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Research Article

Diagnostic Yield of Cartridge Based Nucleic Acid Amplification Test (CBNAAT) in Pulmonary Tuberculosis with People Living with HIV (PLHIV) Compared to Sputum Microscopy

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Abstract

Introduction: *Tuberculosis is a highly infectious disease which is caused by Mycobacterium tuberculosis. Tuberculosis is the most common opportunistic infection in PLHIV, and the risk of death in HIV-TB co-infected individuals are high .In 2017, among HIV-negative people, estimated deaths caused by TB were 1.3 million (range, 1.2–1.4 million) and there were an additional 3,00,000 deaths from TB (range, 2,66,000–3,35,000) among HIV-positive people. we have taken up the study of comparing detection of mycobacterial tuberculosis bacilli in sputum by Xpert/RIF and method in fluroscent smear pulmonary tuberculosis and rifampicin resistance with a special focus on PLHIV*

Aims and Objectives

- *To compare the sputum microscopy and cartridge based nucleic acid amplification test (CBNAAT) in diagnosing pulmonary tuberculosis with people living with HIV (PLHIV).*
- *To determine whether there is any correlation between CD4 counts in terms of detection with these two techniques among plhiv patients.*

Materials and Methods: *One hundred PLHIV patients with age >18 years with symptoms suggestive of tuberculosis who met the criteria were selected. All patients included in the study underwent a detailed history and clinical examination. CD4 lymphocyte counts of all the patients were determined by flow cytometry. Two Sputum samples were collected at least 1ml for each patient, in 2 sterile containers. One was sent for AFB smear examination under fluorescent microscopy. One sputum sample of 1 ml was collected in a sterile falcon container and was analyzed by CBNAAT on Xpert® MTB/RIF manufactured by Cepheid, endorsed by WHO (2010).Data analysis was done using Microsoft Excel software. Diagnostic yield of sputum microscopy and CBNAAT were compared with McNemar test.*

Observations and Results

- *Total 100 PLHIV patients with presumptive PTB were included in the study. Sputum microscopy detected 21 cases and sputum for CBNAAT detected 52 cases. Smear-positive CBNAAT negative cases were 4. A total number of PLHIV patients with pulmonary tuberculosis were 56.*

- *CD4 cell count upto 100 cells/ml was seen in 16 patients and PTB was detected in 11(68%) cases by CBNAAT. In 19 patients with CD4 COUNT 101- 200 cells/ml, PTB was detected in 14(73.68%) cases by CBNAAT COUNT >500 cells/ml, PTB was detected in 7(26.92%) cases by CBNAAT. Mean CD4 count was 355.84±281.25 cells/ml. CBNAAT positive and sputum microscopy positive cases were 17.CBNAAT negative and sputum microscopy positive cases were 4.CBNAAT positive and sputum microscopy negative were 35.P-value was 0.0031 (<0.05) which was significant. Out of 52 CBNAAT detected cases, 48 were rifampicin sensitive and 4 were rifampicin resistant.*

Conclusion: *CBNAAT is to be indicated as a primary diagnostic test in PLHIV with presumptive TB.CBNAAT detects pulmonary TB in PLHIV with greater efficacy than sputum microscopy, also helping in early diagnosis in less than 2 hours. It also detects rifampicin resistance with high specificity and can be used for screening for MDR-TB so that early therapy can be started, thus decreasing the incidence of MDR-TB.*

Keywords: *PLHIV, sputum microscopy, CBNAAT, Pulmonary tuberculosis, CD4 count.*

Introduction

Tuberculosis is a highly infectious disease which is caused by Mycobacterium tuberculosis. Tuberculosis is the most opportunistic infection in PLHIV, and the risk of death in HIV-TB co-infected individuals are high .In 2017, among HIV-negative people, estimated deaths caused by TB were 1.3 million (range, 1.2–1.4 million) and there were an additional 3,00,000 deaths from TB (range, 2,66,000–3,35,000) among HIV-positive people . The World Health Organisation (WHO) TB statistics of India in 2016 gave an estimated incidence of 2.79 million TB cases and incidence of TB among HIV patients was 87,000.Among HIV-negative people, estimated deaths caused by TB were 4.5 lakh people. There were an additional 12,000 deaths from TB among HIV-positive people. Standard sputum-based methods to detect PTB are sputum microscopy and culture. However, in HIV-TB coinfected individuals, there is scanty sputum production, decreased number of bacilli in sputum due to lack of caseous necrosis, and high incidence of non-tubercular mycobacterial infection. The specificity and sensitivity of sputum microscopy as a diagnostic tool are reduced by these factors. These shortcomings can be eliminated by sputum culture for mycobacteria. But it is a slow test usually taking 4 - 8 weeks and not economical for screening purposes [5]. Due to the above reason, there is a delay in the initiation of treatment especially for drug-resistant forms of TB. In PLHIV, the pooled sensitivity of Xpert MTB/RIF for smear-negative culture-positive TB was 61% (95% CrI, 42–79%) when compared with 97% (95% CrI, 91–99%) for smear-positive culture-positive TB which was a statistically significant result. Therefore, in PTB-PLHIV patients, the smear-positive disease was more mostly diagnosed using Xpert MTB/RIF than those with smear-negative disease. Hence we have taken up the study of comparing detection of mycobacterium tuberculosis bacilli in sputum by Xpert /RIF and fluroscent smear method in pulmonary tuberculosis and rifampicin resistance with a special focus on PLHIV.

Aims and Objectives

1) To compare the sputum microscopy and cartridge based nucleic acid amplification test (CBNAAT) in diagnosing pulmonary tuberculosis with people living with HIV (PLHIV).
2) To determine whether there is any correlation between CD4 counts in terms of detection with these two techniques among PLHIV.

Materials and Methods

Study Design: A Hospital-based cross-sectional observational study.

Source of Patients: PLHIV patients attending Government Hospital for Chest and communicable diseases and ART centre, Visakhapatnam, Andhra Pradesh who met the criteria were selected.

Study Setting: Government hospital for chest and communicable diseases and ART centre, Visakhapatnam, Andhra Pradesh

Sample Size: 100.

Inclusion Criteria

- Patients willing to participate in the study.
- One hundred PLHIV with age >18 years of age with symptoms suggestive of tuberculosis. (a cough >2 weeks plus one other symptom such as night sweats, fever, unintentional weight loss)

Exclusion Criteria

- Extrapulmonary TB
- Patients aged <18 years
- Diabetes mellitus
- malignancy
- Who are on immunosuppressive therapy.
- Patients not willing to participate in the study.

Procedure

All patients included in the study underwent a detailed history and clinical examination.CD4 lymphocyte counts of all the patients were determined by flow cytometry. Two Sputum samples were collected at least 1ml for each patient, in 2 sterile containers. One was sent for AFB smear examination under fluorescent microscopy. One sputum sample of 1 ml was collected in a sterile falcon container and was analyzed by CBNAAT on Xpert® MTB/RIF manufactured by Cepheid, endorsed by WHO (2010). The Xpert MTB/RIF assay is rapid molecular assay which can be used with minimal hands-on technical time, enabling diagnosis of TB and also a simultaneous assessment of rifampicin resistance in less than 2 hrs. It uses real-time PCR (rt-PCR) technology to diagnose TB and also to detect rifampicin resistance using unprocessed specimens, regardless of the smear status. This assay is conducted in a simple and fully automated cartridge-based system. Data analysis of the study was done using Microsoft Excel software. Diagnostic yield of sputum microscopy and CBNAAT were compared with McNemar test.

Ethical Consideration

Informed consent was taken from all the patients before enrollment into the study. The study was done after obtaining approval from the institutional ethics committee

Observation and Results

100 PLHIV cases were subjected to sputum microscopy and sputum for CBNAAT. Sputum microscopy detected 21 cases and sputum for CBNAAT detected 52 cases. Only sputum microscopy was positive and CBNAAT negative in 4 cases. A total number of PTB in PLHIV were 56 cases.

Age and Sex Distribution in PTB-PLHIV Patients

Among 56 PLHIV patients with pulmonary tuberculosis,43 patients (76.79%) were males, 13 (23.21%) were females [Table-1]

Table – 1 Age and Sex Distribution in PTB-PLHIV Patients

AGE IN YEARS	MALE	FEMALE
18-20	0	0
21-40	24	10
41-60	18	3
>60	1	0
TOTAL	43	13

Comparison of Sputum Microscopy and CBNAAT in PTB-PLHIV Patients

Out of 100 cases, CBNAAT positive cases were 52 and sputum microscopy positive cases were 21.CBNAAT positive and sputum microscopy positive cases were 17. CBNAAT negative and sputum microscopy positive cases were 4. CBNAAT positive and sputum microscopy negative were 35. The p-value is 0.0031(<0.05) which was significant. CBNAAT shows significantly higher detection rate when compared to sputum smear microscopy (52% vs 21%).

Table-2 Comparison of Sputum Microscopy and CBNAAT in PTB-PLHIV Patients

	CBNAAT	
Sputum Microscopy	**Positive**	**Negative**
Positive	17	4
Negative	35	44

CD4 Counts and Their Relationship with CBNAAT Results

CD4 COUNT up to 100 cells/ml was seen in 16 patients and PTB was detected in 11(68%) cases by

CBNAAT. In 19 patients with CD4 COUNT 101-200 cells/ml, PTB was detected in 14(73.68%) cases by CBNAAT. In 24 patients with CD4 COUNT 201-350 cells/ml, PTB was detected in 13(54.17%) cases by CBNAAT. In 15 patients with CD4 COUNT 351-500 cells/ml, PTB was detected in 7 (46.67%) cases by CBNAAT. In 26 patients with CD4 COUNT >500 cells/ml, PTB was detected in 7(26.92%) cases by CBNAAT [Table- 3]. Mean CD4 count was 355.84 cells/ml (SD±281.25 cells/ml).

CD4 Counts and their Relationship with Sputum Microscopy and CBNAAT

CBNAAT showed a significantly higher detection rate when compared to sputum smear microscopy in patients with a CD4 count of less than 350/ml. However, in patients with a CD4 count above 350/ml, there was no significant difference between smear microscopy and CBNAAT in detecting Mycobacterium tuberculosis (TABLE 4). In fact, both the diagnostic modalities showed very good concordance with κ values of 0.727 and 0.898.

CBNAAT and Drug Resistance

Out of 52 CBNAAT detected cases, 48 were rifampicin sensitive and 4 were rifampicin resistant [Table-5].

Table – 3 CD4 Counts and their Relationship with CBNAAT Results

CD4 Count (CELLS/ML)	CBNAAT Positive	CBNAAT Negative	Total
UPTO 100	11	5	16
101-200	14	5	19
201-350	13	11	24
351-500	7	8	15
>500	7	19	26

Table – 4 CD4 Counts and their Relationship with Sputum Microscopy and CBNAAT

CD4 COUNT (CELLS/ML)	SPUTUM MICROSCOPY POSITIVE	CBNAAT POSITIVE	Sputum Microscopy Positive & CBNAAT Negative	Total PTB PLHIV Patients	Total PLHIV Patients	p- value
UPTO 100	2	11	1	12	16	0.0032
101-200	4	14	2	16	19	0.0029
201-350	4	13	1	14	24	0.0145
351-500	5	7	0	7	15	0.7104
>500	6	7	0	7	26	1
TOTAL	21	52	4	56	100	0.0001

Table – 5 CBNAAT and Drug Resistance

Rifampicin Sensitive	Rifampicin Resistant	Total
48	4	52

Discussion

The main reasons for high mortality in tuberculosis patients are due to lack of proper diagnosis in the correct time. With HIV and TB co-infection, this is particularly important because the detection rates are very low. Especially in high HIV prevalent areas, there is an immediate need to implement the latest diagnostic modalities for the detection of tuberculosis.

Conventional sputum microscopy has decreased sensitivity in PLHIV patients due to scanty sputum production, lack of caseous necrosis leading to a decreased number of bacilli in sputum. The role of CBNAAT with a potential to diagnose tuberculosis and rifampicin resistance within 2 hours is promising. The Xpert MTB/RIF assay is rapid molecular assay which can be used with minimal hands-on technical time, enabling diagnosis of TB and also a simultaneous assessment of rifampicin resistance in less than 2 hrs. It uses real-time PCR (rt-PCR) technology to diagnose TB and also to detect rifampicin resistance using unprocessed specimens, regardless of the smear status. This assay is conducted in a simple and fully automated cartridge-based system. Rifampicin resistance is particularly amenable to the rapid molecular detection since >95% of the rifampicin-resistant strains contain mutations located in the 81 bp core region of RNA polymerase rpoB gene, which encodes the active site of the enzyme in the bacteria. Mutations which occur in this 81bp core

region of rpoB gene are highly suggestive of rifampicin resistance. The susceptible isolates will have the same wild-type nucleotide sequence most of the time. The basis for the detection of rifampicin resistance is the difference between the first cycle threshold (early CT) and the last cycle threshold (late CT) M. tuberculosis-specific beacon (ΔCT). The rifampicin resistance was seen when ΔCT was >3.5 cycles and sensitive if ≤3.5 cycles. The assay terminates after 38 cycles. The assay is deemed as indeterminate for rifampicin resistance when the first probe cycle threshold (CT) is >34.5 cycles and the last probe has a cycle threshold (CT) of>38 cycles [11].

In PLHIV, the pooled sensitivity of Xpert MTB/RIF for smear-negative culture-positive TB was 61% (95% CrI, 42–79%) when compared with 97% (95% CrI, 91–99%) for smear-positive culture-positive TB which was a statistically significant result. Therefore, in PTB-PLHIV patients, the smear-positive disease was more mostly diagnosed with TB using Xpert MTB/RIF than those with the smear-negative disease[12].The pooled sensitivity by univariate analysis was 95% (95% CrI, 90–97%) and the pooled specificity was 98% (95% CrI, 97–99%) by using Xpert MTB/RIF to detect rifampicin resistance.

1 Among 56 patients with PTB-HIV co-infection, the mean age of patients was 39.5 ± 8.99 years. The age group of thepatients varied from 19 to 66 years. Most of the patients (60.71%) were from the age group of 21 to 40 years, followed by the age group of 41 to 60 years which was about 37.5%.. In the study by **Patel et.al**[17] 34% were below 30 years of age, 64% were in the age group of 31-50 years and only 2% were above 50 years of age. In a study by **Christopher et. al**[19] in Nigeria, 32% of the patients were below 30 years' age, 56% were in the age group of 31-50, 8% were 51-65 years and 4% above 65 years.

2. In the present study of 56 PTB PLHIV, 76.79% were males, 23.21% were females. The striking male-predominance noted in the present study was similar to that observed in other studies In a study by **Patel.et.al**[17], 82% of the patients were male and 18% were female and in the study by **Praveen Kumar et.al**[18] male patients were 90.5 % and female were only 9.5%. But **Purushottam et.al** [16] observed that male patients were 58% and female were 42%. But in a study conducted by **Christopher et.al**[19] in Nigeria females were predominant with 57.4% females and 42.6% males co-infected with HIV-TB.

3. In the present study, CBNAAT was positive in 52 cases (52%) and microscopy was positive in 21 (21%) cases. In **R Dewan et al study**[5], 11 out of 100(11%) were positive by sputum microscopy for acid-fast bacilli and 40 (40%) were positive by CBNAAT. In **S. Subbarao et al** study[13], 7 out of 191 PLHIV patients (3.66%) were positive by sputum microscopy for acid-fast bacilli and 54 (28.2%) were positive by CBNAAT. In **Gabriella et al** study[13], among the sample size of 131 patients, 45 (34.4%) had TB. CBNAAT detected 44 out of 45 cases and smear microscopy detected 31 out of 45 cases. Two patients had false positive CBNAAT, one of them was also smear- positive. So total CBNAAT detected were 46 (35.11%) and microscopy detected were 32 (24.43%) cases. In **Prem Parkash Gupta et al** study[15], LED-Fluorescent Microscopy for AFB detected 8 (26.67%) cases and CBNAAT detected 17 (56.67%) cases out of 30 PLHIV patients.

Table – 6 Diagnostic yield of Sputum Microscopy and CBNAAT in PTB- PLHIV Patients in Different Studies

STUDY	**Sputum Microscopy (%)**	**CBNAAT (%)**
Present study	21	52
R Dewan et al study	11	40
S.Subbarao et al study	3.66	28.2
Gabriella et al study	24.43	35.11
Prem Parkash Gupta et al study	26.67	56.67

CBNAAT helps in increased case detection to diagnose pulmonary tuberculosis in PLHIV as compared to conventional sputum microscopy. Thus, this helps to diagnose tuberculosis early and initiation of anti-. Tuberculosis treatment Therefore,

CBNAAT is to be indicated as a primary diagnostic test in PLHIV with presumptive pulmonary tuberculosis.

4. In the present study we got a higher detected CBNAAT positivity was 68% in patients with CD4 count up to 100 cells/ml, when compared to the **R. DEWAN et.al** study[5], where it was 43.75% and in patients with CD4 count of 101-200 cells/ml detected CBNAAT positivity was 73.68% when compared to R. DEWAN et.al study it was 41.67%.In patients with CD4 count of 201-350 cells/ml CBNAAT positivity was detected 54.17% in the present study when compared to R. DEWAN et.al study it was 37.5%.In patients with CD4 count of 351-500 cells/ml CBNAAT positivity was detected 46.67% in the present study when compared to R. DEWAN et.al study it was 50%.In patients with CD4 count greater than 500 cells/ml CBNAAT positivity was detected 26.92% in the present study when compared to R. DEWAN et.al study it was 20%.

Higher proportions of tuberculosis is seen in PLHIV patients with CD4 count <200/µl.

Therefore, low CD4 counts can also be used as a marker for suspicion of severe forms of tuberculosis.

Monitoring of CD4 cell count is still important soon after diagnosis with HIV, before beginning HIV treatment. It provides important information about disease progression and the immune system.

Table– 7 CD4 Count and their Relationship with CBNAAT Result in Different Studies

	Present Study			R.Dewan et al Study				
CD4 Count (Cells/Ml)	**CBNAAT Positive**	**CBNAAT Negative**	**Total**	**CBNAAT Positive(%)**	**CBNAAT Positive**	**CBNAAT Negative**	**Total**	**CBNAAT Positive (%)**
Upto 100	12	4	16	75.00	14	18	32	43.75
101-200	16	3	19	84.21	10	14	24	41.67
201-350	14	10	24	58.33	9	15	24	37.50
351-500	7	8	15	46.67	5	5	10	50.00
>500	7	19	26	26.92	2	8	10	20.00

5. In the present study, CBNAAT detected 4 (7.69%) rifampicin resistant cases out of 52 CBNAAT positive cases. In **R Dewan et al** study[5], CBNAAT detected 10 (25%) rifampicin resistant cases out of 40 CBNAAT positive cases .In **Gabriella et al** study[13], CBNAAT detected 3 (6.52%) rifampicin resistant cases out of 46 CBNAAT positive cases In **Prem Parkash Gupta et al** study[15], CBNAAT detected 2 (11.76%) rifampicin resistant cases out of 17 CBNAAT positive cases .

Limitations of the Study

1) Detection was not confirmed with the gold standard of detection, the culture of Mycobacterium Tuberculosis
2) Rifampicin resistance not confirmed with line probe assay or culture.
3) It was a hospital-based study but not community-based study.

Conclusion

CBNAAT is to be indicated as a primary diagnostic test in PLHIV with presumptive TB. CBNAAT detects pulmonary

TB in PLHIV with greater efficacy than sputum microscopy, also helping in early diagnosis in less than 2 hours. It also detects rifampicin resistance with high specificity and can be used for screening for MDR-TB so that early therapy can be started, thus decreasing the incidence of MDR-TB.

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