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Original Research Article

Prevalence of tuberculosis/human immunodeficiency virus co-infection and diagnostic accuracy of some biomarkers used in the diagnosis of pulmonary tuberculosis

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Abstract

Purpose: *To determine the prevalence of tuberculosis (TB)/human immunodeficiency virus (HIV) co-infection among people living with HIV and to evaluate the diagnostic accuracy of some biomarkers used in the diagnosis of pulmonary TB.*

Methods*: The study was a cross-sectional study comprising 290 HIV-positive subjects. Sputum samples collected were analyzed using microscopy and GeneXpert for the detection of TB and antibiotic resistance. Blood samples were collected for adenosine deaminase (ADA) and lactate dehydrogenase (LDH) assays using standard laboratory procedures.*

Results*: The prevalence of TB/HIV co-infection in this study was 6.6 % using GeneXpert and 4.8 % using acid-fast bacilli (AFB) smear microscopy. All cases of TB/HIV co-infection were sensitive (100 %) to rifampicin. Furthermore, of the 19 cases of TB/HIV co-infected subjects, 47.4 % were females, while 52.6 % were males. ADA, LDH, and LDH/ADA ratio were significantly higher ($p < 0.001$) in subjects co-infected with TB/HIV compared with HIV-positive subjects only. The sensitivity and specificity of ADA were 85% and 58 %, LDH was 67 % and 74 %, while LDH/ADA ratio was 66 % and 89 %, respectively.*

Conclusion: *The prevalence of TB/HIV co-infection is still relatively high and constitutes a major public health concern, considering the devastating effect on the community.*

Keywords: *Biomarkers, Co-infection, GeneXpert, HIV, Tuberculosis*

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INTRODUCTION

Mycobacterium tuberculosis is the cause of tuberculosis (TB), a chronic, infectious, and highly contagious disease that is challenging to treat. The two most common causes of infectious disease-related deaths globally are tuberculosis (TB) and human immunodeficiency virus (HIV), which frequently coexist [1]. The burden of HIV/TB co-infection varies significantly between nations and regions; nevertheless, sub-Saharan Africa has long been known to have the highest burden of co-morbidity, where the co-infection still has a devastating impact on the human population [2]. The frequency of HIV/TB co-infection varies greatly; among TB patients, the prevalence of HIV ranged from 3.8 to 72.3 % [2], while among HIV-positive patients, the prevalence of TB varied from 2.9 to 64.5 %. In a systematic review and Meta-analysis done in

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Nigeria, the pooled prevalence of TB/HIV coinfection was 25.8 %. The highest coinfection prevalence of 34.3 % was recorded among the North Central States of Nigeria, while the lowest prevalence of 19.3 % was recorded among the Southeastern states of Nigeria [3].

The World Health Organization (WHO) reports that TB continues to be the primary cause of mortality and morbidity among individuals living with HIV, resulting in 1.2 million TB-related deaths among seronegative individuals and 208,000 fatalities among HIV-positive patients. This is true despite advancements in prevention, diagnosis, and treatment options. According to estimates from the World Health Organization, 8.2 % of the 10.0 million (range, 9.0–11.1 million) cases of tuberculosis were HIV-positive [4]. Furthermore, tuberculosis remains the leading cause of death among people living with HIV, accounting for approximately 30 % of AIDS-related deaths [4]. The main risk factor for tuberculosis infection developing into tuberculosis disease is HIV. Thus, in people living with HIV, TB must be prevented, detected, and treated early.

While several countries have made significant strides toward mitigating the TB/HIV co-morbidity, global standards for testing for TB in HIV patients and providing antiretroviral medication to HIV-positive individuals have not been met [5]. The risk of developing active tuberculosis and the death rate related to TB are both significantly increased by HIV infection. As a result, TB accelerates the course of HIV infection and raises the chance of contracting additional opportunistic infections. Moreover, TB is the primary cause of death for those who are HIV-positive. The TB/HIV epidemic has therefore grown more severe due to the impact of one infection on the natural history and pathophysiology of the other. Hence, the need to determine the prevalence of HIV/TB co-infection and associated biomarkers used in their diagnosis justified this study. The study aimed to determine the prevalence of HIV/TB co-infection among people living with HIV attending the directly observed treatment, short-course (DOTS) Clinic in a tertiary institution in Lagos State, and to evaluate the diagnostic accuracy of some biomarkers used in the diagnosis of pulmonary TB.

METHODS

Study design

This study was a cross-sectional study performed on specimens from human subjects. It was conducted from November 2023 to February 2025, at the DOTS Clinic, Lagos University Teaching Hospital, Lagos State. Sputum samples from HIV-infected individuals were collected and analyzed using the Ziehl-Neelsen staining technique to detect the presence of acid-fast bacilli (AFB) and GeneXpert for the detection of TB and antibiotic resistance, while blood samples were collected for adenosine deaminase (ADA) and lactate dehydrogenase (LDH) assays. Sample size was calculated using Eq 1 as shown below.

$N = Z^2pq/d^2$ …….. (1)

Where; N= the desired sample size; z = is a constant given as 1.96; p = expected prevalence (25.8 %; [3]); q = 1 – p; d = acceptable error (5%)

Two hundred and ninety (290) samples were collected and used for this study. The subjects were HIV-positive subjects attending the DOTS clinic at Lagos University Teaching Hospital. Ethical approval for the study was obtained from the Health Research and Ethics Committee of Afe Babalola University, Ado-Ekiti, and Lagos University Teaching Hospital (LUTH), Lagos State. Information on age, sex, clinical diagnosis, relevant medical history, and other variables pertinent to the study was obtained from participant interviews and clinical records. Additional clinical information, including duration of symptoms, treatment status, previous tuberculosis treatment, HIV status, antiretroviral therapy, or other variables, was documented where available. Eligibility was assessed before enrollment by reviewing participants' clinical information and applying the prespecified inclusion and exclusion criteria. Written informed consent was obtained from participants aged 18 years and above before enrollment. For participants aged 15 – 17 years, written assent was obtained from the adolescents, together with written permission from their parent or legally authorized guardian. The study was explained in language appropriate to the participants' level of understanding, and participation was voluntary. Participants and their parents or guardians, as applicable, were informed of the study procedures, potential risks, anticipated benefits, confidentiality protections, and their right to decline or withdraw without affecting their clinical care.

Inclusion and exclusion criteria

Male and female HIV-infected individuals aged 15 – 65 years who visited the DOTS clinic at Lagos University Teaching Hospital and gave their consent were included. Individuals with

other underlying health conditions outside HIV, those who did not meet the inclusion criteria and those who did not give their consent were excluded.

Sample collection

Sputum sample

A suitable specimen container (a clean, wide-mouthed, leak-proof container) was given to each participant. Participants were led to an outside sample collection booth where they were asked to take a deep breath, exhale two or three times, and then cough deeply from the chest. Sputum was collected using an open jar that was positioned next to the mouth. Following collection, the lid was firmly fastened on. A labeled container was given so that a second sample could be collected the same day, one or two hours after the first sample was obtained. However, in cases where it was not feasible to collect the second sample the same day, it was collected early the following day and brought to the clinic. A smear was made from the first sample before it was used for the GeneXpert test and a second smear was made from the second sample.

Blood sample

Five milliliters (5 mL) of whole blood was equally collected from each subject into a plain tube and allowed to clot. It was then centrifuged at 3000 rpm for five minutes and the serum was aliquoted and stored at -20 °C until analysis.

Analytical methods

HIV Screening

HIV rapid screening was done using the Determine™ rapid diagnostic kit.

Identification of Acid-Fast Bacilli (AFB)

Identification of acid-fast bacilli (AFB) was done using the GeneXpert technique and the Ziehl-Neelsen staining technique. A smear was prepared directly from the patient specimen prior to processing and all specimens were processed in a microbiology biosafety cabinet under containment level 3 conditions [6].

Lactate dehydrogenase (LDH) and adenosine deaminase (ADA)

These were determined spectrophotometrically. The principle of LDH estimation is based on the oxidation of lactate by NAD+ to produce pyruvate and NADH, catalyzed by LDH. The rate of rise of NADH yields the catalytic concentration measured spectrophotometrically at 340 nm. On the other hand, the principle of ADA estimation is based on the enzymatic deamination of adenosine to inosine and ammonium, catalyzed by ADA. Using the glutamate dehydrogenase (GLDH) linked reaction, the rate of decrease of NADH at 340 nm is used to calculate the catalytic concentration.

Statistical analysis

Statistical analysis was done using SPSS version 25. The results of this study were presented in tables and charts. Data were presented as frequency, simple percentage, mean and standard deviation. Comparison was made using chi-square and Student's t-test and values of $p < 0.05$ were considered significant. Receiver operating characteristic (ROC) curve analysis was performed to assess the discriminatory ability of the selected biomarkers. The optimal cut-off values were determined using the Youden index (sensitivity + specificity − 1), with corresponding area under the curve (AUC), 95% confidence intervals, sensitivity, and specificity calculated.

RESULTS

Table 1 shows the demographic characteristics of the subjects studied. Two hundred and ninety (290) HIV-positive subjects were recruited for this study, comprising 41.38 % males and 58.62 % females. Age group classification showed that 13.10 % belonged to age group 15 – 30 years, 38.28 % belonged to age group 31 – 40 years, 28.62 % belonged to age group 41–50 years, while 20.0 % belonged to age group 51 years and above. With respect to macro examination, 27.24 % were salivary, 11.03 % were mucopurulent, 43.10 % were mucosalivary, 13.79 % were mucoid, while 4.83 % were purulent. Table 2 shows the frequency distribution of HIV/TB co-infection based on method of diagnosis and rifampicin sensitivity. The results obtained showed that a total of 19 (6.6%) of TB cases were detected. AFB detected 14 of the 19 GeneXpert-positive cases (73.7 %). There was no significant difference ($p > 0.05$) between the two methods of TB detection in HIV subjects. All 14 AFB-positive samples were also GeneXpert-positive. GeneXpert identified five additional positive samples among those that were AFB-negative. Thus, GeneXpert had a higher observed positivity rate than AFB smear microscopy. No AFB-positive/GeneXpert-negative results occurred. With respect to sensitivity to rifampicin, all cases of HIV/TB co-

infection were sensitive (100 %) to rifampicin in HIV/TB co-infected subjects using the GeneXpert technique.

The results obtained showed that out of the 290 HIV-positive subjects examined, 6.6 % were co-infected with TB, while 93.4 % did not have TB. Furthermore, of the 19 cases of HIV/TB co-infected subjects, 47.4 % were females, while 52.6 % were males. Similarly, age-specific prevalence was 7.9 % (3/38), 5.4 % (6/111), 7.2 % (6/83), and 6.9 % (4/58) for age groups 15 – 30, 31 – 40, 41 – 50, and > 51 years, respectively. Age group 15–30 years has the highest rate and the groups do not differ significantly (chi-square p = 0.94) (Figure 1). Table 3 shows the mean values of lactate dehydrogenase (LDH) and adenosine deaminase (ADA) in subjects co-infected with HIV/TB compared with HIV alone. The mean values of LDH (U/L) in subjects co-infected with HIV/TB and HIV-positive subjects were 484.89 ± 27.39 and 411.63 ± 32.23; ADA (U/L) was 43.70 ± 1.71 and 41.02 ± 2.11, while LDH/ADA ratio was 11.09 ± 1.69 and 9.57 ± 1.27, respectively. LDH and LDH/ADA ratio were significantly higher (p < 0.05) in subjects co-infected with HIV/TB compared with HIV-positive subjects. As shown in Table 4 and Figure 2, the ROC curve analysis identified the optimal cut-off values for ADA, LDH, and LDH/ADA ratio as follows: > 56 U/L (area under the curve [AUC] = 0.682; p = 0.003), > 550 U/L (AUC = 0.766; p = 0.001), and > 8.5 (AUC = 0.834; p = < 0.001). The sensitivity and specificity of ADA were 85 % and 58 %; LDH was 67 % and 74 %, while LDH/ADA ratio was 66 % and 89 %, respectively.

Table 1: Demographic characteristics of the subjects

Variable	Frequency	Percentage (%)
Gender		
Male	120	41.38
Female	170	58.62
Age (years)		
15 – 30	38	13.10
31 – 40	111	38.28
41 – 50	83	28.62
51 years and above	58	20.00
Macro examination	79	27.24
Salivary	32	11.03
Mucopurulent	125	43.10
Mucosalivary	40	13.79
Mucoid Purulent	14	4.84

Table 2: Frequency distribution of HIV/TB co-infection based on method of diagnosis and rifampicin sensitivity

Item	AFB	GeneXpert	*P*-value
HIV/TB co-infection	14 (4.8%)	19 (6.6%)	0.470
Rifampicin			
Sensitive	-	19 (100%)	-
Resistant	-	0%	-

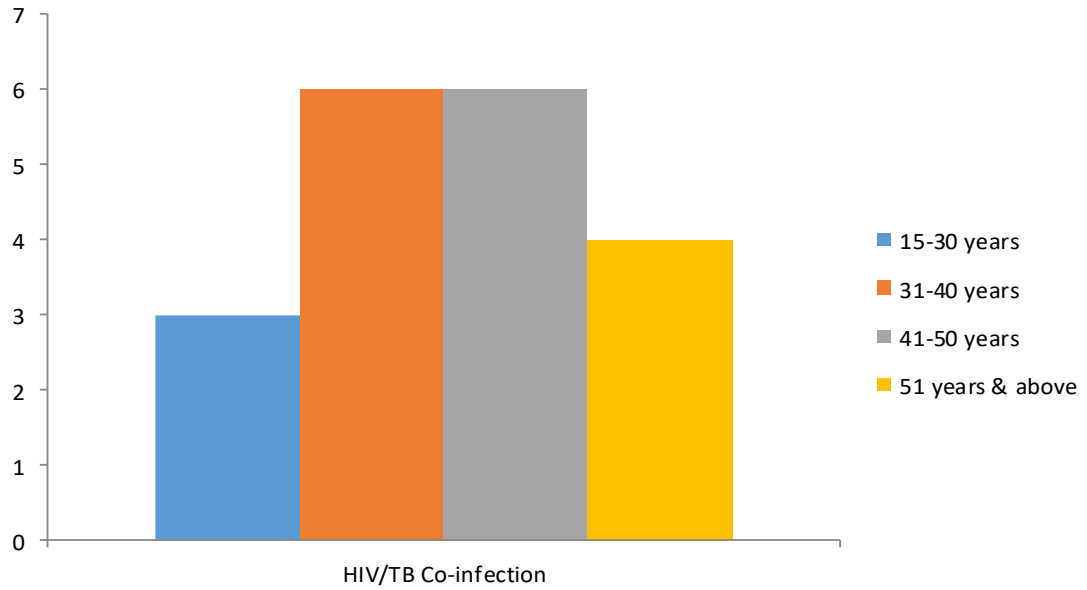

Figure 1: Frequency distribution of TB/HIV co-infection with respect to age. *P < 0.05

Table 3: Lactate dehydrogenase and adenosine deaminase in TB/HIV co-infection compared with HIV alone

Variable	HIV (Mean ± SD) (N = 271)	TB/HIV (Mean ± SD) (N = 19)	t-value	*P*-value
LDH (U/L)	411.63±32.23	484.89±27.39	11.131	<0.001
ADA (U/L)	41.02±2.11	43.70±1.71	6.494	<0.001
LDH/ADA ratio	9.57±1.27	11.09±1.69	3.845	<0.001

Keys: LDH – Lactate dehydrogenase; ADA – Adenosine deaminase

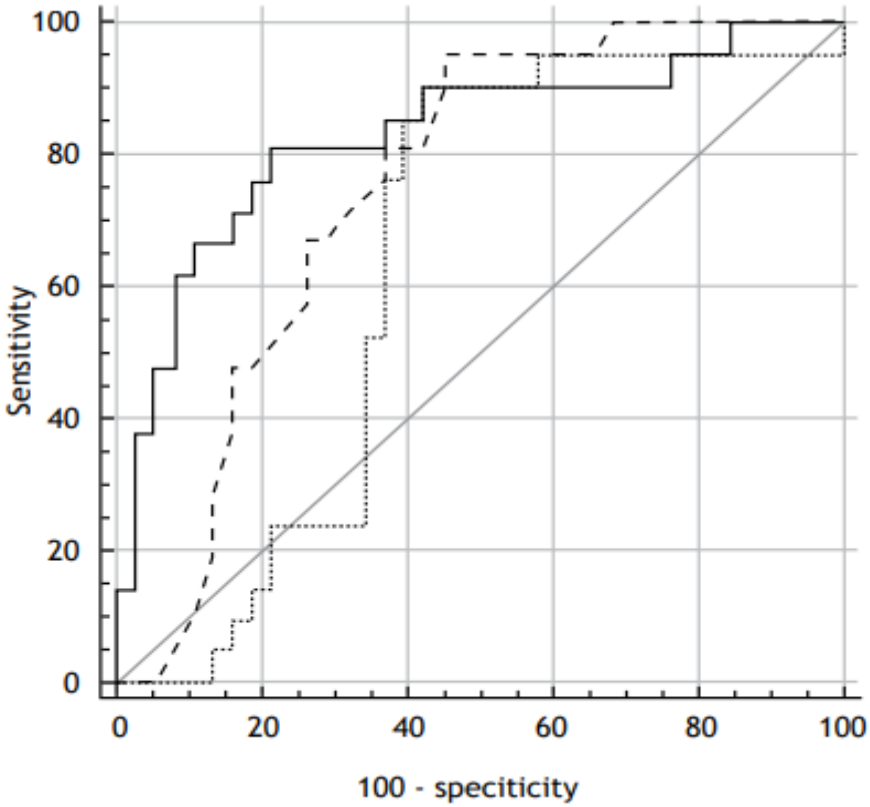

Figure 2: ROC curve analysis of the determination of the adenosine deaminase level (dotted line), lactate dehydrogenase (dashed line), and LDH/ADA ratio (solid line)

Table 4: Characteristics of the tests and their best cutoffs

Parameter	Cutoff	AUC	p-value	Sensitivity (%)	Specificity (%)
ADA (U/L)	> 56	0.682	0.003	85	58
LDH (U/L)	> 550	0.766	0.001	67	74
LDH/ADA ratio	> 8.5	0.834	<0.001	66	89

Keys: LDH – Lactate dehydrogenase; ADA – Adenosine deaminase

DISCUSSION

The prevalence of HIV/TB co-infection in this study was relatively high at 6.6 % which is lower than the 10.7 % reported in another part of Nigeria [7]. Patients visiting an infectious disease hospital in Kano, Northwest Nigeria, had a reported 14.7% prevalence of pulmonary TB [6]. According to studies conducted in Southeast Nigeria [8], Calabar [9], and Bayelsa [10], the prevalence of HIV/TB co-infection was 17.2 %, 24.8 %, and 32 %, respectively. Nwadioha *et al* [11] observed a 21.5 % prevalence of TB among patients in Makurdi, whereas Anígilájé *et al* [12] reported a 19.8 % prevalence of TB among HIV-positive patients in Benue State. Improved lifestyle, increased community education, increased TB case detection and treatment, isoniazid preventive therapy (IPT), TB infection control, and early antiretroviral therapy (ART) initiation [7] are some of the possible explanations for the low prevalence of TB/HIV co-infection in this study. However, the disparities in sample sizes, monitoring systems,

and cooperation on TB/HIV control programs among nations and regions may be the reason for the disparity in prevalence in the various studies.

This study confirmed findings from other studies [5] that GeneXpert has high sensitivity and specificity for detecting TB infection in clinical specimens. The prevalence of TB in individuals with HIV was 4.8 % by smear microscopy and 6.6 % by GeneXpert MTB/RIF. With GeneXpert, tuberculosis is diagnosed early enough to allow for proper treatment and to stop the spread of the disease. In addition, GeneXpert detects tuberculosis cases more quickly, accurately, and precisely than other methods. The diagnostic accuracy of the GeneXpert assay in detecting TB infection was supported by a study among HIV/TB co-infection and rifampicin-resistant tuberculosis (RR-TB) conducted in South Africa with a prevalence of 17.3 %, although the difference between positive TB cases by AFB smear microscopy and GeneXpert was not statistically significant [13]. In a similar study, Habte *et al* [14] reported that GeneXpert assisted in household contact tracing and that the GeneXpert MTB/RIF detection rate of cases of TB was 35.9 % as opposed to 12.8 % by smear microscopy [14].

The findings of this study indicated that while co-infection was more common in men than in women, there was no significant difference in the frequency distribution of HIV/TB co-infection by gender. The report that women are less likely than men to be co-infected with HIV and develop TB is in line with other findings that men are more likely to develop TB during antiretroviral therapy (ART) and at all immunological levels [8]. This could be a result of inherent genetic heterogeneity in responsiveness to ART or gender differences in attitudes toward ART adherence. According to gender, the prevalence of TB/HIV was 41.1 % and 58.9 % among male and female participants in a comparable study conducted in Yenagoa, Bayelsa State [10]. Male patients had a co-infection rate of 25.6 % and female patients had a co-infection rate of 24.8 % in a prior study conducted in Lagos, Nigeria [11]. Kooffreh *et al* [9] reported a TB prevalence of 35.6 % in males and 28.6 % in females in Ikot-Ekpene, Cross River State. Zingué *et al* [15] found that among male and female individuals in Burkina Faso, the prevalence was 10.3 % and 20.3 %, respectively.

In this study, age group 15 to 30 years had a higher HIV/TB prevalence than the other age groups. This outcome was in line with the report of other studies conducted in Nigeria [8]. The high rate of HIV/TB co-infection in this age group may be caused by the high number of HIV cases in this age group, which is in line with findings from a previous study [9]. Furthermore, a significant prevalence of HIV/TB co-infection was found by Enoch *et al* [10] among sexually active, fertile, and birthing individuals between the ages of 34 and 44. Although there is no age limit on contracting TB, Gberindyer *et al* [16] noted that younger individuals tend to be more gregarious and hence more likely to come into contact with someone who has TB. Older people may take ART drugs more consistently, leading to quicker immunological stabilization. However, elderly individuals may have an early aging of the immune system [16].

This study found that 0 % of HIV/TB co-infected patients were resistant to rifampicin. This is in contrast to a previous study in Southeast Nigeria that found 5.5 % of patients were resistant to rifampicin [8]. A previous study in Nigeria reported 2.18 % of patients were resistant to rifampicin among HIV/TB coinfected patients [5]. Some African countries also reported higher rates of rifampicin resistance [15]. The low prevalence of rifampicin resistance found in this study when compared to other studies conducted in Nigeria and other African nations could be the result of variations in sample size, study location, and other factors not taken into account. Furthermore, Mulu *et al* [17] clarified that variations in the likelihood of HIV acquisition, exposure to anti-TB medications, and national TB control programs could account for variances in the rate of rifampicin resistance among various authors.

The study reported that there was no significant difference ($p > 0.05$) in the mean ADA values between co-infected HIV/TB subjects and HIV subjects, but the ADA levels were greater than 40 U/L in both groups. Similarly, in most studies, an ADA level ≥ 40 U/L in a lymphocytic exudate obtained via thoracentesis has been the most widely accepted indicator for a diagnosis of tuberculous pleural effusion [18]. Due to the lack of additional cost and intervention, the use of this criterion in the differential diagnosis may be satisfactory. Furthermore, the employment of ADA as a biomarker in clinical diagnosis is also facilitated by its high sensitivity and specificity. This finding is consistent with previous research [18–19] that found ADA levels higher than 40 U/L in individuals with co-infections of HIV and TB. On the other hand, patients with co-infection of HIV and TB had considerably higher LDH and LDH/ADA ratios ($p < 0.05$) than patients with HIV alone. This difference may be attributed to the distinct clinical characteristics of the two illnesses

[18]. In patients with co-infection with both HIV and TB, an increase in LDH levels typically signifies endothelial damage and pulmonary or pleural tissue injury. This result is in line with other research that found that patients with HIV/TB co-infection had significantly higher levels of ADA, LDH, and the LDH/ADA ratio [19].

Researchers and clinicians have underscored the necessity of supplementary examinations in the identification of tuberculosis cases, particularly in patients for whom the disease may present with diagnostic challenges. The World Health Organization recommends target sensitivity and specificity values for screening tests of 90 % and 70 %, respectively, and minimum specificity of 98 % for novel diagnostic tests for tuberculosis in patients [19]. In this study, the best results were obtained in terms of sensitivity by LDH and LDH/ADA ratio, and the best results were obtained in terms of specificity by LDH/ADA ratio. With the exception of the LDH/ADA ratio, both biomarkers demonstrated excellent overall sensitivity and somewhat low specificity, which supported their utility in the diagnosis of pulmonary tuberculosis (PT). This result is consistent with other research that found that the diagnostic biomarkers for pulmonary tuberculosis, ADA, LDH, and the LDH/ADA ratio, have good sensitivity and specificity [20].

This study had some limitations. First, a small number of TB-positive participants were recorded among the HIV-positive subjects, which may affect the precision of the diagnostic accuracy estimates and proposed cut-off values. Additionally, the single-center, cross-sectional design may limit the generalizability of the findings. Larger, multicenter studies are recommended to validate the diagnostic performance and clinical utility of the evaluated biomarkers.

CONCLUSION

The study demonstrated that the prevalence of HIV/TB co-infection remains relatively high and continues to pose a significant public health challenge due to its associated morbidity, mortality, and potential for community transmission. The findings further revealed the superiority of GeneXpert as a diagnostic tool for early and accurate TB diagnosis in immunocompromised patients. The findings of this study also suggest that LDH and LDH/ADA ratio may serve as valuable adjunct biomarkers in the diagnosis and monitoring of tuberculosis among HIV-infected patients. Overall, the study underscores the importance of improved diagnostic strategies, early detection, and integrated HIV/TB control measures in reducing the burden of co-infection and improving patient outcomes.

DECLARATIONS

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None.

Conflict of interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. NJO, BOO and AHE conceived and designed the study. NJO, VOA, ETO, and OOE collected and analyzed the data. EAO wrote the manuscript. BOO and AHE supervised the entire research. All authors read and approved the manuscript before submitting for publication.

Ethical Approval

It was obtained from the Health Research and Ethics Committee of Afe Babalola University, Ado-Ekiti (ABUADHREC/12/02/2024/258), and Lagos University Teaching Hospital (LUTH), Lagos State (ADM/DSCST/HREC/APP/6705).

Availability of data and materials

The data used and/or analyzed during the current study are available from the corresponding author.

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