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Efficacy of Fluorescence Microscopy in the Diagnosis of Tuberculosis in Guyana

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Authors' contributions

This work was carried out in collaboration between all authors. Author RK did the study design and wrote the protocol. Authors DJ, LS and GS did the statistical analysis and literature searches while analyses of study were by authors RK and DJ. All authors read and approved the final manuscript.

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ABSTRACT

Background: Tuberculosis is one of the potentially serious human diseases and is still one of the major causes of mortality. It ranks as the second most leading cause of death from a single infectious agent, after the human immunodeficiency virus (HIV).
Objective: The aim of the study was to evaluate the efficacy of Fluorescence microscopy (FM) technique to determine sensitivity in detecting TB between HIV positive and HIV negative patients in a poor resource country.
Methods: The study was a cross sectional and blind assessment on 50 suspected cases of TB among HIV positive and HIV negative patients using FM method against Zeihl Neelsen (ZN) staining method. Culture results were considered as gold standard.
Results: Of the total 50 specimens examined by ZN, FM and culture method 32%, 40% and 38% were found positive by ZN, FM and culture respectively. FM was sensitive to ZN on several aspects. The difference in their case detection rates were statistically significant (χ^2 = 35.3, p<0.001). In detecting overall patients for TB, FM method showed sensitivity of 90.0% (95% CI 68.3-98.5) over ZN method 75.0% (95% CI 50.9-91.3) with a kappa value of 0.83 (p≤0.05). FM method showed excellent sensitivity, sensitivity, PPV and NPV all with 100% (95% CI 48.0-100) among HIV-TB patients and an excellent kappa value of 1 (p≤0.05).

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Conclusion: This study presented greater sensitivity of FM method over conventional ZN staining method in detecting TB among HIV positive patients. Fluorescence microscopy can be widely used even in peripheral laboratories where culture facilities are not available.

Keywords: HIV-TB; fluorescence microscopy; AFB staining.

1. INTRODUCTION

Tuberculosis (TB) is one of the world's deadliest diseases with one third of the world's population infected with TB. In 2013, 9 million people around the world became sick with TB disease and around 1.5 million TB-related deaths worldwide. Globally in 2013, an estimated 480, 000 people developed multidrug resistant TB (MDR-TB) [1,2,3]. One-fourth of the deaths were associated with HIV infection and most of it in resource-limited settings (RLS) where the burden of HIV infection is high [4,5].

Guyana has one of the highest tuberculosis (TB) burdens in South America, and TB prevalence has steadily risen over the past two decades. Recent study on assessment of *Mycobacterium tuberculosis* from twelve Caribbean territories showed Guyana with higher proportion of drug resistant strains and relatively high TB-HIV co infections [6]. With increase mortality rate due to TB, an accurate and prompt tuberculosis (TB) diagnosis is crucial in disease control. Conventional culture method on solid medium for detection of TB and MDR-TB is time consuming and takes several months to report the results. Commercially available liquid culture systems, although having short turnaround time (TAT), are not accessible in settings where the need is greatest and are prohibitively expensive and require laboratories with advanced infrastructure [7,8,9]. Given the context of the emerging MDR-TB and extensively drug-resistant TB (XDR-TB) strains, there is an urgent need in resource-limited settings for a new, high-performing, inexpensive, and rapid diagnostic method for effective detection of TB and drug-resistant TB [10]. The recent advances in molecular biology and a better understanding of the molecular basis of drug resistance in TB, have given us new tools for rapid diagnosis [11].

This study, therefore effort to evaluate the effectiveness of Fluorescent microscopy technique as against Ziehl Neelsen staining (ZN) technique in detection of acid-fast bacilli (AFB) among TB patients in Guyana.

2. METHODS

2.1 Study Design

A total of 50 sputum samples were collected from the Georgetown Chest Clinic and the National Public Health Reference Laboratory during April 2013 to June 2013 using a simple random sample to assess the effectiveness of FM technique (which uses phenolic acridine orange florescent staining) as against ZN staining of sputum smears. Data was examined based on treatment history and results of HIV test (serologic) at the time of TB diagnosis. All four methods were used for patients suspected of pulmonary tuberculosis - Petroff's method, ZN staining, FM technique and culture on modified Lowenstein-Jensen media (gold standard) for detection of *Mycobacterium tuberculosis*.

2.2 Data Analysis

All data analysis was done using SPSS 21.0 software. Sensitivity, specificity, predictive values, and kappa statistics (with 95% confidence intervals) were performed to compare the two diagnostic methods and the resistance profile. A kappa value of 0.70 was considered excellent. For diagnosis and drug sensitivity testing of TB, culture method still remains the gold standard and was used as the reference standard for all analysis in this study. A p-value of 0.05 was considered significant and all probabilities were two tailed.

3. RESULTS

A total of 50 samples were collected in this study, 20% (10) were tested positive for HIV and 80% (40) were tested HIV negative. Overall, the mean patient age was 38 (95% CI 35.2-42.2) with 52% (26) collected from male patients and 48% (24) were collected from female patients (Table 1).

Of the total 50 specimens examined by ZN, FM and culture method, 32%, 40% and 38% were found positive by ZN, FM and culture respectively. FM was found to be more sensitive over ZN. It showed a statistically significance

among their case detection rates (χ^2 = 35.3, p<0.001). The sensitivities, specificities, predictive, and kappa values of the overall outcome is shown in Table 2. The ZN technique was less sensitive compared with the fluorescence technique as against gold standards. Sensitivity for ZN and FM techniques were 75% (95% CI 50.9-91.3) and 90% (95% CI 68.3-98.5) respectively were as the specificity for both techniques were recorded as 96.7% (95% CI 82.7-99.4) which was statistically significant (p≤0.05). A kappa value of 0.83 was recorded for the overall study.

Table 1. Shows the status of the patients in the study

Female	24	48%	
Male	26	52%	
Age group			
<20	2	4.0%	
20-29	9	18.0%	
30-39	19	38.0%	
40-49	12	24.0%	
>50	8	16.0%	
HIV positive	10	20%	
HIV negative	40	80%	P≤0.05

Table 2. Performance of ZN and FM technique for detecting TB among HIV positive and HIV negative patients

Overall	ZN	95% CI	FM	95% CI
Sensitivity	75.0%	50.9-91.3	90.0	68.3-98.5
Specificity	96.7%	82.7-99.4	96.7	82.7-99.4
PPV	93.8%	69.7-99.0	94.7	73.9-99.1
NPV	85.3%	69.0-95.0	93.6	78.5-99.0

Kappa =0.83; p≤0.05

Comparison of ZN and FM technique among HIV negative patients recorded sensitivity of 85.7% (95% CI 57.2-97.8) with both methods and a specificity of 96.2% (95% CI 80.3-99.4) and 88.5% (95% CI 69.8%-97.4%) were recorded respectively (Table 3). Kappa value was recorded as 0.83 with a statistical significance of p≤0.05.

Table 4 shows comparison of ZN and FM technique among HIV positive patients. FM technique recorded a sensitivity of 100% (95% CI 48.0-100) and only 60% (95% CI 15.4 -93.5) sensitivity was recorded with ZN method among HIV positive group. Specificity and PPV was recorded 100% with both methods. An excellent kappa value of 10 with statistically significance (p≤0.05) was recorded.

Table 3. Performance of ZN and FM diagnostic methods for detecting TB among HIV negative patients

HIV negative	ZN	95% CI	FM	95% CI
Sensitivity	85.7%	57.2-97.8	85.7%	57.2-97.8
Specificity	96.2%	80.3-99.4	88.5%	69.8-97.4
PPV	92.3%	63.9-98.7	80.0%	51.9-95.4
NPV	92.6%	75.7-98.9	92.0%	73.9-98.8

Kappa=0.83; p≤0.05

Table 4. Performance of ZN and FM diagnostic methods for detecting TB among HIV positive patients

HIV positive	ZN	95% CI	FM	95% CI
Sensitivity	60.0%	15.4-93.5	100%	48.0-100
Specificity	100%	48.0-100	100%	48.0-100
PPV	100%	30.5-100	100%	48.0-100
NPV	71.4%	29.3-95.5	100%	48.0-100

Kappa=10; p≤0.05

4. DISCUSSION

The AFB staining method is the most common and supportive method in the diagnosis of TB infection in resource-limited settings. Although AFB staining method is a much cheaper, simple, and rapid method but also possess a low specificity and variable sensitivity [12]. Culture based diagnosis is considered as gold standard method as compared to sputum smear which also include an increased sensitivity. Because of the high costs and the requirement of equipment's of some conventional tests like MGIT and BACTEC, it is not practical to use such methods in developing nations. In a study done in Guyana for diagnosis of MDR-TB (Multi drug resistant- TB) among HIV patients by the NRA and Hain LPA, showed acceptable correlation and that HIV infection does not affect drug susceptibility testing [13]. These methods are expensive and difficult to carry in a resource limited setting.

Agreement rates among HIV-positive patients were exceptional (100% sensitivity and Kappa value 1) were observed with FM technique. FM proved to be more reliable than the ZN method in many studies with a major advantage that it enabled the detection of positive smears, which were overlooked with ZN stained smears containing low-density bacilli [14,15]. The use of FM significantly increases the diagnostic value of the smear, particularly where there are low-density bacilli, which may escape detection on ZN stained smears. However, to be considered

smear positive a specimen needs to contain approximately 10 mycobacteria per milliliter [16]. The sensitivity of sputum microscopy in HIV infection ranges from 43 to 51 per cent, and in many resource-limited settings with high rates of co-infection, the sensitivity may be much lower [17,18,19].

Methods that improve speed or sensitivity include fluorescence microscopy and alternative specimen processing methods, such as concentration, bleach sedimentation and same-day sputum collection (so-called front loading) strategies. Any procedure for digestion or liquefaction followed by centrifugation, prolonged gravity sedimentation, or filtration increases sensitivity by 13 to 33 per cent over direct microscopy, when culture is used as the reference standard [20,21,22,23,24].

Co-infection with HIV leads to many challenges in both the diagnosis and treatment of tuberculosis. With increase in rates of drug resistant tuberculosis, including multi-drug (MDR-TB) and extensively drug resistant TB (XDRTB) mortality has increased. Sputum smear microscopy in HIV-infected patients are not highly sensitive therefore newer diagnostic tests are urgently required that are cheaper, sensitive, specific and easy to use in remote and resource-constrained settings [25]. The treatment is very important in prevention of TB and/or HIV which can only be done with a very effective diagnostic tool. Thus an effective diagnostic tool is very crucial in diagnosing TB in early stage and in HIV patients to reduce mortality and spread of infection.

5. CONCLUSION

The study concludes that the FM method is quite cost effective in terms of both time and expense and especially for the laboratories handling large number of sputum specimens it will be of greater benefit. FM method is also more reliable than ZN in diagnosing TB among HIV patients.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that the study was approved by the ethics committee at Ministry of Health and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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