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THE SUPREMACY OF GENEXPERT OVER AFB STAIN IN PULMONARY AND EXTRAPULMONARY TUBERCULOSIS

Pulmonology

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ABSTRACT

Introduction: Tuberculosis (TB) has existed for millennia and remains a major global health problem . GeneXpert assay has constituted a major breakthrough for tuberculosis diagnostics, providing a rapid, accurate way of identifying TB patients

AIM: To compare the diagnostic yeild of Genexpert with AFB stain in suspected cases of pulmonary and extrapulmonary cases of TB

MATERIALS AND METHODS: We retrospectively reviewed the respiratory samples of suspected pulmonary tuberculosis (Bronchoalveolar lavage and Sputum) and extrapulmonary samples of 187 patients from June 2017 to June 2018 for ZN stain and GeneXpert.

RESULTS: Of the total 187 cases studied, 138 samples were Pulmonary and 49 were Extrapulmonary. A total of 68 cases were diagnosed with Pulmonary Tuberculosis. Among pulmonary samples, an additional 5 samples in BAL and 18 in sputum were detected using genexpert. Among 49 extrapulmonary specimens, 28 were biopsy specimens and 21 were fluid specimens. Amidst biopsy and fluid specimens, 18 samples were gene xpert positive,but only 4 were positive with AFB stain.

DISCUSSION: According to our study, Gene Xpert has a higher yield than AFB smear in respiratory and extrapulmonary specimens. The result of this study shows that GeneXpert can increase more than four times the rapid diagnosis of extrapulmonary tuberculosis compared to AFB stain.

CONCLUSION: Gene Xpert could replace smear microscopy as the initial diagnosis of tuberculosis due to its high detection rate in pulmonary and extrapulmonary specimens and also helps detecting unsuspected cases of Primary MDR TB.

KEYWORDS

Genexpert, AFB stain,Tuberculosis

INTRODUCTION

Tuberculosis (TB) has existed for millennia and remains a major global health problem causing ill-health for approximately 10 million people each year and is one of the top ten causes of death worldwide. In 2017, TB caused an estimated 1.3 million deaths among HIV-negative people, and there were an additional 300000 deaths from TB among HIV-positive people. There were an estimated 10.0 million new cases of TB equivalent to 133 cases per 100000 population [1].

The incidence of TB in INDIA is approximately 2.2 million out of 9.6 million new cases annually, accounting for about a quarter of worlds TB cases. Hence the expeditious detection of M. tuberculosis and rifampin (RIF) resistance in infected patients is very critical for disease management, because of the high risk of transmission from person to person and emergence of MDR-TB and extensively drug resistant tuberculosis.[2]. Smear microscopy has poor sensitivity inspite of the fact that it is rapid and cost effective.[2]. Thus, rapid detection of tuberculous bacilli, which is necessary for early treatment initiation, improving patient outcomes, depends on nucleic acid amplification techniques. As conventional laboratory techniques and culture which is the gold standard are time consuming for the diagnosis of tuberculosis, GeneXpert MTB/RIF (Xpert) assay has constituted a major breakthrough in diagnosing tuberculosis, especially in high burden , low income countries like India.[3]. With the advent of GeneXpert technology, it has changed the norms with a recent systematic review showing pooled sensitivity of 88% and pooled specificity of 98% [4,5,6].

This versatile diagnostic test is an automated, catridge based nucleic acid amplification system that works on real-time PCR and needs operators with minimal technical expertise. It allows the diagnosis of tuberculosis and concurrent assessment of rifampicin resistance within 2 hours duration with minimal biohazards.[7] This test not only provide the advantage of rapidity of diagnosis but also detect even low MTB genomic copies in various specimens.[8] The Xpert MTB/RIF purifies and concentrates the Mycobacterium tuberculosis bacilli from samples, isolates genomic material and amplifies the genomic DNA by PCR.[9,10]

The Xpert MTB/RIF was specifically developed and optimized for testing sputum samples. At present, evaluations of the assay have extended to a variety of nonrespiratory clinical samples from patients with EPTB.[7] However, comparing with pulmonary disease, it is difficult in doing this test in EPTB samples due to the diversity of the clinical samples, insufficient tissue for analysis and extraction of MTB DNA from samples due to its paucibacillary nature.[7]

When the confirmation of TB diagnosis is missed, it negatively impacts both the patients and the tuberculosis control by increasing mortality and morbidity and also increases the transmission of MDR–TB when undiagnosed and ineffectively treated. Therefore these techniques not only provide the advantage of rapidity of diagnosis but also detect even low MTB genomic copies in various specimens.

AIM

To compare the diagnostic yield of Genexpert with AFB stain in suspected pulmonary and extrapulmonary cases of TB

MATERIALS AND METHODS

This is a Retrospective observational study conducted in the Department of Pulmonary medicine, Saveetha medical college and hospital, Chennai between the period of June 2017 – June 2018. The study population included all the suspected cases of Pulmonary and extrapulmonary TB. Total sample size was 187. Outcome measures of the study was to determine the yield of MTB in Genexpert over AFB staining so that all the suspected cases who were smear negative are identified and initiated on treatment early , thus improving the quality of treatment care and reducing the morbidity of the patients. All basic statistical calculations like percentage calculation, development of graphs was done using spread sheets.

According to the suspected localization of the tuberculosis infection, body specimens or fluids from these patients were sent to the Microbiology/ Pathology labs in our hospital for AFB (Ziehl Nielson) staining. Pulmonary and extrapulmonary fluid samples and sputum specimens were collected in a falcon tube was sent to Thiruvallur GH in a cold chain box for GeneXpert/RIF assay. Solid biopsy specimens were collected in sterile containers / falcon tube with saline and was sent to ITM, Chetpet for GeneXpert/RIF assay in a cold chain box.

Pulmonary specimens included both sputum and bronchial wash .Bronchial wash was done in smear negative suspected TB patients with the help of Fibre optic Bronchoscope .The collected samples were sent to Microbiology laboratory of the hospital and Thiruvallur GH in a cold chain box in sterile containers and Falcon tube.

Inclusion criteria

Patients with clinical suspicion of pulmonary or extrapulmonary tuberculosis including symptoms of cough with or without expectoration for >2 weeks, weight loss, fatigue, haemoptysis and loss of appetite and extrapulmonary manifestations.

Exclusion criteria

1. Samples received without clinical history
2. Samples in which genexpert was not done
3. Patient with history of lung malignancies or fungal infections

RESULTS

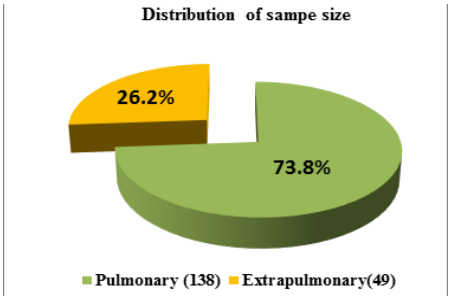

Figure 1: Distribution of sample size

PULMONARY SAMPLES

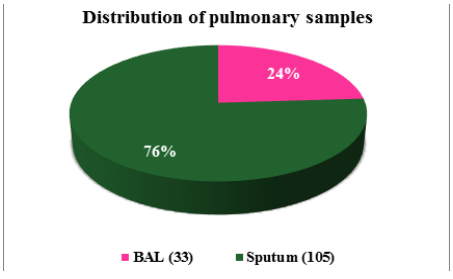

Figure 2 Distribution of Pulmonary samples

DISTRIBUTION OF BALAFB VS GENEXPERT

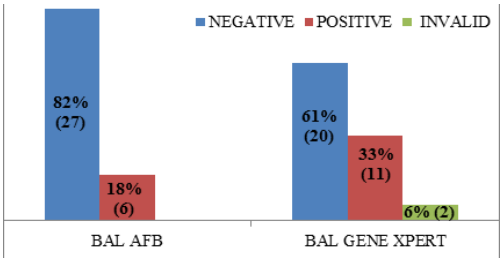

Figure 3 Distribution of BALAFB vs Genexpert

COMPARISON OF BALAFB WITH GENEXPERT

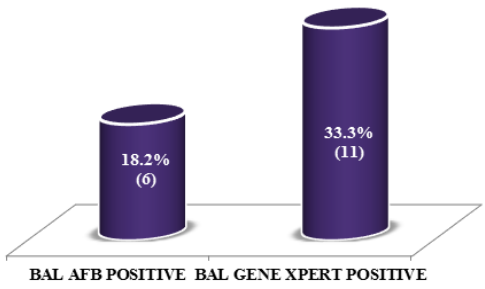

Figure 4 Comparison of BALAFB vs Genexpert

DISTRIBUTION OF SPUTUM AFB

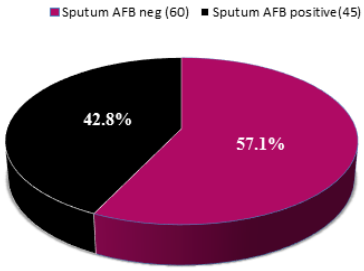

Figure 5 Distribution of Sputum AFB stain

DISTRIBUTION SPUTUM GENE XPERT

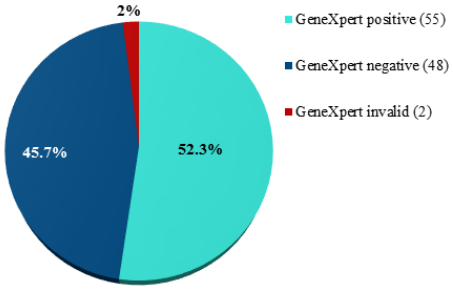

Figure 6 Distribution of Sputum GeneXpert specimens

COMPARISON OF SPUTUM GENE XPERT VS SPUTUM AFB

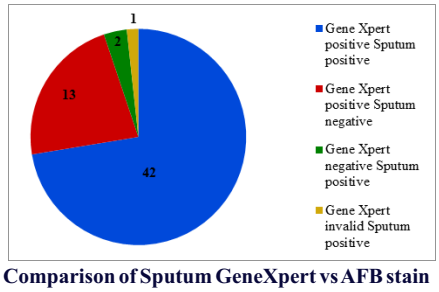

Figure 7 Comparison of Sputum GeneXpert vs AFB stain

RIFAMPICIN SENSITIVITY VS RESISTANCE

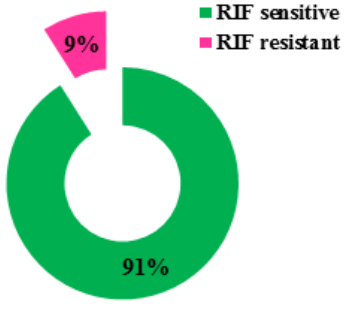

Figure 8 Rifampicin sensitivity vs resistance

EXTRAPULMONARY BIOPSY SPECIMENS

Table 1: showing the distribution of extrapulmonary biopsy specimens and comparison with GeneXpert and AFB stain

SAMPLES	N (%) Total-28	AFB Stain		Gene Xpert	
		Negative	Positiv(2)	Negative	Positiv(9)
OMENTAL BIOPSY	2 (7.1%)	2	0	2	0
PLEURAL BIOPSY	2 (7.1%)	2	0	2	0
ENDOMETRIAL BIOPSY	2 (7.1%)	2	0	1	1
INTESTINAL BIOPSY	2 (7.1%)	2	0	2	0

SINUS TRACT BIOPSY	1 (3.5%)	1	0	0	1
SUBTALAR JT BIOPSY	1 (3.5%)	1	0	0	1
TRANSPEDICULAR BIOPSY	3 (10.7%)	3	0	2	1
ADNEXAL MASS BIOPSY	1 (3.5%)	1	0	0	1
BREAST BIOPSY	1 (3.5%)	1	0	1	0
LYMPH NODE BX/FNAC	11 (39.2%)	9	2	7	4
INCISIONAL BIOPSY	1 (3.5%)	1	0	1	0
CERVIX BIOPSY	1 (3.5%)	1	0	1	0

EXTRAPULMONARY FLUID SPECIMENS

Table 2: showing the distribution of fluid samples and its comparison with GeneXpert and AFB stain

SAMPLES	N (%) (21)	AFB STAIN		GENE XPERT	
		Negative	Positive	Negative	Positive
ASCITIC FLUID	2 (9.5%)	2	0	2	0
PUS	9 (42.8%)	8	1	3	6
CSF	3 (14.2%)	3	0	2	1
SYNOVIAL FLUID	1 (4.7%)	1	0	1	0
PLEURAL FLUID	5(23.8%)	5	0	3	2
PERITONEAL WASH	1 (4.7%)	1	0	1	0

Of the total 187 cases studied, 138 (74.7%) samples were Pulmonary and 49 (25.8%) were Extrapulmonary (figure1). Pulmonary samples included both BAL – 33 samples (24%) and sputum – 105 (76%) (figure2). Out of the 33 BAL samples 11 cases were positive in total including GeneXpert and AFB stain. All the AFB positive cases were GeneXpert positive and hence an additional 5 cases were detected using GeneXpert (15.2%) (figure3,4). Among the total 105 sputum samples, 45 (42.8%) cases were positive with AFB stain while 60 (57.1%) were negative (figure 5). In sputum samples, 55 (52.3%) cases were positive with GeneXpert while 48 (45.7%) cases were negative and 2 cases gave invalid report (figure 6). Hence 42 sputum samples were positive in both GeneXpert and AFB stain, 13 cases were GeneXpert positive but sputum negative , 2 were GeneXpert negative but smear positive and 1 GeneXpert report was invalid but sputum smear was positive (figure 7). Additionally 13 (24%) cases were detected using GeneXpert in sputum specimens. Rif resistance was observed in 5 (9%) samples (figure 8).

Among the 68 positive pulmonary cases, 65 cases were positive by GeneXpert with 18 (27%) cases being detected additionally by Gene xpert.

Extrapulmonary specimens accounted to 49 (25.8%) of the total 187 cases studied. A total of 28 (57.1%) biopsy specimens were included in the study and AFB stain was positive in 2 specimens only while 9 cases were positive with GeneXpert . Therefore an additional of 7 cases were detected with the help of GeneXpert . Out of the total 21 (42.9%) fluid samples, AFB stain was positive in one sample while 9 samples were positive with GeneXpert . Pus yielded a better result than other specimens.

Among the Extrapulmonary samples a total of 18 (37%) cases were positive with GeneXpert while only 3 specimens were positive with AFB stain.

DISCUSSION

Here we are aiming at detecting the diagnostic yield of GeneXpert over AFB stain both in pulmonary and extrapulmonary samples. The World Health organization has recommended the use of gene Xpert in pulmonary and extra pulmonary samples for the rapid detection of MTB [11].

The results of our study showed that Gene Xpert has a higher yield than AFB smear in respiratory and extrapulmonary specimens. Gene xpert is one step ahead in detecting positive cases in samples which go undetected by AFB smear. These data suggests that smear microscopy detected TB in lesser patients than GeneXpert MTB/RIF assay. Smear microscopy might miss specimens with low bacilli/ml, as direct smear microscopy needs at least 10,000 bacilli/ml of sputum for direct microscopy to show a positive result[12,13]

Among the Pulmonary samples, GeneXpert helped to diagnose 27% of the cases which were smear negative. A study conducted by Narute S et al showed that GeneXpert was helpful to detect 40% of smear negative pulmonary samples [14]. The high percentage may be due to their smaller sample size of 100 when compared to our which was 187. MTB/Rif assay was positive in 65 of the 68 positive cases yielding a diagnostic accuracy of 96%. This was comparable to the study done by Barnard DA et al in which 36 cases were positive in GeneXpert out of the 39 positive cases with an accuracy of around 92%.[15] . The study done by Koegelenberg C et al also showed a similar result in which 37 specimens were positive with GeneXpert out of the total 42 positive cases which they confirmed with culture[16].

Another study done by Monika et al identified 24.7% positivity by using Gene Xpert in pulmonary samples , while our study showed 49.3%, possibly because we have included more number of sputum specimens than BAL when compared to their samples which had 21 sputum and 149 BAL[17]

As per this study GeneXpert also proved helpful in detecting more extrapulmonary cases than the histopathology and AFB stain. The GeneXpert MTB/RIF assay has a better diagnostic potential for specimens, such as pus and aspirates, which is not easily diagnosed by other laboratory techniques. Findings of the study aids the use of GeneXpert MTB/RIF assay in routine EPTB diagnosis [18].Similar studies were carried out by Lawn and Zumla [7] who employed the GeneXpert MTB/RIF assay for diagnosis of EPTB.

The study included both biopsy and fluid specimens. Among the extrapulmonary samples tissue biopsy had a higher yield of 18.4% (9) which included 4 (8.2%) lymph node samples, followed by pus 12.2% (6) and pleural fluid 4% (2). These results were comparable to the study done by Stephen Lawn et al [7] in South Africa which showed the output of GeneXpert was more in biopsy specimens followed by pus. Another study by Shamma Shetye et al showed that out of the total 268 samples, the positivity rate was more for tissue biopsies or fine-needle aspirates accounting to 35% and yield of pus was 21% [18].

Our study showed an increase of 17.6% (33) in diagnosing TB which was similar to study done by Gerardo et al which shows the use of Xpert MTB/RIF assay increased the number of positive results by 16.5% [19]

One of the most important factor was, 5 (6%) cases were found to have Rifampicin resistance, among which 3 were Primary MDR TB .This was similar to a study by Shagufta Iram et al which showed 6.5% Rif resitance [20]. This helped in early initiation of appropriate treatment for MDR TB without delay and decreasing the spread of MDR TB and its mortality.

LIMITATION

We did not perform culture for mycobacteria to rule out the possibility of false positive results, however Xpert MTB/RIF have shown specificities above 95% in previous studies [21]

CONCLUSION

Gene Xpert could replace smear microscopy as the initial diagnosis of tuberculosis due to its high detection rate in pulmonary and extrapulmonary specimens and also helps in detecting unsuspected cases of Primary MDR TB. It is particularly useful for our country where the incidence of tuberculosis is high, as it provides a simple, reliable, and cost-effective diagnostic modality

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