Original Research Article

NON-TUBERCULOUS MYCOBACTERIA INFECTION AMONG PEOPLE WITH HIV IN RIVERS STATE, NIGERIA

ABSTRACT

Background: The incidence of pulmonary non-tuberculous mycobacterial (NTM) disease is rapidly increasing in regions with high tuberculosis burden. The relative proportion of mycobacterial infections in HIV patients varies in different geographical areas, indicating the background frequency of latent *Mycobacterium tuberculosis*.

Aim: An assessment of NTM infections among people living with HIV was carried out in Rivers State Nigeria.

Methods: A sample of 260 people living with HIV presenting with symptoms suggestive of tuberculosis was recruited for the study. Direct smears of the collected sputum samples were made on clean, grease-free slides, dried and stained by the Ziehl Neelsen (ZN) method to confirm sputum AFB positivity. The samples were cultured using the Lowenstein-Jensen media according to established protocols.

Results: The results showed a 2.7% prevalence of NTM and a 61.2% prevalence of *Mycobacterium tuberculosis* infection. Logistic regression shows that factors associated with increased risk of NTM infection include; Age <40 (OR:2.7; 0.3 – 23.4), Persons with at most primary educational background (OR: 3.4; 0.7 – 15.9), Unemployment (OR: 0.5 – 12.4) and previous treatment for TB (OR: 2.4; 0.4 – 13.2).

Conclusion: The study showed a considerable occurrence of NTM infection among persons suspected to have TB, hence there is a need to improve the spectrum of diagnosis for mycobacterial diseases in order to improve treatment outcomes and control the likely occurrence of drug resistant mycobacterial infections.

Keywords*: Culture, Non-tuberculous Mycobacteria, HIV, Rivers State*

1. INTRODUCTION

Tuberculosis (TB) is the deadliest infectious disease in the world which disproportionately affects low-and-middle-income countries (LMICs) where diagnostic resources and treatment options are limited[1, 2]. The incidence of pulmonary non-tuberculous mycobacterial (NTM) disease is also rapidly increasing in these regions traditionally dominated by *Mycobacterium tuberculosis* (MTB) infections. This poses significant diagnostic and treatment challenges, since these two diseases are often indistinguishable clinically or by sputum smear microscopy (SSM), which is still the most commonly used TB diagnostic tool in LMICs. Consequently, NTM-infected patients are easily misdiagnosed as TB patients, and multidrug-resistant (MDR) TB is unable to be accurately identified, thus leading to unnecessary TB treatment for months[3–5] . These issues complicate the management of patients and contribute to the worsening of the current TB and NTM epidemiological features including development of drug resistant strains[6–8]. It is therefore critical to develop improved diagnostic tools to accurately distinguish these two different pathogens that have many similar clinical and epidemiological features but have different treatment regimens. Tuberculosis usually develops in immunocompromised patients or in patients with pre-existing lung disease, but immunocompetent subjects are also susceptible to NTM infection[2, 9, 10]. Chronic pulmonary disease is the most

common clinical manifestation of nontuberculous mycobacterial disease[5, 11]. The major symptoms of cough, haemoptysis, fever, and/or weight loss are similar to those associated with tuberculosis[2, 9, 10]. For several years, Nigeria has remained in the league of the highest TB burdened nations of the world and is currently ranked 4th globally[2]. Sputum smear microscopy is still routinely employed for TB diagnosis at Directly Observed Treatment Short-Course (DOTS) Centers in Nigeria. The diagnostic algorithm entails the interpreting presence of acid-fast bacilli (AFB) in sputum smear microscopy as TB. This algorithm does not differentiate *Mycobacterium tuberculosis* complex (MTC) from nontuberculous mycobacteria (NTM), which are ubiquitous environmental mycobacteria increasingly isolated from immune competent and immune compromised hosts[12–14]. Misdiagnosis impacts TB prevention and treatment. Because the clinical implications and therapeutic options for NTM infection differ significantly from those for tuberculosis, accurate differentiation of TB from NTM infection is critical to avoid under- or overtreatment of either condition, while also considering the potential patient care and economic consequences[4, 14–16]. The study assessed the prevalence of NTM infection among persons living with HIV that present with symptoms suggestive of tuberculosis in Rivers State, Nigeria.

2. METHODS

2.1 Study Area

The study was conducted in the directly observed treatment short-course (DOTS) and HIV clinics, located at the University of Port Harcourt Teaching Hospital (UPTH), Central Chest Clinic, Rivers State Ministry of Health (CCH) and the Braithwaite Memorial Specialist Hospital (BMSH) in Rivers State, Nigeria.

2.2 Study Sample

Two hundred and sixty (260) subjects with symptoms suggestive of TB (chronic cough > 2 weeks) who presented at the HIV clinics, of the hospital were consecutively recruited for the study within a six-month period.

2.3 Specimen collection

Duplicate sputum samples (spot and early morning) were collected in sterile, wide-mouthed bottles from patients that were able to produce. The samples were concentrated according to the Petroff method [17]. The collected sputum samples were preserved in a refrigerator (4^{o}C) at the site of collection and subsequently moved in a cooling box daily to the Medical Microbiology Laboratory of UPTH for initial processing.

2.4 Specimen Analysis

Direct smears of the collected sputum were made on clean, grease-free slides, dried and stained by the Ziehl Neelsen (ZN) method to confirm sputum AFB positivity. The stained slides were stored in slide boxes. The samples were subjected to decontamination procedure, ZN staining and AFB microscopy. The decontaminated sputum samples were subjected to both molecular Line Probe Assay (LPA) (MDRTB*plus* by HAIN Lifescience Germany) for the genotypic identification of MTB and its drug susceptibility testing and duplicate samples were cultured on LJ media, incubated at 37°C for 6-8 weeks and subjected to morphological examination and biochemical testing, using established procedures[1, 17].

2.5 Data Analysis

The data collected was analysed with the Epi Info v7 software (CDC, USA). Descriptive analysis was done with frequencies and percentages as appropriate. Logistic regression was used to assess the

association of demographic characteristics and clinical characteristics with the occurrence of Non-Tuberculous Mycobacteria (NTM). The analyses were done at a 95% confidence interval, a p-value of less than 0.05 was considered significant.

RESULTS

Table 1 shows the demographic distribution of the study participants. Age group distribution showed that 21.5% were between 36 – 40 years, followed by persons between 26 – 30 years (20.8%) and persons between 31 – 35 years (19.2%). The average age of the participants was 37.19 ± 9.57 years old. While 48.1% were male and 51.9% were female, 8.5% resided in rural areas and 91.5% resided in urban areas.

Table 1: Socio-Demographic Data of Respondents

Variables	Frequency (n =260)	Percentage (%)
AGE GROUP		
<21	4	1.5
21-25	15	5.8
26-30	54	20.8
31-35	50	19.2
36-40	56	21.5
41-45	27	10.4
46-50	20	7.7
51-55	13	5.0
56-60	9	3.5
>60	4	1.5
UD	8	3.1
AGE		
Mean ± SD	37.19 ± 9.57	
SEX		
Male	125	48.1
Female	135	51.9
RESIDENCE		
Rural area	22	8.5
Urban area	238	91.5
EDUCATIONAL LEVEL		
Primary	53	20.4
Secondary	131	50.4
Tertiary	55	21.2
No formal education	21	8.1
OCCUPATION		
Public servant	34	13.1
Private employment	41	15.8
Trading/business	98	37.7
Unemployed	87	33.5

Table 2 shows the results of the Mycobacterial detection tests using the Line probe assay and culture techniques. There were 7 (2.7%) NTMs and 159 (61.2%) of the 260 study participants.

Table 2: *Mycobacterial* detection using LPA and Culture techniques

Method	Result	Frequency (n=260)	Percentage (%)
LPA	Positive	157	60.4
	Negative	92	35.3
	Indeterminate	11	4.2
Solid Culture (n=11) (LPA Indeterminate)	NTM	7	2.7
	M. tuberculosis	2	0.8
	Inconclusive	2	0.8

NTM: Non-Tuberculous *Mycobacteria;* ***MTB:*** *Mycobacterium tuberculosis*

Table 3 shows the Logistic regression of socio-demographic factors and clinical factors with the observed NTM growth. Factors associated with increased risk of NTM infection include; Age <40 (OR:2.7; 0.3 – 23.4), Persons with at most primary educational background (OR: 3.4; 0.7 – 15.9), Unemployment (OR: 0.5 – 12.4) and previous treatment for TB (OR: 2.4; 0.4 – 13.2).

Table 3: Association of demographic and Clinical Factors with NTM growth

Variables	NTM (n=7,%)	Non-NTM (n= 253, %)	Chi-square (p-value)	O.R (95%C.I)
Age Groups				
<40	6(85.71)	173(68.38)	0.95 (0.329)	2.7 (0.3 – 23.4)[R]
≥40	1(14.29)	80(31.62)		
Gender				
Female	4(57.14)	131(51.78)	0.79 (0.779)	1.2 (0.2 – 5.6) [R]
Male	3(42.86)	122(48.22)		
Education				
At most Primary	7(100.00)	70(27.67)	2.91 (0.088)	3.4 (0.7 – 15.9) [R]
At least Secondary	3(42.86)	183(72.33)		
Residence				
Urban	7(100.00)	231(91.30)	0.66 (0.415)	NA
Rural	0(0.00)	22(8.70)		
Employment status				
Unemployed	4(57.14)	83(32.81)	1.81 (0.178)	2.7 (0.5 – 12.4) [R]
Employed	3(42.86)	170(67.19)		
Cough>2 weeks				
Yes	6(85.71)	235(92.89)	0.51 (0.472)	0.46 (0.1 – 4.0)
No	1(14.29)	18(7.11)		
Fever				
Yes	4(57.14)	178(70.36)	0.56 (0.452)	0.56 (0.1 – 2.5)
No	3(42.86)	75(29.64)		
Weight Loss				
Yes	6(85.71)	218(86.17)	0.01 (0.973)	0.9 (0.1 – 8.2)
No	1(14.29)	35(13.83)		
Hemoptysis				
Yes	1(14.29)	41(16.21)	0.19 (0.892)	0.8 (0.1 – 7.3)

No	6(85.71)	212(83.79)		
Feeling sick and tired				
Yes	6(85.71)	210(83.00)	0.03 (0.850)	1.2 (0.1 -10.5) [R]
No	1(14.29)	43(17.00)		
Previously Treated for TB				
Yes	2(28.57)	35(13.83)	1.19 (0.274)	2.4 (0.4 – 13.2) [R]
No	5(71.43)	217(85.77)		

O.R: Odds Ratio, 95% C.I: 95% Confidence interval, [R]Risk is increased (O.R>1)

3. DISCUSSION

The effective detection and diagnosis of mycobacteria play an important role in the control and prevention of tuberculosis and tuberculosis like diseases. Failure to characterize acid fast bacilli (AFB) positive NTM lung infections has led to their misclassification and to mistake in treatment for pulmonary tuberculosis in developing countries. The current study showed a 2.7% prevalence of NTM among persons living with HIV and suspected to have tuberculosis. This is relatively lower compared to a similar Nigerian study[3] which reported a 9.5% prevalence of NTM among persons with pulmonary tuberculosis. Similarly, a Ghanaian study reported an 8% prevalence of NTM among persons living with HIV[17]. About 15% of cases who sought clinical treatment for tuberculosis in Nigeria had pulmonary disease that were reportedly caused by NTM infection[18]. The relatively lower prevalence observed in the current study may be attributed to the detection technique utilized in the current study as the Line Probe Assay was used to screen the samples before subsequent culture for the detection of NTMs. The findings of the current study have potential far reaching public health implications of TB misdiagnosis in patients infected with NTM. Invariably, these patients are treated with anti-TB drugs including scarce and costlier second line drugs in those with presumed relapse and multidrug-resistant tuberculosis (MDR-TB). The findings of the current study indicate that persons <40 years were 2.7 times more likely to be infected with NTM. This is consistent with the findings of similar studies which showed that Non-tuberculous mycobacterial infections tend to be more common among middle-aged persons or people with pre-existing immunocompromised conditions[8, 19, 20]. It was also shown that persons with at most primary educational background were 3.4 times more likely to have NTM infection. This is consistent with reports of similar studies which indicated that lower education and socioeconomic status have been reported to be common predictors for tuberculous or TB related infections and poor treatment outcomes[10, 21–23].

Persons with previous treatment for TB were also 2.4 times more likely to have NTM infection. Previous exposure to tuberculosis treatment has also been linked with an increased likelihood of recurrence or drug resistance especially in instances where treatment adherence is relatively poor[3, 11, 20]. Associated toxicities such as isoniazid hepatotoxicity may be life threatening while symptomatic NTM remains untreated. An essential component of NTM treatment (macrolide) is not included in any TB regimen. In addition, the public health estimates of TB and MDR-TB are correspondingly distorted by these misdiagnoses coupled with unnecessary expenditure of healthcare resources[5, 12, 24].

4. CONCLUSION

The study showed a considerable occurrence of NTM infection among persons suspected to have TB, hence there is a need to improve the spectrum of diagnosis in order to improve treatment outcomes and control the likely occurrence of drug resistant mycobacterial infections.

5. Limitations of the Study

Automation (MGIT/BACTEC) was not used for the isolation of the Mycobacteria and the NTM isolated were not speciated.

6. Ethical Approval and Consent

Ethical approval for the study was obtained from the Ethics Committee of all study areas, prior to commencement of the study. Willing signed informed consent was obtained from all participants before they were included in the study and personal information of all subjects were kept confidential. Individuals diagnosed with tuberculosis were referred to the attending physicians for appropriate treatment and management.

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