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Diagnosis of Pulmonary Tuberculosis Using Genotype MTBDRplus Assay in Three Local Government Primary Health Centres of Osun State, Nigeria- a Pilot Study

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Abstract

Study background: Tuberculosis including multi-drug resistant form is not adequately diagnosed in spite of the fact that it constitutes a major public health challenge. This study was carried out to obtain data on laboratory diagnosis of pulmonary tuberculosis at the three local government areas in Osun state, Nigeria.

Methods: Six month pilot study was carried out at Iwo, Irewole and Ede North LGAs, all located in Osun state, Nigeria. Socio-demographic and clinical information of the subjects were obtained using a pre-tested questionnaire. Sputum samples were collected from symptomatic pulmonary tuberculosis patients, stained with Zeihl-Neelsen (Z-N) reagents and cultured on egg-based Lowenstein-Jensen medium. The medium was incubated at 37°C for eight weeks. Acid Fast Bacilli was confirmed by repeat Z-N staining. Drug resistance testing of the isolates was done using Genotype MTBDRplus. Serum was screened for HIV test using recombinant ELISA. Those that were screened positive were retested using Capillus HIV 1 and 2.

Results: Of the 75 subjects studied, seven (9.3%) were over 60 years while the majority, 45 (76.0%) were aged 21-60 years. Thirty-four (45.3%) had their sputum positive for acid fast bacilli (AFB) while 24 (32.0%) were culture positive. Among the 24 isolates, 6 were identified to be non tuberculos mycobacteria (NTM) by molecular methods. The HIV prevalence rate was observed to be 18.6% among all the study participants. Six out of 34 AFB positive persons (17.6%) were also positive for HIV specific antibody, while eight (19.5%) of the AFB negative persons were positive for HIV. There is no significant difference in the incidence of TB between the HIV infected and uninfected groups (p>0.05).Among the 18 mycobacterial isolates (excluding NTM), one strain (5.5%) was found to be resistant to rifampicin and isoniazid (Multi-Drug Resistant-MDR) while three were resistant to isoniazid alone..

Conclusion: This pilot study reveals the existence of MDR-TB and warrants well designed studies to ascertain the magnitude of the problem.

Keywords: Tuberculosis; Multi-drug resistant; Osun state; Nigeria

Introduction

Tuberculosis (TB) continues to be a major threat worldwide despite the existence of antituberculosis drugs for the past 60 years [1]. About 80% of the estimated 9.4 million, new TB cases arising each year occur amongst the 22 countries with the highest global TB burden [1]. With an incidence of 460 smear positive TB cases per 100,000 inhabitants in 2009, Nigeria ranks fourth among these countries [2]. The TB burden in Nigeria is further strengthened by the relative high prevalence of HIV co-infection; as according to the World Health Organization (WHO) [2], about 18% of all TB patients tested were positive for HIV.

Certain important milestones are paramount in the fight to stop the spread of TB globally. These include: early diagnosis, prevention of the spread of the disease, effective treatment with anti-TB drugs and prevention of development of multidrug-resistant bacteria strains. In Nigeria as well as other high burden countries, these milestones have not been adequately addressed [1,3]. Thus, quick and reliable identification of the causative organism and early detection of drug-resistant strains are prerequisites to the effective treatment and confinement of the disease.

The primary diagnostic tool in most of the disease endemic countries remain smear microscopy with few centers having capacity for culture with little or no facilities for drug resistant testing [4,5]. Among the countries in sub-Saharan Africa with high burden of the disease, only few had national surveillance data on MDR-TB [2]. In Nigeria, information on the burden of MDR-TB especially in relation to risk factor like HIV infection is not readily available. Thus this study was carried out to provide preliminary data on MDR-TB in three local government areas in Osun state.

Materials and Methods

This six month cross sectional epidemiological study (January-June, 2011) was carried out at three designated DOTS centers in three Local Government primary health centers in Osun state (Iwo, Irewole and Ede North); the TB laboratory of the University College Hospital (UCH), Ibadan and the TB laboratory of the Nigerian Institute for Medical Research (NIMR), Lagos, Nigeria.

The three LGAs studied have a population of about one million [6]. The TB laboratory in UCH is a designated facility for isolation of Mycobacterium Tuberculosis Complex (MTBC) in the Southwestern part of

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the country while TB laboratory in NIMR serves as National TB reference Laboratory with capacity for detection of MDR-TB.

Of the 105 subjects recruited, only 75 (71.4%) gave their consent and participated in the study. The study protocol was approved by the Ministry of Health, Osun state. Verbal and written consent was obtained from the subjects before enrollment into the study. Those who refused to give written consent were excluded.

Socio demographic characteristics and medical history of the subjects were obtained by questionnaire.

Laboratory Investigations

Smear microscopy and Culture on solid media

Two sputum specimens (on the spot and early morning) were collected from each of the clinically diagnosed confirmed pulmonary TB patients. The specimens were transported to the TB laboratory, UCH for immediate processing. Each sample was smeared, air-dried, fixed and stained with Zeihl-Neelsen (Z-N) reagents. A known positive Acid Fast Bacilli (AFB) and slide stained with egg albumin were used as positive and negative controls respectively. The results were read according to the grading system of the International Union against TB and Lung diseases [7].

For isolation of the organism, sputum was decontaminated using N-Acetyl L-cysteine /Sodium Hydroxide (NALC/NaOH) method [8]. The resulting solution was mixed by vortex mixer. About one ml from the mixture was inoculated onto freshly prepared Lowenstein-Jensen (LJ) medium, incubated at 37°C for eight weeks. *M. tuberculosis* strain H37Rv and sterile LJ medium were used as positive and negative controls respectively. Evidence of contamination was determined by looking out for growth before two weeks of incubation while suspicious isolates were confirmed by colonial morphology, Z N reaction and standard biochemical tests [9]. Patients revealed the presence of AFB by smear or cultures were referred to the chest physician for management.

Drug resistance testing

The culture isolates were subjected to further confirmation as MTBC and then drug resistance testing to rifampicin and isoniazid using Genotype MTBDRplus (HAIN Lifescience, GmbH, Germany). The entire technique was divided into three parts namely: DNA extraction from positive isolates on LJ slope, PCR amplification and hybridization using MTBDRplus dedicated incubator. The entire procedure was carried out according to the manufacturers' instructions [10].

HIV testing

About 5 ml of venous blood was collected from each subject. Serum was screened for HIV using recombinant ELISA kit (Human Biochemical and Diagnostic Laboratories, Germany). Those that were screened positive were retested using Capillus HIV 1 and 2 (Cambridge diagnostic). Positive ones were appropriately counseled and referred to the nearest antiretroviral center for management.

Data analysis

Data was coded and analyzed using statistical software SPSS version 10.0 (SPSS Inc, Chicago, IL, USA). Frequency tables were used to describe demographic characteristics and laboratory variables while Chi square test was used to measure the association between categorical variables.

Results

Demographic characteristics

Consent was sought from 105 participants, of whom 75 subjects (71.4%) gave consent and participated in the study giving a male to female ratio of 0.80 to 1.00 respectively. Only seven (9.3%) were over 60 years while the majority (76.0%) were aged 21-60 years. Of the 75 subjects, six (8.0%) were professionals while 10 (13.3 %) were unemployed (Table 1).

Laboratory data

Thirty-four (45.3%) of the 75 subjects had their sputum samples positive for AFB on smear microscopy while 41 (54.7%) were negative. Twenty four (32.0%) samples were positive by culture, 48 (64.0%) were negative while three (4.0%) were contaminants.

Among the study participants, 24 (32.0%) were positive for both smear microscopy and culture while 24.0% were smear negative but positive for culture (Table 2). Prevalence of HIV positivity among the study population was 14 (18.6%). Of the 34 subjects with smear positive samples, six (17.6%) were HIV positive while eight (19.5%) out of the 41 smear negative ones were positive for HIV. There was no HIV difference ($p>0.05$). Large proportions of subjects with smear negative and culture positive samples and those with only positive culture samples were also HIV positive (33.3%) (Table 2).

Of the 24 isolates tested for drug resistance, one (5.5%) was found

Variable	Sex		
	Male No (%)	Female No(%)	Total No (%)
Age (yr)			
<20	09 (42.9%)	12 (57.1%)	21 (100.0%)
21-40	11 (47.8%)	23 (52.2%)	34 (100.0%)
41-60	10 (76.9%)	03 (23.1%)	13 (100.0%)
>60	02 (28.6%)	05 (71.4%)	07 (100.0%)
Total	32 (42.7%)	43 (57.3%)	75 (100.0%)
Occupational status			
Professionals	02 (33.3%)	04 (66.7%)	06 (100.0%)
Skilled	05 (83.3%)	01 (16.7%)	06 (100.0%)
Unskilled	20 (37.7%)	33 (62.3%)	53 (100.0%)
Unemployed	07 (70.0%)	03 (30.0%)	30 (100.0%)
Total	34 (45.3%)	41 (54.7%)	75

Table 1: Demographic characteristics of study subjects by sex.

Test	Result No (%)	HIV positivity No (%)
Smear Microscopy		
Positive	34 (45.3%)	06 (17.6%)
Negative	41 (54.7%)	08 (19.5%)
Total	75 (100.0%)	14 (18.6%)
Culture	24 (32.0%)	08 (33.3%)
Positive	48 (64.0%)	04 (8.3%)
Negative	03 (4.0%)	02 (66.7%)
Contaminant	75 (100.0%)	14 (18.6%)
Total		
Combined Smear and Culture		
Smear positive Culture positive	24 (32.0%)	06 (25.0%)
Smear negative Culture negative	23 (30.7%)	01 (4.3%)
Smear positive Culture negative	10 (13.3%)	01 (4.3%)
Smear negative Culture positive	18 (24.0%)	06 (33.3%)
Total	75 (100.0%)	14 (18.6%)

Table2: Smear microscopy and culture results by HIV seropositivity.

to be MDR-TB, three (16.7%) showed mono resistance to isoniazid while one (5.5%) was inconclusive. Six (25.0%) were Non Tuberculosis Mycobacteria (NTM) (Figure 1).

Discussion

Globally, ineffective TB detection and rise of drug resistant strains in the presence of high prevalence of HIV infection have led to calls for dramatic expansion of laboratory diagnostic capacities in countries where the two diseases are endemic [2,11]. Majority of the study subjects (76%) were in middle age group. This is in agreement with findings from other studies showing that TB predominately affects patients who are in their productive age [12-14]. This study's positivity rate of 45.3% for smear microscopy and 32.0% for culture were higher than what were earlier reported in this center [15,16]. This might be attributed to the fact that the study populations were symptomatic PTB subjects as against asymptomatic health care workers screened in the two previous studies [15,16]. Moreover, the subjects were recruited directly from their communities through the nearest primary health centers unlike the previous studies which were hospital based.

Fourteen (18.6%) of the subjects were HIV positive. This figure compares favorably with the national statistics of 21% [2]. This implies that DOTS centers can serve as an important entry point for HIV diagnosis, care and support. A significant percentage of subjects with culture positive samples were also HIV positive (Table 2). This further reinforce the fact that TB is one of the most common opportunistic infections among HIV-infected individuals, and co-infected individuals are at high risk of death [17,18]. Coordination and cross–talk between the two national health programmes are crucial not only to improve the outcome of HIV-infected TB patients but also to control the burden of TB in Nigeria. Those subjects who had their samples negative on smear microscopy but positive for culture present a great diagnostic challenge to the National TB Control Programme as culture facilities are only available in few centers in the country. These are most likely TB/HIV co-infected subjects (Table 2).

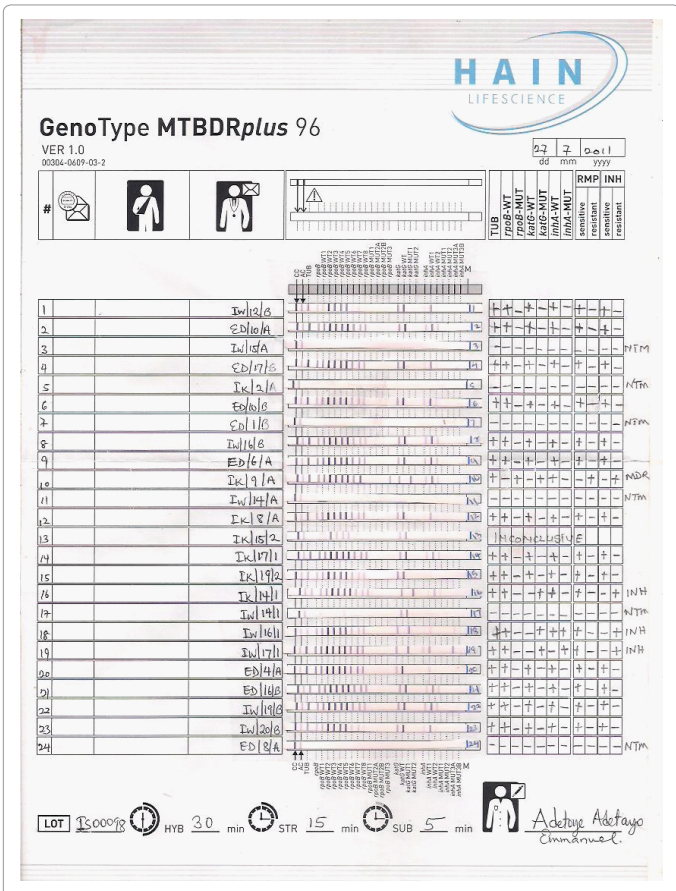

Figure 1: Drug susceptibility results.

Inability to do chest radiography examinations is one of the limitations of the study. This was due to financial constraints. Chest X ray may appear normal in 7-14% of patients with HIV/TB co-infections [19,20], and at the same time miss a substantial proportion of individuals with sub –clinical disease, often seen in advanced HIV immunosuppression [1,22].

Seventy five percent of the isolates were confirmed as MTBC while six (25.0%) were NTM. The MTBC comprises *M. tuberculosis, M.africanum* and *M. bovis*. These three mycobacteria species are AFB and indistinguishable by smear microscopy which is the WHO diagnostic tool available in most of the disease endemic countries including Nigeria. The need to employ molecular typing tool to distinguish between members of MTBC cannot be over emphasized because some species may be associated with serious diseases in humans [23].

About three quarter (72.2%) of the MTBC isolates were susceptible to isoniazid and rifampicin while one was resistant to both drugs (MDR-TB isolate). The exact cause of the emergence of this MDR strain is unknown; however; poor drug compliance could be a possible reason.

The findings of this study show that MDR-TB is present in Osun state. This necessitates a well design study to ascertain the actual burden of MDR-TB in the community.

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