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Research Article

DIAGNOSTIC POWER OF GENEXPERT MTB/RIF ASSAY FOR RAPID DIAGNOSIS OF TUBERCULOSIS AND DETECTION OF RIFAMPINCIN RESISTANCE IN SMEAR NEGATIVE SPUTUM SAMPLE OF SUSPECTED CASE OF TUBERCULOSIS IN TERTIARY CARE HOSPITAL, JAMNAGAR, GUJARAT

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Abstract- Tuberculosis is a widespread, infectious disease caused by various strains of Mycobacteria usually *Mycobacterium tuberculosis*. Tuberculosis typically attacks the lungs and can also affect other parts of the body. An active TB infected patient's cough, sneeze, or otherwise transmit through respiratory fluids are the common cause for the TB through the air. This study, which was conducted in tertiary care hospital, Jamnagar. There were 2 sputum samples, one spot supervised and one early morning collected and transported from various centres to TB culture-DST laboratory, Total 448 Smear Negative Sputum (Suspected case of *Mycobacterium tuberculosis*) samples were received during the study period and proceeded by GeneXpert MTB/RIF Assay. Out of total 448 smear negative sputum samples, in 142 sputum samples *Mycobacterium tuberculosis* Bacilli (MTB) were detected, Out MTB positive cases 12(8.45%) sputum samples resistant to Rifampicin and 103(72.53%) and 39(27.47%) cases from male and female gender respectively. highest number of MTB positive cases were found in age group of 21-30 years which were 38(26.8%) followed by 31-40 years which were 37(26%). Out total 448 Smear Negative Sputum 74 HIV positive sputum samples, in 18 samples *Mycobacterium tuberculosis* Bacilli (MTB) were detected. The study revealed the GeneXpert MTB/RIF Assay test offers a potential solution for improving early MTB diagnosis.

Keywords- *Mycobacterium tuberculosis, GeneXpert MTB/RIF Assay, Smear negative sputum, Rifampicin, HIV positive*

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Introduction

Tuberculosis is a disease caused by bacteria that are spread from person to person through the air. Tuberculosis usually affects the lungs, but it can also affect other parts of body, such as the brain, the kidneys or the spine. In most case, TB is treatable and curable; however, person with TB can die if they do not get proper treatment [1-3]. Tuberculosis is the second most common cause of the death from infectious disease after those due to HIV/AIDS [4]. Antibiotic resistance is a growing problem in multiple drug-resistant tuberculosis infection. Primary resistance occurs when a person becomes infected with a resistance strain of tuberculosis. Primary resistance occurs when a person becomes infected with a resistance strain of tuberculosis. A person with fully susceptible tuberculosis may develop resistance during therapy because of inadequate treatment, not taking the prescribed medications appropriately, or using poor quality medication [5].In addition to being fast acting, the test can determine if there is resistance tuberculosis and is accurate in those who are also infected with HIV [9]. Multidrug resistant tuberculosis poses a formidable challenge to TB control due to its complex diagnostic and treatment challenges. The annual global Multidrug resistant tuberculosis burden is estimated at around 4,90,000 cases, or 5% of the global TB burden; however, less than 5% of exiting Multidrug resistant tuberculosis, the emergence of extensively drug resistant TB. Extended drug resistant tuberculosis, potential institutional transmission, and rapid mortality of Multi resistant tuberculosis and Extended drugs resistant tuberculosis patients with HIV co infection have highlighted the urgency for rapid screening, methods. Conventional methods for bacteriological culture and drugs susceptibility testing are slow and cumbersome, requiring sequential procedures for isolation of *mycobacterium* from clinical specimens, identification of *Mycobacterium tuberculosis* complex, and in vitro testing of strains susceptibility to anti tuberculosis drugs. During this time patients, may be ill-treated, drug resistant strains may continue to spread of resistance may occur. Recent technologies for the rapid detection of anti-tuberculosis drug resistance have therefore become a first line method in tuberculosis research and development, and molecular line probe assays focused on rapid detection of Rifampicin are most advanced [10].

Material and Methods:

This study was conducted in the tertiary care hospital, from August 2017 to September 2018. All samples were collected after detail review of clinical history and laboratory findings. Different samples collected were smear negative sputum, HIV Positive sputum sample, There were 2 sputum samples, one spot supervised and one early morning collected in sterile screw cap wide mouth falcon tube and transported from various centers to TB culture – DST laboratory with cold chain maintained. Total suspected samples received during study period and proceeded for Xpert MTB/RIF Assay.

Principle of the procedure

The GeneXpert Dx System integrates and automates sample processing, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR and reverse transcriptase PCR. The system consists of an instrument, personal computer, barcode scanner, and preloaded software for running tests on collected samples and viewing the results.

The system requires the use of single-use disposable GeneXpert cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is eliminated. Xpert MTB/RIF includes reagents for the detection of tuberculosis and RIF resistance as well as a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. The primers in the Xpert MTB/RIF assay amplify a portion of the rpoB gene containing the 81 base pair "core" region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with RIF resistance [6,7].

Sample preparation

Collect a minimum of 1 mL sputum per specimen. Subject must be in comfortable sitting or standing position. Specimens should be held at 2–8ºC prior to processing whenever possible.

Sputum Decontamination method

The sputum samples were further preceded for decontamination by NALC/NAOH procedure.

NALC/NAOH procedure preparation

Sputum specimens were proceeded as soon as possible or refrigerated them at 2-4ºC. Prepared a written check list of materials required and arranged supplies, reagents and specimen in a BSC. Sputum collected in 50ml sterile, plastic, screw capped centrifuge tubes. Appropriate volume of NALC-NAOH added in the next step for specimen standardization. An equal volume of NALC NAOH solution added with sputum specimen and tighten cap. Gently vortexed each tube at a moderate speed for not more than 20 seconds. Inverted each tube 5 times to ensure that the NALC- NAOH solution contacted the entire inner surface of the tube. The tubes stood at room temperature (20-25ºC) for 15min for decontamination. Diluted the specimen with pH 6.8 phosphate buffer to the 50ml mark. In the BSC, load the diluted specimens into aerosol free safely centrifuge cups. Centrifuge at 3000x g for 20min at 4°C. After centrifugation, opened the safety cups in the BSC, and carefully pour off the supernatant from each tube into a splash proof discard container contained a suitable disinfectant. Use a new pipette for each specimen, re suspended the sediment in 1-2ml of sterile phosphate buffer (pH 6.8) using a 1 or 3 ml in transfer pipette. Preparing the cartridge: Start the test within 30 minutes of adding the sample to the cartridge. Using the sterile transfer pipette provided, aspirate the liquefied sample into the transfer pipette until the meniscus is above the minimum mark. Open the cartridge lid. Transfer sample into the open port of the Xpert MTB/RIF cartridge. Be sure to load the cartridge into the GeneXpert Dx instrument and start the test within 30 minutes of preparing the cartridge.

Interpretation of results

The results are interpreted by the GeneXpert DX System from measured fluorescent signals and embedded calculation algorithms and will be displayed in the "View Results" window. Lower Ct values represent a higher starting concentration of DNA template; higher Ct values represent a lower concentration of DNA template [8].

Results and Analysis

This study was conducted in tertiary care Hospital from August 2017 to September 2018.

Smear Negative Sputum Samples

Total 448 Smear Negative Sputum (Suspected case of *Mycobacterium tuberculosis*) samples were received during the study period.

Prevalence of MTB positive cases in smear negative sputum samples:

Out of total 448 smear negative sputum samples, in 142 sputum samples *Mycobacterium tuberculosis* Bacilli (MTB) were detected by GeneXpert MTB/RIF Assay. So, prevalence of MTB positive cases was 31.69%.

Table-1 *Shows prevalence of Mycobacterium tuberculosis*

SN	Total	MTB Detected	Prevalence
1	448	142	31.69%

Fig-1 Prevalence of MTB in Smear Negative Samples

Table-2 *shows Rifampicin sensitivity in MTB positive case.*

SN	Total Positive MTB	Rifampicin	
		Sensitive	Resistance
1	142	130 (91.55%)	12(8.45%)

Out 142 MTB positive cases 130(91.55%) sputum samples found sensitive to Rifampicin and 12(8.45%) sputum samples resistant to Rifampicin.

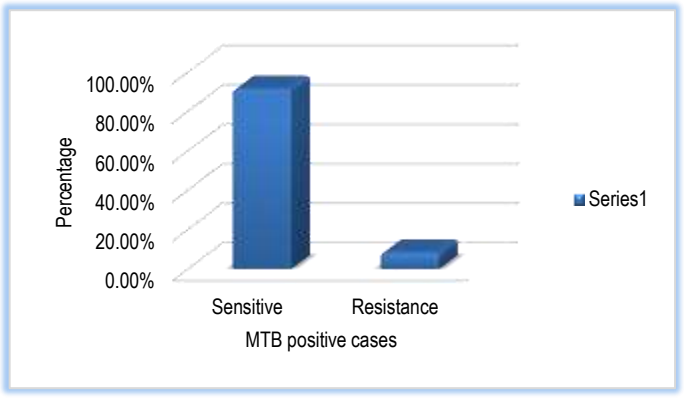

Fig-2 Rifampicin sensitivity in MTB positive cases

Table-3 *shows gender wise distribution of MTB positive cases*

SN	Gender	Positive for MTB	Percentage
1.	Male	103	72.53%
2.	Female	39	27.47%
	Total	142	100%

Out of 142 positive MTB cases, 103(72.53%) and 39(27.47%) cases from male and female gender respectively.

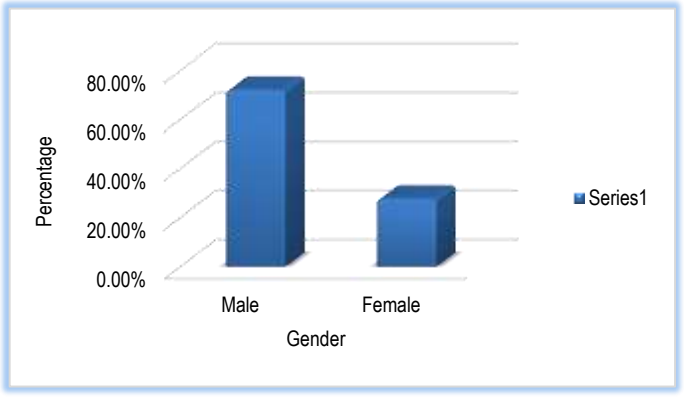

Fig-3 Gender wise Distribution

Table-4 *shows sex wise distribution of rifampicin sensitivity in MTB positive cases*

SN	Gender	Total MTB Positive	Rifampicin	
			Sensitive	Resistant
1	Male	103	97(94.2%)	06(5.8%)
2	Female	39	33(84.6%)	06(15.3%)
	Total	142	130 (91.55%)	12(8.45%)

Table-5 *shows age wise distribution of MTB positive cases and rifampicin sensitivity.*

SN	Age (In Years)	Total MTB Positive	Rifampicin	
			Sensitive	Resistant
1.	1-10	00 (0%)	00	00
2.	11-20	14 (9.9%)	13	01
3.	21-30	38 (26.8%)	35	03
4.	31-40	37 (26%)	31	06
5.	41-50	22 (15.5%)	21	01
6.	51-60	24 (16.9%)	23	01
7.	61-70	05 (3.5%)	05	00
8.	>70	02 (1.4%)	02	00
9.	Total	142 (100%)	130	12

Out of 142 smear negative MTB positive cases, highest number of MTB positive cases were found in age group of 21-30 years which were 38(26.8%) followed by 31-40 years which were 37(26%).

Smear negative sputum samples of HIV positive patients

Out of total 448 smear negative sputum samples, 74 samples were from HIV positive patients.

Prevalence of MTB positive cases in smear negative HIV Positive sputum samples

Table- 6 *shows prevalence of MTB in HIV positive smear negative cases.*

SN	Total HIV Positive Cases	MTB Detected	Prevalence
1	74	18	24.32%

Out 74 HIV positive sputum samples in 18 samples *Mycobacterium tuberculosis* Bacilli (MTB) were detected by GeneXpert MTB/RIF Assay. So, prevalence of HIV positive cases was 24.32%.

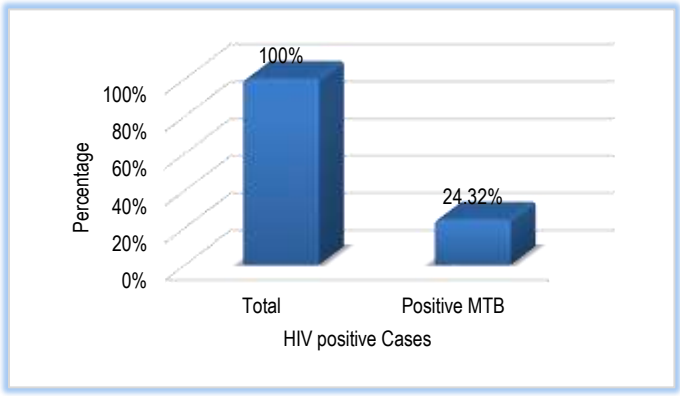

Fig-4 Prevalence of HIV Positive sputum samples

Rifampicin sensitivity in HIV positive MTB positive cases

Table-7 *shows Rifampicin sensitivity in HIV positive cases.*

SN	Total MTB Positive	Rifampicin	
		Sensitive	Resistance
1	18	16(88.9%)	02(11.1%)

Out of 18 positive MTB cases, 16(88.9%) sputum samples were sensitive to Rifampicin and 02(11.1%) sputum samples were resistant to Rifampicin.

Discussion

All samples were collected after detail review of clinical history and laboratory findings. Different samples collected were smear negative sputum, HIV Positive sputum sample. Total 448 Smear Negative Sputum (Suspected case of *Mycobacterium tuberculosis*) samples were received during the study period. Out of total 448 smear negative sputum samples, 74 samples were from HIV positive patients.

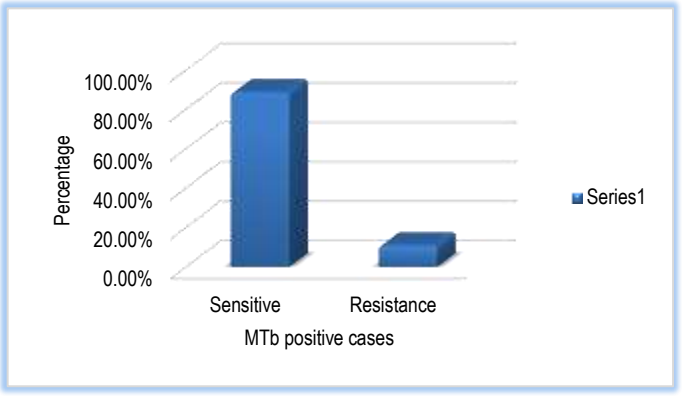

Fig-5 Rifampicin sensitivity in MTB positive cases

Table-7 Comparison of Prevalence of MTB positive case with different study

SN	Study	Study Period (Year)	Prevalence
1	Present Study	2017-18	31.69%
2	Reechaipichitkul W et al [11]	2010-14	21.19%
3	Poojan Shrestha et al [12]	2013	21.32%
4	Thapa A et al [13]	2014	26.81%

In present study, the prevalence of MTB positive cases in smear negative sputum samples was 31.69% which compared with study of Thapa A et al [13] prevalence was 26.81%, Reechaipichitkul W *et al* [11] prevalence was 21.19%, Poojan *et al* [12] prevalence was 21.32%.

Table-8 Comparison of rifampicin sensitivity in MTB positive cases with different study

SN	Study	Rifampicin	
		Sensitive	Resistant
1	Present Study	91.54%	8.45%
2	Raghu Prakash [14]	90.65%	9.35%
3	Sepiso K [15]	94.08%	5.9%
4	Okonkwo RC et al [16]	83.6%	9.5%

In present study, the Rifampicin sensitivity pattern of MTB positive patients, 130 (91.54%) cases were sensitive to rifampicin and 12 (8.45%) cases were resistant to rifampicin which compared with study of Raghu Prakash *et al* [14] in which 90.65% and 9.35% cases were sensitive and resistant to rifampicin respectively, compared with study of Sepiso K [15] in which 94.08% and 5.90% cases were sensitive and resistant to rifampicin respectively and also compared with study of Okonkwo RC *et al* [16] in which 83.6% and 9.5% cases were sensitive and resistant to rifampicin respectively.

Table-9 Gender wise comparison of MTB positive case with different study

SN	Study	Male	Female
1.	Present Study	102/302 (33.77%)	39/146 (26.71%)
2.	Thapa A [13]	27/98 (27.55%)	10/40 (25%)

In present study, the gender wise distribution of MTB positive cases in smear negative sputum samples which were 33.77% and 26.71% in male and female respectively which compared with study of Thapa A *et al* [13] prevalence in male and female patients were 27.55% and 25% respectively.

Table-10 Age group wise comparison of MTB positive cases with different study

SN	Age (In Year)	Present Study	Dhanya et al [17]
1.	0-20	9.9%	9.09%
2.	21-30	26.8%	18.18%
3.	31-40	26%	18.18%
4.	41-50	15.5%	21.21%
5.	51-60	16.9%	15.15%
6.	>60	4.9%	18.18%

In present study majority, positive MTB cases were belonging to middle age groups between 21-50 years of age which is very similar to Dhanya et al [17] study.

Conclusion

There are many conventional techniques available for diagnosis of tuberculosis now days which are microscopy, solid culture, liquid culture, line probe assay, but this study shows that the Genexpert/RIF Assay test helpful to epidemiological purpose to detect more smear negative sputum and HIV positive MTB patient. The Genexpert/RIF Assay test offers a potential solution for improving early MTB diagnosis. The Genexpert/RIF is automated cartridge based nucleic acid amplification test, easy for use in peripheral labs and clinics by unskilled personnel. This test detects almost all smear positive MTB patients and about three quarters of the smear negative MTB patients and concurrently testing for rifampicin resistance, thus identifying patients who need second line drug treatment.

Application of research: The use of GeneXpert is improve the diagnosis of HIV-associated TB compared to microscopy because in HIV positive patient's tubercle bacilli load not enough for detection by smear microscopy.

Research Category: Medical microbiology

Abbreviations:

MTB- *Mycobacterium tuberculosis*, HIV-Human Immunodeficiency virus

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Ethical approval: IEC/Certi/112/2017 (ECR/6/INST/GUJ/2013, Office of the Drug Controller General,India).

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