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Original Article

Prevalence of Multi-Drug-Resistant Tuberculosis among Human Immunodeficiency Virus and Nonhuman Immunodeficiency Virus-Positive Pulmonary Tuberculosis Patients of Two Referral Hospitals in Southeast Nigeria

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Abstract

Introduction: Multidrug-resistant tuberculosis (MDR-TB) is a type of TB that is resistant to the two most effective first-line drugs: rifampicin and isoniazid and it remains a major public health threat, particularly in developing countries. **Objectives:** To assess the MDR-TB prevalence among human immunodeficiency virus (HIV) and nonHIV positive pulmonary TB patients of two referral hospitals in southeast Nigeria. **Materials and Methods:** Sputum specimens of individuals presenting with a cough of >2 weeks duration were screened by Ziehl–Neelsen technique for the presence of acid-fast bacilli (AFB). **Results:** A total of 103 subjects with AFB-positive sputum samples were recruited from the two referral hospitals and HIV-1/2 antibodies were screened using serial algorithm method. The positive sputum samples were subjected to Xpert MTB/RIF assay (GeneXpert®, Cepheid, USA) and cultured on the Lowenstein–Jensen medium for 42 days at 37°C. Drug susceptibility testing was done on the isolates using the nitrate reduction assay. Eighty-three (80.6%) of the isolates were obtained from culture after suspected colonies were subjected to morphological, biochemical, and immunological tests and of the 83 (80.6%) samples analyzed using Xpert MTB/RIF assay 45 (67.2%) were rifampicin-resistant. The prevalence of culture-positive TB was higher in the HIV-negative sub-population (82.02%) when compared with the HIV-positive participants (71.40%). The rate of MDR-TB was high among HIV-positive patients though not statistically significant. HIV positive patients showed prevalence of (66.70%), whereas HIV-negative patients had (42.60%). **Conclusions:** The World Health Organization estimated that 26% of patients with TB infection in Nigeria are HIV-positive and the alarming evidence of MDR-TB prevalence in this study calls for close monitoring of the prevalence of drug resistance, especially in HIV-infected population.

Keywords: Human immunodeficiency virus, multidrug-resistant tuberculosis, patient, prevalence, tuberculosis

Introduction

Human immunodeficiency virus (HIV), multi-drug resistant tuberculosis (MDR-TB) is emerging as major challenge facing TB control programs worldwide, particularly in Asia and Africa,[1] and TB has been causing great suffering to human beings throughout recorded history.[2] The World Health Organization (WHO) has documented that MDR-TB is emerging as a major challenge for TB control programs and is becoming extensively widespread today worldwide, even in high-income countries with low TB incidence. It is

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a challenge not only from a public health point of view but also in the context of the global economy, especially in the absence of treatment for MDR-TB at national programs level in developing countries.[3] The magnitude of MDR-TB is not known precisely because of the lack of prevalence information from all countries. Most sub-Saharan African countries have been unable to carry out the necessary laboratory investigations because of the absence of proper equipment to identify the *Mycobacterium tuberculosis* strains resistant to the two drugs used in the first-line treatment.[4] People living with HIV are at a higher risk of developing MDR and extensively drug-resistant TB (XDR TB) associated with increased mortality and greatly reduced survival time.[5] HIV and MDR-TB are equally balanced deadlier combinations.[6] Even though the impact of HIV infection on MDR-TB is of great public health importance, the relationship between the two infections is not yet clearly understood. Nigeria is ranked the 4th among 22 high-burden TB countries, and it is estimated that 26% of patients with TB infection in Nigeria are HIV positive.[7]

Global surveillance program showed variation in the magnitude and trends of drug resistance in different countries. Migration of population has also been reported to strengthen the transmission dynamics of TB as well as antimicrobial drug-resistant organism.[8] However, the implementation of directly observed therapy strategy in Nigeria since 1993 has achieved a case detection rate of 30% and treatment success rate of 79%, which is still below the global target of 70% detection and 85% cure rate, respectively.[9]

Based on the existing facts, MDR-TB has reached alarming levels worldwide with the emergence of strains that are virtually untreatable with the existing drugs. Drug-resistant strains, along with HIV/AIDS, are causing the biggest challenge to efficient management and control of TB and the report of an outbreak of XDR-TB in South Africa[10] with its extremely high case fatality rate, has drawn wide attention. It has been indicated that MDR-TB is likely to be more prevalent in Africa than previous reports indicated; hence, this study assessed MDR-TB prevalence among HIV non-HIV positive pulmonary TB patients of two referral hospitals in Southeast Nigeria.

Materials and Methods

Settings

This study was conducted at Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi and St Patrick's Hospital, Mile 4 Abakaliki. Nnewi is the second-largest city in Anambra State and is home to nearly 388,805 residents. Abakaliki is the capital of Ebonyi state and has a population of 149,683 persons.[11] NAUTH is a tertiary health institution and serves as a site for the treatment and management of both TB and HIV patients. It is also a referral center for both cases. St Patrick's Hospital, Mile 4 Abakaliki is a faith-based health facility and offers both antiretroviral therapy and TB care to patients. The minimum sample size was calculated using the formula stated by Charan *et al*.,[12] and a total of 103 sputum smear-positive acid-fast bacilli (AFB) samples were collected for the study.

Ethical approval

Ethical approval for this study was obtained from NAUTH research and ethics committee. Consent was obtained from each participant, and participants' confidentiality was maintained throughout the study. Participants received no financial motivation for their involvement in the study. Participants were free to withdraw from the study at any point, and their withdrawal would not affect their treatment. This study was conducted between January 2015 and September 2016.

Sample collection and analysis

About 2 ml of venous blood sample from smear-positive AFB participants' was collected in plain tube, allowed to clot and the serum separated and screened for the presence of HIV-1/2 antibodies using serial algorithm method. Determine®, Unigold® and Stat-Pak® HIV test kits were used according to the manufacturer's instruction.[13]

Consenting, eligible participants were screened for the presence of AFB in their sputum. Two sputum samples (spot and early morning) were collected in sterile screw cap universal containers from each participant on 2 consecutive days and stained by Ziehl–Neelsen's (ZNs) method.

Progressively, early morning mucoid or mucopurulent sputum specimen was collected from each participant with smear-positive AFB test result into a sterile screw cap universal bottle. The specimen was then stored in the refrigerator until transported to the TB reference laboratory of Dr. Lawrence Henshaw Memorial Hospital (DLHMH) in Calabar, Cross River State. Transport was done within 72 h of collection.

After appropriate sample preparation, two Lowenstein–Jensen (LJ) medium slants were cultured for each sample. Tubes were loosely capped and incubated as such at 37°C for 1 week in a slanted position to ensure even distribution and absorption of inoculum. After 1 week, tubes were incubated upright for up to 6 weeks, and the caps tightened. An in-house strain H37RV and an uninoculated tube were used as a positive and negative control, respectively.

After colonies were confirmed by ZN staining for acid-fastness, niacin test was carried out on each inoculated and control tubes. The formation of yellow color was interpreted as a positive reaction; the absence of color was regarded as negative reaction for the production of Niacin. catalase test, p-Nitrobenzoic acid (PNB), and TB Ag MPT64 rapid test was carried out in this study, and *M. tuberculosis* identification was based on its slow growth rate, no pigmentation, no growth on LJ medium-containing PNB, niacin production, catalase-negative at 68°C, and positive Ag MPT 64 test.

Drug-susceptibility testing was carried out on all confirmed *M. tuberculosis* colonies and nitrate reduction assay method was used.[14]

GeneXpert MTB/RIF assay for the detection of rifampicin resistance was carried out on the sputum samples of the participants. Sputum sediments were mixed with sample buffer in a ratio of 1:3 in a screw cap tube and screwed tightly. The tube was vortexed for 20 s. The sample was incubated at room temperature for 10 min. After 10 min, the sample was vortexed again for 20 s and incubated at room temperature for 5 min. After incubation, 2 ml of the sample was inoculated into the GeneXpert cartridge. The cartridge was scanned into the GeneXpert machine and allowed to run for 2 h. After 2 h, the test result was read off the screen of the GeneXpert machine monitor.

Data analysis

A standard questionnaire was completed for each recruited patient to collect demographic parameters. Data were compiled and summarized using Microsoft Excel and analyzed using Statistical Package for the Social Sciences, SPSS version 20, SPSS Inc., Chicago, IL, USA. Frequencies were calculated as percentages. Comparison of categorical variables and significance testing was made with Chi-square test. Value of $P < 0.05$ was considered statistically significant.

Results

A total of 103 subjects with AFB-positive sputum samples were recruited from the two referral hospitals and HIV-1/2 antibodies were screened. Results of culture positivity based on human immunodeficiency virus status of participants is shown in Table 1. The prevalence of culture-positive TB was higher in the HIV-negative sub-population compared with the HIV-positive participants [Table 1]. Prevalence of multi-drug-resistant tuberculosis with respect to human immunodeficiency virus positivity is shown in Table 2. The rate of MDR-TB was high among HIV-positive patients though not statistically significant. HIV positive patients showed prevalence of 66.70% whereas HIV-negative patients had 42.60% [Table 2]. Drug susceptibility testing was done on the isolates; Assessment of drug resistance with respect to human immunodeficiency virus status are shown in Table 3. Eighty-three of the isolates were obtained from culture after suspected colonies were subjected to morphological, biochemical, and immunological tests and of the 83 (80.6%) samples analyzed using Xpert MTB/RIF assay 45 (67.2%) were rifampicin-resistant. The resistance pattern of isolates with respect to human immunodeficiency virus positivity are detailed in Table 4.

Table 1: Culture positivity based on human immunodeficiency virus status of participants

HIV status	Culture positive (%)	Culture negative (%)
HIV negative	82.02	17.98
HIV positive	71.40	29.60

HIV: Human immunodeficiency virus

Table 2: Prevalence of multi-drug-resistant tuberculosis with respect to human immunodeficiency virus positivity

HIV status	MDR-TB isolates (%)	Non-MDR-TB isolates (%)
HIV negative	42.60	57.40
HIV positive	66.70	33.30

HIV: Human immunodeficiency virus, MDR-TB: Multi-drug-resistant tuberculosis

Table 3: Assessment of drug resistance with respect to human immunodeficiency virus status

HIV status	Resistant isolates	Percentage of resistant isolates	Resistant rate (%)	*P*
Positive	6	9.0	60.0	0.076
Negative	61	91.0	83.6	

HIV: Human immunodeficiency virus

Table 4: Resistance pattern of isolates with respect to human immunodeficiency virus positivity

Drug pattern	Number of resistant isolates	
	HIV positive	HIV negative
Rifampicin only	0	1
Ethambutol only	0	1
Isoniazid only	0	8
Streptomycin only	0	3
Rifampicin and ethambutol	0	2
Rifampicin and streptomycin	1	8
Isoniazid and ethambutol	1	3
Isoniazid and streptomycin	0	4
Isoniazid and rifampicin	1	4
Rifampicin, isoniazid, and ethambutol	0	2
Streptomycin, isoniazid, and rifampicin	1	7
Strptomycin, rifampicin, and ethambutol	0	3
Streptomycin, isoniazid, and ethambutol	0	2
Streptomycin, isoniazid, rifampicin, and ethambutol	2	13

HIV: Human immunodeficiency virus

Discussion

In the 1980s, it was realized that TB had not only ceased to decline in the developed countries, notably, the USA but also was actually increasing, particularly in major cities.[15] Subsequently, it was realized that the disease was out of control and increasing at an alarming rate across most of the poorest regions of the world, especially Africa due to HIV/AIDS[16,17] and today, Nigeria is ranked the 4th among 22 high burden TB countries and worthy of note is the fact that TB remains one of the killer diseases among children, adults, and people living with HIV.[18]

Based on the existing facts, it is estimated that 26% of patients with TB infection in Nigeria are HIV positive[18] and this with the alarming evidence that MDR-TB can be transmitted is of great concern and calls for close monitoring of the prevalence of drug resistance, especially in HIV-infected population.

MDR-TB has reached alarming levels worldwide with the emergence of strains that are virtually untreatable with the existing drugs. Drug-resistant strains, along with HIV/AIDS, are causing the biggest challenge to efficient management and control of TB. MDR-TB which is caused by *M. tuberculosis* strains that do not respond to standard therapies not only poses problems for the treatment of individuals but also for the control of TB in populations as it represents lapses in public health.[19] Although the evidence for HIV as a specific risk factor for MDR-TB is variable, HIV has been associated with gastrointestinal malabsorption of anti-TB medications.[4]

The rate of MDR-TB was higher among HIV-positive patients though not statistically significant. HIV as a risk factor for MDR-TB has not been demonstrated in studies. No significant association between HIV and MDR-TB was demonstrable in Nnewi[8] Furthermore, in a review of HIV as a risk factor for MDR-TB,[20] reported that not a single study in Africa demonstrated an association between HIV infection and MDR-TB. It was observed that HIV increases the chances of transmission of MDR-TB rather than leading to inadequate treatment.[21]

Scientifically, several biological mechanisms linking-drug-resistant TB to HIV infection have been suggested. Drug malabsorbtion, especially rifampicin and ethambutol in HIV-infected patients can lead to drug resistance.[22] However, in this study, HIV infection had no significant association with drug resistance and this agrees with Lukoye *et al*.,[23] who stated that evidence showing HIV as an established independent risk factor for drug resistance has not been documented. Unfortunately, people think that HIV infection is a risk factor for drug resistance.

Conclusions

According to the National drug resistance survey in 2012, the prevalence rate of MDR-TB in Nigeria is about 2.9% (WHO, 2013). However, MDR-TB in Africa, including in Nigeria, is more prevalent than previously reported. Given the limited health care funding and substantial incidence of HIV in Nigeria, even a relatively low but increasing tide of MDR-TB can lead to disastrous consequences for the country. This study demonstrated that there is association between MDR-TB and HIV though not statistically significant, calls for strong considerations of HIV/TB control program.

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Authors' contributions

This study was carried out in collaboration between all authors. They have developed their assigned parts of the manuscript and reviewed the other parts. All authors reviewed and agreed the final version of the manuscript.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Compliance with ethical principles

The study was approved by the Research Ethical Committee of Nnamdi Azikiwe University Teaching Hospital Nnewi and all participants provided informed consent and participants' confidentiality was maintained throughout the study.

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