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Full Length Research Paper

Sensitivity of direct smear microscopy for the diagnosis of TB in high HIV prevalent population

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Diagnosis of tuberculosis (TB) amongst HIV patients is a great challenge due to the low density of Acid Fast bacilli (AFB) in their sputum. The study was conducted to determine the sensitivity of direct smear microscopy (DSM) for TB diagnosis in HIV endemic setting using culture as a gold standard. During the period of study, 549 TB suspects were screened, and they comprised of 396(72%) HIV-positive and 153(28%) HIV-negative patients. Overall, TB positive rate was 221(40.3%), the rate by culture and DSM were 155(28.2%) and 12(2.2%) respectively. Among HIV-positive patients, the overall TB positive rate was 151(38.1%), while the rate by culture and DSM were 120(30.3%) and 7(1.7%) respectively (p = 0.0001). The overall positive rate for HIV-negative was 70(45.8%) and the rate by culture was 35(22.9%) and DSM was 5(3.3%) with p = 0.0004. The sensitivity of DSM in HIV- positive and negative population was 21.5 and 53.8% respectively. Findings from this study showed the low case detection rate of DSM as compared to culture, more especially in HIV positive persons. To improve case detection, we recommend the use of culture as back up to enhance the performance of DSM especially in HIV positive persons.

Key words: Tuberculosis, HIV, acid fast bacilli, direct smear microscopy, culture.

INTRODUCTION

Establishing a definitive diagnosis of TB in HIV-infected patients has remained a challenge world wide. The situation is even worrisome in resource-limited settings of Sub-saharan Africa where a significant number of HIV cases occur. Diagnosis of TB in this region is basically by DSM (Wilkinson and Sturm, 1997; Gebre et al., 1995).

This technique is relatively inexpensive, widely available and able to identify infectious cases (Cattamanchi et al., 2009; Behr et al., 1999). However, the overall sensitivity, for identifying TB infection in the general population is 35 to 70% while sensitivity in HIV-infected cases is further reduced to 20% (Elliott et al., 1993; Wood, 2007; Matee et al., 2008).

Culture of *Mycobacterium* sp. from clinical specimen is regarded as gold standard for TB diagnosis (Getahun et al., 2007). This technique is usually not employed in resource-limited settings because of issues associated with infrastructural requirement and funding (Apers et al., 2003; Hudson et al., 2000).This study was aimed at determining the sensitivity of direct smear microscopy for the diagnosis of TB in high HIV prevalent setting.

MATERIALS AND METHODS

Study center

The study was carried out at the Nigerian Institute of Medical

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Table 1. Age and sex distribution of the study population.

Age groups (years)	Males n (%)	Females n (%)
0 - 14	3(0.5)	2 (0.4)
15 - 24	31 (5.6)	23(4.2)
25 - 34	114(20.8)	111(20.2)
35 - 44	117(21.3)	45 (8.2)
45 - 54	52(9.5)	31(5.6)
55 - 64	6 (1.1)	8(1.5)
≥ 65	5 (0.9)	1(0.2)
Total	328 (59.6)	221 (40.3)

Research (NIMR) Yaba Lagos, Nigeria. NIMR has several divisions and units including the DOTS/TB clinic, HIV clinic and the National TB Reference Laboratory (NTBRL) where this study was carried out. The NIMR-HIV clinic refers HIV-positive patients who are TB suspects to NTBRL for diagnosis and treatment monitoring. In addition, patients (from other parts of the country) with complicated TB cases are usually referred to NTBRL for rapid and improved laboratory investigations.

Ethical approval and study population

Permission to carry out the study was granted by the NIMR ethical committee. Only those patients that gave written informed consent were enrolled in the study. Inclusion criteria included male and female TB suspects of all age groups that consented to HIV counseling and testing. Exclusion criteria included patients without symptoms of TB, and the TB suspects with unknown HIV status, who refused to undergo HIV testing after counseling. Sampling was non-randomized as all eligible TB suspects who presented at NTBRL were enrolled. Patients' bio-data and history of disease was obtained with a questionnaire.

Specimen collection

This was a six month prospective study carried out in NIMR between January 2009 to June 2009. Five hundred and seventy one TB suspects that visited NIMR DOTS clinic were instructed on how to produce good quality sputum. Each patient produced 2 sputum samples (Spot and early morning) in sterile wide mouthed, screw capped containers. Samples when collected were maintained at temperatures between 4 to 8°C. All samples were processed within 24 h of collection.

Laboratory methods

Laboratory diagnosis of TB was done by DSM and solid culture. Air dried smears were stained by Ziehl Neelsen (ZN) staining technique. Each smear was examined and quantified as per protocol. Sputum samples were decontaminated with 4% NaOH and concentrated by centrifugation at 3000 g for 15 min. Sediments were re-suspended in 2 ml phosphate buffer (pH 6.4) and 3 drops of the sediment inoculated unto slopes of Lowenstein Jensen (LJ) medium. The innoculated LJ slopes were incubated in the dark at 37^{o}C and examined on the third day for contamination and weekly for up to 8 weeks for growth of mycobacteria. Rapid HIV screening was done according to method described by Kongnyuy et al. (2009).

Quality assurance

Internal quality control for DSM included the use of positive (1+) and negative control slides in controlling the staining reagents and procedures. All slides were read by experienced microscopist and stored properly for external quality control. Quality control measures for culture included monitoring of equipment, reagents and performance indicators such as contamination rates and mycobacterium recovery rates.

Statistical analysis

Data obtained in this study was analyzed using Epi Info 3.5.1. Significance limits were set at the 95% probability level.

RESULTS

Out of 571 patients that submitted specimens for AFB smear and culture, 22 patients had contaminated cultures and were excluded from the study. Thus, a total number of 549 patients were enrolled. Gender distributions were 328(59.6%) males and 221(40.3%) females, with male to female ratio of 1.5: 1. (P = 0.5344). Age range of the patients was between 4 and 80 years. About 225 (40.9%) of the study population was within 25 to 34 year age group. Children between the ages of 0 to 14 years and elderly ≥ 65 years had the lowest prevalence rate of 0.9 and 1.1% respectively (Table 1). Proportion of patients with HIV infection is depicted in Figure 1. In all, 396 (72%) of the study population were infected with HIV while 153 (28%) were HIV negative.

Table 2 shows the culture and DSM results of the HIV-positive cases. Among this group, there were 151 (38.1%) positive cases of TB. Of which 120 (30.3%) were culture-positive DSM-negative and 7 (1.7%) were culture-negative DSM-positive (p = 0.0001).

The culture and DSM results of the HIV-negative cases are shown in Table 3. Seventy patients (45.8%) from this group were TB positive. Of this, 35 (22.9%) were culture-positive DSM-negative while 5 (3.3%) were culture-negative DSM-positive (p = 0.0004). In all, 221 (40.3%) patients from both HIV-positive and negative populations had TB.

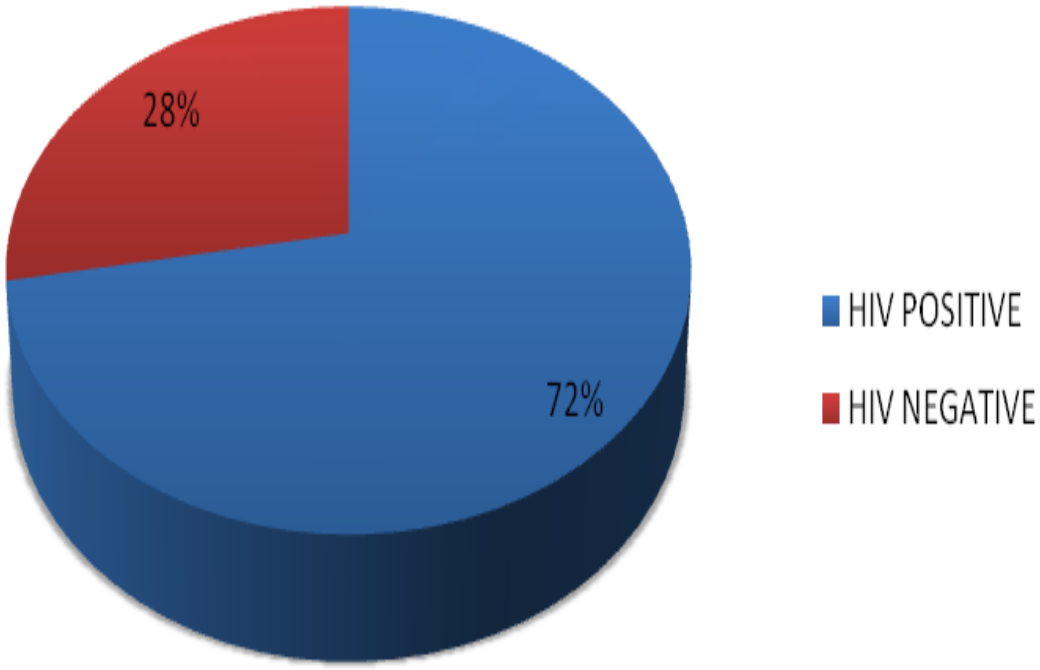

Figure 1. Proportion of the study population with HIV infection.

Table 2. Culture and DSM results of 396 HIV-positive cases.

Variable	Culture-positive (n = 144) DS M n (%)	Culture-negative (n = 252) DSM n (%)
Positive	24 (6.1)	7 (1.7)
Negative	120 (30.3)	245 (61.9)

Table 3. Culture and DSM results of 153 HIV-negative cases.

Variable	Culture-positive (n = 65) DSM n (%)	Culture-negative (n = 88) DSM n (%)
Positive	30 (19.6)	5 (3.3)
Negative	35 (22.9)	83 (54.2)

DISCUSSION

A TB burden of 40.3% was recorded in this study. This appears higher than reports from studies done in other parts of the country. Studies done in Lagos and Edo state demonstrated lower prevalence rates of 23 and 21 % respectively (Nwobu et al., 2004; Ilorin et al., 1998; Idigbe et al., 1997). Similarly report by Nwachukwu et al. (2009) demonstrated a TB prevalence rate of 16.8% in Abia state. However, a higher prevalence rate of 65% was reported in Kano by Taura et al. (2008). Reason for high TB rate in the study may be because of the category of patients that visited the study center. NIMR as a NTBRL has the capacity to carry out additional TB laboratory investigations other than TB screening by AFB smear microscopy. Consequently patients that visited the study center were mostly confirmed TB cases who were referred not necessarily for TB diagnosis but for Drug susceptibility testing. Another possibility for high TB prevalence among the study population was the high HIV positivity rate of 72%. Increasing number of HIV patients with latent TB infection are fast developing active disease associated with clinical signs and symptoms due to the suppressed immunity caused by HIV (Haskins et al., 2009). Since TB screening is carried out on only symptomatic individuals, it then follows that a good number of TB- HIV co-infected patients with undiagnosed TB would be screened and captured as TB-positive

cases.

The male preponderance in this study could be attributed to gender difference in behavior, social and economic factors. Smoking and abuse of alcohol result in decrease immunity and increase propensity to develop progressive form of TB (Holmes et al., 1998). Men may perhaps be more vulnerable to TB infection than females due to their high drinking and smoking habits. Social isolation due to stigma associated with TB affects both sexes, but the consequences may be harsher for females. Studies have demonstrated that female patients were at risk of divorce, marital breakdown and harassment by in-laws, while the unmarried ones were concerned about reduced chances of marriage (Johansson et al., 2000; Balasubramanian et al., 2004). Male patients on the other hand were concerned principally with loss of income and economic hardship. This social stigma may prevent females from seeking medical help. Highest TB prevalence occurred among the 25 to 54 year age group. This is the economically productive age group, who must work to earn a living. There is high possibility of getting infected and transmitting the disease to other members of their families and fellow workers due to factors such as poverty, overcrowding, malnutrition and lack of adequate medical care in the country.

The study demonstrated a TB/HIV co-infection rate of 27.5%. This finding is in agreement with reports from other studies. Ilorin et al. (1998) reported a 34% HIV prevalence rates among the AFB positive population. Similarly, Idigbe et al. (1997) showed TB/HIV co-infection rate of 28% among prison inmates in Lagos state. On the other hand, Umeh et al. (2007) recorded a lower TB/HIV prevalence rate of 12.6% in a referral chest clinic in Nasarawa State. Reports from studies done in other parts of the world have demonstrated higher HIV prevalence among TB case than that seen in the general population (Mohanty and Basheer, 1995; Sharma et al., 2003). More so, African countries with high HIV prevalence rates were usually those with high rate of TB (Godfrey-Faussett and Ayles, 2003). The linear association between HIV and TB strongly demonstrated that HIV could be a major risk factor in the recent TB upsurge.

The findings of this study showed that about 30.3% of the HIV-positive and 22.9% of the HIV-negative patients would have been missed by using only DSM for TB diagnosis. DSM was more sensitive in HIV-negative than in HIV- positive populations. Previous studies had also reported increased sensitivity of DSM in HIV-negative cases (Colebunders and Bastians, 2000; Aderaye et al., 2007). This is not surprising since the minimum number of bacilli needed for detection in stained smear is 5,000 to 10,000 per mL of sputum against 10 to 100 viable bacilli per mL by culture method (Oludiran et al., 2008; Wood, 2007). It then means that many sputum samples from HIV-positive patients will be smear negative due to low rate of caseation necrosis that results in lower number of AFB in their airways (American Thoraxic Society, 2000). In addition, many patients with suspected pulmonary tuberculosis do not produce sputum spontaneously (Aderaye et al., 2007), the situation is even worse in TB patients co-infected with HIV. Most often than none, poor quality sputa are produced by these co-infected patients. The usefulness of DSM depends largely on the quality of the sputum specimens and performance quality of the laboratory. Poor sputum quality often results in AFB smear negative results (Hirooka et al., 2004). This somewhat explains the high rate of smear negative results recorded in the study.

The sensitivity of DSM in this study was lower than that found in Tanzania (61.8%) despite the high HIV-positivity rate of the study population in Tanzania (Matee et al., 2008). Reasons for this could be the concentrated smear and florescence microscopy method used for the study in Tanzania. Reports have shown that smear concentration method and fluorescent stains improved the sensitivity of AFB smears (Miorner et al., 1996; Shinnick and Good, 1995; Woods and Witebsky, 1995) leading to increased detection of TB cases (Bruchfeld et al., 2000; Steingart et al., 2006; Aderaye et al., 2007).

Conclusion

The result aforementioned strongly demonstrated that the use of only DSM for TB diagnosis in HIV endemic area would certainly result to under diagnosis. About 30.3% of the HIV-positive and 22.9% of the HIV-negative patients would have been missed by using only DSM. Missing out these TB patients would consequently result in delay diagnosis; prolong spread of TB in the community and under estimation of TB burden. Therefore, for increased case finding, culture technique is highly recommended as back up to enhance the performance of direct smear microscopy particularly in high HIV prevalent setting.

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