Research Article

Prevalence and Risk Factors of *Mycobacterium tuberculosis* and *Human immunodeficiency* Virus Coinfection Among Patients on Antiretroviral Therapy in a Specialist Hospital, Lokoja

Martin-Luther Oseni Okolo[1], Monday Eneojo Akor[1], Cornelius Arome Omatola[1*], Zainab Penninah Ochada[1], Ugbane Eleojo Okolo[2], Benjamin Mudi Idache[3], Andrew Isiramen[4], Julius Akor Omatola[5] and Moses Adaji David[6]

[1]Department of Microbiology, P.M.B.1008, Prince Abubakar Audu University, Anyigba, Nigeria; [2]Federal Medical Center, Lokoja, Nigeria; [3]Department of Health and Physical Kinetics, Prince Abubakar Audu University, Anyigba, Nigeria; [4]Department of Microbiology, Ambrose Alli University, Ekpoma; [5]Department of Statistics, Kogi State Polytechnic, Lokoja; [6]Department of Biotechnology Science and Engineering, University of Alabama, Huntsville, United States of America.

Abstract | Worldwide tuberculosis (TB) prevalence increased as a result of the *Human Immunodeficiency Virus* (HIV) epidemic. Nevertheless, the widespread use of efficient antiretrovirals has lately prompted the tendency to reverse. This study was carried out to detect *Mycobacterium tuberculosis* using GeneXpert in HIV seropositive patients attending Kogi State Specialist Hospital, Lokoja. The study was done using 325 confirmed HIV patients (86 males and 239 females). A structured questionnaire was administered and 325 patients who consented were enrolled in the study. Sputum specimen was collected from each patient, processed, and examined using Xpert® MTB/RIF Assay Version 4. The prevalence of HIV/TB was found to be 6 (1.85%). Infection was more prevalent in the age group 26-35 years (3.92%) and among the females (83%) than their counterpart males (17%). Co-infection was found only among the married (2.43%). A higher prevalence (1.89%) was found in patients from monogamous families (2.41%) and in patients residing in rural areas compared to urban dwellers (1.65%). Occupation-specific prevalence showed that farmers had the highest prevalence (2.56%) followed by those who identified as traders (2.16%) and civil servants (1.79%). Yet among patients who identified as students, no incident of TB was observed. Patients with secondary education had the highest prevalence (3.08%) followed by patients with tertiary education (1.58%). Patients with no formal education and primary education had no co-infection. Between the sociodemographic factors that were assessed and the HIV patients who had TB infection, there was no statistically significant correlation (P>0.05). The rate of HIV/TB co-infection, though low in this study, could worsen the clinical outcomes in affected patients. Therefore, there should be increased public health awareness of TB and HIV transmission and prevention in the study area.

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***Correspondence** | Omatola Cornelius Arome, Department of Microbiology, P.M.B.1008, Prince Abubakar Audu University, Anyigba, Nigeria; **Email:** omatolac@gmail.com
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Introduction

Tuberculosis (TB) remains a common infection in young people, the elderly, or people with a weakened immune system (WHO, 2020a). Tuberculosis had long been declared a "global health emergency" by the world health organization yet, remains an unending malady (Lawn and Zumla, 2011). Today, one challenge faced in curbing the tuberculosis burden is the incidence of HIV- associated tuberculosis. Over 7.1 million individuals worldwide are now suffering from TB, which is a minor rise from 7.0 million in 2018 but a substantial increase from 6.4 million in 2017 and 5.7–5.8 million every year during 2009–2012 (WHO, 2020b). In 2019 alone, 69% of notified TB patients were coinfected with HIV, up from 64% in 2018. Moreover, rifampicin resistance was acquired in 61% of those with diagnosed pulmonary TB, up 10% from the prevalent rate in 2018. Actualizing the global control of TB will require early and precise diagnosis of drug-resistant and drug-sensitive TB. Though, delays in case reporting or identification, and TB underdiagnosis have been identified as major hurdles in countries where patients experience significant geographical and socio-economic challenges while accessing hospital care, conditions increasing the chances of transmission and perpetuation of the epidemic (Adhikari *et al*., 2022).

The transmission of TB is influenced by numerous factors such as host susceptibility, the index case, bioaerosols' behavior, the environment in which transmission occurs, and pathogen-associated factors (Adhikari *et al*., 2022). By using prognostic and diagnostic markers to focus on preventative and curative medications, it is possible to identify infected individuals who are at risk of developing the disease and treat them. Notwithstanding, TB/HIV co-morbidity has accelerated the TB epidemic, posing serious programmatic and therapeutic challenges globally (Adhikari *et al*., 2022).

Being the most prevalent opportunistic infection (OI) among HIV-infected people and the leading cause of mortality in co-infected people, TB, HIV/TB coinfection continues to be a significant public health concern worldwide (Lawn and Churchyard, 2009). For individuals infected with HIV, the risk of developing TB is 18-fold higher than the general population (WHO, 2020b). In HIV-endemic areas, viral coinfection with TB is still a significant contributor to the incidence of active TB in HIV-endemic regions because of the immunosuppressive nature of HIV/AIDS (Bruchfeld *et al*., 2015). Persons with HIV (PLWH) on antiretroviral therapy (ART) nevertheless have higher rates of TB incidence despite the fact that ART can minimize the incidence of TB both at the individual and population levels (Lawn *et al*., 2006). Again, the infection of a host by *Mycobacterium tuberculosis* imparts negatively on the host's immunological response to HIV, thus accelerating the disease progression from HIV infections to AIDS (Bruchfeld *et al*., 2015). Reports have shown that TB/HIV co-infection as well as rifampicin resistance is on the increase annually (WHO, 2020b), thus posing treatment and management challenges in clinical practice.

In Kogi State, the endemicity of HIV infection is well documented in the general population and among different selected groups (Omatola *et al*., 2019a, b, 2018, 2020a). However, there is a dearth of information regarding the viral coinfection with TB in Kogi State. Therefore, a study to understand the actual prevalence and predisposing risk factors of TB among people living with HIV is important to inform policy formulation for effective planning and implementation of intervention strategies for preventing TB and HIV co-infection in the study area.

Materials and Methods

Study area and design

The study was conducted at Kogi State Specialist Hospital, Lokoja. Lokoja is a city in Nigeria, situated at the confluence of the Niger and Benue Rivers. Lokoja, the capital of Kogi State is located at latitude 7°45'N-7°51'N and longitude 6°45'E and lies at an altitude of 45 to 125 meters above sea level. Lokoja has a total area of 1, 230 sq mi (3,180 km^2) (Omatola *et al*., 2020b). The Hospital provides diagnostic and treatment services for a substantial number of patients within and beyond the State. This study was a cross-sectional hospital-based study. Three hundred and twenty-five (325) HIV seropositive patients who attended Kogi State Specialist Hospital, Lokoja between July and December 2021 were included in this study. The inclusion criteria included HIV seropositive patients aged 16-65 years who were willing to participate in the study. All the participants provided informed consent prior to the

commencement of sample collection. Ethical approval for the study was obtained from the Research Ethical Committee of Kogi State Specialist Hospital, Lokoja with the authorization number KSSH/RECA/966/VOL. 1/55.

Data collection and laboratory investigations

A structured questionnaire was used to collect information on the socio-demographic profile, clinical treatment of HIV, and a presumptive diagnosis of tuberculosis of the patients. Patients presumptive to TB infection were positive for at least one of these four cardinal symptoms; persistent cough for more than two weeks, sweats during night sleep, fever, and weight loss. Sputum and blood samples were collected from each of the 325 patients. The samples were collected with the assistance of trained hospital personnel. Sera from blood samples were used for HIV assays using Abbot determine and Uni-gold rapid HIV test kits. For all the subjects, sputum microscopy for TB was done. From each participant, a spot sample was obtained on the same day and a morning sample on the next day. Tuberculosis was confirmed if any one of the sputum samples, spot or morning sample, in microscopy was found positive for *M. tuberculosis*. Negative samples from sputum microscopy were further tested using gene Xpert MTB/RIF. HIV patients whose samples were positive for *M. tuberculosis* in Xpert MTB/RIF test were regarded as confirmed TB patients. In accordance with the testing guidelines of the National Tuberculosis Programme, a repeat of gene Xpert MTB/RIF tests was carried out for sputum samples indicating Rifampicin resistance.

Statistical analysis

The statistical analysis was performed using INGRAPH software. All values were expressed in the form of percentages, mean, and standard deviation. The chi-square test was used to assess for a statistical association between qualitative variables and HIV/TB infection. Statistical significance was set at P≤0.05.

Results and Discussion

Age distribution of HIV/TB Patients

A total of 325 confirmed HIV patients attending Kogi State Specialist Hospital, Lokoja participated in the study. Eighty-six (26.5%) of the respondents were males while 239 (73.3%) were females. Of the 325 confirmed HIV seropositive respondents, 6 (1.85%) were positive for TB by GeneXpert analysis, with a female-to-male rate of 5(83%) vs 1(17%). Table 1 shows the age distribution of HIV/TB co-infected patients attending Kogi State Specialist Hospital, Lokoja. The highest prevalence rate was found between the ages 26-35 at 3.92% followed by ages 36-45 years with a prevalence of 1.59 % while ages 16-25, 46-55, 56-65, and above 65 years had no co-infection rate. The respondents ages were distributed evenly, with no discernible differences (P=0.5315).

Table 1: *Age distribution of HIV/TB patients.*

Age	Number of participants	HIV/TB co-infection n (%)	P value	Chi-square
16-25	10	0 (0)	0.5315	4.126
26-35	102	4 (3.92)		
36-45	126	2 (1.59)		
46-55	55	0 (0)		
56-65	31	0 (0)		
>65	1	0 (0)		

Table 2: *Socio-demographic profiles of HIV/TB co-infected patients.*

Variable	No. of Participants	HIV/TB Coinfection n (%)	P	Chi square
Marital status				
Single	28 (8.62)	0 (0)	0.5968	1.884
Married	247 (76)	6 (2.43)		
Divorced	17 (5.23)	0 (0)		
Widow	33 (10.15)	0 (0)		
Type of family				
Monogamous	264 (81.23)	5 (1.89)	0.3551	1.603
Polygamous	61 (18.77)	1 (1.64)		
Occupation				
Student	22 (6.77)	0 (0)	0.9349	0.8263
Civil Servant	112 (34.46)	2 (1.79)		
Trader	139 (42.77)	3 (2.16)		
Farmer	39 (12)	1 (2.56)		
Others	13 (4)	0 (0)		
Educational status				
Primary	4 (1.23)	0 (0)	0.8634	0.7415
Secondary	65 (20)	2 (3.08)		
Tertiary	253 (77.85)	4 (1.58)		
None	3 (0.92)	0 (0)		
Place of residence				
Urban	242 (74.46)	4 (1.65)	0.2912	2.032
Rural	83 (25.54)	2 (2.41)		

Socio-demographic profile and HIV/TB co-infection

An HIV/TB coinfection rate of 2.43% was observed among the married subjects. There was no co-infection among the singles, divorced, and widows. Although, there was no statistically significant difference between marital status and HIV/TB occurrence (P=0.5968). Two hundred and sixty-four (81.23%) of the respondents were from monogamous families while 61 (18.78%) were from a polygamous family. The highest co-infection rate (1.89%) was found among those from monogamous families. There was no significant difference between the rate of co-infection and the various forms of families (P=0.3551). The highest HIV/TB prevalence was found among farmers (2.56%) followed by traders (2.16%) and Civil servants (1.79%). However, the difference was not significant (P=0.9349). Participants with secondary education had a higher co-infection rate (3.08%) than those with tertiary education (1.58%). There was no co-infection among patients with other levels of education (Table 2). A higher number (242) of the respondents live in urban areas. However, a higher co-infection rate was found among those residing in rural areas (Table 2). Though, there was no significant difference between HIV/TB coinfection and places of residence (P=0.2912).

Risk factors associated with HIV/TB co-infection

Of all the 325 respondents undergoing antiretroviral treatment, 6 (1.85%) were co-infected. Co-infection rate was higher (4.69%) among respondents who had been treated for 2-5 years (Table 3) than in other treatment periods. Participants with less commitment to HIV treatment were significantly more infected with TB than the more committed individuals (P=0.0159). Six (6) of the respondents claimed they had been diagnosed with at least one opportunistic infection while 319 had not been diagnosed with the opportunistic infection. The rate of co-occurrence was highest (16.67%) among those who had been diagnosed with the opportunistic infection. There was however no significant difference between those who have suffered an opportunistic infection or not (P=0.4899). A small fraction of the population comprising 3.38% and 7.08% had previous TB infections and served as caregivers to TB patients, respectively. The rate of co-infection was highest (27.27% and 13.04%) in the respective populations. There was no statistical significance among those who had previous TB infection or among those who have served as caregivers to TB patients in time past (P>0.05).

Table 3: *Risk factors associated with HIV/TB co-infection.*

Risk Factors	Number of respondents (n= 325) (%)	umber of co-infected respondents (n=6) (%)	P value
Those on treatment for HIV			
Yes	325 (100)	6 (1.85)	0.5000
No	0 (0)	0 (0)	
Duration of treatment (in years)			
1-2	9 (2.77)	0 (0)	0.2361
2-5	64 (19.69)	3 (4.69)	
5-10	177 (54.46)	3 (1.69)	
>10	75 (23.08)	0 (0)	
Commitment to treatment			
61-80	23 (7.08)	2 (8.70)	0.0159
81-100	302 (92.92)	4 (1.32)	
Improved health status			
Yes	321 (98.77)	6 (1.87)	0.4919
No	4 (1.23)	0 (0)	
Previous opportunistic infection since treatment			
Yes	6 (1.85)	1 (16.67)	0.4899
No	319 (98.15)	5 (1.57)	
Previous TB infection			
Yes	11 (3.38)	3 (27.27)	0.4836
No	314 (96.62)	3 (0.96)	
Lived/stayed with TB patient before			
Yes	23 (7.08)	3 (13.04)	0.4575
No	302 (92.92)	3 (0.99)	

Co-infection with HIV and tuberculosis (TB) continues to be a significant global public health issue. This study was conducted to detect *Mycobacterium tuberculosis* and associated risk factors of transmission in HIV seropositive patients attending Kogi State Specialist Hospital, Lokoja, Kogi State, Nigeria. Out of the 325 sputum specimen examined, six (6) were positive for TB by GeneXpert, given an overall prevalence of 1.85%. The overall prevalence of HIV/TB co-infection from this study is lower when compared to 3.7% recorded among HIV-positive patients attending COCIN Rehabilitation Center in Mangu, Jos, Plateau State Nigeria (Banda *et al.*, 2021), 6.4% in Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State (Okonkwo *et al.*, 2015), 9.6% in a study conducted among patients attending HIV Clinic at Jos University Teaching Hospital, Jos Plateau State (Agbaji *et al.*, 2013), 13.8% (Olaniran *et al.*, 2011) and 14% (Okonkwo *et al.*, 2015) reported from studies conducted at Obafemi Awolowo

University Teaching Hospital Ile-Ife and DOTS the center in Military Hospital, Port Harcourt, Rivers State Nigeria. The prevalence obtained from this study is much lower than the rate reported in a similar study conducted at the University of Benin Teaching Hospital at 33.9% (Effiong and Nwakaego, 2015) and 34.4% was revealed in a study conducted at Dalhatu Araf Specialist Hospital Lafia, Nassarawa State (Gyar *et al*., 2014). The differences in the prevalence may be due to differences in sample size, sampling techniques, selection criteria, and population densities in the study areas and locations. Furthermore, government and international intervention programs over the years, level of awareness and education on TB, and prevalence of HIV/AIDS could also play a significant role in the differences observed in TB prevalence among HIV/AIDS patients in the different studies.

In relation to age, the prevalence of TB in HIV-infected patients was highest (3.92%) in the age group 26-35 followed by 1.59% in the age group 36-45. The prevalence of HIV/TB co-infection from this study is lower than those obtained by other researchers. Enoch *et al.* (2021) reported a prevalence of 33.9% in DOTS in Bayelsa State, Nigeria. In analogous works, the prevalence of HIV/TB co-infection was reported highest in the age group 21-40 at 13.8%, 18.2%, and 46.9% (Zingué *et al*., 2018; Fekadu *et al*., 2015; Bankole *et al*., 2014). Additionally, Gyar *et al.* (2014) reported a prevalence of 34.4% in the age group 31-40 years in Nassarawa State, Okonwo *et al.* (2015) reported a prevalence of 21.2% among the age group <30 years in Enugu State, and Olaniran *et al.* (2011) reported 5.8% among the age group 21-30 years in Ogun State. Despite the differences in the prevalence reported, the fact that the young (26-35 years) had the highest burden of the parameters determined (HIV/TB co-infection). Although the results may be affected by the method of detection, procedures for taking samples, the study's location, and knowledge of precautionary measures for TB in the locality, the level of social activities related to the age category of 26–35 years may potentially lead subjects within the age group to TB and HIV infections.

The prevalence of HIV/TB co-infection by gender from this study male 1 (17%), female 5(83%) reveals that females have a higher co-infection rate compared to their male counterparts. This is coherent with a similar study carried out in Yenegoa Bayelsa State; the prevalence by gender of 58.9% among females and 153(41.1%) among males (Enoch *et al*., 2021). Other findings within Nigeria that are congruent to the findings of this study are those reported by (Agbaji *et al*., 2013; Effiong and Nwakaego, 2015; Okonkwo *et al*., 2015). Research in other African countries also support the findings of this study; female 69% in Amahar region of Ethiopia and males 31.1% (Fekadu *et al*., 2015); female 20.3% and males 10.3% in Burkina Faso (Zingué *et al*., 2018). However, related works in different parts of Nigeria are not in agreement with the higher prevalence reported in this study with respect to gender. The prevalence of HIV/TB co-infection was higher in males 37.3%, 25.6%, and 44.6% as opposed to the female counterparts (26.8%, 24.8%, and 29.6%) in Bayelsa, Lagos, and Akwa-Ibom State respectively (Enoch *et al*., 2021; Nwanta *et al*., 2011; Kooffreh *et al*., 2016). Further, reports of the following authors (Banda *et al*., 2021; Olaniran *et al*., 2011; Okonkwo *et al*., 2015; Musa *et al*., 2015) do not agree with the result of the findings here.

Occupation-specific prevalence of HIV/TB co-infection is highest among the farmers (2.56%). This can be a result of inadequate schooling, poor awareness of TB mitigation strategies, and poor health-seeking behaviour. The findings of this study are consistent with a similar study in Jos where farmers had the highest prevalence of 5.2% (Banda et al., 2021), but differs from other researchers (Okonkwo *et al*., 2015; Olaniran *et al*., 2011; Gyar *et al*., 2014; Agbaji *et al*., 2013 among others). The public health implication of this high prevalence among farmers is that the co-existence of HIV/TB infection will affect the health of the patients and consequently their productivity. This will lead to increased food insecurity, especially because there are very few farmers in the study population thus, the need to control TB in the study area cannot be over-emphasized. Concerning marital status, all patients co-infected for HIV/TB are married, giving a significant difference in the co-infection rates among the married compared to the singles, divorced, and widows. This is contrary to previously documented reports of more HIV/TB co-infection among the single as 32.8% reported by Oladeinde *et al.* (2014) in Edo and 13.6% by Musa *et al.* (2015) in Kano. Although this study recorded a significant difference in the prevalence of TB in HIV-infected patients among the marital classes, Banda *et al.* (2021) did not record any difference in the prevalence of HIV/TB among the married and singles. This study recorded

a slightly higher prevalence among patients from monogamous families (1.89%) when compared to patients from polygamous families (1.64). Fekadu *et al.* (2015) reported a prevalence of 158 (27.7%) of TB, HIV/TB co-infection among HIV-infected people in Amahora, Ethiopia. It was observed that individuals with greater education levels had a lower prevalence of TB, HIV, and HIV/TB coinfection. A similar study by Enoch *et al.* (2021) which recorded a high prevalence among those with primary and non-formal education has been documented. However, this study reveals the highest prevalence in patients who have secondary education as opposed to the aforementioned. Those who have higher education are subjected to more social circumstances at work, school, and in other facets of life. As a result, individuals may be more susceptible to TB infection given that the disease is air-borne and that sexual activity is the primary transmission route of HIV. Lack of health education on the modes of transmission of TB might have predisposed the naive educated patients (Enoch *et al.*, 2021). Furthermore, the prevalence rate was higher in patients who resided in rural settlements (2.41%) when compared to urban dwellers. This could also be attributable to insufficient health campaign programs and education in rural areas and the lack of accessibility to standard and well-equipped healthcare centers in rural areas. The findings from this study are opposed to reporting of Gyar *et al.* (2014) who reported the highest prevalence of 42.7% among Urban dwellers.

All patients who participated in the study were on antiretroviral therapy (ART) and each claimed to be committed to therapy at a minimum rate range of 61-80% since their therapy began. However, co-infection was found among the subset of patients who claimed a 61-80% commitment rate when compared to 4(1.32) patients who claimed an 81-100% commitment rate. This result may be suggestive that the drug regimen used for the treatment of patients may not be appropriate to their current clinical disease state. Also, a less accurate viral load analysis with subsequent effect on the follow-up and treatment may be the gap for the patients as shown and demonstrated in China (Cui *et al.*, 2017).

The study also revealed co-infection among patients who had been on antiretroviral therapy for between 2 to 5 years. Patients on therapy for less than 2 years and above 10 years had no record of HIV/TB co-infection. This is congruent with the study that reported a reduction of the tendency to acquire TB disease in the first year (1-2 years) of HAART (VanRie *et al.*, 2011). However, it is not in agreement with the study in Burkina Faso which reported a 0.18 per 100 py TB incidence rate after the first year of ART (Dembele *et al.*, 2010).

The observed rate of 4.69 in years 2-5 of HIV therapy is twice as high as the 1.6 to 2.2 per 100 py observed in years 2-4 (Johannesburg) of ART (VanRie *et al.*, 2011) but comparable to the 3.2 to 4.5 per 100 prevalence rates in years 2-4 (Cape Town) of ART in South Africa (Lawn et al., 2011). A very small percentage of 1.23 of the respondents claimed antiretroviral therapy for HIV did not improve their health status. HIV/TB co-infection was not found in the said population which could be indicative that other factors (other than a compromised immune state) such as smoking, poor nutrition, and previous contact with TB-infected patients among others may be responsible for the co-infection 6(1.87) found among those who claimed improvement of health status following ART therapy.

History of previous opportunistic infection appears to be a very important risk factor for acquiring TB disease among HIV patients. The chances of HIV/TB co-infection are significantly affected by the history of opportunistic infection. A prevalence of 16.67% was observed among patients who had been previously affected by any opportunistic infection. Results from this study are supported by the studies of the following researchers (Kibret *et al.*, 2013; Temesgen *et al.*, 2019; Abdu *et al.*, 2021). Social behaviour, socioeconomic factors, education and awareness of TB preventive strategies, immunological state of the patient, and health-seeking behaviour are some plausible factors affecting the results in either way. The prevalence of HIV/TB co-infection with relation to previous TB infection is highest (27.27) among HIV patients who had been previously exposed to TB infection. This agrees with the work of Adejumo *et al.* (2017) in Lagos and Payam *et al.* (2007) in San Fransisco. Possibly, TB treatment and relapse as well as treatment of recurrent TB may explain the high TB prevalence rates among these patients.

The history of living in the same house with a family member or friend with active TB disease was associated with latent TB infection (Haley *et al.*, 2008; Koppaka *et al.*, 2003). According to prior research, 24% of West African TB patients had a family

history of the disease, compared to 10% of controls (Lienhardt *et al*., 2005) and our result that household exposure to a known TB case being a significant risk factor for TB disease is consistent with that finding. Moreover, in comparison to 11% of controls, 45% of patients reported household contact with a known TB case (Hill *et al*., 2006). This discovery has major implications for public health. Increasing household size was discovered in prior research (Hill *et al*., 2006) and TB risk factors have been linked to overcrowding in several other studies conducted in a range of contexts (Mangtani *et al*., 1995; Coker *et al*., 2006). Thus, as people share the same air space and are in close proximity to one another, living in the same home as TB patients is a known risk factor. Hence, household exposure by way of caregiving, household size, and overcrowding, lack of or poor awareness is possible factors responsible for the resulting outcome.

Conclusions and Recommendations

This study establishes the prevalence of HIV/TB co-infection (1.86%) in the study group (HIV seropositive patients attending Kogi State Specialist Hospital, Lokoja, Kogi State Nigeria). There is a low prevalence of HIV/TB co-infection. However, the usage of Antiretroviral drugs is an important parameter in the prevalence observed in the study population. This identifies a dual endemic problem of concern in Lokoja and its environs and calls for stringent public health measures to eradicate tuberculosis through therapeutic and prophylactic campaigns as Human Immunodeficiency Virus associated Tuberculosis remains a major public health problem in Nigeria. Thus, it is imperative to curb the spread of TB in other to avoid further morbidity, co-morbidity, and mortality due to TB infection. Timely case detection of Tuberculosis by making more available GeneXpert molecular machines in health facilities both in the rural and urban areas is recommended. Secondly, HIV patients should be routinely screened for TB even without the presentation of TB presumptive signs. Also, to successfully eradicate TB from the population, intervention campaigns that focus on education and awareness of TB transmission and prevention should be regularly organized by the government, its agencies, health personnel, and international bodies burdened with health matters. Also, we urge increased TB screening, treatment, and prevention as a means of diminishing the number of TB in HIV-positive individuals as well as enhanced antiviral therapy coverage and monitoring of HIV patients.

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Novelty Statement

The present study provided evidence of TB/HIV co-infection in a population endemic for HIV/AIDS. Our findings indicating greater predisposition of farming occupation to TB among people living with HIV/AIDS, especially in the economically active age group, may be raising concern to food insecurity in the area.

Author's Contributions

MOO and MEA designed the study and obtained ethical approval. MEA collected samples. MOO, MEA, ZPO, JI, BMI, and UEO ran an assay. MEA, CAO, MOO, MAD and JAO analyzed the data. CAO, AI, and MEA wrote the draft manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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