

**DIAGNOSIS OF TUBERCULOSIS AND DRUG RESISTANT TUBERCULOSIS IN PLHA
(REGISTERED AT ART CENTRE TIRUNELVELI) USING CB-NAAT**

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ABSTRACT

Background: Tuberculosis is the commonest opportunistic infection seen in Patients infected with HIV and Pulmonary TB is the commonest form of the disease. The aim of this study was to assess the role of CBNAAT in diagnosing tuberculosis and drug resistance in TB in HIV infected persons and its comparison with routine sputum AFB microscopy. **Methods:** Cross sectional descriptive study. 200 HIV infected patients were recruited for the study and the prevalence of pulmonary tuberculosis was assessed both with sputum AFB microscopy and sputum CBNAAT. Extrapulmonary tuberculosis was identified with relevant tests. The epidemiological details of the patients were obtained and tabulated to derive the conclusions. **Results:** The prevalence of Tuberculosis in HIV infected individuals as per this study was found to be 15%. The overall prevalence of Pulmonary TB in the given population is 13% and extra pulmonary is 2%. This study also suggests that meals with HIV have a higher incidence of Tuberculosis. The study also revealed that sputum CBNAAT is more efficacious than sputum microscopy for detection of TB and the prevalence of rifampicin resistance in TB among HIV infected individuals was 10%. **Conclusion:** Sputum CBNAAT is a valuable test for diagnosing tuberculosis both in HIV infected and non infected people and it has an additional advantage of identifying rifampicin resistance thereby making it an effective screening tool for MDR TB.

KEYWORDS: Tuberculosis, HIV, PLHA, CB-NAAT.

INTRODUCTION

HIV associated tuberculosis remains a major public health challenge. Tuberculosis is the most common opportunistic infection in HIV infected individuals and co-infected individuals are at high risk of death. WHO recommends tuberculosis screening at the time that HIV infection is diagnosed. Pulmonary tuberculosis is the most common form of TB disease HIV positive and HIV negative patients with active pulmonary TB generally manifest similar clinical features, namely cough, fever, night sweats, hemoptysis and weight loss. Sputum based methods are standard to diagnose pulmonary tuberculosis that includes sputum microscopy and culture, but in PLHA patients very much scanty sputum production and there is no caseous necrosis leading to very low number of bacilli in the sputum. These factors decreased the sensitivity of test.

To overcome these disadvantages culture and sensitivity for sputum and drug sensitivity testing used as the diagnostic tool, But this test take around 4-8 weeks for the results to come, so cannot be used for the screening purpose. There is also a delay in starting ATT and

chance spreading MDR-TB is more, so now a day's PCR based methods like CB-NAAT is available for M. tuberculosis detection. The advantage of this tests are highly sensitive, results will get within 100 min, and also we can detect the rifampicin resistance.

Its role in diagnosing TB in PLHA has not studied widely in India. So this study to evaluate the role of CB-NAAT in diagnosis TB in PLHA and its comparison with Sputum AFB microscopy. Based on this aim of our study is to estimate the prevalence of pulmonary tuberculosis in PLHA using CB-NAAT also to estimate the prevalence of drug resistant tuberculosis in PLHA diagnosed to have tuberculosis using CB-NAAT and comparison of efficacy of CB-NAAT with that of Sputum microscopy and AFB in diagnosing tuberculosis.

MATERIALS AND METHODOLOGY

This study is done in 200 PLHA patients who have registered at ART centre, Tirunelveli. The study period is from October 2014 to September 2015. A total of 200 sputum samples of the patients with symptoms suggestive of pulmonary tuberculosis including both new

cases and on treatment were received. All specimens were collected in pre-sterilized falcon tubes with three layer packing system, after through rinsing of the oral cavity with clean water. Samples along with prescribed Performa containing details of patients like Name, Address, Age, Sex, HIV status, and Name of the referring centre was received in the Microbiology Laboratory.

TB detection was done by Xpert MTB/RiF assay, made by Cepheid-Sunnyvale-USA. Sputum specimens were processed according to the GeneXpert Dx system operator manual given by Central TB division, Government of India, Guidance document for use of cartridge based nucleic acid amplification test (CB-NAAT) under RNTCP. The assay is designed for extraction, amplification and identification of *rpoB* gene of *M. tuberculosis* as it accounts for more than 95% of mutations associated with rifampicin resistance), ensuring high degree of specificity by use of three specific primers and 5 unique molecular probes. The

number of positive beacons, their detection timing (indicated by rise of fluorescent signal above a predetermined baseline cycle threshold) and the results of sample processing controls, allow the test to distinguish among the following results: no TB; TB detected, rifampicin resistance detected; TB detected, no rifampicin resistance detected; TB detected, rifampicin resistance indeterminate; and an invalid result. Xpert MTB/RIF cartridge is a disposable, single self-enclosed test unit in which all steps of NAAT i.e. Sample processing, PCR amplification and detection are automated and integrated. The manual steps involved in the assay are adding reagent to liquefy sputum and sample loading.

OBSERVATION AND RESULTS

In our study group, most of the patients were in age group of 41-50 followed by 31-40 years while rest of cases was mostly above 50 years., In our study most of the patients were male which is around 66 percent of cases while rest were female patients.

Table 1: Age distribution.

AGE GROUPS	NO.OF PLHA	PERCENTAGE
< 30	10	5
30-40	67	33
41-50	75	37
51-60	39	20
>60	9	5

Next we analyzed the number of patients who were on ART and in our study around 110 patients were on ART and rest were not on ART which may be due to their normal CD4 levels.

We next analysed the age wise distribution of patients who are on ART and not on ART and there was not much difference in distribution except in age group of 41-50 where most of patients were on ART.

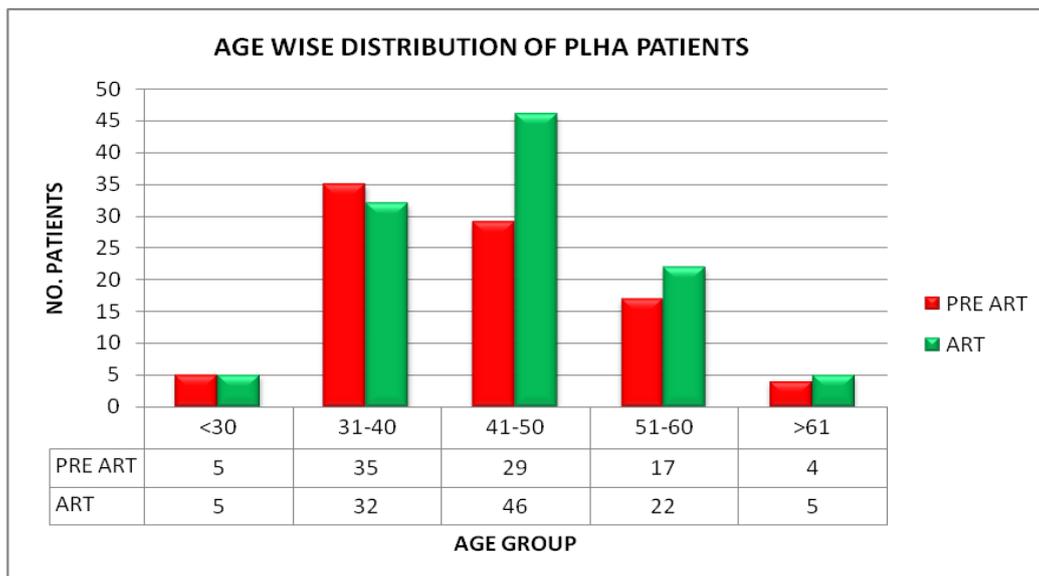


Chart 1: Age wise distribution of patients on ART.

Next we evaluated the TB Prevalence in our study group and among 200 patients 31 patients had Tb and among them 26 had Pulmonary TB and 5 had extra-pulmonary TB., Among this 25 cases were male and 6 cases were

female. Among 25 male patients with TB 21 had Pulmonary TB and 4 had extra-pulmonary TB, Similarly among six female patients 5 had PTB and 1 had Extrapulmonary TB.

Table 2: Prevalence of TB.

	NO.OF.PLHA	PERCENTAGE
TB	26	13%
EPTB	5	2%
NO TB	169	85%
TOTAL	200	100%

Coming to age wise distribution of TB most of cases were in 41-50 age group where 14 patients fall in this group among which 11 had PTB and 3 had EPTB, 9 patients had TB in 31-40 age group among which 7 had

PTB and 2 had EPTB. While there were only PTB in other age groups, like 2 cases in less than 30 years, 6 cases in 51-60 age group.

Table 3: Age wise distribution of TB.

		TB		Total
		NO	YES	
AGE	<30	8	2	10
	30-40	58	9	67
	41-50	61	14	75
	51-60	33	6	39
	>60	9	0	9
Total		169	31	200

Also we analysed the prevalence of TB in ART and PRE-ART patients in our study group where 12 patients had TB in patients on ART while 19 patients had TB

patients in Pre –ART group. Among 12 patients in ART group 9 had PTB and 3 had EPTB. Similarly among 19 patients in Pre-ART 17 had PTB and 2 had EPTB.

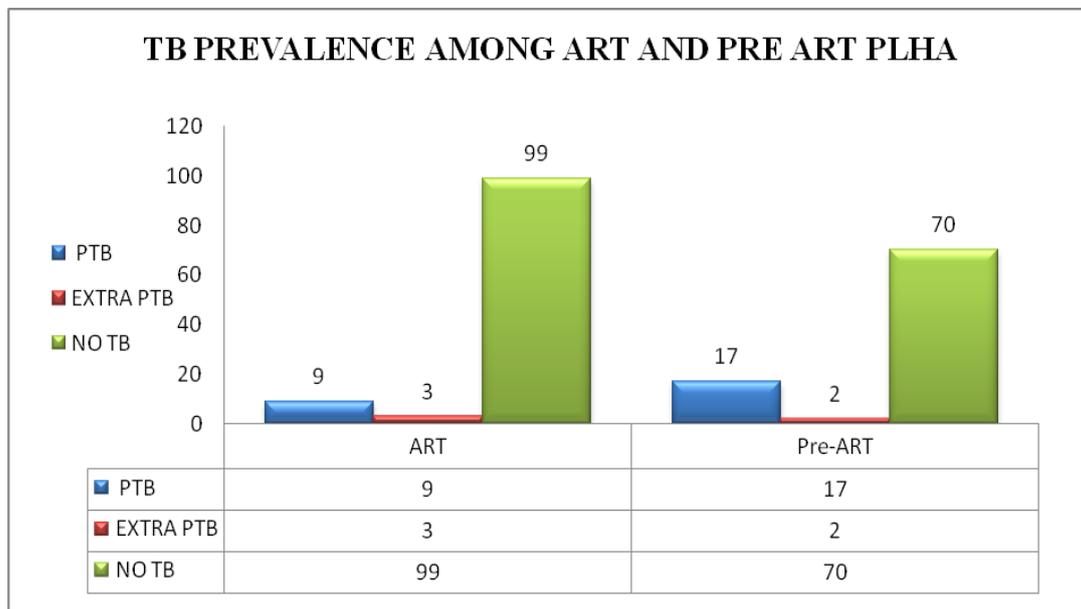


Chart 2: TB prevalence in relation with ART.

Out of 26 pulmonary TB patients 25 has got MTB detected using CB-NAAT in sputum. But Sputum microscopy for AFB was positive in 21 patients. So when we comparing the efficacy of both these tests using Mc NEMAR test- the P value come as 0.219, that means sputum microscopy for AFB is inferior to CB-NAAT in diagnosing tuberculosis. So CB-NAAT is more efficacious than Sputum microscopy for AFB. As CB-NAAT can also detect the rifampicin resistance. In this Study out of 31 TB patients 3 has got rifampicin resistance (that is out of 26 PTB patient 3 has got and in 5 EPTB patients no rifampicin resistance detected). So

Prevalence of Rifampicin resistance in TB in PLHA is 10% (in PTB 12% and in EPTB 0%).

DISCUSSION

Tuberculosis is the most common opportunistic infection in PLHA. Sputum microscopy was the key to diagnosis of tuberculosis in PLHA, but due to poor yield and so much other factors now a days we are using PCR based diagnostic tools like CB-NAAT. We can also find out the rifampicin resistance by these methods. The studies comparing these two (that is sputum microscopy and CB-NAAT) are limited, by this So this study “Diagnosis

of tuberculosis and drug resistant tuberculosis in PLHA (REGISTERED AT ART CENTRE TIRUNELVELI) USING CB-NAAT done in 200 PLHA patients who have registered at ART centre, Tirunelveli.

Out of 200 PLHA 132 patients were males and 68 were females, so 68% of study population constituted by males. Men account for a greater proportion of the epidemic's burden vis-à-vis women at 61% and 39%, respectively.^[1] Coming to the age wise distribution of PLHA, all patients in my study comes between the age group of 20-70 years and most number of patients comes between 30-50 yrs, which come around 70% of total study population. Out of 200 patients 110 patients (55%) on ART and rest 90 patients (45%) were in Pre-ART stage. The adult HIV prevalence in India has declined to an estimated 0.31% (0.25–0.39%) in 2009 against 0.36% (0.29–0.45%) in 2006. Among the high prevalence states, the HIV prevalence has declined in Tamil Nadu to 0.33% in 2009 and other states show either a plateau or a slightly declining trend over the time period 2006–2009.^[2] There are states in the low prevalence states where the adult HIV prevalence has risen over the last 4 years. The estimated number of PLHIV in India is 2.4 million (1.93–3.04 million) in 2009. Of which, 39% are women, children under 15 years of age account for 4.4% of all infections, while people aged 15–49 years account for 82.4% of all infections.^[3]

The one of the main aim of this study was to find out the prevalence of TB in PLHA. Out of 200 PLHA patients 31 found to have tuberculosis. So the estimated prevalence of the tuberculosis is 15%. Out of 31 tuberculosis patients 26 having pulmonary tuberculosis and rest of the 5 patients having extra-pulmonary tuberculosis. In extra-pulmonary tuberculosis 3 having tuberculouspleural effusion and 2 patient having TB lymphadenitis. Prevalence of pulmonary TB comes around 13% and that of extra-pulmonary TB is 2%. HIV-infected persons have approximately 8 times higher risk of developing TB than persons without HIV. Early diagnosis and prompt initiation of TB treatment help to reduce the burden of TB in HIV people.^[4,6] In a study done in Gujarat in patients coming to ART centre with the prevalence was around 17.8 which is similar to our study.^[5]

Out of 132 male patients 20% having tuberculosis, that is 26 patient, and out of 68 female patients 7% having TB that come around 5 patients. In the 26 male patients with TB, 22 are having pulmonary TB and 4 are having extra-pulmonary TB. In female TB patients 4 having pulmonary TB and 1 having extra-pulmonary TB. When we calculate P-value which comes around 0.022, that is statistically significant. So gender has relationship with TB, males more commonly affected by TB in PLHA similar to previous studies.^[7] Where as a study done in Africa had different results in contrast where the proportion of female respondents who had TB/HIV-co

infection accounted for a larger proportion in the sample 107(69%) compared to male respondents 48(31%).^[8]

When we considering the relationship of age groups and tuberculosis in PLHA; P value come as around 0.627, this is not statistically significant. So TB has on relationship with age groups in PLHA. Similarly in one another study it was found that the majority of respondents were in age ranged between 31-40 years. A recent national survey report revealed that 58% of prevalent TB cases in Ethiopia are less than 35 years of age.^[9,10]

When we taking the occurrence of pulmonary TB in patient on ART and in Pre-ART stage, 17 out of 90 pre-ART patients and 9 out of 110 ART patients has got PTB, when calculated the P value its come around 0.022, which is also statistically significant. So whether patient on ART or Pre-ART stage has got relationship with TB in PLHA. Total prevalence of TB in ART patient is 12 out of 110 PLHA having TB(Out of which 9 having pulmonary TB and 3 having Extra-pulmonary TB) and in Pre-ART stage 19 out of 90 patient has got TB(17 having PTB and 2 having EPTB). So in ART 21% has got tuberculosis and in Pre-ART 11% has got Tuberculosis. So TB is more prevalent in Pre-ART group.

Out of 26 pulmonary TB patients 25 has got MTB detected using CB-NAAT in sputum. But Sputum microscopy for AFB positive in 21 patients. So when we comparing the efficacy of both these tests using Mc NEMAR test- the P value come as 0.219, that means sputum microscopy for AFB is inferior to CB-NAAT in diagnosing tuberculosis. So CB-NAAT is more efficacious than Sputum microscopy for AFB. As CB-NAAT can also detect the Rifampicin Resistance. In this Study out of 31 TB patients 3 has got rifampicin resistance(that is out of 26 PTB patient 3 has got and in 5 EPTB patients no rifampicin resistance detected). So Prevalence of Rifampicin resistance in TB in PLHA is 10%(in PTB 12% and in EPTB 0%). This is similar to previous studies which proved in a low-resource high-burden setting, CBNAAT may have greatest impact where the clinician's pretest confidence in TB is low and empirical treatment has not been started. This is because MTB-D results will lead to appropriate initiation of treatment and MTB-ND results may enable clinicians to hold-off treatment.^[11]

CONCLUSION

CB-NAAT efficacy to detect Mycobacterium tuberculosis has been already demonstrated in various studies. It has got advantage of diagnosing tuberculosis in PLHA with increased case detection rate and with lesser time to diagnose. In addition to that it can also detect rifampicin resistance with high specificity, so we can use this method as a screening tool for MDR-TB and start the patient on CAT-IV anti-tuberculosis drugs as early as possible. By this means we can reduce the risk of transmission of MDR-TB as the conventional methods

like sputum culture and drug sensitivity tests takes 4-8 weeks for the diagnosis of MDR-TB. And also to conclude the estimated prevalence of TB in PLHA (registered at ART centre, Tirunelveli) using CB-NAAT is 15%, and out of the all TB patients' prevalence of MDR-TB is 10%.

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