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A STUDY OF RIFAMPICIN RESISTANCE AMONG TUBERCULOSIS CASES ATTENDING RNTCP CENTER GGH, VIJAYAWADA.

KEYWORDS: *RNTCP, PLHIV, MDR-TB, CBNAAT.*

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ABSTRACT **Aims and objectives:** To study the Rifampicin resistance among CBNAAT diagnosed cases of Tuberculosis and to compare the data of HIV reactive cases with their CD4 counts.

Materials and Methods: This is a prospective study conducted at GGH, Vijayawada. 2347 samples from suspected TB cases were submitted for CBNAAT (Xpert MTB/RIF assay). The data was analysed in relation to CD4 counts among PLHIV & Extra pulmonary cases, suspected paediatric TB & smear negative cases.

SUMMARY: Among the 2347 tested patients 269 were positive for TB. Out of 269 positives 235(87.36%) are HIV reactive & 34(12.63%) are HIV non reactive. Rifampicin resistance is 3.40 %(8) among HIV reactive & 32.35%(11) among HIV non reactive cases. An attempt is made to correlate these results with CD4 counts in HIV reactive patients.

Background and Objective: Tuberculosis (TB) is one of the commonest opportunistic infection and the leading cause of death in HIV patients in developing countries. HIV infection is a well recognised risk factor for both activation of initial tuberculosis infection and reactivation of latent infection.[1] Diagnosing tuberculosis (TB) in people living with HIV/AIDS (PLHIV) is challenging as sputum microscopy is negative in more than half of the patients due to lack of caseous necrosis. Sputum culture is a slow method which takes 4 - 8 weeks for growth of the Mycobacteria. Delayed treatment for TB in PLHIV is associated with increased mortality. The role of a newly launched catridge based nucleic acid amplification test (CBNAAT) with a potential to diagnose TB and rifampicin resistance within 2 hours in PLHIV is promising.[2]

INTRODUCTION

India accounts for one fourth of the global TB burden i.e 2.2 million out of 9.6 million new cases annually. In India, more than 40% of population is infected with Mycobacterium tuberculosis.

	Incidence	Prevalence	Mortality
Global	9.6 million (176/lakh/year)	13 million (227/lakh/year)	1.1 million (21/lakh/year)
India	2.2 million (167/lakh/year)	2.5 million (195/lakh/year)	2.2 lakhs (17/lakh/year)

Source: Global TB Report 2015

TB now ranks alongside HIV as a leading cause of death worldwide. TB kills more adults in India than any other infectious disease.

In India, every day: More than 6000 develop TB disease and more than 600 people die of TB (i.e. 2 deaths every 5 minutes). India has the highest burden of both TB and MDR TB and second highest of HIV associated TB based on estimates reported in Global TB Report 2015. An estimated 71,000 cases of MDR-TB emerge annually from the notified cases of pulmonary TB in India. Based on sub-national DR surveys carried out in three states of India, 3% among new TB cases and 12%-17% among previously treated TB cases have MDR-TB.[3]

In 1993, a revised strategy called DOTS to control tuberculosis was pilot tested and as a result of tremendous success in this pilot project RNTCP was launched in 1997 with WHO recommended DOTS strategy. By March 2006 entire country has been covered under the programme.[4]

Since RNTCP programme started; there are number of newer initiatives incorporated into the programme to control Tuberculosis. RNTCP has announced 'universal access to quality TB diagnosis and treatment in the community' with a target of 'reaching the unreached' as its new goal and target in the new National Strategic Plan(NSP)2012-2017.

MDR-TB suspect criteria as per current programme guidelines:-

Criteria A

(1A)- All failures of new TB cases

(2A)- Smear positive previously treated cases who remain smear positive at 4th month onwards

(3A)- All pulmonary TB cases who are contacts with known MDR-TB cases

Criteria B

(4B)- All smear positive previously treated pulmonary TB cases at diagnosis

(5B)-Any smear positive follow up result in new or previously treated cases

Criteria C

(6C)-All smear negative previously treated pulmonary TB cases at diagnosis

(7C)-HIV TB co-infected cases at diagnosis.

TB-HIV collaborative activities between RNTCP and NACP started first time in 2001 and in 2007 patients with high risk behaviour who are not responding to anti TB treatment were promoted for selective referral to HIV counselling and testing centre(ICTC).

Three' I 'S of HIV-TB:

1.Intensified TB case finding followed by high-quality AntiTB treatment

2.Isoniazid Preventive Therapy(IPT)

3.Infection control in HIV care setting.

RNTCP has developed a guidance document for the use of CBNAAT technology. Currently 80 such CBNAAT machines deployed across the country(2015.)[4]

Materials and Methods : The study was conducted at RNTCP Centre GGH ,Vijayawada. The study period is from August 2015 to August 2016. Expectorated Sputum samples & appropriate fluid samples from extra pulmonary tuberculosis patients were sent to RNTCP.

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A total of 2347 clinically suspected cases of TB were subjected to CBNAAT assay. The patients came with symptoms of both pulmonary and extra pulmonary tuberculosis, paediatric TB & smear negative cases, including both new cases and on treatment were included.

CBNAAT is a semi-quantitative nested real time PCR in-vitro diagnostic test.

Figure 1 : CBNAAT instrument

Standard Assay Procedure of Gene Xpert:
The assay utilizes single-use plastic cartridges with multiple chambers that are preloaded with liquid buffers and lyophilized reagent beads necessary for sample processing, DNA extraction and heminested rt-PCR. Clinical sputum samples (or decontaminated sputum pellets) are treated with sodium hydroxide and isopropanol-containing sample reagent (SR). The SR is added to the sample (currently recommended at a 3:1 ratio for sputum pellets and a 2:1 ratio for unprocessed sputum samples) and incubated at room temperature for 15 min. The treated sample is then manually transferred to the cartridge which is loaded 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. The test platform employs a sonic horn that inserts into the cartridge base to cause ultrasonic lysis of the bacilli and release of the genetic material. The assay then amplifies a 192 bp segment of the rpoB gene using a hemi-nested rt-PCR reaction. Mycobacterium tuberculosis is detected by the five overlapping molecular probes (probes A–E) that collectively are complementary to the entire 81 bp rpoB core region. M. tuberculosis is identified when at least two of the five probes give positive signals with a cycle threshold (CT) of d"38 cycles and that differ by no more than a prespecified number of cycles. The basis for detection of rifampicin resistance is the difference between the first (early CT) and the last (late CT) M. tuberculosis-specific beacon (ÄCT). The system was originally configured such that resistance was reported when ÄCT was >3.5 cycles and sensitive if less than d"3.5 cycles.[5]

Results and Discussion:
Table 1: TB positives identified by CBNAAT Assay (n=2347)

CBNAAT ASSAY	HIV Reactive		HIV Non -Reactive		Total
NO Tested	2239	95.39%	108	4.60%	2347
NO Positive	235	87.36%	34	12.63%	269

A total of 2347 samples were tested by CBNAAT. Among these 2239(95.39%) samples were HIV reactive and 108 (4.60%) were from HIV non reactive cases. Out of total 269 positive cases 235 (87%) are HIV reactive and 34(13%) are HIV non reactive cases.

Table 2: Types of cases tested by CBNAAT as per RNTCP guidelines other than PLHIV.

NO TESTED	EXTRA PULMONARY CAESES	6C OLD TB TREATED	7C HIV WITH OLD TB TREATED	PEDIATRIC CASES
Total Tested	36	55	31	36
Percentage of the total tested	1.5%	2.3%	1.3%	1.5%

Out of 2347 tested more cases are from PLHIV followed by 6C, Extrapulmonary cases & Paediatric cases.31 cases belong to 7C category.

Table 3: Rifampicin Resistance pattern among HIV reactive & HIV non-reactive cases

Categories	Rifampicin Sensitive		Rifampicin Resistance		Total
HIV Reactive	227	96.59%	8	3.40%	235
HIV Non-Reactive	23	67.64%	11	32.35%	34
Total	250	92.93%	19	7.06%	269

This study attempted to compare the Rifampicin resistance among HIV reactive and HIV non reactive patients. A high rate of resistance i.e 32.35% is seen in HIV non reactive cases, compared to HIV reactive cases which is 3.4%.Thiis may be due to the reason that the non reactive cases tested were old TB cases who already received anti tuberculosis treatment and are mostly treatment failures.

Table 4: No of Deaths among CBNAAT POSITIVE cases in relation to HIV Reactivity

Categories	Deaths among Rifampicin Sensitive Cases	Deaths among Rifampicin Resistant Cases	Total
HIV Reactive	2	2	4
HIV Non-Reactive	2	2	4
TOTAL	4	4	8

In this table we tried to compare the mortality among HIV reactive cases and Non reactive cases with that of Rifampicin resistance & sensitive cases and there was no much difference observed.

Table 5: CBNAAT POSITIVITY AMONG HIV REACTIVE CASES IN RELATION TO CD4 COUNT& RIFAMPICIN RESISTANCE

CD_4	NO OF TB POSITIVES	NO OF RIFAMPICIN RESISTANT CASES
UPTO 100	34	1
101-200	44	2
201-350	45	1
351-500	18	NIL
›500	13	1
CD4 count available	81	3
TOTAL	235	8

In table 5: an attempt is made to correlate the TB positivity by CBNAAT assay with CD_4 count of the HIV reactive cases and resistance pattern to Rifampicin.

There are more number of TB positive cases in patients with CD_4 counts below 350 in comparison to CD_4 counts above 351.Rifampicin resistance is not in correlation with CD_4 counts, drug resistance will develop due to non compliance with the treatment regimen than the immune status of the patient.

RNTCP centre, of GGH, Vijayawada is a medical college level centre, and the referral cases are from other RNTCP centres for CBNAAT assay. CD_4 counts were not available for those patients who are from other centres.

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Conclusion: Xpert MTB/RIF may be the foremost choice among all molecular diagnostic tests but it has its own limitations. Resistance to RIF is taken as a surrogate marker for MDR-TB, but certain strains may exhibit only mono-resistance to RIF that may not warrant full line MDR therapy, thus, leading to over-estimation of the MDR-TB cases. Likewise study from Mumbai, India demonstrated how specimens with rifampicin results reported as sensitive by Gene Xpert could be resistant to isoniazid. Other drawbacks of Xpert MTB/RIF are requirement of stable electric power supply, temperature control and annual calibration of instrument. Regardless of all these limitations, addition of Xpert MTB/RIF assay to the present set of diagnostic modalities for TB on account of its unambiguous, rapid results, and high sensitivity and specificity will facilitate early diagnosis.[7]

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