PRIMARY MULTI-DRUG RESISTANT TUBERCULOSIS AMONG HIV SEROPOSITIVE AND SERONEGATIVE PATIENTS IN ABEOKUTA, SOUTHWESTERN NIGERIA

Ejilude Oluwaseun, Akinduti Paul Akinniyi, Oluwadun Afolabi

Department of Medical Microbiology & Parasitology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.
**Corresponding Author: Ejilude Oluwaseun , seunejilude@yahoo.com, +2348055220754.

ABSTRACT

Background : The occurrence of drug multi–resistant *Mycobacterium tuberculosis* among the HIV seropositive patients has raised global public health concern. This study was, therefore, undertaken to determine the prevalence of multi-drug resistant tuberculosis(MDR-TB) among HIV seropositive and seronegative patients in Abeokuta, Nigeria.

Methods: A total of five hundred and four (504) sputum samples from deep coughs were collected from TB suspects who came to register for the first time at public health facilities in Abeokuta, Nigeria. Sputum samples were decontaminated using N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) and cultured on Lowenstein Jensen (L J) medium. Each isolate was culturally and biochemically characterized by standard methods and their drug susceptibility tests (DST) to first line anti-tuberculosis drugs (isoniazid, rifampicin, streptomycin and ethambutol) were performed by 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% was seropositive while 7.9% of the 215 females were seropositive. The occurrence(62.5%) of TB in HIV patients was found to be associated with HIV ($P<0.05$). The overall prevalence of multi-drug resistant *Mycobacterium tuberculosis* among TB patients was found to be 5.8%. MDR-TB was found to be

significantly associated with HIV seropositive patients having 32% rate when compared to HIV-seronegative rate of 2.2% (P<0.05).

Conclusion: From the study, TB prevalence was found to be high among the studied population , while MDR-TB was relatively high in TB patients especially among the HIV seropositive patients.

Keywords: sputum, PTB, MDR-TB, HIV.

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INTRODUCTION

Despite efforts to control the pulmonary tuberculosis (PTB) epidemic, there were an estimated 9.4 million incident cases of the disease worldwide in 2009 (1). The HIV epidemic and the emergence of anti-TB drug resistance represent serious threats for achieving the stop TB partnership's goal of eliminating TB as a public health problem by 2050 (2).

Multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB) strains, defined as strain that is resistant to isoniazid and rifampicin, cause most concern because morbidity and mortality rates are higher than in TB caused by drug-sensitive strains (1).MDR-TB pose an increasing challenge to international health(3),particularly in the context of HIV infection. The incidence of MDR-TB disease was estimated to be 0.5 million in 2007, with prevalence of as many as 2 million cases worldwide (4).

Drug resistance in a new TB case: Presence of resistant strain of *M.tuberculosis* in a newly diagnosed TB patient who has not previously been treated with TB drugs (or therapy of less than 1 month duration). These patients were likely to have been infected with a strain that was already drug resistant. These cases are sometimes referred to as "primary drug resistance" (5).Drug resistance in previously treated TB case: Presence of a resistant strain in a TB patient who has previously received at least I month of TB therapy. These cases are likely to have been initially infected with a drug-susceptible *M.tuberculosis* strain, but during the course of anti-tuberculosis treatment, drug resistance emerged (sometimes referred to as "secondary drug resistance") (5).

The HIV pandemic presents a massive challenge for global TB control.The prevention of HIV and TB, the extention of WHO Directly Observed Treatment Short-course (DOTS) programs, and a focus effort to control HIV-related TB in areas of high HIV prevalence are matters of great urgency(6). Timely identification of patients with MDR-TB enables rapid initation of adequate treatment, thus preventing the patient from spreading the disease and from acquiring further resistance (6).

However, due to some reported cases of MDR-TB in many parts of the world and in-view of the fact that no data exist on the prevalence of MDR-TB in Abeokuta, SouthWestern Nigeria, this study was therefore undertaken to determine the prevalence of primary MDR-TB in Abeokuta, southwestern Nigeria, so as to plan for effective MDR-TB management among patients especially those co-infected with HIV.

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 isoniazid(0.2µg/ml),rifampicin(40µg/ml),streptomycin(10µg/ml) and ethambutol(20µg/ml) on Lowenstein Jensen medium by proportion method(12). Quality control strain-H37RV was included in each batch of testing. All compounds were obtained from Sigma (St. Louis, MO, USA).

Definition of MDR-TB: We defined MDR strains according to the World Health Organisation definition of MDR-TB (1).Multi-drug resistance *M.tuberculosis* was defined as resistance to at least isoniazid and rifampicin.

Poly-drug resistance *M.tuberculosis* was defined as resistance to more than one drug but not isoniazid not isoniazid and rifampicin at the same time (1).

Mono-drug resistant *M.tuberculosis* was defined as resistance to only one drug (1).

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/IC version 10.1.The x^2 test was used to calculate p value when appropriate.

RESULTS

Patients recruitment started January,2011 and was completed in May,2011.Of 504 samples cultured, 69(13.8%) were positive,and 7(1.4%) were contaminated. Table 1 shows the prevalence rate of *M. tuberculosis* by age. Highest *M.tuberculosis* prevalence rate of 6.6% was found among age group 20 – 34 years, while the least rate of 0.6% was recorded by age group 65-79 years.

Table 1. Prevalence rate of MTB by age

Age group	Positive rate n(%)	Negative rate n(%)	NTM n(%)
Less than 5yrs	0(0.0)	3(0.6)	0(0.0)
5-19yrs	5(1.0)	47(9.4)	0(0.0)
20-34yrs	33(6.6)	147(29.4)	3(0.6)
35-49yrs	23(4.6)	103(20.6)	2(0.4)
50-64yrs	5(1.0)	78(15.6)	2(0.4)
65-79yrs	3(0.6)	36(7.2)	1(0.2)
80-94yrs	0(0.0)	13(2.6)	0(0.0)
Total	69(13.8)	427(85.4)	8(1.6)

$p<0.05$; Key: NTM – Non Tuberculos Mycobacteria

The prevalence of HIV infection was found to be 40(7%) . HIV seropositive males recorded significantly higher rate of 17.3% as against 8.8% recorded by HIV seropositive females (P<0.05). Smokers recorded significantly higher rate of 44.0% when compared to 7.1% recorded by non-smokers(P<0.05). The occurrence of TB among HIV patients was found to be associated with HIV infection (P<0.05)(Table 2). The overall prevalence of multi-drug resistant M. tuberculosis among TB patients was found to be 4(5.8%).MDR-TB was found to be significantly associated with HIV seropositive patients having a rate of 32% when compared to HIV seronegative rate of 2.2%(Table 3).

Table 2. Factors contributing to prevalence of pulmonary mycobacterium tuberculosis(PMTB)

	PMTB positive				
Factors	**N**	**n**	**(%)**	**X^2**	**P value**
Sex					
Male	289	50	17.3	5.98	<0.05
Female	215	19	8.8		
Smoking					
Smokers	82	36	44.0	14.71	<0.05
Non-Smokers	422	30	7.1		
HIV					
Seropositive	40	25	62.5	11.02	<0.05
Seronegative	464	44	9.5		

Keys: N,Number of sujects; n,number positive; X^2,Chi-square.

Table 3. Anti-tuberculosis drug resistance Versus HIV status

Resistance	HIV Seronegative		HIV Seropositive	
	n_1	(%)	n_2	(%)
All Sensitive	35	(79.5)	10	(40)
Monoresistance	5	(11.5)	4	(16)
Polyresistance	3	(6.8)	3	(12)
Multiresistance	1	(2.2)	8	(32)
Total	44	(100.0)	25	(100.0)

P<0.05

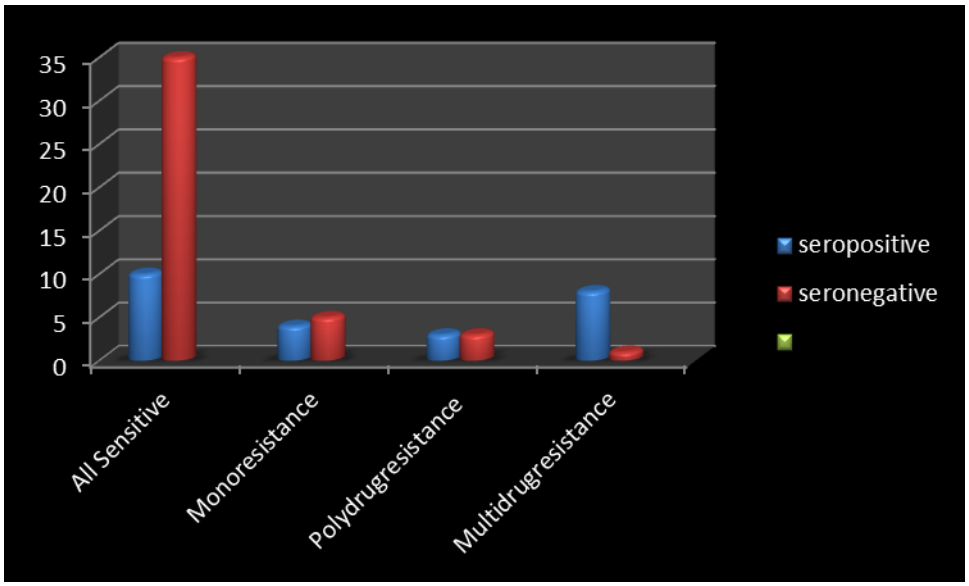

Figure 1. Anti-tuberculosis drug resistance Versus HIV status

DISCUSSION

Tuberculosis is a disease with deep social and economic roots. Low- income people with large families, living in dense urban communities with deficient housing conditions, have a high probability of becoming infected, developing active disease, and dying from TB (13).However, the prevalence of (13.8%) obtained in this study is in agreement with 13% reported by Cadmus *et al*.,(2001)(13) in Ibadan, SouthWestern, Nigeria. This suggests that TB is endemic in this locality probably due to poor TB control practice and non-compliance with preventive guidelines. The prevalence of PTB among different age groups was highest (6.6%) in age group 20 – 34 years. This finding is similar to 7.2% rate reported by Schaaf *et al.*, (14) among age group 25 – 35 years in Tygerberberg, South Africa. Accordingly, TB has its highest burden among young adults (15).

Significantly higher PTB rate of 44.0% found among smokers when compared to 7.1% rate recorded by non-smokers ($P<0.05$), is slightly lower than 50% PTB rate reported by Sarah Yang (17). Benolwitz (18) reported that smoking increases the risk of *M.tuberculosis* infection to disease, and the risk of death among TB patients. Condition that increase exposure to TB, such as overcrowding, long waiting times in clinics, sharing of facilities, and open multiple-bed wards, are common in medical institutions in Abeokuta, Nigeria.

The HIV prevalence of 7.9% obtained in this study is higher than 3.9% reported by FMOH(2009)(19).However, the high prevalence obtained was due to increase in 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 17.3% HIV prevalence rate obtained from males is significantly higher than 8.8% 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 (20). 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.

The emergence of multi-drug resistant *M.tuberculosis* is a problem of global concern.Present study gave an overall prevalence of 5.8% MDR-TB among HIV seropositive and seronegative patients in Abeokuta.This is in agreement with 6.0% reported by Beatriz *et a*l.(21) in Southwestern Columbia . This high MDR-TB rate could be due to spontaneous mutation of *M.tuberculosis* in the infected patients(22), or infection with drug resistant strains of *M.tuberculosis*(23).Moreover,poverty,ignorance and lack of quality health services could also be responsible for the high prevalence.There was a significant association between MDR-TB and HIV seropositive patients having 32.0% rate when compared to HIV seronegative rate of 2.2%($P<0.05$). However, this is in agreement with high prevalence of MDR-TB reported by Van Pie *et al.,* (2006)(4) in a community in Cape Town, South Africa. Futhermore, this result is contrary to the report of Dye (7) and Tracevska *et al.,* (11).Tracevka *et al*.,(11) reported that among all opportunistic diseases associated with HIV/AIDS, the distinctive feature of TB lies mainly in its airborne dissemination to other patients, to health-care workers and to the entire community. Poverty, social inequities, difficult access to public health situation that is hampering the international efforts aimed at controlling both diseases. Moreover, a complex biological interplay occurs between *M.tuberculosis* and HIV in the co-infected host that results in the worsening of both pathologies.HIV promotes progression of *M.tuberculosis* latent infection to disease and, in turn, *M.tuberculosis* enhances HIV replication, accelerating the natural evolution of HIV infection (23).

Recommendations and conclusion

The prevalence of multi-drug resistant *M.tuberculosis* was relatively high among TB patients especially HIV seropositive patients. This is a threat to TB control programme in Abeokuta, Nigeria. The high HIV seroprevalence in this TB patient population is of great concern in terms of patients management and public

health perspective. We recommend that DOTS and DOTS-plus TB control programs control programs should be integrated with well functioning HIV management programs to ensure that ARVs are widely administered to limit susceptibility to TB disease.Further study is needed to detect *M.tuberculosis* drug resistance genes.

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Competing interests

The authors declare that they have no financial or personal relationship(s) which may influenced them inappropriately in writing this article.

Authors' contributions

All authors have made substantial contribution to the manuscript.E.O. designed the study,as well as performing the experiments and writing the manuscript.A.P.A. provided the technical advised and assisted with data analysis and O.A. contributed to the study design, supervised the laboratory testing during the study and critically revised the paper.

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