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Comparison of Microscopic Methods with CBNAAT in Suspected Pulmonary Tuberculosis Patients among HIV Seropositive

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ABSTRACT

Introduction: Tuberculosis is the most common opportunistic infection in people with HIV. The two pathogens namely *Mycobacterium tuberculosis* and HIV potentiate one another, accelerating immunological deterioration and causing premature death if untreated. Accurate diagnosis of disease remains the biggest obstacles to global tuberculosis control. Direct sputum smear microscopy is the cornerstone of tuberculosis diagnosis worldwide. RNTCP is currently using CBNAAT to diagnose tuberculosis and to find out rifampicin resistance.
Objective: To compare the conventional microscopic techniques with CBNAAT for early diagnosis and treatment of co-infection patients.
Methods: This study involved 220 HIV seropositive with symptoms suggestive of tuberculosis. Spot and early morning sputum samples were collected and were subjected to Z-N staining, fluorescent staining (before and after adding sample reagent for the purpose of homogenization) and CBNAAT.
Results: Among these 220 patients, HIV-TB coinfection was more common in males between 31-40 years with CD4 cell count less than 200 cells/µl. out of 220 sputum samples, 29(13%) samples were positive by CBNAAT, among them 15 by fluorescent staining whereas, only one sample was positive for *Mycobacterium tuberculosis* by all three methods.
Conclusion: CBNAAT was found to be better method compared to conventional microscopic methods and among the microscopic methods, fluorescence staining is better technique.

Keywords: *Mycobacterium tuberculosis*, HIV, HIV-TB coinfection, Z-N staining, fluorescent staining, CBNAAT

INTRODUCTION

Tuberculosis (TB) is a major cause of morbidity and mortality and it is a significant global public health problem worldwide. [1,2]According to the World Health Organization over the past two decades, tuberculosis remains a major global health problem among developing countries. [3] Tuberculosis, has been reported to be a significant problem of the poor across the world in developed and developing countries contributing to the disease poverty trap. [4,5] Twenty five percent of global annual TB incidents occur in India making it highest Tuberculosis burden country.[6]

TB has been the top 10 causes of death worldwide, ranking above HIV/AIDS as one of the leading causes of death from an infectious disease.[7]The WHO End TB Strategy aims to reduce TB deaths by 90% and to cut new cases by 80% between 2015 and 2030, and to ensure that no TB-affected family faces catastrophic costs due to TB.[8] Patients with TB infection, coinfected with HIV, have a 20-30 times higher risk of developing tuberculosis diseases during their lives. [9] In the individual host, the two pathogens namely *Mycobacterium tuberculosis* and HIV potentiate one another, accelerating immunological

deterioration and causing premature death if untreated. [10]

TB can occur at any stage of CD4 T cells depletion but it is common during the early stage when the CD4 T cells are relatively normal.[11] HIV/AIDS fuels the tuberculosis epidemics in many ways, such as promoting progression to active tuberculosis, increasing the risk of reactivation of latent tuberculosis infection, as well as increasing chance of tuberculosis infection once exposed to tubercle bacilli.[12]Tuberculosis in HIV/AIDS patients is curable if it is diagnosed accurately and treated promptly, this need special attention due to the complexity of the diagnosis and treatment involved in tuberculosis and HIV/AIDS confection. [13]

Accurate diagnosis of disease remains one of the biggest obstacles to global tuberculosis control.[14] Inadequate diagnosis results in poor patient outcomes and contributes to sustained TB transmission. Delayed diagnosis of TB increases the risk of both mortality and of Immune Reconstitution Inflammatory Syndrome in patients receiving ART, and is also associated with TB transmission in health facilities and in the community.[15]

The majority of national TB control programmes, particularly in low- and middle-income countries, still rely predominantly on sputum microscopy for diagnosis. Direct sputum smear microscopy is the cornerstone of tuberculosis (TB) diagnosis worldwide.[16]

Microscopy has the advantage of being inexpensive, relatively rapid to perform, and specific in most settings. The sensitivity of sputum microscopy in HIV infection ranges from 43 to 51 percent.[17]Revised National TB Control Programme (RNTCP) is also currently using Xpert MTB/RIF to diagnose pulmonary TB, pediatric TB, extra pulmonary TB and rifampicin resistance and Multi Drug Resistance [18, 19]

The Gene Xpert has shown a lower limit of detection of 100 acid-fast bacilli (AFB) exceeding other mycobacterial nucleic acid amplification assays, with overall sensitivity around 98 and 75% in smear-positive and smear-negative respiratory samples.[20] The stigma associated with tuberculosis with its link to HIV/AIDS, poor adherence associated with high pill burden in case of coinfection, high mortality in HIV/AIDS and tuberculosis coinfected patients and difficulties in integrating tuberculosis and HIV/AIDS in one control program complicate the whole tuberculosis, HIV/AIDS management.[21, 22]

As sensitivity and specificity varies from place to place this study is done to compare the sensitivity and specificity of microscopic method with that of CBNAAT in HIV and tuberculosis co -infection in our hospital.

AIMS AND OBJECTIVES OF THE STUDY

1. To study the validity of sputum microscopy in detecting *Mycobacterium tuberculosis* among HIV seropositive.
2. To compare the results of conventional staining with that of fluorescent staining.
3. To compare the direct smear microscopy with that of treating the sample with NALC NaOH.
4. To compare the results of conventional staining methods with CBNAAT

MATERIALS AND METHODS

This comparative study involved 220 patients of various ages, who had attended ART centre and were referred to department of Microbiology. All patients were investigated for the presence *Mycobacterium tuberculosis* in HIV seropositive both by conventional sputum microscopy method which included Z-N staining, Fluorescent staining and by molecular method CBNAAT.

Method of collection of specimens

After explaining the patient about the study, a written informed consent was obtained from each patient following which complete history was taken and noted in the Proforma prepared for the purpose.

Patient was given three wide mouthed container and instructed to take a deep breath and then cough, to bring out the expectorant and spit into the container without mixing with saliva.

They were requested to give two spot sputum samples and one early morning sample. One spot sample was processed for CBNAAT whereas one spot and early morning samples were processed for Z-N staining and fluorescent staining.

Sputum microscopy

Two smears were prepared from one of the spot sample before and after adding sample reagent (NALC NaOH). Each of these smears were stained with Z-N stain and fluorescent stain respectively. Similar procedure was followed for early morning sample. Staining methods were followed as per RNTCP guidelines.

Cartridge Based Nucleic Acid Amplification Technique (CBNAAT)

One of the spot sample was processed for CBNAAT

GeneXpert MTB/RIF assay is a nucleic acid amplification (NAA) test which simultaneously detects DNA of *Mycobacterium tuberculosis* complex (MTBC) and resistance to rifampin (RIF) (i.e. mutation of the rpoB gene) within 2 hours, compared. [23]

Clinical sputum samples are treated with sodium hydroxide and isopropanol-containing sample reagent (SR). The SR is added to the sample and incubated at room temperature for 15 min. The treated sample is then transferred to the cartridge which is placed into the Gene Xpert instrument. Subsequent processing is fully automated. The cartridge incorporates a syringe drive, a rotary drive and a filter upon which *M. tuberculosis* bacilli are deposited after being liberated from the clinical material.

RESULTS

Out of 220 clinical sputum samples studied*M. tuberculosis* was positive in 29 (13.18%) (fig.1). Fig.2 shows male preponderance with 55% and it is also explains that most of the suspected cases are between 31-40 (30.9 %) years of age. Table 1 explains presenting symptoms of our study group which were as follows fever, weight loss (20.9%), followed by cough, breathlessness (18.6%), followed by only fever(13.6%) and cough (12.3%). CD4 count could be performed only on 130 cases. CD4 count was between 201-300 cells/µl in 23.8% of cases (Table 2).

All samples were subjected to Z-N staining, Fluorescent staining andCBNAAT. 29 sputum samples were positive by CBNAAT, among them 15 by fluorescent staining whereas, only one sample was positive for *Mycobacterium tuberculosis* by all the three methods (fig.3).

M. tuberculosis was positive by staining technique in CD4 cell count less than 100cells/µl, whereas CBNAAT showed positive results irrespective of CD4 cell count (figure 4). Rifampicin resistance was detected in 2isolates (6.9%) by CBNAAT out of 29 confirmed cases (figure 5).

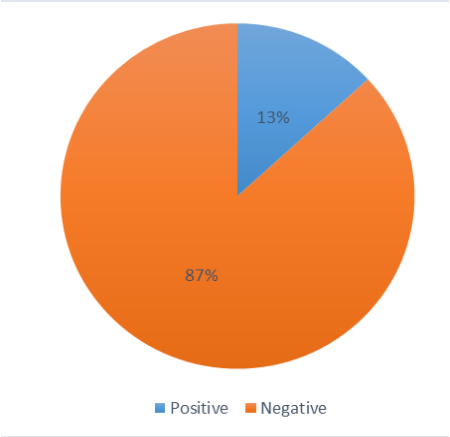

Figure 1. Results of *Mycobacterium tuberculosis* in HIV seropositive

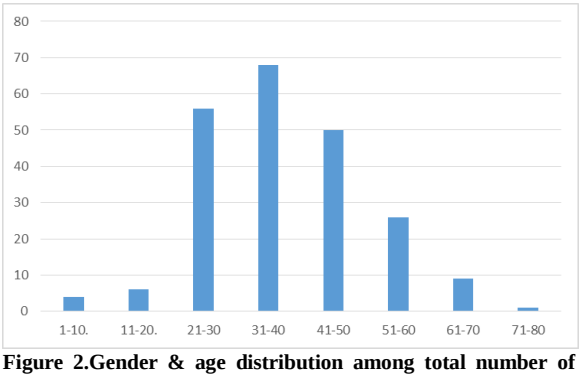

Figure 2.Gender & age distribution among total number of cases

Table 1. Distribution of presenting symptoms among cases

Symptoms	Number of patients	Percentage (%)
Cough	27	12.3
cough since 2 months	15	6.8
cough since more than 2 months	6	2.7
cough, breathlessness	41	18.6
cough, fever,weight loss	4	1.8
cough, hemoptysis	24	10.9
Fever	30	13.6
fever since 2 months	23	10.5
fever, cough	3	1.4
fever, cough, hemoptysis	2	0.9
fever, weight loss	45	20.5
Total	220	

Table 2.CD4 cell count among study group

CD4 cell count	Number of patients	Percentage (%)
Less than 100	24	18.5
101-200	21	16.1
201-300	31	23.8
301-400	24	18.5
401-500	17	13.1
501-600	10	7.7
More than 600	3	2.3
Data not available	90	
Total	220	

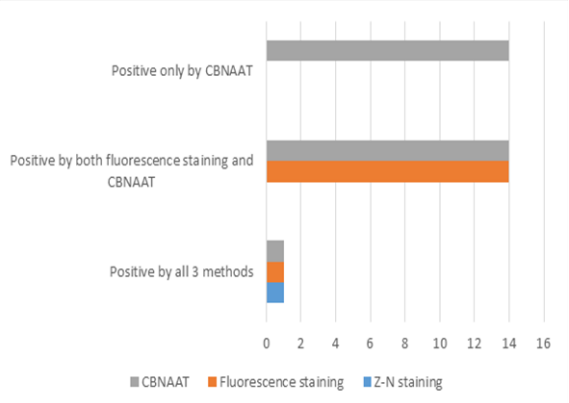

Figure 3. Results of various methods used for detection of *Mycobacterium tuberculosis*

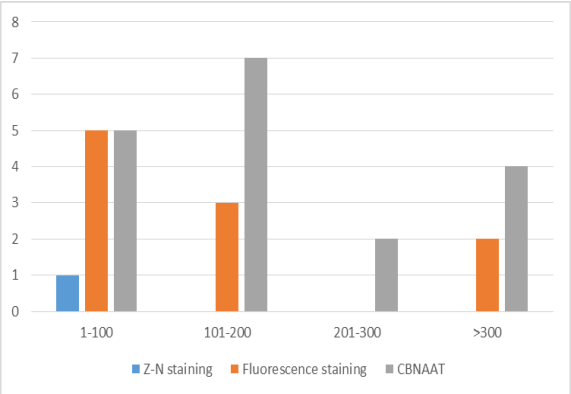

Figure 4. Figure showing distribution of CD4 cell count among confirmed cases.

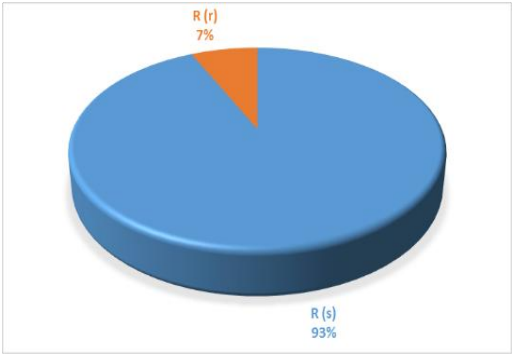

Figure 5.Rifampicin susceptibility detected by CBNAAT

DISCUSSION

Tuberculosis is a leading problem throughout the world. The prevalence of tuberculosis among HIV seropositive varies greatly worldwide and within geographic areas, and is rapidly increasing due to potentiation of their pathogenesis. It is important to detect *Mycobacterium tuberculosis* in HIV patients to help early diagnosis and management.

In this study sputum samples from HIV seropositive with 220 suspected tuberculosis cases were subjected to different detection methods like microscopy and CBNAAT. The results of these methods are compared with each other and statistically evaluated considering CBNAAT as gold standard. The results are also compared and discussed with other studies.

Z-N staining

In our study Z-N staining showed 0.45% positive cases when CBNAAT is considered as gold standard, whereas in studies conducted by Agarwal et al., [23] 2.2% cases were positive by ZN staining, 7.3% by Jain et al.,[24] and 13.38% by Mittal et al.,[25] Z-N staining done after adding sample reagent for the purpose of homogenization did not detect *Mycobacterium tuberculosis*. Our study detected Mycobacterium tuberculosis in less cases by Z-N staining compared to other studies

Sensitivity of fluorescence staining is 6.8% in our study which correlates with conducted by Getachew *et al*. [27] whereas, study conducted by Chaidir *et al*[26] in 2013 and by Workineh *et al*[28] in 2017showed

75.5% and 4.4% sensitivity respectively. Sensitivity of fluorescence staining when CBNAAT as gold standard was 48.28%.

Sensitivity of CBNAAT in our study is 13.63%, it is less compared to other studies such as by Dewan *et al*[29] in 2015 40%, Verma *et al* [28]in 2016 is 30.90% at Kota, India. Study conducted by Basavaraj *et al* [30] at Raichur, India in 2016 showed 22.31%, Mittal *et al*[25] in 2017 at Gorakhpur, India showed 34.5% whereas study conducted by Bajrami *et al* [7] at Kosovo in 2016 showed 82.3% of sensitivity. Sensitivity in South India is decreased compared to North India and other countries, as it is shown in Raichur also.

CONCLUSION

CBNAAT was found to be better method compared to conventional microscopic methods, among staining techniques, fluorescence staining is better in detecting *M. tuberculosis* when compared to Z-N staining. In these staining techniques we also compared by adding sample reagent for homogenization of sample but we found that there is reduction in detection rate. So smear study following addition of sample reagent is not useful.

We also observed that coinfection was more common among males of mid adulthood and in those who showed CD4 cell count less than 200 cells/µ l.

Since early detection can prevent disease transmission and early patient recovery, molecular technique like CBNAAT help in early diagnosis and early treatment. As CBNAAT is simple and rapid technique that can be used to detect such infection and also helps to know Rifampicin susceptibility which in turn helps to start appropriate treatment.

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