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## Rapid Molecular Detection of *Mycobacterium Tuberculosis* and Rifampicin Resistance among Suspected Tuberculosis Patients in Otukpo, Benue State Nigeria

Ochayi, A. S. <sup>1</sup>, Amuta, E.U. <sup>1</sup>, Ikpeama, O.J. <sup>2</sup>

Department of Microbiology, University of Mkar, Benue State <sup>1</sup>, Brigade Medical Centre Sokoto <sup>2</sup>.

Corresponding Author: ikpeama35@gmail.com/ +234-806-261-9025

### Abstract

Pulmonary tuberculosis (PTB) is a common opportunistic infections (OI) inextricably bound to the Human Immuno-deficiency Virus and Acquired Immune Deficiency Syndrome (HIV/AIDS). This study was carried out to determine the prevalence of *Mycobacterium tuberculosis* and Human Immuno-deficiency Virus (MTB/HIV) co-infection and to evaluate the current level of Rifampicin (RIF) resistance in Otukpo local Government Area. Sputum samples were collected for the detection of *Mycobacterium tuberculosis* and rifampicin resistance using MTB/RIF geneXpert Analyzer. On spot screening was performed for HIV type 1 and type 2 (HIV1/2) following the Nigerian HIV serology algorithm. Blood samples were collected from positive cases for Cluster of Differentiation 4 (CD4<sup>+</sup>) estimation, White Blood Cells (WBC) count and Haemoglobin (HB) analysis respectively on a BD FACSCount (Becton Dickinson Fluorescence-Activated Cell Sorting count) analyzer and Sysmex analyzer. The project was carried out between December, 2014 and June, 2015 in a Centre for Integrated Health Program (CIHP) supported Anti-Retroviral Therapy and Directory Observing Treatment Short-course (ART/DOTS) clinic hosted by the General Hospital, Otukpo. MTB was detected in 62% (n=249/401) of the total samples analyzed. MTB/HIV co-infection was found in 31.1% (79/249) and RIF resistant cases was found in 13.2% (33/249). The age groups of 21-30 (25.7%) and 31-40 (31.7%) years had the highest number of MTB/HIV co-infection and RIF resistance respectively. Prevalence with respect to age distribution and MTB/HIV co-infection were not statistically significant with respect to gender (p>0.05). Also, RIF resistant cases were more in the re-treated cases 87.9% (29/33) than new cases 12.1% (4/33). Furthermore, there was a significant association between low CD4<sup>+</sup> count (64.6%) with MTB/HIV co-infection (p<0.05). White blood cell count of most participants with co-infection was

within normal range (4.5-10 x 10<sup>9</sup>/l). Similarly, 97.5% (77/79) of co-infected persons had normal haemoglobin (8-17g/dl). MTB/HIV co-infection was most prevalent among farmers 62% (49/79), followed by commercial motor cyclist (Okada riders) 13.9% (11/79) and then students 5.1% (4/79). Creation of more Molecular Laboratories with rapid diagnostic capability is advocated to help understand the real burden of TB and Drug resistance pattern among TB patients living in Otukpo and her environ.

**Keywords:** Molecular Detection, *Mycobacterium Tuberculosis*, Rifampicin Resistance Otukpo, Benue State, Nigeria.

### Introduction

Tuberculosis (TB) is a major public health problem in Nigeria, a country of 169 million people, with the country currently ranking 10<sup>th</sup> among 22 high TB burdened countries of the world and fourth highest in Africa (after South Africa, Ethiopia and DR Congo) (First National TB Prevalence Report, 2012). Prior to 2012, there was no national survey to determine the prevalence of tuberculosis disease (TB) in Nigeria. Estimates of the burden of TB in the country relied on indirect assessment by the World Health Organization (WHO) based on existing TB surveillance information, which in itself is affected by the completeness of TB notification and instances of TB under-diagnosis (First National TB Prevalence Report, 2012). The World Health Organization (WHO, 2002) identified TB as the leading cause of death among People Living with HIV/AIDS (PLWHA) accounting for 14-54% of HIV/AIDS deaths globally (WHO Stop TB Department Estimates, 2002). In attempt to curb the trend of TB infection in the country, the Federal Ministry of Health in 2003 adopted the WHO's

Directly Observed Treatment Short-Course (DOTS) Strategy. DOTS services were being implemented in the 36 States and the Federal Capital Territory (FCT). The total TB patients detected through the program were 46,596 while 28,173 were smear positive (guideline for TB/HIV Related Conditions, 2009). Since then (2003) the overall case notifications have consistently increased due to DOTS expansion in the country. This increase plateaued in 2008 despite the intensified approach to preventive measures of the National Tuberculosis and Leprosy Control Programme. Out of 97,853 notified TB cases in 2012, 52,901 (59%) were confirmed through smear microscopy, 32,972 (37%) were based on a clinical diagnosis (smear-negative), and only 4, 432 (5%) were extra-pulmonary TB. Some variation was observed in the pattern of TB case notification in the states and the six geo-political zones of the country. Case notification for all forms of TB in the North West zone was 47 per 100,000 population; in the North East zone, 65 per 100,000 population; in North Central zone, 80 per 100,000; in the South West zone, 71 per 100,000; in the South-South zone, 52 per 100,000 population; and in the South East zone, 41 per 100,000 population (First National TB Prevalence Survey 2012). The overwhelming number (80%) of TB case notification recorded in the North Central zone could be attributed to the strong association of TB with HIV/ AIDS that was highly prevalent in Benue and neighboring North Central States as at then (First National TB Prevalence Survey, 2012).

Tuberculosis (TB) and human immunodeficiency virus (HIV) disease are among the leading causes of infectious disease-associated mortality worldwide. The duo has been inextricably bound together from the early years of the HIV/AIDS epidemic. Their dangerous synergy affects all aspects of each disease, from pathogenesis and the epidemiologic profile; to clinical presentation, treatment, and prevention; to larger issues of social, economic, and political consequence. It is estimated that infection due to *Mycobacterium tuberculosis* is present in one-third of the world's population (about 2 billion people). There is an estimated eight to ten million new cases of active TB each year worldwide, causing roughly 3 million death (Wikipedia.com).

In parallel, although HIV is only a recent pathogen of humans, it is estimated that 75 million (63million-89million) people have become infected with HIV/AIDS (UNAIDS Factsheet, 2014). During the past years, 5 million adults and children

were estimated to have been newly infected with HIV. Although TB-related death in people living with HIV have dropped by 36% since 2004, TB remains the leading cause of death among people living with HIV (UNAIDS Factsheet, 2014).

Both the TB and HIV/AIDS global pandemics are constantly undergoing changes and still evolving especially at the points of their interaction. The harmful collaboration of TB and HIV/AIDS has added greatly to the suffering and loss of life caused by each disease (Gerald *et al.*, 2007). In the western world, the rate of new *M. tuberculosis* and HIV infections has reduced. Conversely, the numbers of individuals living with HIV infection have increased. In contrast, most resource-limited countries, like Nigeria where high rates of *M. tuberculosis* infection already exist, both the incidences and the prevalence of HIV/AIDS and TB have continued to dramatically increase (Gerald *et al.*, 2007).

More worrisome is the growing recognition of multidrug-resistant TB (MDR-TB) and the recently described extensive drug-resistant TB (XDR-TB), associated with a very high mortality rate among HIV-infected individuals. This may further undermine TB control efforts in resource-poor countries (Neel *et al.*, 2007). Otukpo Local Government Area of Benue State is one of the highest endemic pockets of HIV/AIDS at the sub-national levels in Nigeria and there is little or no published investigation on tuberculosis and mycobacteria resistance patterns. Only few researches have been conducted to determine the prevalence of *Mycobacterium tuberculosis*. It became imperative therefore to determine the burden of tuberculosis and the current level of drug (Rifampicin) resistant mycobacteria infection in this part of the country. Data obtained will serve as reference information to health institutions and policy makers. The aim of this study is to find out the burden of co-infection of *Mycobacterium tuberculosis* and HIV/AIDS and the prevalence of Rifampicin resistance among tuberculosis patients in Otukpo, Benue State, Nigeria.

## Materials and Method

### Description of Study Area

### Population Structure and Distribution

Otukpo is one of the oldest Local Government Area in Benue State. It is made up of four districts and thirteen council wards. The inhabitants of the area are predominantly Idoma, the second largest ethnic

group in Benue State North Central Nigeria. The people speak the Idoma language though slight dialectical variations exist. The population of the local government Area based on the 2006 National Population Commission census was 261, 666. Otukpo is located in the North by Apa Local Government, North-East by Gwer and Obi Local Government, South and South-West by Ado and Ohimini Local Government while it shares a common boundary with Olamaboro Local Government of Kogi State to the West. The main occupation of the people is farming though a few of the indigenes engage in petty trading and other commercial ventures (Umunna, 2015).

### **Study Design and Subjects**

The study was cross-sectional in design. A total of 401 sputum samples was collected and analyzed in General Hospital Otukpo, the host site of the CIHP supported ART/DOTS clinic. The samples were collected from December, 2014 to June, 2015. Presently, this is the only GeneXpert Centre for testing drug resistance in the Area. The study included all patients suspected of pulmonary TB (New cases and re-treated cases) and all HIV positive clients who consented to examination. Blood samples was also collected and screened for each patient for the presence of the Human immune-deficiency virus (HIV) according to the Nigerian National HIV/AIDs screening algorithm. All ages were included in the study. The study was approved by the ethics committee of the hospital.

At regular clinic days, ward patients, out patients and patients Transferred in (T.I) presenting symptoms typical of PTB and HIV cases (who may have failed mycobacterium detection by the traditional AFB smear microscopy method) come to access medical care in the hospital. On arrival, patients are physically examined and screened for symptoms of pulmonary TB, like chest pain, cough lasting more than 2 weeks, fever, night sweats, and weight loss. Upon identification, patients were counseled and those who provided written informed consent were recruited into the study. Consented patients were queried about their TB history to assess their treatment exposure prior to the study and provided sterile sample bottles for a spot sputum sample. All patients with AFB-positive samples or symptoms typical of tuberculosis infection as well as people living with HIV/AIDS (PLWHA) were enrolled in the study irrespective of age.

### **Sample Preparation and Processing**

Sputum MTB/RIF (Nucleic Acid Amplification

Technique)

#### **Principle:**

The Xpert MTB/RIF detects DNA sequences specific for *Mycobacterium tuberculosis* and Rifampicin resistance by polymerase chain reaction. The Xpert® MTB/RIF purifies and concentrates *Mycobacterium tuberculosis* bacilli from sputum samples, isolates genomic material from the captured bacteria by sonication and subsequently amplifies the genomic DNA by PCR. The process 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 (Van Rie *et al.*, 2010).

#### **Sample preparation Procedure:**

- With the aid of paper towel soaked with 5% Lysol solution, the lid of sample container was unscrewed.
- 2 volumes of sample reagent (NaOH and Isopropanol) was added directly to 1 volume of the sample in the collection container.
- The lid was carefully replaced.
- With the aid of a disinfected paper towel firmly wrapped around the lid, sample was shaken vigorously 20 times and incubated at room temperature for 10 minutes.
- Sample was again shaken vigorously 20 times and incubated for 5 min.
- An MTB/RIF cartridge was labeled with participant's ID
- 2 ml of the sample was pipetted into the cartridge (Center for Clinical Care in Nigeria 2014) GeneXpert Training Manual, 2014)

#### **Sample Processing**

- The sample was processed by clicking on the 'create test' button on the GeneXpert visual display unit.
- With the aid of a barcode reader, the MTB/RIF cartridge was scanned and sample information was keyed into the system.
- The 'start' button was clicked and green signal light appeared on all four GeneXpert modules indicating the machine readiness to start operation.
- The cartridges were loaded into the machine and module doors closed.
- Test result was generated in 120 minutes and sent to a remote printer for printing (CCCRN GeneXpert Training Manual, 2014).

## Determination Of CD4 Count Cells (BD FACSCount)

### Principle

When whole blood is added to the reagents, fluorochrome-labeled antibodies in the reagents bind specifically to lymphocyte surface antigens. The instrument is fitted with laser light which on contact with the fluorochrome-labelled cells, fluoresce. The fluorescent light provides the information for the instrument to count the cells (BD FACSCount system User Guide Version 1.0, 2004).

### Sample Preparation and Processing

- Each reagent tube was labeled with appropriate subject's accession number
- With the aid of a vortex machine, each tube was shaken vigorously (Vortex) upside down for 5 seconds then placed upright and vortexed for another 5 seconds.
- With the aid of a "curing" station, each reagent tube was opened and placed in the BD workstation.
- Each EDTA BD Vacutainer tube containing subject's sample was inverted 10 times to adequately mix blood.
- With the aid of an automatic Pipette, 50 $\mu$ l of whole blood was transferred into each reagent tube changing pipette tips between tubes-reverse pipetting.
- Each tube was capped and vortex upright for 5 seconds and incubated in the dark for 60 minutes at room temperature.
- After incubation, each tube was uncapped and 50 $\mu$ l of fixative solution was dispensed into each tube changing pipette tips in-between tubes.
- Each tube was recapped and vortexed upright for 5 seconds
- Each sample tube was then placed on the sample holder and CD4 cells were estimated by the BD FACSCount auto analyzer.

## Determination of Blood Parameters (Sysmex KX-21N)

### Principle

The KX-21N performs speedy and accurate analysis of 18 full blood count parameters in the blood. It employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block using the DC detector method. The RBC and Platelets are

taken by the RBC detector block also using the DC detector method. The HGB detector block measures the haemoglobin concentration using the non-cyanide haemoglobin method.

### Procedure

- With the aid of a Fisher automatic mixer, samples were properly mixed to ensure homogeneity.
- The Vacutainer plug was removed with care to avoid blood spillage.
- With the sample tube set to the machine sample probe, appropriate sample volume was aspirated into the machine for analysis.
- Result of analysis was shown on a liquid crystal display (LCD) Screen and a hard copy printed out.

### HIV Screening

This was done using the serial antibody testing panel of the Nigeria National Algorithm for HIV serology adopted by the Centre for Integrated Health Program (CIHP) and approved by the Centre for Disease Control (CDC).

### Statistical Analysis

A computer program SPSS version 16.0 (Statistical Package for Social Science) was used for data analysis. A frequency distribution table and prevalence percentage was used for different estimation using standard formulae. A p-value of  $\leq 0.05$  was considered significant in all statistical comparisons.

### Result

In this study, 401 sputum samples was obtained randomly from patients who were attending the ART/DOTs clinic located in General Hospital Otukpo and supported by the Centre for Integrated Health Program (CIHP). Blood sample was also collected and screened for each patient for the presence of the Human Immuno-deficiency Virus (HIV). *Mycobacterium tuberculosis* (MTB) was detected in 249/401 (62.9%) samples. Out of the 249 MTB positive samples, MTB/HIV co-infection was detected in 79/249 (31.1%) and RIF resistant cases was 33/249(13.1%). The age groups 21-30 years (25.7%) and 31-40 years (31.7%) had the highest number of MTB/HIV co-infection and RIF resistant MTB. The difference was however not significant ( $p>0.05$ ) as shown in Table 1. There was no significant difference in prevalence of MTB/HIV co-infection with respect to gender ( $p>0.05$ ) even though there were more males 57.0% (45/79) than female 43.0 % (34/79) as shown in

table 2. Also, the RIF resistant MTB positive cases was more in the re-treated patients 87.9% (29/33) compared to new patients 12.1% (4/33) ( $X^2=28.701$ , df:3;  $p<0.05$ ) as shown in table 3. There was a significant association of low CD4<sup>+</sup> ( $X^2=21.962$ , df: 3;  $p<0.05$ ) and MTB/HIV co-infection. A significant 64.6% (51/79) of the patients had low CD4<sup>+</sup> (<450 cells/ $\mu$ l) while 35.4% (28/79) had normal CD4<sup>+</sup> ( $\geq 450-1500$ ) count. There was no case of high CD4<sup>+</sup> (> 1500cells/ $\mu$ l) count in the study as shown in table 4. White Blood Cells count of most participants was within normal count level (3.5-10  $\times 10^9/l$ ) ( $X^2=8.280$ , df: 3;  $p<0.05$ ). A total of 14 % (11/79) had low WBC, 83.5% (66/79) had

normal WBC while only 2.5% (2/79) had high WBC count as shown in table 5. Similarly, 97.5 % (77/99) of co-infected persons had normal haemoglobin (8-17g/dl) level, 2.5% (2/79) had high HB (>17g/dl) while none had low HB (<8g/dl) ( $X^2=8.682$ , df: 3;  $p<0.05$ ) as shown in table 6. MTB/HIV co-infection was most prevalent among farmers 62 % (49/79), followed by commercial motor cyclist (Okada riders) 13.9% (11/79) and lowest among student 5.4% (4/79). There was a significant association between occupation and the proportional distribution of MTB/HIV co-infection in MTB patients ( $X^2=54.02$ , df: 10;  $p<0.05$ ).

**Table 1. Age distribution of MTB Patients in Otukpo Local Government Area**

Age (Years)	MTB (%) / RR (%)	MTB/HIV (%) / RR (%)	Total (%) / RR (%)
<1-10	1 (50.0) / 1(50.0)	2(0.8) / 0 (0.0)	0 (0.0) / 0 (0.0)
11-20	19 (82.6) / 4 (17.4)	23(9.2) / 1(100)	0 (0.0) / 1 (0.4)
21-30	137 (57.8) / 27(42.2)	64 (25.7) / 4 (30.8)	9 (69.2) / 13 (5.2)
31-40	51 (64.6) / 28 (35.4)	79 (31.7) / 1 (7.1)	13 (92.9) / 14 (5.6)
41-50	28 (71.8) / 11(28.2)	39(15.7) / 0 (0.0)	3(100.0) / 3(1.2)
51-60	20 ( 80.0) / 5 (20.0)	25 (10.0) / 0 ( 0.0)	1 (100.0) / 1(0.4)
61-70	12 ( 85.7) / 2(14.3)	14(5.6) / 0 ( 0.0)	1(100.0) / 1 (0.4)
71-80	1 (50.0) / 1(50.0)	2(0.8) / 0 (0.0)	0 (0.0) / 0 (0.0)
$\geq 81$	1 (100.0) / 0(0.0)	1(0.4) / 0 (0.0)	0(0.0) / 0 (0.0)
<b>Total MTB (%)</b>	170 (68.3)	79(31.7)	249(100.0)
<b>Total RR (%)</b>	6 (18.2)	27 (81.8)	33(100.0)

**Legend**

MTB= Mycobacterium Tuberculosis  
 RR= Rifampicin Resistance  
 HIV= Human Immunodeficiency Virus

**Table 2 Proportional Distribution of HIV among MTB patients according to gender.**

Gender	MTB/HIV	MTB	Total (%)
Male	45(31.5)	98(68.5)	143(57.4)
Female	34(32.1)	72(67.9)	106(42.6)
<b>Total(%)</b>	<b>79(31.7)</b>	<b>170(68.3)</b>	<b>249(100.0)</b>

$X^2=6.69$ ;  $df=3$ ,  $p>0.05$

**Legend**

MTB= Mycobacterium Tuberculosis

RR= Rifampicin Resistance

HIV= Human Immunodeficiency Virus

**Table 3. Treatment distribution of patients with RIF Resistant MTB in Otukpo Local Government Area**

Age (years)	RIFAMPICIN RESISTANT		Total (%)
	Re-treated	New cases	
1-10	0 (0.0)	0 (0.0)	0 (0.0)
11-20	0 (0.0)	1(100.0)	1(3.0)
21-30	11(84.6)	2 (15.4)	13 (39.4)
31-40	13 (92.9)	1(7.1)	14 ( 42.4)
41-50	3(100.0)	0 (0.0)	3 (9.1)
51-60	1(100.0)	0 (0.0)	1 (3.0)
61-70	1(100.0)	0 (0.0)	1 (3.0)
71-80	0(0.0)	0(0.0)	0(0.0)
<b>Total(%)</b>	<b>29(87.9)</b>	<b>4(12.1)</b>	<b>33(100.0)</b>

$X^2=28.7$ ;  $df=3$ ,  $p<0.05$

**Table 4. CD4<sup>+</sup> distribution in patients with MTB and HIV Co-infection in Otukpo Local Government Area.**

Infection	CD4 COUNT ESTIMATION			Total (%)
	Low	Normal	High	
MTB/HIV	51(64.6)	28(35.4)	-	79(31.7)
MTB	41(24.1)	129(75.9)	-	170(68.3)
<b>Total(%)</b>	<b>92(36.9)</b>	<b>157(63.1)</b>	<b>-</b>	<b>249(100.0)</b>

$X^2=21.96$ ;  $df=3$ ,  $p<0.05$

**Legend**

MTB= Mycobacterium Tuberculosis

RR= Rifampicin Resistance

HIV= Human Immunodeficiency Virus

**Table 5: WBC distribution in MTB and HIV Co-infected patients in Otukpo Local Government Area**  
**WHITE CELL COUNT ESTIMATION**

<b>Infection</b>	<b>Low (&lt;500 cells/ul)</b>	<b>Normal (500-1500 cells/ul)</b>	<b>High (&gt;1500 cells/ul)</b>	<b>Total (%)</b>
MTB/HIV	11(14.0)	66(83.5)	2(2.5)	79(31.7)
MTB	9(5.3)	158(92.9)	3(1.8)	170(68.3)
<b>Total(%)</b>	<b>20(8.0)</b>	<b>224(90.0)</b>	<b>5(2.0)</b>	<b>249(100.0)</b>

$X^2=8.28$ ;  $df=3$ ,  $p<0.05$

**Legend:** MTB= Mycobacterium Tuberculosis RR= Rifampicin Resistance  
HIV= Human Immunodeficiency Virus

**Table 6. HB Level in patients with MTB and HIV Co-infection in Otukpo local Government Area**  
**HAEMOGLOBIN B ESTIMATION**

<b>Infection</b>	<b>Low (&lt;7g/dl)</b>	<b>Normal (7-18g/dl)</b>	<b>High (&gt;18g/dl)</b>	<b>Total (%)</b>
MTB/HIV	- (-)	77(97.5)	2 (2.5)	79(31.7)
MTB	2 (1.2)	162(95.3)	6(3.5)	170(68.3)
<b>Total(%)</b>	<b>2(0.8)</b>	<b>239(96.0)</b>	<b>8(3.2)</b>	<b>249(100.0)</b>

$X^2=8.68$ ;  $df=3$ ,  $p<0.05$

**Legend:** MTB= Mycobacterium Tuberculosis RR= Rifampicin Resistance  
HIV= Human Immunodeficiency Virus

**Table 7. Tuberculosis Infection based on age and Occupational groups**

<b>Age Group (Years)</b>	<b>Commercial Cyclist</b>	<b>Farmer</b>	<b>Retiree</b>	<b>Traders</b>	<b>Student</b>	<b>Pupil</b>	<b>Total</b>
<1-10	0(0.0)	0(0.0)	0 (0.0)	0(0.0)	1(100.0)	0(0.0)	1(1.3)
11-20	1(25)	0	0 (0.0)	0 (0.0)	3(75.0)	0.(0.0)	4(5.0)
21-30	5(18.5)	18(66.7)	0(0.0)	4(1.6)	0(0.0)	0 (0.0)	27 (34.2)
31-40	3 (10.7)	21(75.0)	0(0.0)	4(14.3)	0(0.0).	0(0.0)	28 (35.4)
41-50	2 (18.2)	8(72.2)	0(0.0)	1(9.1)	0(0.0)	0(0.0)	11(13.9)
51-60	0(0.0)	2(2.5)	3(60.0)	0(0.0)	0 (0.0)	0(0.0)	5 (6.3)
61-70	0(0.0)	0(0.0)	2(100)	0(0.0)	0(0.0)	0(0.0)	2(2.5)
>71	0 (0.0)	0 (0.0)	1(100)	0 (0.0)	0 (0.0)	0 (0.0)	1(1.3)
<b>Total</b>	<b>11(13.9)</b>	<b>49(62.0)</b>	<b>6(7.6)</b>	<b>9(11.4)</b>	<b>4(5.1)</b>	<b>0(0.0)</b>	<b>79(100.0)</b>

$X^2=54.02$ ;  $df=10$ ,  $p<0.05$

**Legend:** MTB= Mycobacterium Tuberculosis RR= Rifampicin Resistance  
HIV= Human Immunodeficiency Virus

## Discussion

The study was set to determine the prevalence of MTB/HIV co- infection among TB patients in Otukpo Local Government area and to assess the current level of MTB RIF resistance in the area. Possible association of blood parameters like the white blood cells population and haemoglobin was estimated along with the effect of the disease on CD4 count.

The prevalence of MTB/HIV co-infection in the study was 31.1% (79/249) with the age distributions showing higher frequency of the infection among the productive and reproductive age groups of 21-30 years old and 31-40 years old. HIV prevalence increased with age to a peak of 11.2% (28/249) among the 31- 40 years old and then declined progressively to 0.4% (1/249) among those 71 years and above. The MTB/HIV co-infection rate of 31.1 % obtained in this study could be compared to 28.12% obtained in Ibadan (Terry *et al.*, 2010). Similar studies from other parts of Nigeria reported prevalence of 12.0% in Ile-ife (Onipede *et al.*, 1999) 14.9% in Kano (Zubairu and Musa, 2009), and 41.2% from a rural tuberculosis referral clinic in Nassarawa State (Grace *et al.*, 2010).

However, our observed prevalence was higher compared to a 1.42% prevalence observed in a previous report (Okonkwo *et al.*, 2013) in Nnamdi Azikiwe University Teaching Hospital, Nnewi. The most probable reason for this variation could be the fact that their study was carried out using PTB suspects whose HIV status were unknown as at the time of presenting for sputum AFB microscopy. Also, Ziehl Neelsen's sputum microscopy diagnostic technique was used for MTB detection. The Ziehl Neelsen's sputum microscopy diagnostic technique can only detect fewer than 60% of all new MTB cases and as low as 20% of HIV/TB infection (IDSA 2007).

Also, gender distribution showed that more male (57.4%) than females (42.6%) were co- infected even though the infection was relatively more prevalent among the feminine gender 32.1% (34/106) compared to male 31.5% (45/143). The high percentage of co-infection recorded among the male gender could probably be due to their early exposure to smoking, alcoholism, outdoor past times and farming activity which is the main economic stay of the people. More men are involved in the farming business whose processes often involves inhalation of dusty particles and carbon polluted air from bush burning and other land preparation

processes.

The prevalence of RIF resistance in the study was 13.2% (Retreated=11.6%; New cases=1.6%). There was significant difference in infection between re-treated and treatment naïve patients in the study. Rifampicin resistance of 1.6% among treatment naïve (new) patients in the present study is lower than the WHO estimated prevalence of 2.2% of cases among the notified newly diagnosed PTB cases. Also our observed 11.6% prevalence of Rifampicin resistance among the re-treated is higher than 9.4% estimated by WHO. The value in this study is however similar to 2.6% and 11.3% reported by Nwadioha and colleagues (2014) in a related study in Makurdi, the Benue State capital. The slight variations in findings may be traceable to the fact that the health facility serves as the only service point accessible to more than ten local government areas in the area coupled with the attending high referral from primary health care centres in the rural communities where there appears to be more presence of MTB because of the low standard of living.

The current study also found a significant association ( $p < 0.05$ ) of low CD4<sup>+</sup> ( $\leq 350$  cells/ $\mu$ l) count with MTB/HIV co-infection. This finding is in agreement with reports from other parts of the country like Calabar, Delta State and Port Harcourt (Erhabor *et al.*, 2010). Our finding is however at variance with a study by Audu and colleagues (2005) which indicated that there was significant relationship between MTB/HIV co-infection and low CD4<sup>+</sup> count in multiply infected participants.

Our findings on haematological parameters such as the white blood cell count (WBC) and Haemoglobin (HB) count however did not correlate with findings from most part of the country as both WBC and HB count in this study was maintained at normal level (WBC  $\geq 6.0 \times 10^9/l \pm 2.0$  and HB 10g/dl  $\pm 2$  respectively) ( $p < 0.05$ ). Our finding is at variance with the very low WBC and HB reported among MTB/HIV co-infection by other studies. This shift could probably be due to increase in the number of treatment centers scattered across towns and rural communities by NGOs and other Government agencies rendering free professional services. Also, the recent upward review of HIV treatment guideline in ART clinics across the country from the previous CD4<sup>+</sup> count of  $\leq 350$  cells/ $\mu$ l bench mark for commencing ART to  $\leq 500$  cells/ $\mu$  may have contributed positively. This result underscores the importance of early diagnosis and commencement of



chemotherapy for co-infected patients to boost their chances of getting cured of pulmonary tuberculosis and prevention of TB in HIV patients.

A very strong association ( $p < 0.05$ ) was found among co-infected participants and farming as a profession. The study showed a higher prevalence among farmers followed by Commercial Motor Cyclist and then Students. This report is in agreement with a study carried out by Terry and colleagues (2010) which reported an association between farming and MTB/HIV co-infection. The most probable explanation lies in the nature and processes of farming which is often labour intensive and exposes farmers to inhaling dusts from dried clogs of soil. Stress, dust and the smoke polluted air from bush burning and other land preparation processes often synergize to mount pressure on the ailing immune system of HIV patients rendering them more vulnerable to PTB infection.

### Conclusion

MTB/HIV co-infection and Rifampicin resistance is prevalent in Otukpo Local Government area of Benue State, North Central Nigeria. Low CD4<sup>+</sup> count was identified by the study as one of the factors strongly associated with MTB/HIV co-infection. This collaboration poses serious threat to the society especially the more productive and reproductive young adults who are supposed to constitute a significant population of the workforce and future leaders of the nation. This report highlights the usefulness of MTB/RIF GeneXpert in the laboratory diagnosis of tuberculosis and Rifampicin resistance especially in individuals with MTB/HIV co-infection.

### Recommendations

We recommend that the distribution of MTB/RIF GeneXpert in all ART clinics across the country to scale up early and accurate TB diagnosis and treatment. We advocate that treatment of HIV patients be commenced early before their CD4 count falls to a level where they become prone to opportunistic infection especially mycobacterium tuberculosis. This will further check disease progression and reduce the risk of *Mycobacterium tuberculosis* to minimum. Other test panels that offer rapid testing and could detect multi resistant *Mycobacterium tuberculosis* is recommended both for preventive purposes and reduction of cost of treatment and management of patients with drug resistant cases. Finally, further investigation of MTB/RIF positive cases using culture is recommended to study the molecular epidemiology of the bacterium.

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