EVALUATION OF THE ANALYTICAL PERFORMANCE OF XPERT MTB/RIF ASSAY IN THE DIAGNOSIS OF TUBERCULOSIS AMONG HIV SEROPOSITIVE AND SERONEGATIVE PATIENTS IN ABEOKUTA, SOUTHWESTERN, NIGERIA

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ABSTRACT

Background: Tuberculosis (TB) remains a major health problem particularly in low/middle-income countries(LMIC). Achieving accurate diagnosis of TB disease is more complex in HIV patients than in subjects with normal immunity. The spread of multidrug resistant TB (MDR-TB), the detrimental convergence with HIV infection and the unavailability of rapid diagnostic tools have contributed to the failure of global control of TB. In a prospective clinical validation, we assessed the diagnostic accuracy of Xpert MTB/RIF assay among HIV seropositive and seronegative patients in Abeokuta.

Methods: A total of five hundred and four (504) sputum samples from deep cough were collected from TB suspects who came to register for the first time at public health centres in Abeokuta according to the guidelines of the National TB and Leprosy Control Programme. The sputum samples were examined using Zeihl-Neelsen staining method, Xpert MTB/RIF assay and their results compared to composite reference standard Lowenstein Jensen(LJ) culture. Detection of rifampicin resistance by Xpert MTB/RIF was compared to the LJ proportion method. Patients were screened for HIV infection by using Determine, Unigold and Stat-Pak HIV test kits and confirmed by Western blot technique. Socio-demographic data of the patients were obtained by administering questionnaires and conducting personal interviews.

Results: Out of 504 patients, 7.9% prevalence of HIV infection was recorded. Of 289 males, 11.4% were seropositive while 7.9% of the 215 females were seropositive. Significantly highest HIV seropositivity rate of 3.6% was recorded among age group 35-49 years when compared to other age groups (P< 0.05). Of 313 culture positive TB cases, Xpert MTB/RIF assay accuaretly detected 310 previously undiagnosed TB cases, resulting in a sensitivity, specificity, positive predictive value and negative predictive value of 98.4%(95% CI 97.5 – 99.4%), 95.9%(95% CI 94.8 – 98.5%), 98.8%(95% CI 92.6- 99.2%) and 94.0% (95% CI 88.5 – 97.2%) respectively. Only 242(77.3%) of 313 active TB cases were ZN smear positive. For rifampicin resistance detection, sensitivity of Xpert MTB was 97.2% (95% CI 96.8 – 98.7%) ; specificity was 99.6%(95% CI 92.6 – 99.8%); positive predictive value was 95.2%(95% CI 92.6 – 97.8%) and negative predictive value was 99.8% (95% CI 88.5 – 99.9%).

Conclusion: In HIV seropositive and seronegative in our population with high clinical suspicion of TB, Xpert MTB/RIF performed well for TB diagnosis and rifampicin (RIF) resistance and outperformed smear microscopy.

Keywords: TB, HIV, Sputum, Diagnosis, Xpert MTB/RIF

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INTRODUCTION

Tuberculosis (TB) constitutes a serious threat to public health in the world, with nearly 10 million new cases and 1.7 million deaths annually(1). The incidence of multidrug resistant (MDR) TB is increasing with almost 0.5 million reported new cases in 2010 (2). The global control of TB and MDR-TB has created an urgent need for timely and effective diagnostic method.

Globally, around 2 million people are infected with *Mycobacterium tuberculosis* (1). Every year almost 9 million people develop active disease and 2 million people lose their lives to the illness. Active tuberculosis is predominantly pulmonary in nature. The route of transmission of pulmonary TB is through air, which makes this a highly transmissible disease. However, given the infectious nature of pulmonary TB, fast and accurate diagnosis is an important element of TB treatment and control.

Mycobacterial culture is a sensitive but slow way to diagnose TB. To halt the disease spread, it is essential that TB- particularly TB that is resistant to several treatment drugs (multi-drug-resistant, or MDR-TB)-is diagnosed quickly. Recently, several nucleic acid amplification technology (NAAT) tests have been developed that rapidly detect *M.tuberculosis* DNA in patient samples and look for DNA changes that make *M. tuberculosis* drug resistant (4). In December 2010, the World Health Organization(WHO) endorsed Xpert MTB/RIF(Cephied, Sunnyvale,CA,USA)- an automated DNA test that detects *M.tuberculosis* and rifampicin resistance(an indicator of MDR-TB) within two hours- for the investigation of patients who might have TB, especially in regions where MDR-TB and HIV infection are common (5).

Molecular detection of TB and rpo B gene mutations associated with RIF resistance greatly speeds the diagnosis of both drug susceptible and MDR tuberculosis. The rapid detection of MTB and RIF resistance allows the physician to make critical patient management decisions regarding therapy (6).

Lack of a rapid, sensitive, and specific diagnostic test for TB, especially in resource poor countries, greatly complicate TB control worldwide. Xpert MTB/RIF, a new diagnostic method based on real time pcr offers advantage compared to microscopy, which has low sensitivity, and culture which has a long turnaround time. However, due to paucity of information on the impact of Xpert MTB/RIF in the diagnosis of TB in this part of the world, this study was therefore undertaken to compare the performance of Xpert MTB/RIF with acid fast bacilli(AFB) smear microscopy and culture(gold standard) in the diagnosis of TB among HIV seropositive and seronegative patients in Abeokuta, southwest Nigeria.

MATERIALS AND METHODS

Areas of Study

A cross-sectional study was designed based on the most recent WHO guidelines for surveillance of drug resistance in TB(7).This study was conducted in the chest unit of Sacred Heart Hospital,Lantoro,Abeokuta, Federal Medical Centre,Idi-Aba,Abeokuta,and General Hospital,Ijaye,Abeokuta,Ogun State, Nigeria. Abeokuta the capital of Ogun State,Nigeria,lies on latitude 7^0 15N and longitude 3^0 25E. The city, which is about 81 km South west of Ibadan and 106 Km North of Lagos, is located on an altitude of about 159 m above sea level. It has a hot humid weather with annual rainfall of 963.3 mm (8). Its population is estimated to be 6,740,843 according to the 2006 census report(8).

Ethical Consideration: Approval was obtained from ethical committee of the hospitals for the study to be carried out.Informed consent was also obtained from the patients as they visited the clinics.

Sample Selection and Collection: Patients diagnosed for pulmonary tuberculosis on the basis of chest x-ray were enrolled in the study.Systematic sampling method was carried out by selecting every third patients with suspected active tuberculosis(New case patients).Patients were considered as new case patients(NCs) if they had never received treatment for TB or had taken anti-TB drugs for <1 months(9).Each selected subject was instructed to produce and submit 3 sputum specimens(from a deep cough) in a standard screw-capped leak-proof sputum container with specific clinic identification number, within 2 consecutive days.The first sputum specimen was obtained on the first contact with the centre(spot specimen) while the second specimen was an early-morning specimen produced at home after cleaning the mouth with water.The third specimen was another spot specimen produced at the clinic when the early morning specimen was submitted.The three specimens were processed at the same time. About 5mls of venous blood was also collected from the patients for HIV serology.

Sample Analysis: Sputum samples were decontaminated using N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) using the method adopted by Granich *et al*.,2005(10). Smears were

made from the sediment pellet on a clean glass slides and stained using Ziehl-Neelsen(ZN) staining method.50µl of the decontaminated sputum samples were cultured on Lowenstein Jensen(LJ) slants in triplicate and sealed with parafilm to prevent contamination and dessication.The inoculated slants were incubated in MSE incubator model 4032 at 37^0C under CO_2 for eight weeks. Each isolate was culturally and biochemically characterized by ZN staining, Niacin accumulation test, Catalase test, growth on PNB medium,arysulphatase test and nitrate reduction test(11).The *M.tuberculosis* isolates were tested against, rifampicin(40µg/ml) on Lowenstein Jensen medium by proportion method(12). Quality control strain-H37RV was included in each batch of testing. Rifampicin drug crystal was obtained from Sigma (St. Louis, MO, USA).

X-pert MTB/RIF Assay

The Xpert MTB/RIF assay is a hemi-nested real time PCR method that amplifies the 81-bp region of the RIF-resistance-determining region of the *rpo*B gene, position 507-533. A sample reagent buffer containing NaOH and isopropanol was added in a 2:1 ratio to the processed sputum ensuring a final volume of at least 2 mls. After 15 minutes of incubation with intermittent hand mixing, 2mls of the liquefied inactivated sample was added to the cartridge that contains the wash buffer, reagents for lyophilized DNA extraction and PCR amplification, and fluorescent detection probes(five for the rpoB gene and one for an internal control,*Bacillius globigii* spores). The cartridge was then placed in the instrument(manufactured by Cepheid GeneXpert® system, Sweeden) module and results were automatically generated within 2 hours and reported as M.tb- detected or – not detected (with semiquantification) and RIF sensitive or resistant.

HIV Testing

HIV counseling(pre and post) and testing was done on consented patients by following national algorithm. Screening test was done using determine HIV kit, reactive sera were further tested using Unigold HIV kit. Stat-Pack was used for inconclusive result and serves as tie-breaker. HIV confirmatory test was performed by western blot technique using immunetics(Qualicode^{TM}HIV-1/2) kit. All tests were performed and interpreted according to manufacturers' instructions.

Data Analysis: Analyses of all data obtained were performed by using STATA 10(StataCorp) version 10.1.The x^2 test was used to calculate p value when appropriate.Sensitivity,specificity, and positive and negative predictive values were calculated using culture as gold standard.

RESULTS

Age versus HIV seropositivity is shown in Table 1.0 .Highest HIV seropositivity rate of 3.6% was recorded among age group 35-49 years, while <5years recorded no incidence.

Figure 1.0 shows sex distribution of HIV infection. HIV seropositive males recorded significantly higher rate of 11.4% as against 7.9% recorded by HIV seropositive females($P<0.05$).

Table 2.0 and 3.0 show the Xpert MTB/RIF Vs ZN and culture status and their comparative performances. Xpert MTB/RIF detected *M.tuberculosis* in 242 out of 243 ZN positive and 68 out of 261 ZN negative patients. Xpert MTB/RIF detected *M.tuberculosis* in 310 out of 313 culture positive patients. Xpert MTB/RIF had higher sensitivity of 98.4% when compared to 59.1% recorded by ZN. The specificity of Xpert MTB/RIF and ZN was found to be 95.9% and 79.8% respectively, while their PPV were 98.8%(95%CI 92.6%-99.2%) and 65.2%(95%CI 54.8 – 68.9) respectively. Xpert MTB/RIF recorded the higher NPV of 94.0%(95% CI 92.6% - 99.2%) when compared to 80.7%(95% CI 76.4% - 84.3%) recorded by ZN .

The distribution of TB/HIV co-infection by different methods is shown in table 4.0. Among HIV seropositive patients, Xpert MTB/RIF had significantly higher positivity rate of 40%, when compared to 17.5% rate recorded by ZN.

Table 5.0 shows the performance characteristics of xpert MTB/RIF compared to drug susceptibity testing(DST) for rifampicin by Lowenstein-Jensen proportion method(reference standard). Out of 310 *M.tuberculosis* positive patients detected by Xpert MTB/RIF, 35(11.3%) were RIF resistant and 275(88.7%) RIF sensitive resulting in 97.2%(95%CI 96.8% - 98.7%) sensitivity and 99.6%(95%CI 98.1%-99.8%)specificity with 95.2%(95% CI 92.6%-97.8%)PPV and 99.8%(95% CI 88.5% - 99.9%) NPV .

TABLE 1 : AGE DISTRIBUTION OF HIV INFECTION

Age	HIV Seropositive n (%)	HIV Seronegative n (%)
< 5 years	0(0.0)	3(0.6)
5 – 19 years	2(0.4)	50(9.9)
20-34 years	14(2.8)	169 (33.5)
35 – 49 years	18 (3.6)	110(21.8)
50 - 64 years	5 (0.9)	80(15.9)
65 - 79 years	0 (0.0)	40 (7.9)
80 - 94 years	1(0.2)	12(2.4)

(P<0.05)

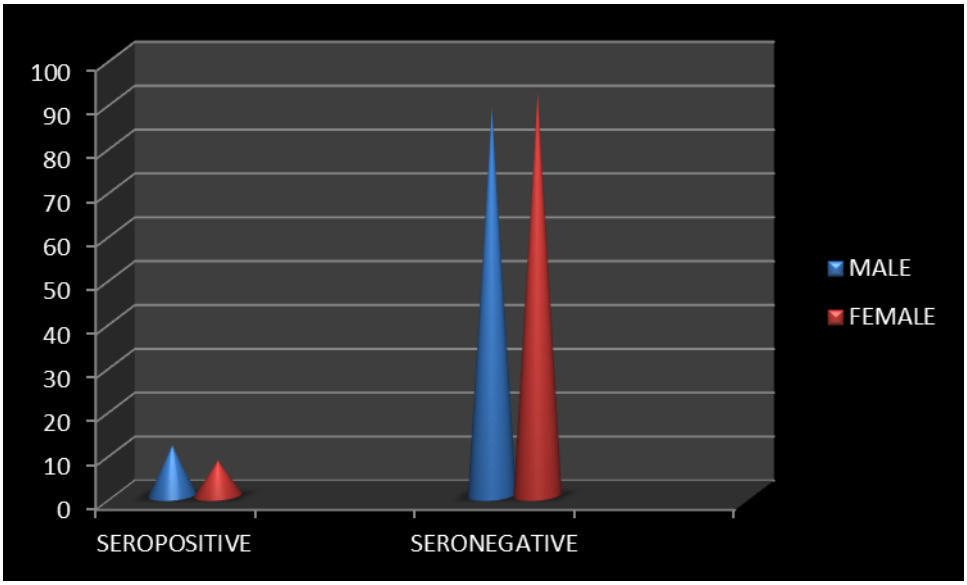

Figure 1.0 : Sex distribution of HIV Infection.

Table 2.0 : Xpert MTB/RIF Vs ZN and Culture Status(reference standard)

Xpert MTB/RIF	ZN	ZN	Culture	culture
	Positive (N=243)	Negative (N=261)	Positive (N=313)	Negative (N=191)
	n(%)	n(%)	n(%)	n(%)
MTB detected	242(99.6)	68(26.1)	310(99.0)	0(0)
MTB not detected	1(0.4)	193(73.9)	3(0.9)	190(99.5)
Total	243	261	313	190

Table 3.0 : Comparative Performance of ZN AND XPERT MTB/RIF PTB Diagnostic tests

Diagnostic Tests	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
ZN	59.1(58.5-59.7)	79.8(72.3-85.8)	65.2(54.8-68.9)	80.7(76.4-84.3)
Xpert MTB/RIF	98.4(97.5-99.4)	95.9(94.8-96.5)	98.8(92.6-99.2)	94.0(88.5-97.2)

Key : CI- Confidence Interval , PPV – Positive Predictive Value, NPV – Negative Predictive Value.

Table 4.0 : Distribution of TB/HIV co-infection by different methods

		PTB diagnostic methods		
HIV Status	N	ZN	Xpert MTB/RIF	Culture
Seropositive	40	7(17.5)	16(40.0)	15(37.5)
Seronegative	464	236(50.9)	294(63.4)	298(64.2)

Table 5.0: Performance Characteristics of Xpert MTB/RIF compared to Drug Susceptibility Testing (DST) for Rifampicin by Lowenstein Jensen(LJ) Proportion Method.

DST Method	N	RIF Resistant n(%)	RIF Sensitive n(%)	Sensitivity% (95% CI)	Specificity% (95% CI)	PPV% (95% CI)	NPV% (95% CI)
Xpert MTB/RIF	310	35(11.3)	275(88.7)	97.2(96.8-98.7)	99.6(92.6-99.8)	95.2(92.6-97.8)	99.8(88.5-99.9)
LJ Proportion	313	36(11.5)	277(88.5)	100(83.9-100)	100(89.8-100)	100(89.2-100)	100(96.8-100)

DISCUSSION

Rapid and effective diagnosis of patients suspected of having TB remains a challenge. The conventional TB diagnostic techniques, including methods based on direct microscopic examination by Ziehl-Neelsen staining and culture(12) have limitations and are thus not always helpful in diagnosing TB(13). Smear microscopy alone, although cheap and easy to perform, has a highly false-negative result and cannot identify drug- resistance (14). Currently. Only 28% of expected incident cases of tuberculosis are detected and reported as smear positive(15). Culture is more sensitive than smear microscopy, culture generally provides results in at least 2-8 weeks(16) requires biosafety measures, and needs specialized laboratory personnel.

One of the latest assay, Xpert MTB/RIF assay, was evaluated in this study. The assay detects M.tuberculosis and rifampicin resistance by PCR amplifying five overlapping probes complementary to rifampicin resistance-determining region(RRDR) of the Mtb rpoB gene and subsequently probes this region for mutations that are associated with RIF-resistance(17).

The HIV prevalence of 7.9% obtained in this study is higher than 3.9% reported by FMOH(2009). Kamran *et al.* (18) reported 12.0% HIV prevalence in Ontario, Canada. However, the high HIV prevalence obtained was due increase transmission rate in Abeokuta. Risk factors such as prostitution, high-risk practice among itinerary workers and unprotected sexual behavior and improper blood screening before transfusion largely contribute to HIV spread.

The 11.4% HIV prevalence rate obtained from males is significantly higher than 7.9% recorded by females ($P<0.05$). Similarly, CDC statistics showed that in 2008, 73% of persons living with HIV infection were male adults or adolescents in Columbia (19). Beyond the statistics of sex-based differences in the infection rates, there are profound differences in the underlying causes and consequences of HIV/AIDS infections in male and female, reflecting differences in biology, sexual behavior, social attitudes and pressure, economic power and vulnerability.

Highest HIV prevalence of 3.6% obtained among age group 35 – 49 years is similar to the some reports (20),that people between 35 and 45 years of age are most affected with HIV/AIDS. High HIV prevalence among age group 31- 40 years in has been reported(22). The reason for this finding is that condom use is not common among this age group. It has been reported that

more than 80% of men and more than 70% of women under 25 years old use condom(22). He also reported that knowledge about HIV/AIDS is lowest among people older than 40 years.

A key advantage of Xpert MTB/RIF assay over smear microscopy is the simultaneous assessment to RIF-resistance. The xpert assay was highly sensitive(97.2%) and specific(95.7%) for RIF resistance. This agrees with some reports (23,24). However, the assay can detect RIF resistance by only probing the *rpoB* gene, and the mutation points in approximately 5% RIF-resistant Mtb isolates occur outside core *rpoB* gene region(24), so it would not be identified by Xpert MTB/RIF assay.

Futhermore, the sensitivity of smear microscopy decreased significantly in HIV-infected compared to uninfected patients. The same pattern was seen in xpert MTB/RIF assay. However, the sensitivity of Xpert MTB/RIF assay was higher than that of smear microscopy.

CONCLUSION

The Xpert MTB/RIF assay fulfills the requirements of rapidly and effectively diagnosing TB and RIF- resistance among HIV infected and uninfected patients, particularly in patients with high pre-test probability of TB.

The implementation of Xpert MTB/RIF will need to be assessed for appropriate management of quality assurance, the adequacy of clinic resources (infrastructural and human), data collection, acceptance by patients and health care providers, and affordability, especially in resource – constrained settings.

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