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Original Research Article

COMPARATIVE STUDY OF CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST ASSAY AND MICROSCOPY FOR DIAGNOSIS OF PULMONARY AND EXTRA PULMONARY TUBERCULOSIS IN PEOPLE LIVING WITH HIV

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Abstract

Background: To compare the diagnostic role of CBNAAT assay with Ziehl–Neelsen and Fluorescent staining microscopy in Pulmonary and Extra Pulmonary tuberculosis in people living with HIV and also to determine the rifampicin resistance tuberculosis in HIV infected individuals by CBNAAT. **Materials and Methods:** A prospective study analysed the results of 100 pulmonary and extra pulmonary samples by Cartridge Based Nucleic Acid Amplification Test assay (CBNAAT), Ziehl Neelsen and Fluorescent Microscopy and the rifampicin resistant pattern. **Results:** Out of 100 samples, 87 were pulmonary and 13 were extra-pulmonary samples. Positivity for Pulmonary samples by CBNAAT, ZN Microscopy and Fluorescence Microscopy found to be 17.2%, 10.3% and 13.79% respectively. Positivity for EPTB samples by CBNAAT, ZN Microscopy and Fluorescence Microscopy found to be 23%, 15.38%, and 23% respectively. **Conclusion:** CBNAAT was found to be a rapid and better method as compared to conventional microscopic methods. Among microscopy techniques, fluorescence microscopy is better at detecting M. tuberculosis when compared to Z-N Microscopy.

INTRODUCTION

Tuberculosis (TB) remains a major cause of ill health and one of the leading causes of death worldwide.[1] According to the Global TB Report 2021, the estimated incidence of all forms of TB in India for the year 2020 was 188 per 100,000 population (129-257 per 100,000 population).[2] Human immunodeficiency virus (HIV) and TB are still considered important causes of mortality and morbidity in developing countries. World Health Organization (WHO) showed that there were 10.4 million incident cases of TB worldwide of which, about 10% were co-infected with HIV and about 1.4 million deaths, of which, 400,000 deaths were among people co-infected with HIV.[3] The prevalence of TB in HIV patients in India is around 17%.[4]

The two pathogens, Mycobacterium tuberculosis and HIV, synergistically affect the host, causing impairment of immunity.[5] HIV coinfection is known to aggravate severity of latent TB and worsens TB infection by 20 times. TB, on the other hand, is the most common cause of death in AIDS patients (1.8 million death).5 HIV-positive people with pulmonary tuberculosis may have the classic symptoms of tuberculosis. But many people have few symptoms of tuberculosis or even fewer specific ones. In addition, upto a fifth of people may have normal chest X-rays. HIV-positive people with tuberculosis may indeed frequently have so-called "subclinical" Tuberculosis. This is often not recognized as tuberculosis and subsequently, there can be delays in both tuberculosis diagnosis.

People living with HIV are more likely to have extrapulmonary tuberculosis. It is much more common in countries with a high HIV prevalence, such as India. A primary factor behind tuberculosis mortality among people with HIV is late diagnosis. WHO Global tuberculosis report highlighted that globally 44% of people with HIV-associated tuberculosis were not diagnosed in 2019. Enhancing the detection of tuberculosis among people living with HIV is therefore critical. Enhanced laboratory

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capacity, particularly with the increased availability of platforms such as chest X-ray and sputum microscopy. HIV-infected persons with TB are more likely to have negative typical X-ray finding of PTB (cavity, upper lobe infiltrates, and focal infiltrates) and negative sputum smears thereby causing delay and missed diagnosis of TB in HIV-infected patients.[6] Unfortunately, in rural areas there is no rapid test for diagnosis. Culture diagnostics and molecular line probe assays are costly with inadequate biosafety measures and insufficient trained laboratory staff making detection of early TB difficult.[7] Microscopy is a rapid, easy to perform, and inexpensive but it detects approximately 50% of the active cases and has low sensitivity (20%-60%) and cannot determine the viability of the bacilli, cannot be used to predict drug sensitivity testing and multi-drug resistance. The Löwenstein–Jensen method, being a culture-based "gold standard test", takes several weeks to provide result, with simultaneous progression of the disease in the meantime. Thus, many active TB patients remain undiagnosed and continue to spread the disease in the community.

Cartridge-based nucleic acid amplification test (CBNAAT) assay is a simplified type of automated real time polymerase chain reaction (PCR) that can detect TB and does screening for multidrug resistance (MDR). It takes 2 to 3 hours to detect MTB and rifampicin resistance from sample.[8] WHO recommends the use of CBNAAT assay as initial diagnostic test for suspected MDR-TB or TB–HIV coinfection.[9] It has sensitivity around 98% in smear-positive and 68% in smear-negative samples. Therefore, the present study was undertaken to compare the diagnostic role of CBNAAT assay in PTB and EPTB in people living with HIV (PLHIV) and to compare the sensitivity of CBNAAT with Ziehl–Neelsen (ZN) and Fluorescent staining microscopy and also to determine the rifampicin resistance tuberculosis in HIV infected individuals by CBNAAT.

MATERIALS AND METHODS

This prospective study was conducted in the Tuberculosis Culture and DST (CDST) laboratory, a national TB Elimination Programme (NTEP) certified laboratory, Department of Microbiology with association with Department of Medicine, Gajra Raja Medical College, Gwalior, Madhya Pradesh, India. A total of 100 pulmonary (sputum) and extra pulmonary (Gastric lavage, Pus, Ascitic fluid, CSF, lymph node, tissue biopsies, Pleural fluid, Gastric aspirate in children) samples which were received at CDST of all PLHIV patients, who were presumptive tuberculosis cases was collected from the OPD of Department of Medicine from May 2021 to July 2022.

Approval of Research Ethics Board

Ethical approval was obtained from the Research Ethics Committee, Gajra Raja Medical College, Gwalior, India (D.No.883-84/IEC-GRMC/2021). Informed written consent was obtained from the respondents.

Inclusion Criteria

Age above 15 Years and features suggestive of PTB and EPTB in PLHIV as per NTEP guidelines.

Exclusion Criteria

Those receiving treatment for TB or HIV-infected individuals already on anti-tubercular therapy and unwilling patients.

Study Procedure

Specimen collection and processing: Two sputum samples were collected of each patient under sterile conditions and also in leak-proof, sterile containers. In the shortest time possible, the samples were processed. In the case of a delay, they were kept at 4°C for no longer than 24 hours before even being processed immediately. All samples were handled in a class II A2 biosafety cabinet. One sample for smear microscopy and another was for CBNAAT, which was diluted with three times reagent, incubated at room temperature and loaded into the CBNAAT cartridge for automated analysis. The following results are obtained: TB not detected, TB detected, rifampicin resistance detected, no rifampicin resistance detected, invalid, indeterminate. Results were obtained within 2 hours.

Ziehl Neelsen staining: A smear was made from each sample and spread on a label, clear glass slide. The slide was then stained by ZN stain and observed under immersion oil after that the smear had been allowed to air-dry and also fixed by heat (100X). Acid-fast bacteria had a bright red appearance and a beaded look.[10]

Fluorescent microscopy: Smears were prepared on frosted slides and completely covered with Auramine O solution. After 20 min, the slides were washed and decolourised with 0.5% acid alcohol solution for 3 min and counter-stained with 0.5% potassium permanganate for 1 minute. Stained smears were examined under LED-FM with 400X magnification and 40 fields were examined. LED-FM results were reported for the presence or absence of AFB using the WHO/International Union Against Tuberculosis and Lung Disease scale, with a positive result corresponding to ≥ 1 AFB per 20x for screening and 40x for confirmation.[11]

CBNAAT: A nucleic acid amplification test (NAAT) which uses real time PCR. It is used in identification and amplification of genomic DNA sequences of MTB and rifampicin resistance. It also detects the mutation in RNA polymerase β (rpoB). It has 89% sensitivity and 99% specificity when used as an initial test to replace smear microscopy. It has a sensitivity of 67% and a specificity of 99% when used as an adjunct for smear-negative microscopy.[9]

Statistical Analysis

The data was recorded in a master chart using a Microsoft excel spreadsheet, and correlation was

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checked. SPSS version 20.0 was used to statistically analyse the data. The sensitivity, specificity, PPV, NPV, and diagnostic accuracy for the diagnosis of TB was calculated for AFB smear microscopy, and CBNAAT and compared with each other. All p-values < 0.05 were considered significant.

A total of 100 HIV positive clinically M. tuberculosis suspected cases were enrolled for this study. Out of these, 87 were pulmonary and 13 were extrapulmonary cases. Among these 87 pulmonary tuberculosis cases, 15 (17.24%) were tuberculosis detected whereas out of 13 extrapulmonary tuberculosis cases, 03 (23.07%) were tuberculosis detected. Out of 87 pulmonary samples, 15, 12 and 09 samples were M.TB detected in CBNAAT, Fluorescence and ZN Microscopy respectively. (Table 1). Positivity for Pulmonary samples by CBNAAT, ZN and Fluorescence Microscopy were 17.2%, 10.3%, and 13.79% respectively. Out of 13 extrapulmonary tuberculosis (EPTB) samples, 03, 02 and 02 were M. TB detected by CBNAAT, Fluorescence and ZN Microscopy respectively.

RESULTS

(Table 1). Positivity for EPTB samples by CBNAAT, ZN and Fluorescence Microscopy were 23%, 15.38% and 23% respectively. Out of a total of 15 pulmonary M.TB, 01 (6.6%) pulmonary sample was rifampicin resistance. (Table 2).

Out of 100 HIV positive samples with clinically suspected TB, the positivity of M.TB detected in HIV positive patients was found out to be maximum in the age group of >60 years (50%), followed by 21-30 years (25%), 11-20 years (20%), 31-40 years (19.35%) and 41-50 years (11.11%), respectively. (Table 3)

Out of 100 samples, 34 were of the females and 66 of the males. M.TB detected by CBNAAT were 2 out of 34 in female patients with 5.8% positivity and 16 out of 66 in male patients with 24.24% positivity, respectively. The ratio came out to be 1.94:1. The p value came out to be 0.024, by Chi Square Test which is significant. (Table 4)

Table 1: Comparison of Tuberculosis detected by CBNAAT and Microscopy (ZN and Fluorescence)

Tuberculosis	CBNAAT	Zeihl Neelsen microscopy	Fluorescence microscopy
Pulmonary	15 (17.2%)	09 (10.3%)	12 (13.79%)
Extra pulmonary	03 (23%)	02 (15.38%)	03 (23%)
Total	18	11	15

Table 2: Detection of Rifampicin Resistance by CBNAAT

CBNAAT	Rifampicin resistance	
	Detected	Not detected
M.TB detected	1	17
M.TB not detected	0	79

Table 3: Age wise Positivity of MTB Detected Samples by CBNAAT

Age	Total Samples	MTB Detected	Positivity
0-10 Yrs	04	0	0
11-20 Yrs	10	02	20.00
21-30 Yrs	24	06	25.00
31-40 Yrs	31	06	19.35
Yrs	18	02	11.11
Yrs	09	0	0
>60 Yrs	04	02	50.00
Total	100	18	0

Table 4: Male-Female Ratio

Sex	Sample tested	MTB detected	Positivity	P value
Female	34	02	5.8%	0.024
Male	66	16	24.24%	
Total	100	18	18%	

DISCUSSION

Tuberculosis occurs at any stage of HIV and it presents differently according to the level of immunosuppression. Most of the HIV patients are paucibacillary, in which involvement of hilar and mediastinal lymph nodes are seen. They also lack cavitation and are smear negative. Sputum culture takes 4-8 weeks for mycobacteria to grow, hence a rapid diagnostic test, CBNAAT cartridge-based nucleic acid amplification test by WHO is recommended. It offers a promising solution to these challenges in detecting presumptive pulmonary tuberculosis with a turnaround time of 2 hours.

A total of 100 HIV-positive samples were taken in this study. Out of which, 87 were pulmonary and 13 were extrapulmonary. Out of 87 pulmonary samples, 15 were detected for TB by CBNAAT with 17.24% positivity. Out of 13 extrapulmonary samples, 3 were TB detected by CBNAAT with 23.07% positivity. CBNAAT positivity (45%) was found to be more than sputum smear microscopy (15%) in a study conducted by Hariom Gupta et

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al,12 In a similar study conducted by Hanumanthraju MV et al.[13] (Acid Fast Sputum) detected positive in 13 (1.07%) samples and CBNAAT in 173 (14.29%) samples.

Our study detected TB in 9 out of 87 HIV-positive samples by ZN Microscopy with 10.3% positivity which was more as compared to the study done by Hanumanthraju MV et al.[13] This discrepancy is found, as in our study HIV positive TB suspected samples were taken as compared to the above-mentioned study, which has included all sputum samples, therefore our study resulting in higher sensitivity. Higher positivity of CBNAAT for PTB and EPTB samples was found to be 17% and 23% respectively in our study which was similar to a study conducted by Sameera Akhtar et al,14 In which for both PTB and EPTB, CBNAAT showed an increase by 7.8 and 11.1%, respectively.

In our study, Out of 87 pulmonary samples 15 were M. TB detected by CBNAAT, 12 by Fluorescence Microscopy, and 9 by ZN Microscopy respectively. Positivity for Pulmonary samples by CBNAAT, ZN Microscopy and Fluorescence Microscopy came out to be 17.2%, 10.3%, and 13.79% respectively. Similar results were concluded in a study by Sameera Akhtar et al.[14] where Sensitivity of CBNAAT for sputum smear–positive and sputum smear-negative TB were 100 and 11.3% respectively. We had greater positivity in both the methods of detection (CBNAAT-17.2% and ZN Microscopy-10.3%) when compared with the study conducted by Hanumanthraju MV et al.13 Out of 1210 samples tested, CBNAAT was positive in 173(14.29%) samples.

Out of 13 EPTB samples, positivity for EPTB samples by CBNAAT, ZN Microscopy, and Fluorescence Microscopy came out to be 23%, 15.38%, and 23% respectively, which was in concordance with the study conducted by Sameera Akhtar et al14 CBNAAT is better for the diagnosis of EPTB and pulmonary TB when compared to microscopy. The above-mentioned study only compared CBNAAT with ZN Microscopy.

In our study, we compared CBNAAT with Microscopy (ZN and Fluorescence), out of which Fluorescence Microscopy was found better than ZN Microscopy. It was found that Fluorescence Microscopy was more sensitive for both pulmonary and EPTB samples. It may be because organisms in Fluoroscence Microscopy offer better contrast, appearing as brilliant yellow against the dark background rendering it easy to detect. In a study done by Ashwini B S et al.[15] CBNAAT was found to be a better method compared to conventional microscopic methods and among the microscopic methods, fluorescence staining is the better technique.

In our study, male preponderance was to be found with a ratio of 1.94:1. In which out of 100 samples, 66 were of males and 34 were of the females. This was found to be similar in a study conducted by Hariom Gupta et al.[12] where the sex ratio was 2.63:1. Male predominance might be due to their risk-taking behavior and migration for employment. Also, the health status of females remains neglected in our society, so even in our study, there were less number of female samples. Most of the time, females are not self-aware and have a tendency to ignore their health issues too. Our study detected MTB by CBNAAT in 2 out of 34 female patients with 5.8% positivity, and 16 out of 66 male patients with 24.24% positivity, respectively. The p-value for which came out to be 0.024, by Chi-Square Test which was found to be significant.

Our study found that the HIV-TB infection was more commonly seen in the age group of >60 yrs. In a similar study done by Ashwini B S et al.[15] HIV-TB coinfection was more common in males between 31-40 years. In a study conducted by Hariom Gupta et al7 Middle-aged 26-45 years (68.12%) old individuals are the most commonly affected. The reason of this variation in result may be due to the immunocompromised state accentuated by the age and HIV.

In our study, only one sputum sample was detected for rifampicin resistance out of 15 M.TB detected samples by CBNAAT, thus resulting in 6.6% sensitivity. In a similar study conducted by D. Pragati Rao et al.[11] out of 231 HIV-positive patients, 59 cases (25.54%) had tuberculosis. 8(13.55%) cases were rifampicin resistant and 51 (86.44%) were sensitive out of all tuberculosis patients. Lesser sensitivity to rifampicin in my study as compared to the above may be due to the low sample size.

CONCLUSION

CBNAAT detects TB in HIV patients with greater efficacy than ZN and fluorescence microscopy, both in PTB and EPTB. It gives early diagnosis in less than 2 hours. Thus, it decreases delayed or misdiagnosed cases, contributing to early start of treatment and thus decreasing the morbidity and mortality rates. It also detects rifampicin resistance and can be used for screening for MDR-TB. Fluorescence microscopy is better microscopy method than ZN Microscopy and males were more infected as compared to females.

REFERENCES

1. Ranjan KP, Ranjan N, Kumar N (April 04, 2023) Molecular Characterization of katG and inhA Mutations by Genotype MTBDRplus Line Probe Assay To Guide Isoniazid and Ethionamide Use for Drug-Resistant Tuberculosis. Cureus 15(4): e37136. doi:10.7759/cureus.37136
2. Bai Y, Wang Y, Shao C, Hao Y, Jin Y: GenoType MTBDRplus assay for rapid detection of multidrug resistance in *Mycobacterium tuberculosis:* a meta-analysis. PLoS One. 2016, 11:e0150321. 10.1371 /journal.pone. 0150321
3. WHO. Global tuberculosis report, 2015. World health organization, Geneva, Switzerland. 2016; WHO/HTM/TB/2015.22.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

4. Newsletter on HIV associated tuberculosis on the rise: India TB Report, DownToEarth .org.in
5. Getahun H, Gunneberg C, Granich R, Nunn P. HIV infection-associated tuberculosis: the epidemiology and the response. Clin Infect Dis 010;50(3, Suppl 3): S201–S207
6. San KE, Muhamad M. Pulmonary tuberculosis in HIV infection: the relationship of the radiographic appearance to CD4 T-lymphocytes count. Malays J Med Sci 2001;8(1):34–40
7. Angella Musewa, Lilian Bulage, Joseph Frank Maganda et al. Turnaround Time for Microbiological Testing of Tuberculosis in Routine Clinical Practice and Time to Patient Initiation on Treatment, Iganga Hospital, Uganda: 2012-2017, 09 September 2021,
8. Al-Mussawi, A.A.a,*; Ali, N.H.a; Abd, A.H.b. Rapid molecular detection of rifampin resistant tuberculosis in Basrah Governorate – South of Iraq. International Journal of Mycobacteriology 4(Suppl 1):p S100, February 2015. | DOI: 10.4103/2212-5531.201443World
9. Health Organization. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicinresistance: Xpert MTB/ RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children: policy update. 2013.
10. Guidelines for programmatic management of drug resistant tuberculosis in India 2021. Downloaded from https://tbcindia.gov.in/showfile.php?lid=3590.
11. Deutsches Institut für Normung Medical microbiology-diagnosis of tuberculosis. Part 32: Detection of mycobacteria by microscopic methods. Berlin: DIN, Beuth Verlag; 1995
12. Gupta H, Pandey RP, Prajapati MK, et al. CBNAAT positivity in sputum of tuberculosis patients with HIV. J. Evolution Med. Dent. Sci. 2019;8(50):3764-3768, DOI: 10.14260/jemds/2019/815
13. H Raju MV, Vinay M. Comparitive study of detection oftuberculosis by sputum microscopy versus cartridge based nucleic acid amplification test. Indian J Microbiol Res 2019;6(3):221-224
14. Akhtar S, Kaur A, Kumar D, Sahni B, Chouhan R, Tabassum N, Akhtar S, Gandhi SG.Diagnostic Accuracy between CBNAAT, TrueNat, and Smear Microscopy for Diagnosis of Pulmonary Tuberculosis in Doda District of Jammu and Kashmir- A Comparative StudyJ Clin of Diagn Res.2022; 16(11):DC08-DC12
15. Ashwini BS, Anuradha K. Comparison of microscopic methods with CBNAAT in suspected pulmonary tuberculosis patients among HIV seropositive. International Journal of Research and Review. 2020; 7(4): 553-559.

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