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Detection and Diagnostic Accuracy of Rapid Urine Lipoarabinomannan Lateral-Flow Assay in Pulmonary Tuberculosis patients in Nigeria

Aneio-Okopi A. Joseph[1,2,*], Okojokwu O. Julius[1], Adetunji Joshua[3], Onoja Annaejane[1], Aminu Folashade[1], Ikwulono Gabriel[4], Ebonyi O. Augustine[2,5], Ejeliogu Emeka[2,6], Audu Onyemocho[7]

[1]Department of Microbiology, University of Jos, Nigeria
[2]AIDS Prevention Initiative in Nigeria (APIN), Jos University Teaching Hospital, Nigeria
[3]Medicom Laboratories of Jos, Nigeria
[4]National AIDS and STIs Control Program (NASCP) , Federal Ministry of Health, Nigeria
[5]Department of Medicine, University of Jos, Nigeria
[6]Department of Paediatrics, University of Jos, Nigeria
[7]Department of Epidemiology and Community Health, College of Health Sciences, Benue State University, Nigeria
*Corresponding author: josephokopi@yahoo.com

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Abstract Background: Tuberculosis (TB) is a public health challenge in both developed and developing countries. Early diagnosis is essential in preventing the further spread of the disease, but the control programs are currently facing a number of constraints and fewer than 25% of all tuberculosis cases especially childhood cases are detected. We aimed to evaluate diagnostic accuracy of a commercially available qualitative immunoassay for the detection of lipoarabinomannan (LAM) antigen of Mycobacteria in human urine by comparing its sensitivity and specificity in TB patients with the AFB and GeneXpert in individuals with presumptive tuberculosis cases. Methods: A cross-sectional study that consecutively enrolled 53 eligible TB adults' patients attending TB Centre, Mangu, Plateau State from February to March 2017. We applied the LAM test on urine collected as a spot and early morning sample. Diagnostic accuracy was analyzed for a microbiological TB reference standard based on Gene Xpert MTB/RIF results and for a composite reference standard including clinical data. Performance of sputum smear microscopy (AFB) was included for comparison. Results: The mean age of the respondents was 41.0±17.0 years.) The male proportion was 36(68.0%) and female was 17(32.0%). The patients with HIV-1 Co-infection were 9(23.8%). Of the 53 patients, the positive testing rate of TB using LAM test was 11 (20.8 %). The proportion of those who tested positive using Gene Xpert was 9(17.0%) and AFB was 33(62.2%), and the sensitivity and specificity were 33.3% and 93.2%, respectively. Negative and positive predictive values were 87.23% and 50.0%, diagnostic accuracy was 83.02%. Conclusion: The study showed great sensitivity of urine LAM test suggesting it could be useful as point of care diagnostic test for presumptive TB cases. Its high negative predictive value suggests a role in screening out uninfected patients; though GeneXpert had superior sensitivity, but the ease of the LAM test holds operational advantage as a screening method, however larger studies are needed to further determine diagnostic accuracy.

Keywords: *Tuberculosis, Lipoarabinomannan point-of-care test, Nigeria*

Introduction

Tuberculosis (TB) poses a serious global public health challenge primarily in developing countries affected by the HIV epidemic. Tuberculosis control programme requires an efficient, early and accurate diagnosis before initiation of therapy [1,2]. The major surface antigen of causative organism (mycobacterium spp) is lipoarabinomannan (LAM), a glycolipid component of the cell wall of mycobacterium and may account for about 15% of the bacterial weight [3]. LAM consists of a mannan polysaccharide backbone with branched oligoarabinosyl containing saccharide side chains; the former is covalently linked to a phosphatidyl inositol lipid moiety. During TB infection LAM in a soluble form is released from metabolically active and degrading bacterial cells, appearing in serum and cleared through the kidneys in urine [3,4].

At present, the diagnosis of an active mycobacterial infection in limited resource countries relies majorly on clinical examination, chest x-ray, and identification of acid-fast bacilli (AFB) in an unprocessed sputum by microscopy. The sensitivity of sputum microscopy to determine active pulmonary TB is somewhat low since about 11,000 bacilli per ml sputum are needed for reliable result. Thus, smear-negative pulmonary TB is a common problem, especially in HIV infected individuals. Various studies classified 24% to 61% of HIV positive tuberculosis patients as smear-negative pulmonary TB [5,6]. This made undiagnosed TB a highly prevalent condition among TB/HIV co-infection patients. In the era of ART, the Mycobacterial culture, which is regarded as the diagnostic gold standard, however, it is time-consuming and unaffordable by many TB endemic countries. However, in resource constrained settings, culturing is still not widespread, because it requires expensive equipment and technical expertise. Consequently, there is an urgent need for rapid, field adapted, inexpensive and accurate tuberculosis diagnostic tools. The Alere urine Determine1 TB LAM Ag rapid test requires only 60 mL of unprocessed urine, and requires little technical skill or no power source, and provides result within 25 minutes at a cost of less than US$3.50 (Alere Determine Insert).

In the last decades, the detection of mycobacterial antigens to diagnose tuberculosis has been subject of various research activities [7]. The fully automated GeneXpert® MTB/RIF (Xpert) assay can provide diagnosis within 2 hours, but wide implementation of this assay is impeded by high costs and requirements for electricity and maintenance. It also separates testing from the clinical interface which may undermine potential impact same-day decision making regarding initiation of TB treatment. This may be particularly problematic in resource-limited settings with a high rate of chronically TB co-infected with HIV patients in whom delays in diagnosis may be associated with increased risk of morbidity and mortality [8].

For the diagnosis of tuberculosis, special attention has been drawn to lipoarabinomannan (LAM), a mycobacterium-specific antigen (lipopolysaccharide) component of the bacilli's cell wall. Mycobacterial antigen in active is released into the blood, passes to renal barrier with little or no major changes. LAM is suspected to be an important virulence factor and therefore a potential drug target and various tests have been developed to detect LAM in serum but none of these test is widely used [9]. The most recent and promising approach was the detection of LAM in urine since the

sample can be easily obtained and its collection is often more accepted than sputum collection or blood samples. The rapid urine LAM assay using unprocessed urine is time saving with an advantage of time-consuming approach requiring concentration and purification of the urine by ELISA [10]. The present study evaluated the commercially available Alere Determine rapid LAM strip to urine from presumptive TB patients to determine its diagnostic value and accuracy with conventional AFB and molecular current gold standard technique GeneXpert MTB-RIF assay.

Materials and methods

A cross-sectional study was conducted from February to March 2017 at TB treatment Centre, Mangu Plateau Sate, Nigeria. Fifty three presumptive TB cases and HIV infected patients were consecutively enrolled and their urine and sputum samples obtained for Urine LAM Alere Determine test (Alere Determine TB-LAM, Waltham, MA, USA) [11], and sputum for the two-reagent cold staining of acid-fast bacilli (AFB) and Gene Xpert method. The collected urine specimens were kept unprocessed at 2-8°C and testing was carried out on the same day. After a urine specimen of 60μL was added to the sample pad, the colloidal gold conjugated antibodies attach to the LAM antigen are released by the specimen from the conjugate pad. This immunological complex is then captured by anti LAM antibodies immobilized on the nitrocellulose membrane and made visible after 25 minutes under standard indoor lighting due to the presence of the colloidal gold label. A positive result (a visible purple/gray line) indicates that LAM antigen of Mycobacteria is present in the sample at or above the detection limit of the test; whereas a negative result (no visible purple/gray line) indicates not present or below detection limit. The test result was read between 25–35 minutes later and graded by comparing the test strip with a reference card. We used the original 2012 reference scale card that consisted of 5 color intensity grades. The test band was graded as zero if no visual band appeared and graded 1 through 5 for a visualized band of equal intensity as those on the reference card. If a faint band was observed with intensity lower than the grade 1 cut-point it was recorded as "faint [12].

In the sputum smear microscopy (AFB), slides were coded to rule out selective bias. Stained smears were microscopically examined which doubled checked by senior experienced laboratory technician. To obtain representative smear, two frosted end slides were prepared, and from the purulent part of sputum sample, extracted, placed on the center of one of the slides using a sterile cotton swab. By holding each slide by its frosted end, the second slide was placed on top of the first slide, and then tried to move the slides against each other in several directions with a rotating motion. The slides were then dried to separate by pulling away from each other horizontally. If the specimen is not evenly spread on both slide smears, the process could be repeated. This cold method was performed as briefly described: prepared smears were flooded with 1% carbol fuchsine, allowed to stand at room temperature for 10 minutes, washed with tap water, decolorized and counterstained with Gabbet's methylene blue for another 2 minutes (previously described [13]. Slides were washed, air-dried and examined using an oil immersion objective [14]. Also, the GeneXpert MTB-RIF assay was performed

on fresh sputum sample according to the manufacturer's specifications (Cepheid, CA, USA) to confirm TB, and results obtained.

Ethical Consideration

The study protocol was approved by the Ethics Committee, University of Jos University Teaching Hospital and permission was obtained from Mangu TB treatment Centre on for accessing the patients and other laboratory results. Urine LAM test results were not used for treatment decision-making but GeneXpert results. The consent of the presumptive TB cases was sought before their urine and sputum were collected for the test.

Statistical Analysis

Statistical analysis was conducted using Epi Info 7.0. Differences in baseline characteristics between groups were analyzed using bivariate logistic regression analysis. Odds ratios with 95% confidence intervals were calculated to measure the effect size. Sensitivity, specificity, diagnostic accuracy value, positive predictive value (PPV) and negative predictive value (NPV) of the LAM, AFB smear tests and GeneXpert as standard were calculated. In all analysis, $P \leqslant 0.05$ was considered statistically significant given the individual corresponding confidence interval at 95%.

Result

Of the 53 participants, 67.9% were males, 32.1% were females; the mean age was 36.0±14.3 years. The rates of infection were lower in male 5(14.0%) than in females (35.3%) (Table 1). The Mycobacteria species were identified in urine specimens by Point-of-care Urine Determine TB LAM Ag (lateral-flow), GeneXpert MTB/RIF and AFB. The positive test rate of Mycobacterium antigen among TB patients by LAM assay was 11(20.8%), GeneXpert, 9(17.0%) and AFB was 6(11.3 %). Among the positive cases, 19-29 years constitutes the highest (22.7%), followed by 30-49 years (19.0%) and ≥50 years (20.0%) (Table 1). Regarding the 7(13.2%) were employed 8(15.1%) unemployed, 13(24.5%) farmers, 15(28.3%) traders and 10(19.0%) were students. The rates of infection in relation to occupation varies; employed (14.3%), unemployed (37.5%), farming (7.7%), trading (26.7%) and students (20.0%). Respondents tested positive for HIV were 21(25.0%) and 5 (23.8%) of them were tested positive to LAM with P=0.86. Regarding duration of cough in relation to diagnosis of TB using LAM test, those with less than 2 weeks history had 1(9.1%) positive result and 10(90.9%) were negative, while those with >2 weeks history of cough had 6(24.0%) positive and 19(76.0%) were negative. The distribution of tuberculosis in relation to history of cough using LAM has a P-Value (0.56).

The evaluation of qualitative Urine LAM testing compared to Genexpert as gold standard was: Sensitivity (77.8%), Specificity (90.9%), Positive predictive value (66.6%), Negative Predictive value (95.2%) and diagnostic accuracy (88.7%). Urine LAM testing was more sensitive than the AFB smear testing. However, both LAM and AFB tests has a diagnostic accuracy of detecting TB cases with value of 88.7% and 83.0% respectively (Figure 1).

Table 1. The distribution of TB patients by socio-demographic factors associated with LAM results

Characteristics	LAM Results		Total (%)	Statistic	P-Value
	Positive (%)	Negative (%)			
Sex					
Male	5(13.9%)	31(86.1%)	36(100.0%)	X^2 3.2	0.07
Female	6(35.3%)	11(64.7%)	17(100.0%)		
Age in Years (Mean ±Sd)			36.0±14.3		
Age group (years)					
19-29	5(22.7%)	17(77.3%)	22(100.0%)	X^2 0.93	0.96
30-49	4(19.0%)	17(81.0%)	21(100.0%)		
50-69	2(20.0%)	8(80.0%)	10(100.0%)		
Total	11(20.8%)	42(79.2%)	53(100.0%)		
Occupation					
Employed	1(14.3%)	6(85.7%)	7(100%)	X^2 3.2	0.52
Unemployed	3(37.5%)	5(62.5%)	8(100%)		
Farming	1(7.7%)	12(92.3%)	13(100%)		
Trading	4(26.7%)	11(73.3%)	15(100%)		
Student	2(20.0%)	8(80.0%)	11(100%)		

Table 2. Analysis of clinical characteristics associated with LAM-results

Characteristics	LAM Result		Total (%)	95% CI	Odds ratio	Test of Significance	
	Positive%	Negative%				Statistic	P-Value
AFB Result						X^2 13.8	<0.001
Positive	9(81.2%)	2(12.5%)	11(100%)	3.1, 295.3	30.3		
Negative	2(18.2%)	51(96.2%)	53(100%)		1		
Gene XPERT Result						X^2 =13.8	<0.001
Positive	6(54.5%)	5(45.5%)	11(100%)	2.2, 300.3	40		
Negative	5(45.5%)	48(90.6%)	53(100%)		1		
HIV Status						X^2 =7.4	0.01
Positive	10 (90.9%)	1(9.1%)	11(100%)	0.29, 25.5	2.7		
Negative	1(9.1%)	52(98.1%)	53(100%)		1		

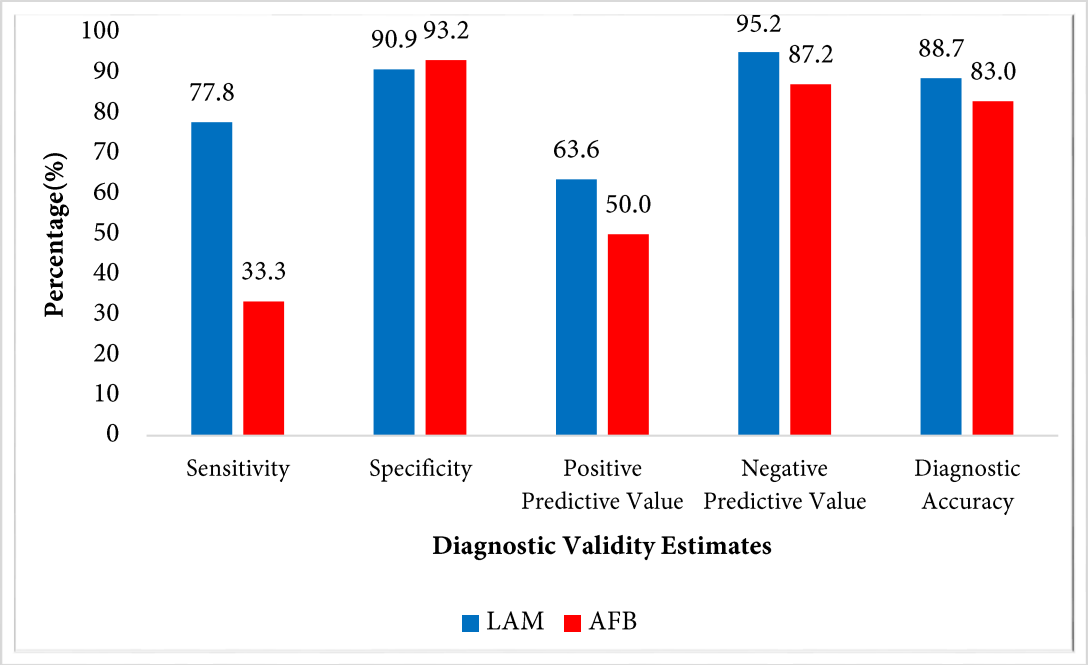

Figure 1. Evaluation the sensitivity and specificity value of urine LAM and sputum AFB Procedure using GeneXpert as the Reference (Gold Standard)

Discussion

In our study, the Determine Urine TB-LAM lateral-flow antigen test was simple to use with the prevalence of TB patients by LAM assay (20.8%), GeneXpert, (17.0%) and AFB (11.3 %). The LAM test showed high positivity of TB (90.9%) in HIV-infected patients, p=0.01, and cough duration of two weeks or more (72.7%, p= 0.001). Even though the CD4 cell count of the HIV patients were not done but the test sensitivity could be increased among patients with low CD4 cell counts. This findings affirm that sensitivity of the LAM test is highest for the HIV/TB co-infected patients. This result is consistent with earlier findings in Cape Town, South Africa and Ghana, West Africa where it was found that LAM test sensitivity increased among HIV patients with very low CD4 cell count (< 100 cells/mm3), [16, 5]. However, many other studies have also evaluated the use of Urine LAM test that have showed increasing sensitivity in very ill patients [17,18,19]. The finding of our study suggests that it could be used as a good screening and rule-in test for TB detection in resource limited settings where GeneXpert may not easily available.

Compared with a diagnostic gold standard of GeneXpert results, the Alere Urine LAM assay was useful for screening for pulmonary tuberculosis in patients with advanced TB and perhaps best used with HIV infected patients with very low CD4 cell counts in a very high burden setting such as Nigeria. Our study showed very high diagnostic validity of Urine LAM test over sputum AFB (Table 2), including the subgroups. These combined results in TB patients with high sensitivity and diagnostic accuracy suggest that the use of LAM is still very promising in very ill TB and HIV-infected individuals. Our results showed that the correlates of HIV status and cough duration of more than two weeks in patients are statistically significant. This study also shows good insights into the diagnostic accuracy of LAM by providing comparing results of sputum smear microscopy and Xpert MTB/RIF, and this suggest that the sensitivity of the LAM assay is adequate to screen for TB

especially in symptomatic patients with cough history of more than two weeks where smear microscope is out of reach in high burden countries like Nigeria. The study strengths was the collection of fresh urine samples and the GeneXpert results, however, it's limitation is the study design (cross-sectional), small sample size, and lack of CD4 cell count result of HIV infected patients.

Conclusions

Although the Urine LAM strip-test is restricted to HIV-infected individuals with advanced immunosuppression, it can be used to target vulnerable population with high-risk of death where desired methods are inadvertently not available. Given the Urine LAM low cost, ease to preform, it has the potential to offer valuable clinical function when combined with sputum AFB in high-burden and inaccessible suburbs healthcare centers where skilled staff are nearly absent.

Conflict of Interest

The authors declare that was no conflict of interest.

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