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COMPARATIVE ASSESSMENT OF DIFFERENT DIAGNOSTIC TECHNIQUES IN THE IDENTIFICATION OF *MYCOBACTERIUM TUBERCULOSIS* IN SUSPECTED CASES OF TUBERCULOSIS

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ABSTRACT

Statement of the Problem: Tuberculosis remains a serious public-health threat in developing countries though it has been eradicated in some advanced countries. This disease constitutes a significant threat to global health, being the second highest cause of morbidity and mortality resulting from infectious agents. Prompt diagnosis of active TB facilitates timely therapeutic intervention and minimizes community transmission. Aim: This study aimed at determining a 'Point of Care' diagnostic tool for pulmonary tuberculosis (PTB) by comparing the efficiency of four different PTB diagnostic tools for different age groups. Methodology: Zeihl Nelson (ZN) staining, culture, Gene xpert (GX) and Lipoarabinomanan (LAM) assay were employed in this study The culture method was used for confirmation. Sputum and urine samples were collected from each of 100 patients symptomatically diagnosed of PTB. Findings: Fifty-seven percent of the population was male while 43% were female. *Mycobacterium tuberculosis* was isolated from 9 (9%) of 100 patients. Similarly, GX detected *Mycobacterium tuberculosis* in 9 (9%) of the patients while the rate of detection using LAM was 10% and with ZN it was 7%. Gene xpert produced no true or false positive and negative result, LAM had one false positive result and ZN had two false negative results. The maximum time frame to generate result was 25 minutes for LAM, two hours for Gene xpert, eight weeks for culture and two days for ZN. Two positive isolates were observed at the same frequency for age group 21-30 and 31- 40 while age groups 1-10, 10-20, 41-50, 50-60 and above has 1 positive result each. Gene xpert had 98.11% sensitivity while LAM had 96.23% and ZN had 86.79%. The choice of 'Point of Care' diagnostic tool is of great concern to clinicians and the general public. Conclusion & Significance: This study identified LAM assay as suitable 'Point of Care' diagnostic and an add-on tool for PTB diagnosis because of its relatively high sensitivity and short maximum time frame to generate result compare to other three diagnostic techniques.

Keywords: Pulmonary tuberculosis, Diagnostic tools, point of care, Lipoarabinomanan, culture, Gene xpert, Zeihl Nelson (ZN) staining, and Add-on test.

INTRODUCTION

Although Tuberculosis (TB) is found all around the world, tuberculosis remains a serious public-health threat in developing countries. This disease constitutes a significant threat to global health being the second highest cause of morbidity and mortality resulting from infectious agents. In 2019, about 10 million people developed TB and 1.4 million died and currently about a quarter of the world's population is infected with M. tuberculosis (Moscow Declaration to End TB, 2019). TB can be very difficult to diagnose. For this reason, the American Thoracic Society (ATS), Infectious Diseases Society of America (IDSA), and Centers for Disease Control and Prevention (CDC) have provided guidance on diagnosing TB in children and adults. Persons with *M. tuberculosis* infection may have no clinical evidence of disease and present asymptomatically, known as latent tuberculosis infection (LTBI). In other patients, the disease manifests symptomatically (having a bad cough that lasts longer than three weeks, having pain in the chest, and coughing up blood or phlegm from deep inside the lungs), known as tuberculosis (TB). Prompt diagnosis of active TB facilitates timely therapeutic intervention and minimizes community transmission (Lewinsohn *et al.,* 2017).

This study was aimed at determining the efficacy of three different diagnostic techniques in identifying the most appropriate diagnostic tool with suitable characteristics for use as a 'point of care' diagnostic tool in pulmonary TB.

MATERIALS AND METHODS

ETHICAL APPROVAL

Ethical approval was obtained from the Sacred Heart Private Hospital, Lantoro, Abeokuta being a Directly Observed Treatment, Short-course (DOTS) centre for the state. Oral informed consent was also obtained from participating patients or/caregivers.

Development of Questionnaire and Selection of patients for the study

A symptom-screening questionnaire was designed to carry out a survey on demographic information, Clinical histories were also obtained through interview and medical record review at enrollment. Selection of subjects was done randomly in order to avoid bias.

Triplicate sputum samples were taken from each of 100 patients suspected of having pulmonary tuberculosis. Ziehl-Neelsen stained smears made from sputum samples were examined by microscopy. Sputum samples were also cultured for isolation of *M. tuberculosis.* Moreover, 2mls of sputum sample void of blood and food particles was taken separately for gene xpert diagnosis. Urine sample (5 ml) was also taken from each suspect for lipoarabinomannan (LAM) diagnosis of tuberculosis.

ZIEHL NEELSEN STAINING AND MICROSCOPY

Each smear sputum sample was prepared on a clean grease-free slide. Smear was allowed to air dry and then heat fixed. The smear was covered with carbol fuchsin stain on flame until vapour rise (i.e. about 60ºC). The heated stain was allowed to remain on the slide for 5 minutes. The stains were then washed off with clean water. The smear was decolourized with 3% v/v acid alcohol for 5 minutes or until the smear was fully decolorized, i.e. pale or pink and then washed thoroughly with clean water. The smear was covered with malachite green stain for 1–2 minutes, washed off with clean water and air -dried. Examination of the smear was done microscopically using the 100X oil immersion objective.

Mycobacterium tuberculosis was identified as acid fast bacilli. These appeared as bright red to intensive purple cells which were straight or slightly curved rods, occurring singly or in small groups or beaded under the microscope.

Urine Lateral Flow LAM Assay

LAM ELISA testing was performed and interpreted according to manufacturer's instructions (Clearview® TB ELISA, Alere Health Services, USA). The strip cover on each Alere Determine™ *TB LAM Ag* test was removed and 10μl of urine sample was placed at the test end of the lateral flow TB LAM Ag stripe. Within 25 minutes, two different marks were observed on the strip indicating a positive result to tuberculosis test or a single mark indicating a negative result.

Culture on Lowenstein Jensen Medium for Isolation of M. tuberculosis

Lowenstein Jensen Medium (LJM) was inoculated with decontaminated and concentrated sputum according to test procedures recommended by the Centers for Disease Control. Inoculated media was incubated in a CO_2 atmosphere at 35-37 ºC. Inoculated LJM slants were incubated away from light for one week with loosened caps to allow the circulation of CO_2 for the initiation of growth. Caps were tightened after one week in order to prevent dehydration of media. The media was examined within five to seven days, and weekly thereafter for up to eight weeks. Slants were examined under light for the appearance of macroscopic growth. Colony morphology on the first day of growth was observed and recorded. Isolates from these cultures were stained with Zeihl Neelsen staining to confirm acid fast isolates.

Protocol for Xpert MTB/RIFAMPICIN Assay

Four mls of quality (containing neither food particles nor blood) sputum sample was collected from patient into a clean calibrated sputum container. Sputum was dissolved in Xpert sample reagent in ratio 1:2. The mixture was mixed by vigorous shaking and allowed to stay for 10 minutes. Shaking was repeated vigorously again and allowed to stay for another 5 minutes. After this, a sterile pipette for each cartridge was used to withdraw 2mls of the dissolved sputum and discharged into the cartridge. The cartridge was then scanned by inserting the cartridge with its content into the Xpert MTB/RIF assay machine and leaving it there for 1hr 45mins. The result was read on the computer system attached to the machine. This test detects MTB in sputum and also determines the sensitivity of the organism to rifampicin.

STATISTICAL VALIDATION AND MEASUREMENT OF MICROSCOPIC SENSITIVITY AND SPECIFICITY

Data were statistically validated by determining the *p* values. The sensitivity, specificity, accuracy, positive- and negative-predictive values were also computed to measure the validity of the tests based on true positive, false positive, true negative and false negative results. Thus, the results of microscopy, LAM and gene Xpert techniques were compared with that of the culture method being the Gold standard as recommended by WHO (2011).

RESULTS

A total of 100 suspects of TB were recruited in all. Fifty three were male while 47 were female. Sputum and urine samples from each suspect were made available for the four different diagnostic tests used. Results for Lipoarabinomannan assay were available in

less than 30 mins, Gene Xpert took close to 2hrs to produce result while sputum smear microscopy took 48 hours. Culture produced result after 8 weeks. A total of nine (9.0%) suspects were positive for *M. Tuberculosis* by culture. Positive samples included five from male and four from female. Lipoarabinomanan assay produced 10 (10.0%) positive results for TB with six from male and four from female. Gene xpert had nine (9.0%) positive results including five from male and four from female. Zeihl Neelsen produced seven (7.0%) positive results with four from male and three from female. All these had no significant difference. There was no significant difference in the prevalence of *M. tuberculosis* as determined by the four diagnostic techniques (Table 1)

Age groups 41-50 (23) and 31-40 (20) were the most frequently represented age groups (Table 2). Using Culture as the gold standard, Gene xpert was able to confirm 9/9 cases, LAM assay had 1 false positive giving 10/9 while ZN had 2 false negative confirming 7/9. Using Wilconxon ranksum test to access the mean rank differences, ZN has 91° ties with a mean rank of 5.50 and 16.50 sum rank for negative ranks with a mean rank of 16.50 and 38.50 sum rank for positive rank. LAM has 90° ties with 4.00 mean rank and 16.50 sum rank for negative ranks with positive rank of 20.00 and 08.00 sum rank. Gene xpert has 100° ties with 1.00 mean rank and 1.00 sum rank for negative ranks with positive rank of 16.50 and 38.50 sum rank (Table 3). LAM, ZN and Gene xpert had 91.0, 93.88 and 98.11 as positive predictive value respectively and 99.03, 96.73 and 99.52 as negative predictive value respectively. In this present study, Gene xpert had the highest sensitivity of 98.11% and specificity of 99.52%. LAM shows sensitivity of 96.23% and specificity of 97.62% while ZN had sensitivity of 86.97% and specificity of 98.57% (Table 4).

Table 1: Sex Distribution of TB Confirmed Cases

Diagnostic tools	Sex	No of Presumptive cases examined	Positive cases	% positive	Negative cases	% Negative	X^2	P^2
CULTURE	Male	53	05	05	48	48	1.664	0.199
	Female	47	04	04	43	43		
Total		100	09	09	91	91		
LAM	Male	53	06	06	47	47		
	Female	47	04	04	43	43		
Total		100	10	10	90	90		
ZN	Male	53	04	04	49	49		
	Female	47	03	03	44	44		
Total		100	07	07	93	93		
Gene xpert	**Male**	53	05	05	48	48		
	Female	47	04	04	43	43		
Total		100	09	09	91	91		

Table 2 Age group distribution of different diagnostic tools of confirmed TB

Diagnostic tools	Age group	Number examined	Positive	Negative	x^2	p- value
Culture	1-10	05	01	04	2.147	0.002
	11-20	07	01	06		
	21-30	19	01	18		
	31-40	20	02	18		
	41-50	23	02	21		
	51-60	16	01	15		
	60 and above	10	01	09		
	Total	100	09	91		
LAM	1-10	05	01	04		
	11-20	07	01	06		
	21-30	19	02	17		
	31-40	20	02	18		
	41-50	23	02	21		
	51-60	16	01	15		
	61 and above	10	01	09		
	Total	100	10	90		
ZN	1-10	05	01	04		
	11-20	07	00	07		
	21-30	19	02	17		
	31-40	20	02	18		
	41-50	23	00	23		
	51-60	16	01	15		
	60 and above	10	01	09		
	Total	100	07	93		
Gene-Xpert	1-10	05	01	04		
	11-20	07	01	06		
	21-30	19	01	18		
	31-40	20	02	18		
	41-50	23	02	21		
	51-60	16	01	15		
	60 and above	10	01	09		
	Total	100	09	91		

Table 3 Wilcoxon Ranksum test for Ziehl-Nelson (ZN) – Culture test

ZN – Culture	N	Mean rank	Sum ranks
Negative ranks	2	5.50	16.50
Positive ranks	7	5.50	38.50
Ties	91		
Total	100		

Where a signifies ZN<Culture, b = ZN Culture and c signifies ZN = Culture

LAM-Culture	N	Mean rank	Sum ranks
Negative ranks	1	4.00	20.00
Positive ranks	9	4.00	08.00
Ties	90		
Total	100		

Gene-xpert	N	Mean rank	Sum ranks
Negative ranks	0	1.00	1.00
Positive rank	0	1.00	1.00
Ties	100		
Total	100		

Table 4 Sensitivity, Specificity and Predictive Values of the Diagnostic Methods

Parameter	VALUE (%) LAM	Ziehl-Nelson Staining	G X
Sensitivity	96.23	86.79	98.11
Specificity	97.62	98.57	99.52
Positive predictive value	91.07	93.88	98.11
Negative predictive value	99.03	96.73	99.52
Positive Diagnostic Likelihood Ratio	40.43	26.54	204.40
Negative Diagnostic Likelihood Ratio	0.04	0.13	0.02

DISCUSSION

This study revealed that more males enrolled for this study than females which might be an index that males are probably more affected with TB than females (though with no significant statistical difference). Nwachukwu *et al.,*(2009); Kehinde and Okesola (2010); Obioma *et al*., (2011); Sani *et al.,* (2015) reported similar cases of more enrollment of males in TB studies. This could be attributed to the social habits of men (for instance, smoking, drug misuse) that predispose them to TB than female. The age groups mostly affected by TB in this study were age groups 31-40years and 41-50years. Sani *et al.,* (2015) observed that people in the age group 11-40years were more affected by TB than other age groups. Similarly, Okonko *et al.,* (2012) reported that TB infection was higher in age group 40 years old and above. This may be due to the fact that these age groups are more exposed to out-door activities and represent the greater proportion of the working force of the population.

The sensitivity and specificity of LAM in this study is greater than the previous study reported by Drain *et al.,* (2016) and this might be attributed to the fact that this study enrolled TB suspects who have not been on any antiretroviral drugs as LAM decreases with such state. The high sensitivity of LAM in this study is in agreement with Drain *et al.,* (2016). This may be attributed to the fact that LAM detects TB better especially in underlying medical conditions such as HIV than Zeihl Nelson due to the pausibacillary nature of the organism. Gene xpert was more accurate in comparison to culture as a diagnostic tool examined in this study, using Culture as the gold standard. However, the sophisticated nature of gene xpert which made it not readily accessible may be a hindrance in its decentralization. Considering the tool that has the most bed-side ability, of the three tools examined in this study, LAM showed the least time in generating result followed by Gene xpert while Zeihl Nelson staining are no longer bedside diagnostic methods since it takes over 24 hours.

CONCLUSION

Early diagnosis and treatment of TB will curb the spread of pulmonary TB and thus, LAM may be best utilized as an add-on test with Zeihl Nelson staining for patients presenting with TB-related symptoms especially in rural centres or locations that are not Directly observed treatment short-course (DOTs). Conflict of interest: None to declare

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