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Evaluation of GeneXpert MTB/Rif Assay and the Conventional Methods for Detection of Pulmonary Tuberculosis in Internally Displaced Persons (IDP) Camps in Gombe State

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Authors' contributions

This work was carried out in collaboration among all authors. Author LM was involved in study design, data collection and manuscript writing. Author KM supervised data collection process and study design. Author GI was involved in manuscript revision and designed the data collection form. All authors read and approved the final manuscript.

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ABSTRACT

Background: Tuberculosis is a global health problem associated with high morbidity and mortality. Rapid diagnosis of tuberculosis is essential for early disease management.
Aim: This study evaluated the performance of gene expert MTB/ RIF assay for the diagnosis of pulmonary tuberculosis and rifampin (RIF) resistance with conventional methods.
Methods: A total of 130 sputum samples from suspected tuberculosis patients were examined from July 2019 to August 2019.
Results: Fifty-nine patients 59(45.4%) were males and seventy-one 71 patients (54.6%) were females. Seventeen patients (13.07%) had tuberculosis. Of the 17 Confirmed tuberculosis patients, 6(35.2%) were ZN positive, 11(64.7%) were GeneXpert positive and 17(100%) were positive to TB Culture. One sample showed false-positive GeneXpert result. The GeneXpert assay achieved

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47.1% sensitivity, 97.3% specificity, 72.7% Positive Predictive Value (PPV) and 92.4% Negative Predictive Value (NPV) while ZN Staining method had 35.3% Sensitivity, 97.27% Specificity, 100% PPV and 91.1% NPV. GeneXpert detected 5(29.41%) Rifampicin resistant TB. The risk factors associated with tuberculosis in this study had HIV (17.6%), Malnutrition (13.8%) and Overcrowding (15.3%).

Conclusion: GeneXpert MTB/RIF assay is a helpful tool for rapid diagnosis and prompt treatment of TB. However, the use of Genexpert does not eliminate the need of conventional microscopy, culture and anti-tubercular sensitivity that are required to monitor the progression of treatment.

Keywords: GeneXpert; TB culture; ZN staining; tuberculosis; malnutrition and overcrowding.

1. INTRODUCTION

Tuberculosis (TB) is one of the major public health threats, competing with the human immunodeficiency virus (HIV) as the cause of death due to infectious diseases worldwide. Although a declining trend in TB incidence, prevalence and mortality has been observed over the late decade, elimination of the disease attenuated global level is still out of reach, and massive resource investment is still required. TB is a poverty-related disease-specific which disproportionately affects the poorest, the most vulnerable and marginalized population groups wherever it occurs. Improving access to diagnosis and care, the basic requirements in the fight against TB, are particularly challenging in these persons [1]. A fresh report released by the National Tuberculosis and Leprosy Control Program, a parastatal under the Federal Ministry of Health, has revealed that Nigeria has been ranked the 4th country with the highest cases of tuberculosis worldwide. The statistics also showed that over 80% of tuberculosis cases in Nigeria were still undetected, while it claims over 1. 5 million lives annually in the country [2]. Equally, Nigeria and India accounted for 48% of global TB deaths among HIV-negative people and for 43% of the combined total TB deaths in HIV- negative and HIV-positive people. Nigeria is among the ten countries that accounted for 77% of the global gap in TB case finding. In 2016, Nigeria notified less than 20% of the total TB cases estimated for that year. More than 80% TB cases in the country are undetected, implying that, there are lots of undiagnosed TB cases in the community which serve as a reservoir for continue transmission of TB" [3]. People living with HIV/AIDS (PLHIV) area extremely high risk of TB, due to the immunological impairment associated to this infection and to frequent co-existence of deprived social conditions.

The risk of active TB is also greater in persons suffering from other conditions that impair the immune system. One million children (0–14 years of age) fell ill with TB, and 250 000 children (including children with HIV associated TB) died from the disease in 2016. Prevention of TB involves screening those at high risk, early detection and treatment of cases, and vaccination with the bacillus Calmette-Guérin (BCG) vaccine [4]. Those at high risk include household, workplace, and social contacts of people with active TB [5]. Treatment requires the use of multiple antibiotics over a long period of time. Antibiotic resistance is a growing problem with increasing rates of multiple drug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) [6].

1.1 Aim

The aim of the study is to evaluate PCR (GeneXpert) in comparison with ZN staining and TB culture for the detection of *Mycobacterium tuberculosis* among TB suspected individuals in IDP camps in Gombe State

2. MATERIALS AND METHODS

2.1 Study Design

This is a cross sectional study of persons suspected to have pulmonary tuberculosis. The study involves the use of ZN Staining method and TB culture (LJ medium) and GeneXpert (PCR) for the diagnoses of TB in persons suspected of having pulmonary tuberculosis.

2.2 Study Area

The study was conducted in Infectious Diseases Hospital Zambuk Gombe from October 2018 to November 2018.

2.3 Study Population

Internally Displaced Persons from IDP camps in Gombe State.

2.4 Inclusion Criteria

1. Internally Displaced Persons suspected to have pulmonary tuberculosis.
2. No history of receiving anti-tuberculous drug within 3 months prior to enrollment.

2.5 Exclusion Criteria

Persons diagnosed with TB and non-IDP were excluded from the study.

2.6 Sample Size Determination

In the calculation of sample size, Prevalence (P) of PTB of 8% was used [7]. Hence, the following formula was used.

$$n = Z^2 pq/ d^2$$

Where,

n= number of sample
Z= level of significant at 95% confidence interval (1.96), P= prevalence rate, q= 1-p
d= tolerable margin of error (5%) = 0.05

$$n = Z^2 pq/ d^2$$

$(1.96)^2 0.08(1-0.08)/0.05^2 = 113$
Attrition rate: 10% of sample size
10% of 113

Therefore, sample size + attrition rate = number of samples to be used in this study
113 = 11.3 = 124.3
Hence, 130 persons with clinical manifestation of TB were used in this study.

2.7 Sampling Technique

The study participants who met the inclusion criteria were selected, examined and interviewed.

2.8 Questionnaire Admin

A questionnaire was administered for all eligible study participants who agreed and filled their consent form to collect demographic, clinical and socio-economical data (age, sex, marital status, etc). Sputum samples were collected in a wide-mouth container. The sputum samples were divided into three portions, one for the ZN staining, one part for TB culture and the other for Genexpert. Laboratory results for sputum smear was categorized into positive or negative, for GeneXpert was also categorized into positive (detected) or negative (Not detected) and likewise TB culture was categorized into positive and negative.

2.9 Laboratory Analysis

2.9.1 Ziehl-neelsen staining method

Thin smear of the sputum was made and was heat fixed by passing the slide 3-4 times through the flame of a Bunsen burner. The slide was placed on a staining rack and strong carbol fuchsin was poured over smear and heated gently at the underside of the slide until fumes appear (without boiling), and was allowed to stand for 5 minutes. The smear was rinsed with water. Twenty (20%) sulphuric acid was poured on the smear and was allowed to stand for 20 seconds. The smear was washed with water and was covered with methylene blue for 2 minutes after which it was washed off with clean water. The back of the slide was wiped clean, and placed on a draining rack for the smear to air-dry. The smear was examined microscopically, using the 100x oil immersion objective.

2.9.2 GeneXpert assay

Sputum samples were collected from patients. The sputum was mixed with the reagent provided with the assay and was allowed to stay for 15minutes. The cartridge containing this mixture was placed in the GeneXpert machine. All processes from this point on was fully automated.

2.9.3 Lowenstein jensen procedure

The sputum sample was Inoculated into the Lowenstein Jensen Media after decontamination and neutralization. It was then Incubated at 37°C in a $C0_2$ atmosphere, Protected from light. The Tubed media was incubated for one week with loosed caps. The Cap was tightened after one week. The media was Examined within five to seven days, and weekly thereafter for up to eight weeks.

2.9.4 Statistical analysis

The data generated was analyzed using statistical package for social sciences (SPSS) for windows, version 23.0 (SPSS Inc., Chicago, IL, USA). Sensitivity, specificity, and positive and negative predictive values were calculated for TB diagnosis using GX. These were compared with the gold standard (conventional culture).

3. RESULTS

Table 1 shows the prevalence of TB among demographic distribution of the study, in which 16.9% of males and 9.85% females had TB. 4.54% of the age group of less than 20 years, 10.3% of 21-40 years and 21.2% of 41-60 years have TB. Among which 15.3% of the married, 13.2% of the singles have TB. There is no statistically significant difference in the prevalence of TB based on age P>0.005.

Table 2 shows the prevalence of TB in IDP camps among demographic variables based on ZN method, GeneXpert and L.J. Culture. Out of 130 sputum sample analyzed from the suspected TB patients, the prevalence of TB among the patients based on gender (sex) showed that female patients had 2.8% with ZN staining method, 4.2% with GeneXpert and 9.9% with TB Culture. The prevalence of TB based on age groups as detected by ZN staining method, GeneXpert and Culture shows that patients aged 21-40 years had the highest prevalence of 8.6% with ZN staining method, 13.7% with GeneXpert and 8.6% with Culture, followed by age group of 41-60 years which had 2.1% with ZN method, 4.2% with GeneXpert and 14.8% with Culture. The age group of under 20 years had 4.5% with GeneXpert and 9.09% with Culture. There is no statistically significant difference in the prevalence of TB based on age P>0.005 as shown in Table 2. The married had the highest prevalence of TB with 5.3% using ZN method, 8.8% with GeneXpert and 15.0% with Culture, followed by the singles who had 7.7% with GeneXpert.

The sensitivity, specificity, Positive predicted value and Negative predictive Value of ZN staining method and GeneXpert are 55%, 97%, 27% and 96.4% for ZN method, 85%,100%,100% and 92.4% for GeneXpert and 100%, 100%, 100% and 100% for LJ Culture method respectively. There is statistically significant difference in the sensitivity, specificity, negative and positive predictive value P<0.05 as shown in Table 3.

Table 4 shows the risk factors associated with Tuberculosis among TB patients. HIV showed to have the highest prevalence of 17.6%, Overcrowding had 15.3% and Malnutrition had 13.8%. There is statistically significant difference in the risk factors associated with tuberculosis P<0.05.

4. DISCUSSION

The GeneXpert MTB/RIF assay has been introduced with the aim to increase the detection of tuberculosis especially in smear negative, extrapulmonary and pediatric age groups. The prevalence of TB in this study is high in aged 41-60 years (21.2%). This is in agreement with the findings of Pennab [8] but contrary with the report by Sani [10] who presented a higher prevalence in a lesser group age.

The observed prevalence of TB in the age group 41-60 years may be attributed to the fact that these groups are sexually active, therefore encountering sexual partners in whom both MTB and HIV are both prevalent and poor hygiene. The age group below 15 years have 4.45% TB prevalence, this can be linked to the age group's

Table 1. Prevalence of tuberculosis based on demographic characteristics of the patients

Variables	Number of samples examined	No. positive to TB	% Prevalence	P-value
Gender				
Male	59	10	16.9	0.00
Female	71	7	9.85	
Age group				
≤20	22	1	4.54	0.150
21-40	58	6	10.3	
41-60	47	10	21.2	
>60	3	0	0.00	
Marital status				
Single	13	2	15.3	0.00
Married	113	15	13.2	
Widow	4	0	0.00	

Key: TB= Tuberculosis, P- Value= Probability value

Table 2. Prevalence of pulmonary tuberculosis in IDP camps based on techniques (ZN, GeneXpert and TB Culture)

Variables	No. of samples examined	No. and % positive by ZN	No. and % positive by GX	No. and % positive by LJ. culture	% prevalence	P-value
Gender						
Female	71	2(2.8)	3(4.2)	7(9.9)	16.9	0.00
Male	59	4(6.8)	8(13.6)	10(16.9)	37.3	
Total	130	6(4.61)	11(8.46)	17(13.07)		
Age groups						
<20	22	0(0.0)	1(4.5)	2(9.09)	13.6	0.263
21-40	58	5(8.6)	8(13.7)	8(8.6)	36.2	
41-60	47	1(2.1)	2(4.2)	7(14.8)	21.1	
>60	3	0(0.0)	0(0.0)	0(0.0)	0.0	
Marital Status						
Married	113	6(5.3)	10(8.8)	17(15.0)	29.2	0.00
Single	13	0(0.0)	1(7.7)	0(0.0)	7.69	
Widow	4	0(0.0)	0(0.0)	0(0.0)	0.00	

KEY: ZN= Ziehl Neelson, GX= GeneXpert, LJ= Loweinstein Jensen, P- Value= Probability Value, TB= Tuberculosis

Table 3. Sensitivity, specificity, positive and negative predictive value of ZN staining and GeneXpert method

Methods used	No of samples	Number of TB detected	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	P-value
Zn staining	130	06	35.3	100	100	91.1	0.00
Genexpert	130	11	47.1	97.3	72.7	92.4	0.00
L.j. culture	130	17	100	100	100	100	0.00

Key: NPV: Negative Predictive Value, PPV: Positive Predictive Value, TB: Tuberculosis; ZN: Zeihl Neelson, P-value: Probability Value, L. J: Lowenstein Jensen

Table 4. Risk factors associated with tuberculosis among patients in IDP camps in Gombe

Risks factors	No. of patients examined	No. positive of samples	% Prevalence	P-value
HIV	130	23	17.6	0.00
Malnutrition	130	18	13.8	0.00
Overcrowding	130	20	15.3	0.00

Key: HIV= Human immunodeficiency virus, IDP= Internally Displaced Person, P value= Probability value

literacy. The incorporation of hygiene practices in their school's curriculum in the levels of primary and secondary school may allow them to effectively receive educational information on MTB prevention from schools and the media. In this study, prevalence in relation to gender, males were shown to have the highest prevalence of TB (16.9%) than the females (9.85%). This result is in agreement with report by Sani [9] but is contrary to the report by Nwachukwu who stated that females have the highest prevalence. The observed high prevalence of TB in males could be as a result of behavioral attitude and also, as a result of indiscriminate use of drugs and its abuse. The difference in MTB diagnosis between the three methods was statistically significant (P<0.05). Higher detection of MTB by TB culture method (100%) was seen in this study compared to GeneXpert (64.7%) and ZN staining (54.5%). The lower prevalence of TB by ZN and GeneXpert can be related to poor quality specimens, not obtaining the proper portion of the specimen during smear preparation and most especially the number of MTB bacilli per ml in the sputum. Culture methods is the gold standard tests for the detection of *Mycobacterium tuberculosis*. In this study, the positive and negative predictive value for ZN method and GeneXpert for the detection of

tuberculosis were 100%, 91.1% and 72.7%, 92.4% respectively.

In this study, ZN stained smears had a sensitivity of 35.3% which is in line with 46% sensitivity of ZN as said by Danish [10] and specificity of 100% compared to 97.27% and sensitivity of 47.1% and 97.3% specificity of GeneXpert assay respectively. Thus, sputum microscopy detected 6(35.3%) which is half of the cases detected by GeneXpert 11(64.7%) and TB Culture detected all the 17(100%) MTB patients. For a sample to be detected by ZN method a bacterial load of 10^4 organisms is required, identification of TB bacilli by GeneXpert requires 131CFU/ml of sample and 10 bacilli/ml for TB culture to detect. [11,12]. GeneXpert detected 1 sample which was smear positive but culture negative. This discrepant result could be attributed to the previous anti tubercular treatment of the patient. Excretion of the residual DNA from the dead bacilli explains the positive GeneXpert and negative culture result [13].

5. CONCLUSION

This study demonstrated good sensitivity and specificity of GeneXpert assay in detecting *M. tuberculosis*. Among the culture methods used in this study, LJ culture showed the highest detection rate which remains the gold standard. The risk factors indicated (HIV, Malnutrition and Overcrowding) increased the risk of TB infection in the IDP camps. This diagnostic test is helpful for rapid diagnosis and prompt treatment of TB, particularly in patients who had a negative sputum acid-fast smear. However, GeneXpert does not eliminate the need of conventional microscopy, culture and anti-tubercular sensitivity that are required to monitor the progression of treatment and to detect resistance to drugs other than rifampicin.

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AVAILABILITY OF DATA AND MATERIALS

The dataset used in this study are available from the corresponding author based on reasonable request.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from the Ethics and Research Committee of the Ministry of Health, Gombe for permission to conduct the study. The ethical approval reference number is MOH/ADM/S/658/VOL.11/112. The research was conducted in conformity with the standard of human experimentation and with the Helsinki Declaration of 1975, as revised in 2000. Patient information was treated with utmost confidentiality. The Study participants bears no financial burden. Informed consent was obtained from all participating subjects in the IDP using standard protocol after the objectives and the procedures were explained to the participants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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