

MYCOBACTERIUM TUBERCULOSIS AND HIV CO -INFECTIONS AMONG PATIENTS ATTENDING A NIGERIAN TERTIARY HEALTH CENTER.

ABSTRACT

AIM. The study is set to determine the prevalence of MTB and Drug Resistant-MTB in the HIV/AIDS endemic community.

METHOD. A three -year retrospective study was conducted from November,2013 to November, 2016 in Benue State University Teaching Hospital, Makurdi Nigeria. Thesubject 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.

RESULT.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 with MTB, by Cepheid GeneXpert MTB/RIF system, while Ziehl Nelseen staining alone detected 2.7%(N=83/2969) ($\chi^2=5.01$; 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 MTB patients, 12.0% (N=22/183) of MTB patients were Rifampicin resistant. Seventy-seven per cent (N=17/22) of the Rifampicin resistant MTB patients was for re treatment, while the remaining 23.0% (N= 5/22) was treatment naïve ($\chi^2=6.01$; df=1; p<0.05). Seventy –one percent(N=130/183) of the patients detected with MTB were HIV sero-positive. All the Rifampicin resistant MTB patients were HIV sero-positive. Among the Rifampicin sensitive MTB 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% MTB with 12.0% Rifampicin resistance MTB in the study wasremarkablein young adult male patients in re-treatment.A hundred per cent total population of Rifampicin resistance MTB patients were HIV sero- positive.

Therefore, we recommendproper education of the youth, especially in sexual habit and availability ofa more robust rapid multi resistance TB

34 drug diagnostic system in order to capture MDR TB and XDR TB across
35 the country, while the HIV program continues.

36 **KEY WORD.** Mycobacterium tuberculosis; Rifampicin resistance; HIV
37 status; Nigeria

38 INTRODUCTION

39 Human Tuberculosis is a re-emerging infectious disease occasioned by
40 HIV/AIDS epidemics and disrupted TB control programs. TB is an opportunistic
41 infection in HIV/AIDS and can occur at any point in the course of progression of
42 HIV infection. The risk of developing TB rises sharply with worsening immune
43 status. Compared with an individual who is not infected with HIV, a
44 person infected with HIV has a 10 times increased risk of developing TB¹. In
45 sub-Saharan Africa, one-third or more of HIV-infected people may develop TB².
46 As HIV infection progresses, CD4⁺ T-lymphocytes decline in number and
47 function. These cells play an important role in the body defense against
48 tubercle bacilli. Thus, the immune system becomes less able to prevent the
49 growth and local spread of *M. tuberculosis*. Disseminated and extra pulmonary
50 disease is more common. Even in HIV-infected patients, PTB is still the
51 commonest form of TB³.

52 Nigeria has a high burden of TB and Multi Drug Resistant tuberculosis
53 (MDR-TB). Drug resistant-TB results in significantly higher mortality rates in
54 HIV-infected patients than drug susceptible TB⁴. World Health Organization
55 currently estimates high burden of MDR TB cases worldwide, particularly in

56 China, India, South Africa, and in former Soviet Union countries⁴. The
 57 emergence of MDR-TB, among both the new smear positive cases and re-
 58 treatment cases has set back some of the progress made in TB control in
 59 Nigeria. Unfortunately, new difficult – to- treat MDR-TB and extensively or
 60 totally drug-resistant tuberculosis cases have been described. Many studies
 61 have found a strong relationship between MTB/ drug resistant TB and
 62 HIV/AIDS^{1,2,3}.

63 Mycobacterium tuberculosis can be diagnosed through the following
 64 techniques such as Ziehl-Neelsen staining, Fluorescence microscopy, liquid and
 65 solid media culture, and molecular techniques (GeneXpert, MTBDRplus,
 66 Multiplex – Nested PCR and others). More recent diagnostic tools include
 67 immune-based assays, skin patch test, rapid culture systems, line-probe assays,
 68 bacteriophage-based assays, molecular beacons and microscopic observation
 69 drug susceptibility assay³.The GeneXpert MTB/RIF introduced by WHO in 2011,
 70 is a novel automated molecular assay recently endorsed by the World Health
 71 Organization for the early diagnosis of both MTB infection and RIF resistance
 72 mutation in the rpoB gene. Laboratory diagnosis of HIV infection is established
 73 by Double ELISA technique (Determine and Unigold rapid tests) by serial
 74 testing strategy and any discordance in the result is settled by using SD Bioline
 75 or Statpack as a tie-breaker.

76 Nigeria has been ranked 10th globally among the high endemic countries
 77 for *TB*, while it also ranked 15th among the 27 heavy burden countries of the
 78 world for Multi –drug –resistant TB according to World Health Organization
 79 Fact sheet on Tuberculosis 2013³. Multi-drug –resistant TB (MDR –TB)
 80 prevalence in Nigeria was recorded 2.9% by National Drug Resistance Survey
 81 commissioned by Federal Ministry of Health, 2010³. Nigeria has HIV national
 82 adult prevalence of 3.6% with estimated 3.3 million people living with HIV,
 83 ranking second among the HIV burden countries on the continent⁵. Benue
 84 state is reputed to be among the high HIV endemic states in Nigeria with 12.7%
 85 prevalence⁵. Some studies have recorded a strong correlation existing
 86 between HIV/AIDS and MTB. Therefore, the need to study the pattern of TB co-
 87 infection among the HIV/AIDS patients in Benue state.

88 **SUBJECTS AND METHOD**

89 Study site and Population

90 Benue state is in the North Central Nigeria and has a teaming population
 91 of about 5 million with its state capital, Makurdi located at the northern part of
 92 the state. The state is surrounded by neighboring states; Enugu state and Kogi
 93 state on the East, Taraba state on the West, Cross River state on the South and
 94 Nassarawa state on the North. Benue state had a total population of 4,253,641
 95 in 2006 census with an average population density of 99 persons per km² with

96 land mass of 34,059 km². Agriculture forms the backbone of the Benue State
 97 economy, engaging more than 70 per cent of the working population. Makurdi
 98 harbors two tertiary centers, namely, Benue State University Teaching Hospital
 99 and Federal Medical Center, Makurdi among other two secondary centers.

100 The 3 –year study was conducted from November ,2016 in Benue State
 101 University Teaching Hospital, Makurdi Nigeria. Approval by the Ethics and
 102 Research Committee of the hospital and written or oral consent of the patients
 103 were sought before sample collection. The study included all patients above
 104 age 15 years suspected of pulmonary TB, treatment-naïve(new) and re-treated
 105 cases irrespective of HIV status. The nature of the study was explained to the
 106 subjects in the simplest language they best understood. Socio-demographic
 107 variables such as address, age, gender, TB treatment status and HIV status on
 108 initial visit were recorded. All ages were included in the study with a mean age
 109 of 32 years.

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

116 **HIV Serology**

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

125 **Smear test**

126 Specimens were digested and decontaminated of other bacteria by the
127 standard *N*-acetyl-L-cysteine–NaOH–Na citrate method⁶. A total of 100µl of
128 each decontaminated sample was placed on a microscope slide, dried, fixed
129 and stained using ZN staining standard procedures. Slides were examined
130 microscopically at ×1000 magnification. A stain was considered positive if it
131 contained at least five bacilli per 300 fields⁶.

132 **GenXpert MTB/RIF System**

133 The GenXpert MTB/RIF detects DNA sequences specific for
134 *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 (*rpoB*) 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.

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.

RESULTS

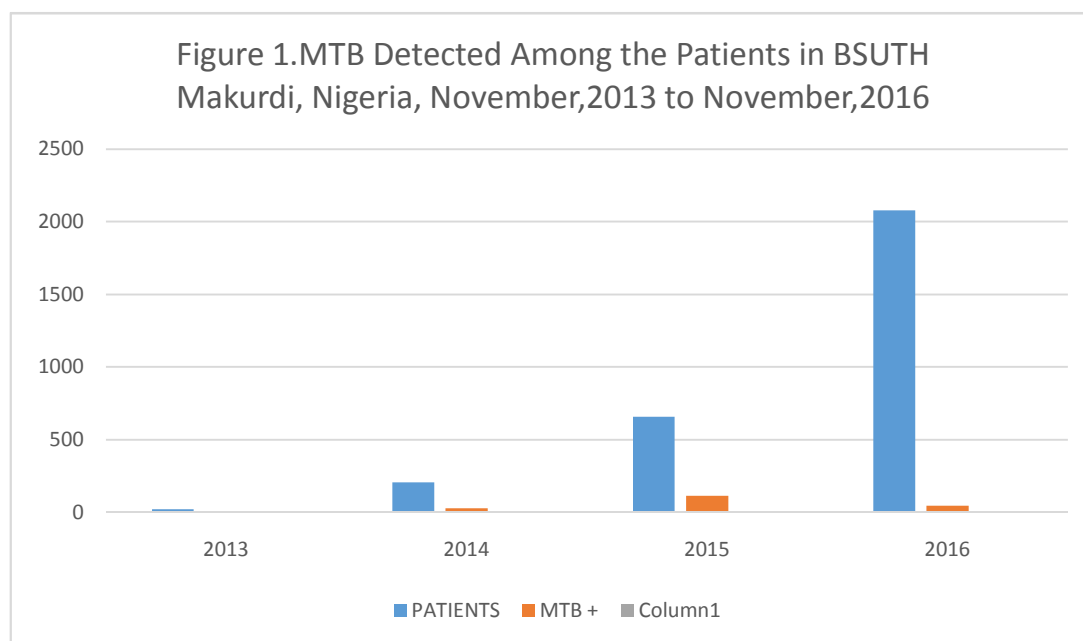
A total of two thousand nine hundred and sixty –nine patients were recruited for the 3 –year study, November, 2013 to November, 2016. The breakdown is as follows: From November, to December, 2013 there were only 21 patients tested with only one positive case of *Mycobacterium Tuberculosis*.

155 In the year,2014, there were 206 patients tested with 25 patients with
156 detected MTB. In 2015, the number of patients tested was 657 with detectable
157 MTB among113. While in 2016, 2079 patients were tested with 44 patients
158 with detectable MTB (Figure1).

159 A total of 183(6.2%) of 2969 patients were positive for MTB by Gene
160 Xpert, while only 44.0%(N=80/183) of the MTB cases was detected by AFB
161 smear test. About seven percent(N=13/183) of the MTB patients were below
162 21 years including adolescents and children; 54.6%(N=100/183) were youths;
163 27.4%(50/183) were young adults; 10.9%(20/183) included adults and the
164 elderly. About fifty percent MTB patients were male. One hundred and twenty-
165 one (66.0%) of the patients were of HIV positive status while rest (44.0%)were
166 of negative status ($\chi^2 = 5.84$; $df=1$; $p<0.05$). There was a significant difference
167 between 88.0%(N=161) Rifampicin sensitive and 12.0% (N=22) resistant MTB
168 cases ($\chi^2 = 7.23$; $df= 1$; $p<0.05$) (**Table1**).

169 Twenty –two (12.0%) of the 183 MTB patients were Rifampicin resistant.
170 Seventy-seven per cent(N=17) of the Rifampicin resistant MTB patients was for
171 re-treatment while the remaining 23%(N=5) was for new treatment ($\chi^2 = 6.01$;
172 $df= 1$; $p<0.05$). Sixteen (72.7%) of the Rifampicin resistant MTB patients were
173 Males compared to 27.3%(N=6) Females (**Table2**). All the Rifampicin resistant
174 MTB patients were HIV sero- positive.

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177 **Table I. Age groups of patients with MTB in BSUTH Makurdi Nigeria from**
 178 **November 2013 to**
 179 **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)

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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)

DISCUSSION

The prevalence of MTB in the study was 6.2%with4.4% 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 are referred to the center. It is obvious that HIV is the major factor that fuels the TB infection and re-activation in this community. The prevalence of co-infection is much higher inmost available studies such as 12.8% in Nassarawa Central Nigeria⁷, 12.0% Ile Ife⁸, 14.5% Oshogbo⁹ and 32.8%in Okada Benin¹⁰, all in Southern Nigeria. Ten percent rate of co-infection with HIV was recorded in Kano Northern Nigeria¹¹. Forty to fifty prevalence rates were found in Kenya,Zambia, Tanzania and South Africa¹², while only less than 2 % in China¹³and Netherland¹⁴.As HIV infection progresses, CD4+ T-lymphocytes decline in numberand function. These cells play an important role in the body

203 defence against tubercle bacilli. Thus, the immune system becomes less able
204 to prevent the growth and local spread of *M. tuberculosis*. Disseminated
205 and extrapulmonary disease is more common. Even in HIV-infected patients,
206 PTB is still the commonest form of TB^{15,1}.

207 The prevalence of MTB Rifampicin Resistance in the study was only
208 12.0% (Retreatment=9.2% ; Treatment naïve=2.8%). The result is similar to a bi
209 -center study done in Makurdi three years ago with 13.9 % RIF resistance
210 (Retreated=11.3%; Treatment naïve=2.6%)³. Some parts of Nigeria had
211 reported RIF resistance prevalence rate of 11.8 to 22%^{16,17}, while, USA,
212 Western Pacific and Europe had reported prevalence of 2.1%, 4.9% and 12%
213 respectively¹⁸ ..

214 Notifiable cases by World Health Organisation (WHO) ranges from 2.2% for new
215 cases and 9.4% for retreatment cases^{18,19} .

216 Only forty-four percent MTB was detected by Ziehl-Neelsen smear
217 technique compared to the 100% MTB detection by Gene Xpert in the
218 study. Sensitivity for smear microscopy in this population was low at 44.0%. A
219 recent Cochrane review found that GeneXpert is more sensitive and specific
220 than microscopy and increases TB detection by 23%²⁰. The accuracy of the
221 diagnostic testing is especially important in patients with HIV co-infection, as
222 smears may be negative in up to 61% of these patients. These findings

reiterate the superiority of GeneXpert over smear microscopy as the initial diagnostic test for TB²⁰. A major advantage of GeneXpert is that it allows for the rapid initiation of second line drugs while awaiting DST and culture²⁰. The shortcomings of smear microscopy may include the followings: (i) It performs poorly in latent infection and in HIV/TB co-infected patients. (ii) Low specificity as it cannot distinguish between pathogenic and environmental mycobacteria. (iii) It is grossly inadequate for diagnosis of extra-pulmonary and paediatric TB. (iv) Its result depends 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 bacilli²¹. The study recorded more number of male gender (112/183) among the detectable TB subjects. This is contrary to most studies, where female is more among the TB population¹⁰. The reason for male preponderance may as well be that men are more exposed to outer environment, high workload and wide range of mobility to acquire the TB bacilli. There is 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 AIDS²². TB and human immunodeficiency virus (HIV) co infection and the

244 exponential increase in drug resistance are greatly responsible for the
 245 resurgence of TB . Other identified factors include neglect of TB control by
 246 governments, poor management of programs, poverty, population growth and
 247 rapid uncontrolled urbanization ¹⁷.TB and HIV/AIDS programs must collaborate
 248 to counteract the impact of HIV on TB.This depends on implementation of the
 249 DOTS strategy and other interventions. In addition to effective TB case-finding
 250 and cure, these interventions include: measures to decrease HIV transmission
 251 (e.g. promotion of condoms, treatment of sexually transmitted infections);
 252 highly active antiretroviral therapy (HAART); TB preventive treatment; and
 253 antibiotic prophylaxis against HIV-related bacterial infections¹⁵.
 254 Full implementation of DOTS-Plus as a step to control MDR-TB will combat an
 255 emerging epidemic^{15,23}.

256 A limitation to the study includes inability to investigate for resistance in
 257 other first and second line anti TB drugs .Misdiagnosing patients as MDR TB
 258 when they are only RIF monoresistant would lead to inappropriate second line
 259 treatment, when such treatment in resource –limited settings is already
 260 limited.

261 There is a need therefore for automated culture and DST in most Nigerian
 262 laboratories and a call for resistance surveillance for XDR TB nationwide.

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