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ORIGINAL ARTICLE

Occurrence of Rifampicin Resistant and HIV Co-Infection Tuberculosis in Ikere-Ekiti, Nigeria

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ABSTRACT

Introduction: Tuberculosis is a major cause of disability and death worldwide. One of the targets of Sustainable Goal Development for 2030 is to put an end to tuberculosis epidemics. This study accessed the occurrence of tuberculosis, its co-infection with HIV and rifampicin resistance in the study area, as well as the reliability of acid fast bacilli (AFB) test in tuberculosis (TB) diagnosis. **Methods:** The study, which was made up of both retrospective and prospective TB investigations, was carried out at the State Specialist Hospital, Ikere–Ekiti, Nigeria, covering April 2014 to March 2017. Sputum samples from 1227 individuals with suspected cases of TB, made up of 496 (40.42%) males and 731 (58.11%) females, were analysed for TB by smear microscopy for AFB and molecular determination using GeneXpert machine. **Results:** A total of 141 (11.49%) individuals were diagnosed tuberculosis positive using the GeneXpert machine, while 78 (6.36%) tested positive by AFB technique. Eleven (7.8 %) of the 141 tuberculosis-positive cases were rifampicin resistant; also a tuberculosis - HIV co-infection rate of 25.53% was obtained. The risk factors associated with TB in the study were smoking, alcoholism, over-crowding and HIV co-infection. Using the GeneXpert as a standard, the AFB's sensitivity, specificity, positive predictive value (PPV) and negative predictive value were 43.26, 98.43, 78.20 and 93.04% respectively. **Conclusion:** The high occurrence of rifampicin resistant tuberculosis and HIV-TB co-infection are of serious concern. The low sensitivity and PPV values, as well as its inability to detect drug resistant TB, undermine the reliability of AFB.

Keywords: HIV-TB co-infection, Tuberculosis, Rifampicin resistance

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INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis and ranks along side with Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) as the leading cause of death worldwide (1). In the year 2017, a total of 10.0 million people with new cases of tuberculosis were reported worldwide, a total of 1.6 million deaths resulted from the disease, and two-thirds of the cases were recorded in eight countries namely India, China, Indonesia, Pakistan, Philippines, Nigeria, Bangladesh and South Africa (1). Tuberculosis remains a major cause of death despite availability of effective treatment (2). One of the targets of Sustainable Goal Development for 2030 is to end tuberculosis epidemics (1).

The Human Immunodeficiency virus infection and tuberculosis often co-infect, forming a lethal combination, each enhancing the severity of the other thereby causing increased mortality (1, 3). An issue that needs global attention presently is the emergence of drug-resistant tuberculosis, which is on the high side in many countries of the world (1, 4).

Many factors contribute to poor treatment outcomes of tuberculosis patients, with HIV playing an important role. HIV co-infection is a very important factor of default and death in tuberculosis patients (5, 6). Delay in tuberculosis diagnosis and unknown HIV status have been identified as important factors of poor treatment outcome (7).

The objectives of this study were to: (a) investigate the occurrence of tuberculosis in Ekiti State Nigeria, (b) compare the efficacy of sputum smear microscopy for acid fast bacilli (AFB) detection to molecular determination using GeneXpert machine technique, in tuberculosis diagnosis, (c) determine the prevalence of Rifampicin resistant tuberculosis and HIV co-infection, and (d) determine the risk factors associated with tuberculosis in Ekiti State, Nigeria.

MATERIALS AND METHODS

The study entailed a comparative use of GeneXpert MTB/RIF machine and sputum microscopy in tuberculosis diagnosis at the Chest Clinic Department of the State Specialist Hospital, Ikere-Ekiti, Nigeria. The hospital serves one of the referral centres for diagnosis and management of both tuberculosis and HIV infection in Ekiti State, Nigeria.

A total of 1227 patients were investigated, made up of 805 and 422 for retrospective and prospective studies, respectively. The retrospective study was carried out by retrieving information from hospital records on cases of tuberculosis diagnosed using the GeneXpert machine and AFB technique. The demographic information of the patients namely, age, sex, and HIV status were obtained. The records of 805 patients that underwent tuberculosis diagnosis between April 2014 and March 2016 were reviewed.

For the prospective study, a total of 422 patients visiting the Chest Clinic of the State Specialist Hospital Ikere-Ekiti, between April 2016 and March 2017, were enrolled for this study, following informed consent. Patients who did not sign the informed consent form and those who declined to participate were excluded from the study. Sputum of suspected TB patients were collected using sterile wide mouth sample container for (a) molecular determination of M. tuberculosis (MTB) using the GeneXpert machine and (b) smear microscopy for acid fast bacilli (AFB) detection. Structured questionnaire was administered to obtain socio demographic data and to assess the predisposing factors of tuberculosis among the patients, such as HIV, smoking, alcoholism, diabetes, malnutrition and overcrowding.

Molecular detection of M. tuberculosis was carried out by the Xpert(R) MTB/RIF assay (8, 9). Sputum samples were aseptically collected in a wide mouth sample container and appropriately labelled, 2 mL of the GeneXpert MTB/RIF reagent was added to the sample after which the lid of the container was replaced and shaken vigorously for 10-20 times and left to incubate at room temperature for 10 minutes. Thereafter, 2 mL of each sample was transferred into appropriately labelled cartridge. The barcodes of loaded cartridge was scanned before being placed into the appropriate module of the GeneXpert machine.

Smear microscopy for acid fast bacilli (AFB) detection M. tuberculosis was carried out by making a smear of sputum to cover 2/3 of the slide, thin enough to be able to read through. It was left to air dry and then heat fixed. The slide was placed on a staining rack across a sink. The entire slide was flooded with Ziehl-Neelsen Carbolfuchsin which was then heated intermittently to produce steam and maintained for 3-5 min. The slide was rinsed off in gentle stream of running water until all excess stain was removed. The slide was then flooded with 3% vol/vol acid-alcohol for 30 seconds to decolorize, after which the slide was rinsed, drained and counterstained with methylene blue for 45 seconds. Following this, the slide was then rinsed, drained and observed under the microscope using the 100x oil immersion objective for Acid-fast organisms (10, 11).

The data generated were subjected to statistical analyses: (a) Chi square statistic and one way ANOVA were used to determine significant difference between variables, (b) The model selection Log-linear Hierarchical algorithm was used to evaluate associations among variables; using the SPSS V-20 software. The effectiveness of AFB compared to GeneXpert MTB was determined by Bayes' theorem (12, 13) using the following parameters: (a) True positive (TP), (b) True negative (TN), False positive (FP), (c) False negative (FN), (d) Sensitivity, true positive rate (TPR) = TP/(TP + FN), (e) Specificity, true negative rate (TNR) = TN/(FP + TN), (f) Positive predictive value (PPV) = TP/(TP + FP), (g) Negative predictive value (NPV) = TN/(FN + TN), (h) False positive rate (FPR) = FP/(FP + TN), (i) False negative rate (FNR) = FN/(TP + FN), (j) False discovery rate (FDR) = FP/(TP + FP), (k) Accuracy (ACC) = (TP + TN)/(TP + FP + FN + TN), (l) The Harmonic mean of precision and sensitivity, F1 score = 2TP/(2TP + FP + FN), (m) Positive likelihood ratio (LR+) = TPR/FPR, (n) Negative likelihood ratio (LR-) = FNR/TNR, and (o) Diagnostic odds rate = LR+/LR-.

RESULTS

A total of 1227 individuals with suspected tuberculosis cases, attending State Specialist Hospital, Ikere-Ekiti, Nigeria, were tested for tuberculosis using GeneXpert and Acid Fast Bacilli (AFB) techniques. The subjects were 4 - 97 (41.80 ± 14.76) years old, made up of 496 (40.42%) males and 731 (59.58%) females. The study consisted of 805 and 422 subjects for retrospective and prospective studies respectively. Six hundred and fifteen (50.12%) of the subjects were HIV positive.

One hundred and forty one (11.49 %) out of the total 1227 subjects were diagnosed tuberculosis positive by the GeneXpert machine, which was found to be significantly (p<0.001) higher than the 78 (6.37%) tested positive by AFB technique (Table I).

Table I: Comparison of results of GeneXpert MTB test and Acid Fast Bacilli (AFB) tests

		GeneXpert MTB Test			χ^2
		Positive	Negative	Total	
Acid Fast Bacilli Test	Positive	61	17	78	191.403 (p < 0.001)
	Negative	80	1069	1149	
	Total	141	1086	1227	

AFB's Sensitivity = 43.26 %
Specificity = 98.43 %
Positive predictive value = 78.20 %
Negative predictive value = 93.04 %

The effectiveness of the AFB test compared to GeneXpert technique in TB diagnosis in this study showed that AFB sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 43.26, 98.43, 78.20 and 93.04%, respectively. The false discovery rate, accuracy, the Harmonic mean of precision and sensitivity, and the Diagnostic odds rate were 0.218, 0.922, 0.557 and 48.15 respectively.

Higher TB infection rate was reported among males than females; male subjects reported 15.12% and female 9.03% by the GeneXpert, while AFB recorded 7.86 and 5.34% positive for male and female respectively. Majority (100) of tuberculosis positive patients were 26-50 years of age (Table II). The result obtained from GeneXpert technique showed that a total of 36 out of 141 positive tuberculosis patients had documented HIV positive results, representing 25.53 % TB-HIV co-infection rate; while the AFB techniques showed that 24 out of 78 TB patients were HIV positive, representing 30.77 % TB-HIV co-infection rate. A total of 11 (7.80 %) out of the 141 positive tuberculosis cases detected by the GeneXpert technique were rifampicin resistant (Table II).

Tuberculosis infection rate was found to be significantly associated with smoking, alcohol consumption, overcrowding, occupation and level of education of the patients, indicating the risk factors. High TB infection rates were obtained among unskilled workers and people without education (Table III).

The tuberculosis infection reported over a period of 36 months, showed monthly fluctuations throughout the period of study (Figure I).

DISCUSSION

Tuberculosis is one of the top 10 causes of death in human beings and it is the leading cause of death resulting from a single infectious agent (1). The study compared the efficiency of GeneXpert machine and sputum smear microscopy testing for AFB in the diagnosis of tuberculosis, as well as, identified the risk factors associated with tuberculosis in Ekiti State Nigeria. In most laboratories in Nigeria, diagnosis of tuberculosis is based on the smear microscopy because the technique is considered to be fast and affordable. However, the AFB technique has been reported to be limited due to its

Table II: Distribution of frequency of tuberculosis along demographic indices and HIV status of patients

Characteristics		GeneXpert				AFB			
		Positive	Negative	Total	P value	Positive	Negative	Total	P value
Sex	Male	75(15.12)	421(84.88)	496	0.046*	39 (7.86)	457(92.14)	496	0.908
	Female	66(9.03)	665(90.97)	731		39 (5.34)	692(94.66)	731	
Age	1-25	15(16.48)	76(83.52)	91		5 (5.49)	86(94.51)	91	
	26-50	100(11.78)	749(88.22)	849	0.213	59(6.95)	790(93.05)	849	0.793
	51-75	23(9.27)	225(90.07)	248		10(4.03)	238(95.97)	248	
	76-100	3(7.69)	36(92.31)	39		4 (10.26)	35(89.74)	39	
HIV	Positive	36(5.85)	579 (94.15)	615	0.011*	24(3.90)	591(96.10)	615	0.455
	Negative	105(17.16)	507(82.84)	612		54(8.82)	558(91.18)	612	
Rifampicin	Resistant	11 (7.80)	ND	11		ND	ND		
	Sensitive	130 (92.20)	ND	ND		ND	ND		

Data from retrospective and prospective studies
Percentages in parentheses
*Significant association
ND = Not done

Table III: Risk factors associated with occurrence of tuberculosis among patients attending State Specialist Hospital Ikere-Ekiti

Characteristic		GeneXpert				AFB			
		Positive	Negative	Total	P value	Positive	Negative	Total	P value
Education	No Education	7(38.89)	11(61.11)	18	0.001*	3(16.67)	15(83.33)	18	0.186
	Primary	0(0)	28(100.00)	28		0(0)	28(100.00)	28	
	Secondary	20(20.20)	79(79.80)	99		11(11.11)	88(88.89)	99	
	Tertiary	21(7.58)	256(92.42)	277		15(5.42)	262(94.58)	277	
Occupation	Skilled	23(8.04)	263(91.96)	286	0.044*	15(5.24)	271(94.76)	286	0.926
	Semi skilled	20(17.09)	97(82.91)	117		12(10.26)	105(88.74)	117	
	Unskilled	5(26.32)	14(73.68)	19		2(10.53)	17(89.47)	19	
Smoking	Smokers	17(22.67)	58(77.33)	75	0.011*	13(17.33)	62(82.67)	75	0.005*
	Non smokers	31(8.93)	316(91.07)	347		16(4.61)	331(95.39)	347	
Alcohol Consumption	Yes	23(20.35)	90(79.65)	113	0.015*	15(13.27)	98(86.73)	113	0.027*
	No	25(8.09)	284(91.91)	309		14(4.53)	295(95.47)	309	
Overcrowding	Yes	12(42.86)	16(57.14)	28	0.047*	5(17.86)	23(82.14)	28	0.202
	No	36(9.14)	358(90.86)	394		24(6.09)	370(93.91)	394	

Data from prospective study only   Percentages in parentheses   *Significant association

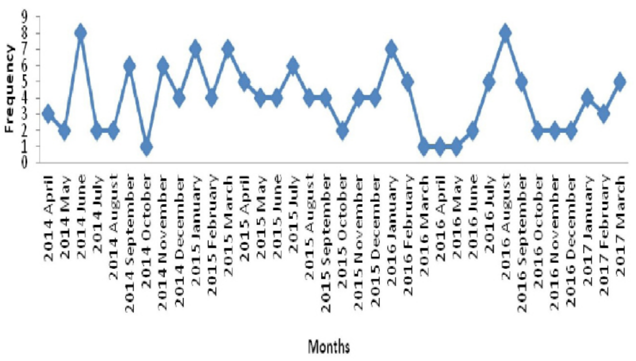

Figure 1: Graphical presentation of reported cases of tuberculosis in State Specialist Hospital Ikere-Ekiti from April 2014 to March 2017

low and variable sensitivity and inability to detect drug resistant Mycobacterium tuberculosis (1).

The risk factors of tuberculosis observed in this study were smoking alcoholism, overcrowding and HIV infection. The present study, reported that 36 out of 141 (25.53 %) of the tuberculosis patients had documented HIV positive results, a TB-HIV infection rate that is far lower than the 60% and 86 % reported for global and African region respectively, by the WHO for the year 2017 (1). Smoking as a risk factor to tuberculosis infection has been well documented (1). The present study observed a low incidence of tuberculosis infection among smokers (22.67%), which is in tandem with the reports by some earlier workers (14, 15, 16); Espinal and co-workers (16) documented 21.8% TB infection among smokers in South Africa. However Singh et al. (17) reported a higher value of 40.3% in Malaysia. The low incidence of tuberculosis among smokers in Ekiti State, Nigeria, reported in this study may be due to the ongoing campaign by Government at all levels to crack down on tobacco smoking in the public, and the views of people in the society that see smokers as irresponsible people, thus young people are being discouraged from smoking. The significant association of tuberculosis with alcoholism reported in this study corroborates earlier report by Hernández-Garduño et al (18), who reported that alcohol consumption was a major contributor to the tuberculosis burden, particularly in the African Region.
The present study reported higher cases of tuberculosis among male folks than the females. In the year 2017, out of the 10.0 million worldwide reported new cases of tuberculosis, 5.8 million (58%) were male adults, 3.2 million (32%) were female adults, while 1.0 million (10%) were children (1). The gender variation may be due to the fact that males are often given to the lifestyle of smoking and alcoholism, which are risk factors of tuberculosis. Tuberculosis is considered as a disease of poverty, illiteracy and low occupation status (1). Low earning power predetermines feeds and feeding ratio and accommodation status. Overcrowding has been identified as factor encouraging tuberculosis spreading in rural communities, poor housing and town planning has been added to the list. According to the 2018 WHO global TB reports (1), evidence abounds that links ending TB with ending poverty, hence advocate for poverty elimination through food security and adequate housing, is essential to tuberculosis elimination worldwide. The findings in present study is in agreement with the earlier works of Farmers (19) and Ojiezeh et al (20), that reported unskilled workers and patients with little or no formal education to be at higher risk of TB infection than their educated counterparts.

The 0.4326 (43.26%) AFB sensitivity value obtained suggested that there were more false negative results produced by the AFB technique, i.e. many positive results were reported negative. However, a high specificity value of 0.9843 (98.43%) indicated that more people without the disease were cleared by the screening test and were declared negative by the AFB screening test. The positive predictive values (PPV) and negative predictive values (NPV) are the proportions of the values that are true positive and true negative, respectively (10, 11). From the results generated, the positive predictive value for the AFB test was found to be 0.7820 (78.20%) i.e. 78.20% of the samples with the disease were predicted to be positive of disease; while NPV value of 0.9304 (93.04%) indicated that 93% of the samples without tuberculosis were correctly predicted to be negative. The low sensitivity and PPV values undermine the reliability of AFB in detection of tuberculosis, while its high values of specificity and NPV makes the technique excellent in eliminating people that do not have the disease.

With The Harmonic mean of precision and sensitivity, F1 score of 0.557, which is closer to 1 than 0, and the diagnostic odds rate of 48.15, which is greater than one (21) indicated that the AFB test is still an effective technique in tuberculosis diagnosis.

This study emphasized on the need to embrace modern technology. The superior efficiency of the GeneXpertMTB/RIF machine over the AFB test, in diagnosis of tuberculosis was further applauded. GeneXpert has been reported to have higher performance in the diagnosis of pulmonary tuberculosis with sputum specimens than AFB method (1, 22). Using the GeneXpert MTB/RIF machine, latent cases of tuberculosis and extrapulmonary tuberculosis (23), as well as resistant infections are detected early and treated accordingly, thereby preventing the patients from being a reservoir of tuberculosis infection.

CONCLUSION

The present study showed that tuberculosis remains a disease of public health concern in Ekiti State, Nigeria, with male folks at a higher risk of infection, due to lifestyle such as smoking and alcoholism, as well as HIV infection, which are the major risk factors in TB infection. Hence, the need to step up the current

Tuberculosis controls strategies in the State. Efforts should be made by Government and non-governmental organizations to campaign against tuberculosis, and treatment of tuberculosis should be provided free or at a highly subsidised rate, so that targets of Sustainable Goal Development for 2030 in putting an end to tuberculosis epidemics can be achieved.

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