafemi Awolowo University Teaching Hospital Complex Oauthc, ILE-IFE. *International Journal of Biological & Medical Research, 2*, 874-877.

Okonkwo, R.C., Anyabolu, A.E. & Onwunzo, M.C., *et al*. (2015). Prevalence of Smear Positive Tuberculosis among HIV-Positive PTB Suspects at the Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria. *World Journal of Medical Sciences, 12*, 248-251.

Pennap, G.R; Makpa, S., & Ogbu, S. (2011). Prevalence of HIV/AIDs among tuberculosis patient seen in a rural clinic in Nigeria. *Trakia Journal of Sciences, 9*, 40-44.

Salami, A, K, & Katibi, I. A. (2006). Human Immunodeficiency Virus associated tuberculosis pattern and trend in the University of Ilorin Teaching. *African Journal of Medical Science*, 35-457.

World Health Organization (2007). UNAIDS; *2007 AIDS epidemic update* (PDF). 16

World Health Organisation. (2020). *Tuberculosis Report*(https://www.who.int/publications/i/items/9+89 240013131.

Citation: Achilike *et al*. (2025). Comparative Assessment of *Mycobacterium tuberculosis* Infection in Advanced HIV Disease Patients in Imo State, Nigeria. *International Journal of Microbiology and Applied Sciences*. *4(3)*: 61 – 66.