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EPIDEMIOLOGY OF SMEAR - NEGATIVE TUBERCULOSIS IN IBADAN, NIGERIA

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Abstract

Inadequate case detection has been identified as one of the reasons for high burden of tuberculosis (TB) in the world especially in poor resourced countries of Africa and Asia. This retrospective laboratory study involving the review of specimens processed at the TB laboratory of the Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan, Nigeria was carried out over a period of five years (January 2006-December 2010) to access the epidemiology of smear- negative TB. Of the 3468 specimens processed, 2,175 (62.7%) were from males while a lower percentage (37.3%)1293 were from females, giving a M:F = 1:0.37. Over half of the specimens, 2,046 (59.0%) were from patients aged 21 to 60 years, 392 (11.3%) from 11 to 20 years, 825 (23.8%) from 60 years and above while 205 (5.9%) were from age 1-10 years. Most of the 2,663 (76.8%) specimens processed were sputum while 201 (5.8%) were gastric washings. Three hundred and nine (8.9%) were smear positive while 392 (11.3%) out of the 3468 specimens processed were culture positive. However, 83 (2.6%) of the 3159 smear-negative specimens were culture positive (false negative) while 66 (21.4%) of the 309 smear- positive specimens were negative for culture (false positive). The majority, 3010 (86.8%) were smear and culture negative while 309 (8.9%) were positive for both tests. Of the 83 false negative specimens, 51 were sputum samples representing (61.4%), 19 (22.9%) were gastric washings while 13 (15.7%) were from extra-pulmonary sites (CSF, aspirates, ascitic fluids, etc). The findings of 2.6% smear-negative but culture positive (false negative) specimens in this study reveals that culture of specimens in addition to smear microscopy from suspected cases is necessary as a diagnostic /confirmatory tool for tuberculosis.

Keywords: Epidemiology**,** Smear negative, TB, Ibadan, Nigeria

Introduction: Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* and is one of the most important infectious causes of human mortality and morbidity worldwide. Inadequate case detection has been identified as one of the reasons for high burden of TB in the world especially in poor resourced countries of Africa and Asia (Maher et al., 1997).

Smear microscopy and isolation of causative organism in pure culture are the two commonly used methods for diagnosing TB. Only few TB diagnostic centers in many of the low-income countries with high burden of the disease have access to culture facilities. This together with the fact that culture for acid-fast bacilli (AFB) requires 6-8 weeks of incubation, limits its usefulness as a first - line diagnostic tool. This situation makes smear examination for AFB the only readily available test for TB diagnosis. However, AFB smear examination is reported to have a sensitivity of 50-60% (Aber et al., 1980), partly because a positive smear requires 5,000 - 10,000 AFB per uL sputum sample whereas culture requires only 10 - 100 AFB per uL (Parry, 1993; Kim et al., 1984). Furthermore, culture can detect infection in about 80% of true cases (Siddiqi et al., 2003).

Smear- negative TB is defined as symptomatic illness in a patient with at least two smear examinations negative for AFB on different occasions in whom infection is later confirmed by culture (Maher et al., 1997). In the absence of readily available culture facilities in high burden countries, most cases of smear - negative TB are diagnosed based on clinical presentations, radiological findings and other laboratory - based indicators (Siddiqi et al., 2003).

In Nigeria however, case detection of TB cases is mainly carried out by AFB smear examination, as the method is available in all the diagnostic centers while isolation of the pathogen in pure culture only exists in some regional reference laboratories including the TB laboratory of the University College Hospital (UCH) and the two national reference laboratories.

This study was carried out to provide data on AFB smear- negative TB in Ibadan, Nigeria.

Materials and methods: This five year laboratory - based retrospective study (January 2006-December 2010) was carried out at the TB laboratory of the Department of Medical Microbiology and Parasitology, UCH, Ibadan, Nigeria. TB laboratory at the UCH is a regional reference laboratory with facilities for smear microscopy and culture. It receives support from Damien Foundation, Belgium through the National TB and Leprosy Control Program of the Federal Ministry of Health, Abuja, Nigeria. Specimens are received within and outside the hospital including adjoining health centers in the South-western part of the country.

Demographic information such as age, sex, tribe, profession and type of specimen from patients who submitted samples during the study period were retrieved from the available hospital register while smear microscopy and culture results were obtained from the laboratory register.

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For suspected cases of pulmonary TB, three sputum samples from each subject were collected into a well- labeled wide mouth container covered with lid while only one specimen - cerebrospinal fluid, lymph node, urine, etc was collected from those with suspected extra-pulmonary TB. The specimens were transported to the laboratory for immediate processing while any sample that was poorly collected for example; sputum that contain saliva was discarded. A direct smear was made from each specimen and stained with Ziehl - Neelson (ZN) reagents (BDH Chemicals Ltd, Poole, England) using a known AFB stained as positive control and a stained slide made of egg albumin as negative control. Quality control of the ZN reagents was included with every staining slide. Results were recorded according to the grading system of the International Union against TB and Lung Diseases (IUATLD) (Enarson, 2000). Each specimen was then decontaminated with 4% sodium hydroxide (NaOH). Concentration was done by spinning in cold centrifuge (IEC CL 30R, Thermo Electron Corporation, UK) at 3,000 revolutions per minute for five minutes.

The sediment was cultured onto Lowenstein - Jensen (LJ) slope (Biomark laboratories, Pune, India) and incubated at 37^0C for a minimum of six weeks and a maximum of eight weeks before it was discarded as no growth. *M. tuberculosis* strain H37Rv and sterile LJ medium were used as positive and negative controls respectively. Suspicious growth on LJ medium were confirmed as *M. tuberculosis* by re-staining with ZN reagents at two, four, six and eight weeks of incubation and by standard biochemical methods e.g oxidase utilization test, tween hydrolysis and growth in the presence of thiacetazone (Barrow and Feltham, 1995). Contamination on LJ medium was determined by looking for visible growth before two weeks of incubation and by carrying out ZN reaction and biochemical tests (Barrow and Feltham, 1995) on growth after two weeks. Molecular and drug susceptibility testing of the isolates were not carried out due to lack of facilities.

Statistical analysis: Data were coded and analyzed using statistical software SPSS version 10.0 (SPSS InC, Chicago, IL). The laboratory variables and demographic characteristics of the patients were described in form of proportions and percentages. Chi square and Fisher's exact tests (where necessary) were used to measure the association between categorical variables.

Results: Three thousand, four hundred and sixty-eight specimens were processed during the five year study period. Of the total specimens processed, 2,175 (62.7%) were from males while a lower percentage(37.3%) 1293 were from females, giving a male to female ratio of 1.00 to 0.37. More than half of the specimens, 2,046 (59.0%) were from patients aged 21 to 60 years, 392 (11.3%) from 11 to 20 years, 825 (23.8%) from 60 years and above while 205 (5.9%) were from children. The majority, 2,663 (76.8%) of the specimen processed were sputum while lower percentage (5.8%) 202 were gastric washings (Table 1).

Table 1: Types of Specimens processed

Type of Specimen	Number	Percentage
Sputum	2,663	76.8%
Gastric washings	202	5.8%
Specimens from extra -pulmonary sites (CSF, Aspirates, etc)	603	17.4%
Total	3,468	100.0%

Three hundred and nine, (8.9%) specimens were reported smear positive while 392 (11.3%) were culture positive. However, 83 (2.6%) of the 3159 smear-negative specimens were culture positive (false negative) while 66 (21.4%) of the 309 smear-positive specimens were negative for culture (false positive). The majority, 3010 (86.8%) were smear and culture negative while 309 (8.9%) were positive for both tests (Table 2).

Discussion: Smear microscopy is the primary mode of TB detection in many resource constrained settings. This often results in a sizeable number of undiagnosed smear-negative individuals leading to delayed anti-TB therapy (Mtel et al., 2005)

From this study, about two-thirds (62.7%) of the specimens processed were from males suggesting that male patients tend to seek medical attention more than their female counterparts. This may be due to the fact that women face more limitations in their travel and financial resources resulting to less accessibility to general health- care services (WHO, 2005).
More than half (59.0 %) of the specimens processed were from patients within the age bracket 21-60 years. This is in agreement with findings from other studies (Erhabor et al., 2003; Ige et al., 2005). This age bracket corresponds to the economic active age group in the community. Furthermore, 5.8% of the specimens processed were gastric washings from children. The relatively low number of specimens from children may be explained by the difficulty in obtaining sputum samples from them as they tend to swallow their sputum. Thus, diagnosis of TB in children has remained a big task worldwide especially in poor resource countries with high burden of the disease**.** The fact that more than three-quarters (76.4%) of the specimens processed were sputum is in line with the fact that pulmonary TB is the commonest form of the disease.

Of the laboratory reports, 309 (8.9%) were smear positive while 392 (11.3%) were culture positive. This finding supports the fact that culture has higher sensitivity than smear microscopy even though it is not statistically validated in this study. Furthermore, 83 of the total specimen processed (2.4%) were smear negative but culture positive. These specimens are probably from smear negative TB patients in whom diagnosis can only be confirmed by isolation of the organism in pure culture. Such specimens contain few mycobacteria (less than 10^4 per uL of specimen) which cannot be detected by smear microscopy as seen in diseased conditions such as TB-HIV co-infection, TB in children and extra-pulmonary TB.

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Table 2: Smear microscopy and Culture results

Test	Result
Smear microscopy	
Positive	309 (8.9%)
Negative	3159 (91.1%)
Total	3468 (100.0%)
Culture	
Positive	392 (11.3%)
Negative	3010 (86.8%)
Contaminants	66 (1.9%)
Total	3468 (100.0%)
Smear microscopy and Culture result	
Smear positive Culture positive	309 (8.9%)
Smear negative Culture positive	83 (2.4%)
Smear positive Culture negative	66 (1.9%)
Smear negative Culture negative	3010 (86.8%)
Total	3468 (100.0%)

False negative: Number of Smear negative but culture positive/ Number of Smear negative X 100 = 83/3159 X 100 = (2.6%)
False positive: Number of Smear positive but culture negative/ Number of Smear positive X 100
= 66/309 X 100 = 21.4%

Concerning the smear negative but culture positive (false negative) specimens, 51 (1.9%) were sputum samples, a higher percentage, 19 (9.4%) were gastric washings while 13 (2.2%) were from extra-pulmonary sites (CSF, aspirates, ascitic fluids, etc) (Table 3).

Table 3: Distribution of Smear negative Culture positive result by Specimen

Specimen	Smear negative Culture positive result	
	No	(%)
Sputum (n = 2663)	51	1.9
Gastric washings (n= 202)	19	9.4
Extra-pulmonary specimens (CSF, aspirates, etc) (n= 603)	13	2.2
Total (n=3468)	83	2.4%

Of the 83 smear negative but culture positive specimens, 51 (1.9%) were sputum. These specimens are most probably from TB/HIV co-infected patients as HIV is commoner in patients with smear negative TB than in those with smear positive disease (Siddiqi et al., 2003). Furthermore, TB is the most common opportunistic infection among HIV -infected individuals, and co-infected persons are at high risk of death (Coebett et al., 2003; Lawn and Churchyard, 2009). The problem of diagnosis of smear negative pulmonary TB has become urgent because the number of patients with paucibacillary lesions in countries with an HIV epidemic is increasing rapidly (Foulds and O'Brien, 1998) although, HIV status of the patients was not determined in this study. The 2.4% smear negative but culture positive cases found in this study confirms the urgency to expand the facilities for culture and confirmation if the goal of zero level (TB anywhere is TB everywhere) will be achieved since it is established that negative smear results do not exclude the presence of less than 10,000 organisms per uL of sputum (Siddiqi et al., 2003).

HIV status of the patients whose specimens were processed was not known because these are not routinely included in the request forms. This is one of the limitations of the study. During the period of study, we noticed that there is poor or inadequate coordination of the TB and HIV activities, thus HIV- TB co-infection were not categorized. However, it is important to note that TB centers can form an important entry point for HIV diagnosis, care and support. Co-ordination between TB and HIV programmes are therefore crucial not only to improve the outcome of HIV -infected TB patients but also to control TB burden in the community.

Another limitation of the study is lack of data on chest X-Rays findings in patients whose specimens were studied. Since this is a purely laboratory study to establish the epidemiology of smear-negative TB, clinical information is not included thus, a joint laboratory and clinical study will give further illumination to studies on TB. Although, Chest X-Rays may not be helpful for diagnosis of smear -negative pulmonary TB in TB/HIV co-infected individuals due to lack of typical radiological findings apart from its financial implications (Siddiqi et al., 2003).

A significant percentage (9.4%) of the smear negative but culture positive specimens were gastric washings obtained from suspected cases of TB in children who are unable to produce sputum necessary for diagnosis. The high percentage of

smear negative TB seen in gastric washings compared with sputum may be explained by the pathophysiology of TB in children in which they tend to present as closed caseous lesions with a relatively small number of mycobacteria compared with open cavities which contain large numbers of mycobacteria as seen in infected adults (Starke, 2001).

Gastric washings gave a high smear negative but culture positive results (9.4%) which suggests that gastric washing does not yield good result and therefore in resource - limited setting where only smear microscopy is the only means of TB diagnosis, more children with TB are likely to be missed. In order to address this situation, the World Health Organization has recommended culture for TB diagnosis in children (WHO, 2008). The tenacious problems of diagnosing TB in children especially in TB endemic settings with limited resources have been previously highlighted (WHO, 2008; Kehinde et al., 2008).

Thirteen (2.2%) of the smear negative but culture positive specimens were from extra-pulmonary sites (CSF, aspirates, ascitic fluids, etc). This is not surprising because these sites tend to have suboptimal number of mycobacteria which are not detected by smear microscopy, thus further justifies the need for culture.

In conclusion, the relatively high prevalence of smear negative but culture positive specimens in this study calls for need to scale up culture facilities in TB laboratories in endemic countries in order to control TB burden in the community.

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