"An Anlytical Study Of Mycobacterial Load In Trunat And Sputum Conversion In Pulmonary Tuberculosis Sputum Smear Positive Patients"

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Abstract
Background: Tuberculosis is one of the major health problem worldwide. TB is one of the 10 causes of deaths in India. Accurate diagnosis and treatment plays major role in reduction in mortality and morbidity of tuberculosis. Truenat (Chip based real -time micro -polymerase chain reaction PCR) plays major role in diagnosis of tuberculosis as well as detection of resistance. In this study bacterial load in truenat (PCR) and sputum conversion is analysed.
Materials And Methods: This Analytical study was conducted at Sri Lalithambigai medical College and Hospital, Adayalampattu, maduravoyal and District TB Centre, Chinna porur, West Chennai, Among 318 patients with sputum smear positive, Age group of 18 years and above, DM, HTN, Smoking, Alcoholic were included in the study.
Result: Most patients were aged between 41-50, Regarding repeat AFB status 13.8% were positive. Bacterial load is highest in age group 51-60, Bacterial load is higher in individual with positive repeat AFB.
Conclusion: Result suggest there is correlation between truenat bacterial load and repeat sputum AFB. Hence monitoring bacterial load in truenat plays vital role in TB treatment and follow up.

Key Words: Tuberculosis, Repeat Sputum AFB, PCR.

INTODUCTION:

Tuberculosis is a major global health problem, Globally the annual incidence of TB estimates about 10 million people of which 2.7 million cases are reported from India. TB kills an estimated 480,000 Indians every year and more than 1,400 every day. India has been engaged in TB control activities for more than 50 years. Yet TB continues to be India's severest health crisis. TB was discovered by Robert Koch On March 24, 1882, at the Berlin Institute for Physiology, Koch announced the discovery of the tuberculosis pathogen – with his lecture on the "Etiology of Tuberculosis" .
GOAL of NTEP is to achieve a rapid decline in burden of TB, morbidity and mortality while working towards elimination of TB in India by 2025. Despite these odds, countries have repeatedly demonstrated that TB can be controlled in the modern era, as long as TB is diagnosed early , treated properly and interrupted transmission .To achieve this (1) Developing reliable and accurate tuberculosis (TB) diagnosis Tool closer to patients is a key priority for global TB control.

Figure 1.Truenat (PCR)

TRUNAT is chip based real time micro polymerase chain reaction for detection of TB and RIF-resistance from Deoxyribonucleic acid that is extracted from sputum sample within an hour.

(8)STEPS IN TRUENAT TEST (PCR):

1)DNA extraction:

I) Collect 2-5 ml pulmonary sputum sample in 50 ml falcon tube/sputum cup and label with patients details.
II) Add 2 drops of liquefaction buffer to the sputum cup, close the lid and swirl gently to mix.
III) Incubate for 10 minutes at room temperature, if sample is not pipettable after 10 minutes incubate for another 5 minutes with swirling at 2 minutes interval.
IV) Transfer 0.5 ml of liquefied sputum to lysis buffer bottle using pipette and add 2 drops of liquefaction buffer to the lysis buffer bottle, swirl gently to mix and incubate for 3-5 minutes.
V) Transfer the entire content of the lysis buffer bottle into the sample chamber of the cartridge and close the cartridge holder.

2. Real-time PCR:

I) The device will beep at the end of the DNA Extraction process (20 minutes),cartridge holder will eject automatically, After this process transfer the sample to master mix tube.

3. Real-time PCR on chip

I) Allow the master mix to stand for 30 seconds to get clear solution and transfer the mastermix tube to the white reaction well of the chip.

4. Detection of resistance:

I) PCR will be completed in 35 minutes, If MTB detected test the same tube for Rifampicin resistance using Truenat MTB-RIF Dx chip this usually takes 55 minutes to complete.

5. Buffers, reagents and mastermixes

I) All buffers and reagents used for nucleic acid extraction and all mastermixes used for PCR are proprietary components of the Truenat MTB kit.

(3)Truenat assays were found to have high diagnostic accuracy. The assays have the potential to be used as a point of care (POC) TB diagnostic tests. The use of rapid molecular tests is increasing across the world, and many countries are phasing out the use of smear microscopy for diagnostic purposes [**3**]. However, microscopy remains a useful diagnostic tool for treatment monitoring. Despite advances in diagnostics, a considerable proportion of TB cases reported to WHO are still bacteriologically confirmed [**3**]. The sputum conversion rate at 2 months is frequently used to evaluate treatment outcomes and effectiveness of a TB control programme. (4) Smear conversion is defined as new smear positive PTB cases who became smear negative after a period of 2 months (Intensive anti -TB treatment) and are therefore no longer infectious .Sputum smear conversion is a key indicator of treatment response and reduced infectivity among bacteriologically confirmed pulmonary tuberculosis (PTB) patients. This sputum conversion is documented in almost all PTB patients and are advised to follow cough etiquette, to wear face mask and to dispose expectorants in safe place.
(.2) Sputum non-conversion is smear positive tuberculosis after 2 months ,despite intensive anti-tubercular therapy for 2 months. (6) Various factors may lead to sputum non-conversion including resistance to anti-tubercular drugs, age, gender, disease severity, non-compliance, drugs unavailability etc. In this study bacterial load in truant and sputum conversion is analysed.

MATERIALS AND METHODS:

In this Retrospective study Patients of 18 years of age and above , All gender, all sputum smear positive TB patients/Truenat positive patients were included. TB patients with DM, HTN, Smoking, Alcoholism were included. Patients below 18 years of age ,Patients with extra pulmonary TB, Patients with negative Truenat were excluded. This study was conducted at Sri Lalithambigai medical College and Hospital, Adayalampattu, maduravoyal and District TB Centre, Chinna porur ,West Chennai.

RESULTS

Table 1. Distribution of patient's characteristics

		Count	Column N %
AGE GROUP	<20	12	3.8%
	21-30	44	13.8%
	31-40	43	13.5%
	41-50	68	21.4%
	51-60	68	21.4%
	61-70	58	18.2%
	>71	25	7.9%

GENDER	FEMALE	80	25.2%
	MALE	238	74.8%
MARITAL STATUS	MARRIED	283	89.0%
	SINGLE	35	11.0%
DIABETES MELLITUS	NEGATIVE	222	69.8%
	POSITIVE	96	30.2%
TOBACCO	NEGATIVE	289	90.9%
	POSITIVE	29	9.1%
ALCOHOL	NEGATIVE	285	89.6%
	POSITIVE	33	10.4%

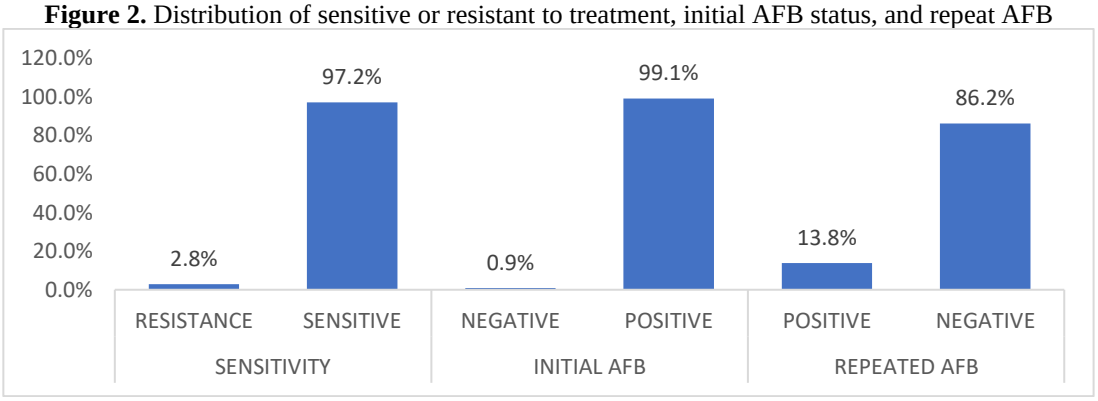
Among 318 patients, 238 (74.8%) were males, and 80 (25.2%) were females. Most patients were aged between 41-50, 68 (21.4%), and aged between 51-60 was 68 (21.4%). 58 (18.2%) patients were aged between 61-70.
283 (89%) patients were married, and 35 (11%) were unmarried. Regarding diabetes mellitus, 96 (30.2%) patients are positive, while 222 (69.8%) are negative. Regarding tobacco, 29 (9.1%) patients are positive, while 289 (90.9%) are negative. Regarding alcohol, 33 (10.4%) of patients are positive, while 285 (89.6%) are negative.

Table 2. Distribution of sensitive or resistant to treatment, initial AFB status, repeat AFB status, and treatment outcome

		Count	Column N %
SENSITIVITY	RESISTANCE	9	2.8%
	SENSITIVE	309	97.2%
INITIAL AFB	NEGATIVE	3	0.9%
	POSITIVE	315	99.1%
REPEAT AFB	POSITIVE	44	13.8%
	NEGATIVE	274	86.2%
OUTCOME	CURED	257	80.8%
	DIED	31	9.7%
	LOST TO FOLLOW UP	7	2.2%
	ON TREATMENT	19	6.0%
	TREATMENT FAILURE	4	1.3%

Regarding sensitivity, 309 (97.2%) of patients are sensitive to treatment, while 9 (2.8%) are resistant. Regarding initial AFB status, 315 (99.1%) patients are positive, while 3 (0.9%) are negative.

Figure 2. Distribution of sensitive or resistant to treatment, initial AFB status, and repeat AFB



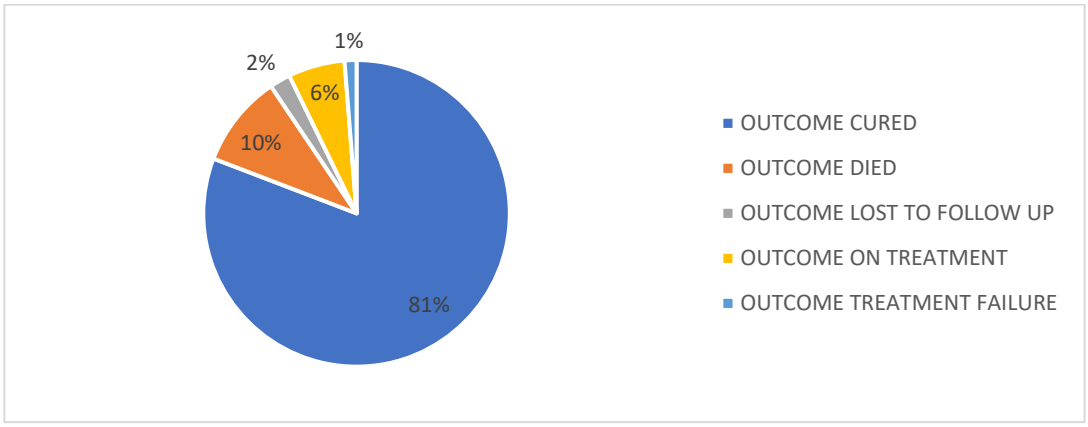


Figure 3. Distribution of treatment outcome

Regarding repeat AFB status, 44 (13.8%) patients are positive, while 274 (86.2%) are negative. In terms of treatment outcome, 257 (80.8%) of patients were cured, 31 (9.7%) died, 7 (2.2%) were lost to follow-up, 19 (6.0%) were on treatment, and 4 (1.3%) had treatment failure.

In terms of treatment outcome, 257 (80.8%) of patients were cured, 31 (9.7%) died, 7 (2.2%) were lost to follow-up, 19 (6.0%) were on treatment, and 4 (1.3%) had treatment failure.
The median bacterial load is highest in males and in tobacco patients. The percentile 25 and percentile 75 values for bacterial load increase with age.

Table 3. Comparison of repeat AFB status, and treatment outcome between bacterial load

		BACTERIAL LOAD			P value
		Median	Percentile 25	Percentile 75	
REPEAT AFB	POSITIVE	825000.00	150000.00	3600000.00	<0.0001
	NEGATIVE	125000.00	5200.00	1300000.00	
OUTCOME	CURED	130000.00	5200.00	1300000.00	0.197
	DIED	560000.00	32000.00	4800000.00	
	LOST TO FOLLOW UP	670000.00	330000.00	2400000.00	
	ON TREATMENT	240000.00	32000.00	1400000.00	
	TREATMENT FAILURE	668000.00	34000.00	1850000.00	

The bacterial load is higher in individuals with positive repeat AFB status than those with negative repeat AFB status. There is a statistically significant difference in repeat AFB between bacterial loads ($p<0.0001$).
In treatment outcome, individuals who died have the highest median bacterial load, while individuals who are cured have the lowest median bacterial load. But there is no significant difference in outcome between bacterial load ($p=0.197$).

CONCLUSION:
Result suggest there is correlation between truenat (Real time PCR) bacterial load and repeat sputum AFB. Higher bacterial load is seen in patients with positive repeat AFB than those with negative Repeat AFB. Hence monitoring bacterial load in truenat plays vital role in TB treatment and morbidity , mortality prevention.

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