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Mycobacterium tuberculosis and HIV Co-infections among Patients Attending a Nigerian Tertiary Health Center

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study is set to determine the prevalence of *Mycobacterium tuberculosis* and Drug Resistant-*Mycobacterium tuberculosis* in the HIV/AIDS endemic community.
Methods: A three -year retrospective study was conducted from November, 2013 to November, 2016 in Benue State University Teaching Hospital, Makurdi Nigeria. The subject criteria included all patients above 15 years, suspected of pulmonary TB, treatment naïve (new) and re-treated cases irrespective of HIV status. The procedures involved Cepheid Gene Xpert MTB/Rif system, Ziehl Nelseen staining and HIV serologic testing.
Results: In a total of two thousand nine-hundred and sixty-nine patients suspected of *Mycobacterium tuberculosis,* only 6.2% (N=183/2969) of patients was detected by Cepheid GeneXpert Mycobacterium/Rifampicin system, while Ziehl Nelseen staining alone detected 2.7%

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(N=83/2969) (X^2= 5.01; df=1; p=0.05). The age groups at risk were 21-35 years and 35-45 years with 54.6% and 27.4% respectively. Among the 183 patients, 12.0% (N=22/183) of patients were Rifampicin resistant. Seventy-seven per cent (N=17/22) of the Rifampicin Resistant *Mycobacterium tuberculosis* patients was for re treatment, while the remaining 23.0% (N= 5/22) was treatment naïve (X^2 =6.01; df=1; p<0.05). Seventy –one percent (N=130/183) of the patients detected with *Mycobacterium tuberculosis* were HIV sero-positive. All the Rifampicin Resistant *Mycobacterium tuberculosis* patients were HIV sero-positive. Among the Rifampicin sensitive patients; 68.3% (N=110/161) had HIV sero-positive status while 31.7% (N=51/161) was HIV sero-negative respectively.

Conclusion: The prevalence of 6.2% *Mycobacterium tuberculosis* with 12.0% Rifampicin Resistance *Mycobacterium tuberculosis* in the study was remarkable in young adult male patients in re-treatment. A hundred per cent total population of Rifampicin Resistance *Mycobacterium tuberculosis* patients were HIV sero- positive. Therefore, we recommend proper education of the youth, especially in sexual habit and availability of a more robust rapid multi resistance TB drug diagnostic system in order to capture MDR TB and XDR TB across the country, while the HIV program continues.

Keywords: Mycobacterium tuberculosis; rifampicin resistance; HIV status; Nigeria.

1. INTRODUCTION

Human Tuberculosis is a re-emerging infectious disease occasioned by HIV/AIDS epidemics and disrupted Tuberculosis (TB) control programs. TB is an opportunistic infection in HIV/AIDS and can occur at any point in the course of progression of HIV infection. The risk of developing TB rises sharply with worsening immune status. Compared with an individual who is not infected with HIV, a person infected with HIV has a 10 times increased risk of developing TB [1]. In sub-Saharan Africa, one-third or more of HIV infected people may develop TB [2]. As HIV infection progresses, CD4+ T-lymphocytes decline in number and function. These cells play an important role in the body defense against tubercle bacilli. Thus, the immune system becomes less able to prevent the growth and local spread of *M. tuberculosis.* Disseminated and extra pulmonary disease is more common. Even in HIV-infected patients, PTB is still the commonest form of TB [3].

Nigeria has a high burden of TB and Multi Drug Resistant tuberculosis (MDR-TB). Drug resistant-TB results in significantly higher mortality rates in HIV-infected patients than drug susceptible TB [4]. World Health Organization currently estimates high burden of MDR TB cases worldwide, particularly in China, India, South Africa, and in former Soviet Union countries [4]. The emergence of MDR-TB, among both the new smear positive cases and re-treatment cases has set back some of the progress made in TB control in Nigeria. Unfortunately, new difficult – to- treat MDR-TB and extensively or totally drug-resistant tuberculosis cases have been described. Many studies have found a strong relationship between MTB/ drug resistant TB and HIV/AIDS [1,2,3].

Mycobacterium tuberculosis can be diagnosed through the following techniques such as Ziehl-Nelseen staining, Fluorescence microscopy, liquid and solid media culture, and molecular techniques (GeneXpert, MTBDRplus, Multiplex – Nested PCR and others). More recent diagnostic tools include immune-based assays, skin patch test, rapid culture systems, line-probe assays, bacteriophage-based assays, molecular beacons and microscopic observation drug susceptibility assay [3]. The GeneXpert MTB/RIF introduced by WHO in 2011, is a novel automated molecular assay recently endorsed by the World Health Organization for the early diagnosis of both MTB infection and RIF resistance mutation in the rpoB gene. Laboratory diagnosis of HIV infection is established by Double ELISA technique (Determine and Unigold rapid tests) by serial testing strategy and any discordance in the result is settled by using SD Bioline or Statpack as a tie-breaker.

Nigeria has been ranked 10th globally among the high endemic countries for *TB,* while it also ranked 15th among the 27 heavy burden countries of the world for Multi –drug –resistant TB according to World Health Organization Fact sheet on Tuberculosis 2013 [3]. Multi-drug –resistant TB (MDR –TB) prevalence in Nigeria was recorded 2.9% by National Drug Resistance

Survey commissioned by Federal Ministry of Health, 2010 [3]. Nigeria has HIV national adult prevalence of 3.6% with estimated 3.3 million people living with HIV, ranking second among the HIV burden countries on the continent [5]. Benue state is reputed to be among the high HIV endemic states in Nigeria with 12.7% prevalence [5]. Some studies have recorded a strong correlation existing between HIV/AIDS and MTB. Therefore, the need to study the pattern of TB co-infection among the HIV/AIDS patients in Benue state.

2. SUBJECTS AND METHODS

2.1 Study Site and Population

Benue state is in the North Central Nigeria and has a teaming population of about 5 million with its state capital, Makurdi located at the northern part of the state. The state is surrounded by neighboring states; Enugu state and Kogi state on the East, Taraba state on the West, Cross River state on the South and Nassarawa state on the North. Benue state had a total population of 4,253,641 in 2006 census with an average population density of 99 persons per km² with land mass of 34,059 km². Agriculture forms the backbone of the Benue State economy, engaging more than 70 per cent of the working population. Makurdi harbors a tertiary medical center, namely, Benue State University Teaching Hospital and other secondary medical centers.

The 3 – year study was conducted from November, 2016 in Benue State University Teaching Hospital, Makurdi Nigeria. Approval by the Ethics and Research Committee of the hospital and written consent of the patients were sought before sample collection. Sample size was obtained by Benneth et al. 1991 with a local prevalence of MTB of 21.5% 3. However, this sample size of 259 was increased to 2969 (N X11) for adequate coverage and proper determinant. The subject criteria included all patients above 15 years, suspected of pulmonary TB, treatment naïve (new) and re-treated cases irrespective of HIV status. The nature of the study was explained to the subjects in the simplest language they best understood. Socio-demographic variables such as address, age, gender, TB treatment status and HIV status on initial visit were recorded.

A Chest clinic with its robust TB diagnostic center was established in November 2013 in Benue State University Hospital, Makurdi Nigeria. The TB diagnostic center comprises GeneXpert machine and Ziehl Nelseen (Acid Fast Bacilli) staining units. The clinical specimens included sputum only. The laboratory investigations were done free of charge courtesy of AIDS Prevention Initiative in Nigeria (APIN)/ PEPFAR & Ministry of Health.

2.2 HIV Serology

The HIV sero-status and confirmation of results are determined by rapid testing according to in-country and WHO HIV testing algorithm where three rapid tests; Determine HIV 1-1/2 (ABBOT Laboratories, illnois USA), SD Bioline HIV1/2 3.0 (Standard Diagnostics, Korea) and UniGold (Trinity Biotech, Ireland) are used. Laboratory diagnosis of HIV infection is established by double ELISA technique (Determine and Unigold) by serial testing strategy and any discordance in the result is settled by using SD Bioline or Statpack as a tie-breaker.

2.3 Smear Test

Specimens were digested and decontaminated of other bacteria by the standard *N*-acetyl-l-cysteine–NaOH–Na citrate method 6. A total of 100 µl of each decontaminated sample was placed on a microscope slide, dried, fixed and stained using ZN staining standard procedures. Slides were examined microscopically at ×1000 magnification. A stain was considered positive if it contained at least five bacilli per 300 fields [6].

2.4 GenXpert MTB/RIF System

The GenXpert MTB/RIF detects DNA sequences specific for *Mycobacterium tuberculosis* and rifampicin resistance by polymerase chain reaction. The Cepheid GeneXpert system is a platform for rapid and simple-to-use nucleic acid amplification tests (NAAT). The sample loaded cartridges are inserted into the Xpert device. The system isolates genomic material from the captured bacteria by sonication and subsequently amplifies the genomic DNA by PCR and identifies all the clinically relevant Rifampicin resistance inducing mutations in the RNA polymerase beta (rpoß) gene in the *Mycobacterium tuberculosis* genome in a real time format using fluorescent probes called molecular beacons. Results are obtained in 90

minutes and very little technical training is required to operate.

2.5 Analysis of Results

Laboratory results and responses from questionnaire were entered into Epi info version 6 statistical software for analysis; chi-square (X^2) was used to compare association between proportions. P-values <0.05 was considered significant at 95.0% confidence level. Findings were presented in tables and figures as applicable.

3. RESULTS

A total of two thousand nine hundred and sixty – nine patients were recruited for the 3 – year study, November, 2013 to November, 2016. The breakdown was as follows: From November, to December, 2013 there were only 21 patients tested with only one positive case of *Mycobacterium Tuberculosis.* In the year, 2014, there were 206 patients tested with 25 patients with detected MTB. In 2015, the number of patients tested was 657 with detectable MTB among 113. While in 2016, 2079 patients were tested with 44 patients with detectable MTB (Fig. 1).

A total of 183 (6.2%) of 2969 patients were positive for MTB by Gene Xpert, while only 44.0% (N=80/183) of the MTB cases was detected by AFB smear test. About seven percent (N=13/183) of the MTB patients were below 21 years including adolescents and children; 54.6% (N=100/183) were youths; 27.4% (50/183) were young adults; 10.9% (20/183) included adults and the elderly. About fifty percent MTB patients were male. One hundred and twenty-one (66.0%) of the patients were of HIV positive status while rest (44.0%) were of negative status (X^2 =5.84; df=1; p<0.05). There was a significant difference between 88.0% (N=161) Rifampicin sensitive and 12.0% (N=22) resistant MTB cases (X^2 = 7.23; df= 1; p<0.05) (Table 1).

Twenty –two (12.0%) of the 183 MTB patients were Rifampicin resistant. Seventy-seven percent (N=17) of the Rifampicin resistant MTB patients was for re-treatment while the remaining 23%(N=5) was for new treatment (X^2 =6.01; df= 1; p<0.05). Sixteen (72.7%) of the Rifampicin resistant MTB patients were Males compared to 27.3% (N=6) Females (Table 2). All the Rifampicin resistant MTB patients were HIV sero-positive.

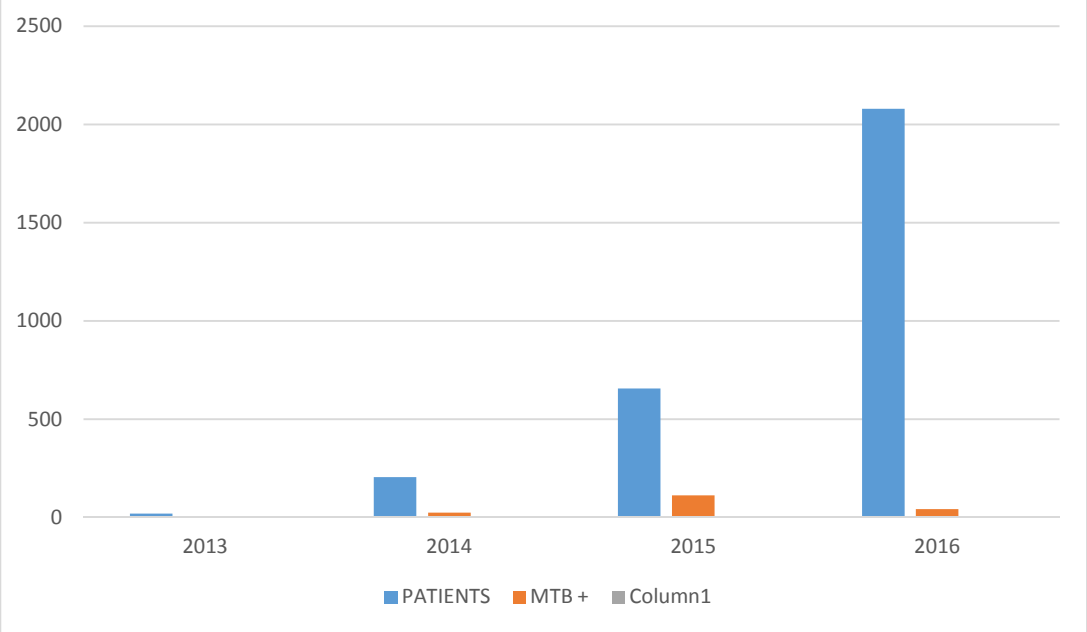

Fig. 1. MTB Detected Among the Patients in BSUTH Makurdi, Nigeria, November, 2013 to November, 2016

Table 1. Age groups of patients with MTB in BSUTH Makurdi Nigeria from November 2013 to November 2016

Age group	MTB	Rif. resistance	Sex :M/F	HIV status: +ve/-ve	Total (%)
<21	13	-	8/5	9/4	13(7.1)
21- 35	100	12	60/40	70/30	100(54.6)
>35- 45	50	9	35/15	30/20	50(27.4)
>45	20	1	9/11	12/8	20(10.9)
Total	**183**	**22**	**112/71**	**121/62**	**183(100)**

Table 2. Treatment distribution of patients with Rifampicin resistant MTB in the study population

Age group (years)	Re- treatment (%)	Treatment-naïve (%)	Sex:M/F	Total (%)
<21	-	-		
21-35	9	3	8/4	12(54.6)
>35 -45	7	2	7/2	9(40.9)
>45	1	-	1/-	1(4.5)
Total	**17(77.0)**	**5(23.0)**	**16/6**	**22(100)**

4. DISCUSSION

The prevalence of *Mycobacterium tuberculosis* (MTB) in the study was 6.2% with 4.4% co-infection rate with HIV in the total 2969 study population. A bi-center study done three years ago in two sister health centers (FMC and NAF Base) in Makurdi recorded an MTB prevalence of 21.5% [3]. An explanation for the drop in MTB prevalence can be attributed to a tertiary health center where only complicated cases were referred to, while the bi-centre study was done in secondary health centers that attended to more number of patients of different nature. It was obvious that HIV was the major factor that fueled the TB infection and re-activation in this community. The prevalence of co-infection was much higher in most available studies such as 12.8% in Nassarawa Central Nigeria [7], 12.0% Ile Ife [8], 14.5% Oshogbo [9] and 32.8% in Okada Benin [10], all in Southern Nigeria. Ten percent rate of co-infection with HIV was recorded in Kano Northern Nigeria [11]. Forty to fifty prevalence rates were found in Kenya, Zambia, Tanzania and South Africa [12], while only less than 2% in China [13] and Netherland [14]. As HIV infection progresses, CD4+ T-lymphocytes decline in number and function [15]. These cells play an important role in the body defense against tubercle bacilli. Thus, the immune system becomes less able to prevent the growth and local spread of *M. tuberculosis* [15,1].

The prevalence of MTB Rifampicin Resistance in the study was only 12.0% (Re treatment=9.2%; Treatment naïve=2.8%). The result was different from a bi –center study done in Makurdi three years ago with 13.9% RIF resistance (Retreated=11.3%; Treatment naïve=2.6%) [3]. Some parts of Nigeria had reported RIF resistance prevalence rate of 11.8 to 22% [16,17], while, USA, Western Pacific and Europe had reported prevalence of 2.1%, 4.9% and 12% respectively [18].

Re treatment cases in the study were previously treated patients who were still sputum smear-positive and had a 9.2% higher risk of RIF drug-resistant TB than the 2.8% RIF drug resistance seen in treatment naïve (or new) patients. These re –treatment cases were the Categories II & IV which included; the Relapse cases, treatment after failure, treatment after default, Chronic and MDR-TB cases. Occasionally, treatment naïve or new patients would present with resistance mostly when they were infected by resistant strains. Notifiable cases by World Health Organisation (WHO) definition recorded from 2.2% for new cases and from 9.4% for retreatment cases [18,19]. It was an interesting observation that in 2016, a 2079 astronomically high number of patients were tested for MTB with only a 44 very few MTB cases detected. An explanation to this might be a change in hospital policy to more advocacy and awareness creation on MTB diagnosis in 2016. Again, tests for

GeneXpert were no longer restricted to Re-treatment cases.

Only forty-four percent MTB was detected by Ziehl Nelseen smear technique compared to the 100% MTB detection by Gene Xpert in the study. Sensitivity for smear microscopy in this population was low at 44.0%. A recent Cochrane review found that GeneXpert was more sensitive and specific than microscopy and increased TB detection by 23% [20]. The accuracy of the diagnostic testing was especially important in patients with HIV co infection, as smears might be negative in up to 61% of those patients. These findings reiterated the superiority of GeneXpert over smear microscopy as the initial diagnostic test for TB [20]. A major advantage of GeneXpert was that it allowed for the rapid initiation of second line drugs while awaiting DST and culture [20]. The shortcomings of smear microscopy might include the followings: (i) It performed poorly in latent infection and in HIV/TB co-infected patients. (ii) Low specificity as it could not distinguish between pathogenic and environmental mycobacteria. (iii)It was grossly inadequate for diagnosis of extra-pulmonary and paediatric TB. (iv) It's result depended on the studious attention of the microscopist. Hence need for other diagnostic tools.

Eighty - two percent highest proportion of GeneXpert positive MTB cases were seen in the youth and young adults. This might be due to riskier sexual behavior, more exposure to the outer environment, high work load and wide range of mobility of young people to acquire the TB bacilli [21]. The study recorded more number of male gender (112/183) among the detectable TB subjects. This was contrary to most studies, where female was higher in the TB population [10]. The reason for male preponderance might as well be that men were more exposed to outer environment, high workload and wide range of mobility to acquire the TB bacilli. There was a devastating synergy observed between the kinetic of both diseases. HIV/AIDS is the most potent re-activator of latent TB into active clinical TB; TB causes rapid progression of HIV infection into clinical AIDS [22]. TB and human immunodeficiency virus (HIV) co infection and the exponential increase in drug resistance were greatly responsible for the resurgence of TB. Other identified factors included neglect of TB control by governments, poor management of programs, poverty, population growth and rapid uncontrolled urbanization [17]. TB and HIV/AIDS programs must collaborate to counteract the impact of HIV on TB. This depends on implementation of the DOTS strategy and other interventions. In addition to effective TB case-finding and cure, these interventions include: measures to decrease HIV transmission (e.g. promotion of condoms, treatment of sexually transmitted infections); highly active antiretroviral therapy (HAART); TB preventive treatment; and antibiotic prophylaxis against HIV-related bacterial infections [15]. Full Implementation of DOTS-Plus as a step to control MDR-TB will combat an emerging epidemic [15,23].

A limitation to the study included inability to investigate for resistance in other first and second line anti TB drugs. Misdiagnosing patients as MDR TB when they were only RIF monoresistant would lead to inappropriate second line treatment, when such treatment in resource –limited settings was already limited.

5. CONCLUSION

A prevalence of 6.2% MTB with 12.0% Rifampicin resistance MTB recorded in our referral tertiary health center was remarkable in young adult male patients in re-treatment. A hundred per cent total population of Rifampicin resistance MTB patients were HIV sero- positive and 77% in re-treatment. MTB detection by smear microscopy (ZN staining) was 44% less sensitive than the GeneXpert in our study.

There is a need for proper education of the youth, especially in sexual habit. Availability of a more robust rapid multi resistance TB drug diagnostic system in order to capture MDR TB and XDR TB across the country is advocated, while the HIV program continues. The importance of automated culture and DST as a control cannot be underscored in most Nigerian laboratories and a call for resistance surveillance for XDR TB nationwide.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee

has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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