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Tuberculosis and Human Immunodeficiency Virus Co-Infection in Rural Eastern Nigeria

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Abstract

Background: HIV promotes progression of TB latent infection to active disease and the relapse of TB in previously treated patients. TB is the leading cause of death in HIV infected patients. Each disease speeds up the progress of the other. This study aims to determine the seroprevalence of the HIV infections, the presence of Mycobacteria species and their drug profile and susceptibility patterns.

Study design: A study population of 805 new subjects presenting with symptoms of bronchopulmonary disorders were studied between January 2011 to June 2012 in health facilities in rural communities in Eastern Nigeria.

This study was made using questionnaire, tuberculosis and HIV tests.

Results: A total of 744 (0.9%) patients were positive for TB and 620(0.7%) for HIV out of which 405 (0.5%) were positive for HIV-1, 215 (0.2%) for HIV-2 and 163 (0.2%) for HIV-1 and HIV-2 antibodies. Correlation of the positivity rates for both HIV and TB showed that 543 (0.6%) of the 744 (0.9%) patients positive for TB were also positive for HIV. Strains of *M. tuberculosis, M. bovis* and other Mycobacteria were associated with pulmonary tuberculosis and their resistance to isoniazide was highest (0.16%), followed by rifampicin (0.15%), streptomycin (0.08%), ethambutol (0.08%) and para-aminosalicyclic acid (0.04%).

Conclusion: Data from this study should be applied to TB/HIV control programmes for effective and proper management of patients as well as formation of a basis for accelerated public awareness of the risk of TB/HIV co-infection in rural communities.

Keywords: Tuberculosis; Human Immunodeficiency Virus (HIV); Co-infection; Rural; Eastern Nigeria

Introduction

The increasing number of new tuberculosis (TB) cases each year, propelled by the 10% annual increase in TB incidence in sub-Saharan Africa is attributable largely to human immunodeficiency virus (HIV) infection.

Co-infection rates in TB-infected patients in some countries are as high as 79% [1].

The HIV epidemic is not merely increasing TB but is also driving a significant increase in the proportion of cases that are smear-negative pulmonary and extrapulmonary; these presentations of TB pose considerable challenges to currently available diagnostic methods and to clinical management. Even when diagnosed, HIV-positive, smear-negative pulmonary TB patients have inferior treatment outcomes, including excessive early mortality [2].

The HIV epidemic is now of such magnitude that meeting initial TB control targets for sub-Saharan Africa would only result in a marginal decline of the annual rise in incidence in the region- from 10% to 7% per year [3].

In order to counter the HIV-driven TB epidemic, WHO and the Stop TB partnership advocate a TB control strategy of expanded scope [4].

These expanded efforts will be central to decreasing the burden of TB in HIV positive persons, and to reversing the alarming rise in African (and global) incidence rates [5].

It is now widely recognized that collaboration between TB and HIV/AIDS disease programmes to provide patient-centered, integrated care and services will be essential to controlling the TB epidemic [1].

This work is carried out in Eastern Nigeria to study the TB and HIV co-infection in the study population, to determine the seroprevalence rate of HIV infection among the TB patients in order to ascertain the HIV infection that is more prevalent in the study population, to detect the presence of Mycobacteria species in the subjects, and to evaluate their drug profile and susceptibility patterns.

Materials and Methods

A total of 805 new cases presenting with symptoms of bronchopulmonary disorders from January 2008 to August 2009 were randomly selected from ten health facilities in rural communities in Eastern Nigeria.

Questionnaire was used for collection of their clinical history and

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biodata. Sputum and sera duplicated samples were obtained from all the patients.

The sputum samples were examined for acid-fast bacilli (AFB) by both Ziehl-Neelsen (ZN) staining, microscopy and culture on Loweinstein-Jensen (LJ) medium. The sputum samples were decontaminated with sodium hydroxide using the modified Petroff's technique in a class one safety cabinet.

Smears were made on clean grease-free slides with part of the resultant final deposits or pellets and later stained using the ZN staining method and examined microscopically for AFB using the ×100 oil immersion objective lens of a binocular light microscope.

The remaining part of the final deposits or pellets were used to inoculate (using a sterile pasteur pipette) on sterile LJ slopes prepared in the laboratory for culture. Prepared cultures showing no growth of *M. tuberculosis* after eight weeks of incubation at 37°C were discarded as negative.

Those with growth were confirmed as acid-fast bacilli by ZN staining using WHO protocol for diagnosis of TB at district level [6, 7].

The cultures with AFB properties were purified by further sub-culturing onto fresh pairs of LJ slopes and incubated at 37°C for 4 weeks. Resultant colonies were further screened for acid-fast properties by ZN microscopy [7] before they are identified by biochemical tests and then tested against anti-TB drugs to determine their susceptibility.

Since there is no completely reliable single test that will differentiate *M. tuberculosis* from other Mycobacteria species, confirmatory tests were done on all preliminary bacilli using biochemical tests. Pure cultures of the isolates were identified based on cultural characteristics (Growth rate, pigment production, Growth on McConkey agar, and results of battery of biochemical tests (Niacin test, Tween-80 hydrolysis, Tellurite reduction, Nitrate reduction, Arylsulfatase, and catalase test).

The pure strains of Mycobacteria isolated from the patients were characterized based on the results obtained from these culture characteristics and biochemical tests. The drug susceptibility pattern of these mycobacteria isolates to anti-tuberculosis drugs was performed with BACTEC MGIT 960 radiometric test and also by the proportion method [8] using LJ medium and the critical proportion taken as 1% [8].

The drugs tested on the organism were isoniazid (INH), Streptomycin (STP), ethambutol, para-aminosalicyclic acid and rifampicin. Susceptibility tests of the mycobacteria to these drugs were performed by preparing drug containing media for the various drugs by incorporating isoniazide (0.2 ug/ml medium), streptomycin(8 ug/ml medium), ethambutol (2 ug/ml medium), para-aminosalicyclic acid (ug/ml medium) and rifampicin (40 ug/ml medium) into LJ medium before inspissation.

Duplicate drug containing tubes were inoculated with 0.2 ml of 10^{-3} and 10^{-5} dilutions of even cell suspensions of each isolate. Duplicate tubes of control medium without drugs were also inoculated. All the inoculated tubes were incubated at 37°C and examined after 4 weeks.

The number of colonies on the control and drug containing media were counted. The critical proportion was taken as 1% bacterial proportion growing on the drug containing medium as compared to the control medium was considered as resistant [8]. The sera samples were screened for HIV-1 and HIV-2 antibodies by using a rapid test kit (Genie 2 HIV 1 and 2 Biorad), ELISA (DIAPRO HIV-1, 2 and group 0) and ELISA (Wellcozyme and Elavia 2 Systems) according to manufacturer's instructions. Positives were confirmed by Western blot (Dupont and Lavblot 2) and ELISA test kit (Diagnostic Bioprobes SRL, Milan, Italy), according to manufacturer's instructions. The veinous blood samples were carefully collected without stasis from each patient. Then, 5 ml of blood collected were immediately put into sterile vacutainer tubes. The serum was obtained after centrifugation of the vacutainer at 2,000 revolutions per minute (rpm) for 60 seconds at room temperature for human immunodeficiency virus testing.

Testing for HIV antibodies was performed using the ELISA test kits according to manufacturer's instructions. The collected sera samples from the patients were tested at least twice with the test kits for the detection, confirmation, serology of HIV-1 and HIV-2.

Confirmed HIV positive sera were collected and serotyped using LAVBLOT 1 and 2 kits. The kits can discriminate HIV-1 and HIV-2 serologically and were used according to the manufacturer's instructions.

The study participants were informed about the results of their laboratory tests through their physicians and were counseled regularly by trained workers at the health facilities.

Data generated were entered and analyzed using EPI-INFO 6.02 software [9].

(Centers for Disease Control and Prevention, Atlanta, USA).

An epitable menu employing a McNemar's corrected chi-square test for 1.2 marched pairs was used to compare the differences.

The chi-square tests with probability values less than 0.05($P<0.05$) were considered to be significant.

Results

TB/HIV Co-infection was highest among 23-34 age groups (21.7%).

They are of child bearing age. The least TB/HIV co-infection was 66-74 age groups (0.2%). A total of 620 (77%) of all the patients were seropositive for HIV out of which 405(1.5%) were positive for HIV-1, 215(2.8%) for HIV-2 and 163 (3.8%) having both HIV-1 and HIV-2 antibodies.

Correlation of the positivity rates for both HIV and TB showed that 543(0.6%) of the 744(0.9%) patients positive for TB were also positive for HIV. On the other hand, only 35(0.04%) of the 71(0.08%) patients negative for TB were positive for HIV. The HIV seroprevalence was 5.6% and 6.3% in TB patients which confirms that infections are emerging progressively.

These results are shown in the table below (Table 1).

Mycobacteria species isolated from the patients were characterized based on results obtained from the culture

Disease conditions	Frequency	Rate of infection (%)
TB (+ve)	744	0.9
TB (-ve)	71	0.08
HIV-1 (+ve)	405	0.5
HIV-2 (+ve)	215	0.2
HIV-1 &HIV-2 (+ve)	163	0.2
HIV&TB (+ve)	543	0.6
Apparently healthy subjects	0	0

Table 1: Distribution of patient categories. Inference: HO is rejected which means that there is a significant difference between TB and HIV status.

Mycobacteria strains isolated	Number of isolates	Rate of isolation (%)
M. tuberculosis	239	3.1
M. bovis	55	0.07
M. avium	29	0.0.3
M. kansassi	33	0.04
M. fortitium	16	0.02
M. xenopi	10	0.01
Total incidence of Atypical Mycobacteria	382	4.8

Table 2: Distribution of mycobacteria species.

Drug/ Nature of resistance (n=744)	Number of resistant isolates	Percentage of resistance (%)
Resistance to one drug	96	0.12
Resistance to two drugs	30	0.04
Resistance to three drugs	18	0.02
Resistance to one or more drugs	120	0.16
Sensitive to all the drugs	169	0.22
Isoniazid	121	0.16
Streptomycin	66	0.08
Para-amino salicyclic acid.	34	0.04
Ethambutol	64	0.08
Rifampin	113	0.15

Table 3: Drug resistant profile of the mycobacteria strains isolated.

characteristics and biochemical tests and the result is outlined in the table below (Table 2).

Over the period of study, strains of *Mycobacterium tuberculosis*, *M. bovis* and other mycobacteria were associated with pulmonary tuberculosis. *M. tuberculosis* is the most prevalent strain isolated.

Drug susceptibility results showed that 169 (0.22%) of the Mycobacteria species were sensitive to all the drugs tested while 120 (0.16%) were resistant to one or more of the drugs tested (Table 3).

Strains of *M. tuberculosis, M. bovis* and other mycobacteria were associated with pulmonary tuberculosis and their resistance to isoniazid was highest (0.16%), followed by rifampin (0.15%), Streptomycin (0.08%), and par -aminosalicyclic acid (0.04%).

Discussion

This study established the type of classical and atypical mycobacteria involved in pulmonary tuberculosis in rural communities in Eastern Nigeria. The classical mycobacteria are called the *Mycobacterium tuberculosis* complex which consists of Mycobacterial species with such high genetic relatedness that they are referred to as a "complex". With the exception of *M. tuberculosis, t*he species rarely cause human disease. Multi-drug resistant tuberculosis (MDRTB) is that TB that is resistant to isoniazid and rifampin. They are more difficult to treat than drug-susceptible TB [10]. Classical atypical mycobacteria are recorded in this study with *M. tuberculosis* and *M. bovis* being most incriminated.

The point of interest is that *M. xenopi* which has not previously been reported in pulmonary infections in Eastern Nigeria was identified in this study.

Furthermore, various strains of atypical mycobacteria constituted 30% of the isolates characterized.

The level of drug resistance recorded in this study is very high. Poor drug compliance due to inadequate and regular supply of drugs as well as poverty and transportation problems is believed to be largely responsible for the drug resistance in the patients.

This study is continous with more recently isolated strains being characterized and tested for drug susceptibility.

HIV-1 patients were 405 (0.5%) and more prevalent in the study population than HIV-2 which was 215 (0.2%). The difference in the seroprevalence rates for

HIV-2 and HIV-1 are significant ($p<0.05$) and tends to suggest that HIV-1 infection is more prevalent in the population studied.

A total of 543 (0.6%) of TB positives were also HIV-seropositive as against 71 (0.08%) TB negative patient.

There was no apparently healthy subject i.e; without TB or HIV. The high HIV prevalence rate in TB positives suggests that there is an association between TB and HIV infection in rural Eastern Nigeria.

Therefore, it is necessary to subject all patients presenting with symptoms of TB to HIV test before they are treated for TB to ensure that appropriate drugs and treatment are given to them.

This study also established the types and frequency of mycobacteria strains involved in pulmonary disorders in the study area so as to determine the sensitivity pattern of the mycobacterial isolates to currently use anti-tuberculosis drugs in rural communities in Eastern Nigeria.

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