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Comparison of Ziehl-Neelsen Technique with Gene-Xpert MTB/RIF Assay in Sputum Samples for Diagnosis of Pulmonary Tuberculosis

KEYWORDS: Ziehl-Neelsen technique, Gene-Xpert MTB/RIF assay, sputum culture

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ABSTRACT **Background:** *Tuberculosis (TB) continues to be a global problem. Rapid, accurate diagnostic test for detection of active tuberculosis is the need of the hour, especially in endemic countries like India. Gene-Xpert assay is an automated real-time PCR method for the diagnosis of pulmonary TB and has the promise to become a complementary method for standard sputum microscopy and culture techniques.*
Methods: *A total of 205 sputum samples of suspected pulmonary TB were studied for a period of 8 months. All the samples are studied by both ZN, Gene-Xpert & culture techniques and results compared with statistical analysis.*
Result: *AFB smear examination showed 94.02% sensitivity among patients with positive Gene-Xpert/RIF assay & culture; 97.3% specificity along with 98.2% PPV and NPV 92.57%.*
Conclusion: *With the high sensitivity, specificity and rapidity Gene-Xpert assay is not only comparable to standard reference methods of sputum microscopy and culture, but also has the potential to emerge as an alternative to these techniques.*

Introduction:
There is an estimated 8.6 million new cases and around 1.3 million deaths per year, TB is still a major health problem. This epidemic is fuelled by MDR-TB.

According to one-estimate, TB affects up to one-third of the world population and is responsible for death of 2 million people each year, with an estimated 4,50,000 cases of multi-drug resistant TB world-wide.[1]

With the emergence of multi-drug resistant TB strains, it is imperative to diagnose TB early and to know drug-sensitivity before starting the anti-tuberculous therapy.

Currently, the world lacks an accurate test that allows early detection of active pulmonary TB. Thus many patients with active TB, in endemic areas are either treated based on clinical grounds or remain undiagnosed and are sources of threat to the community. The need of the hour is rapid, accurate TB diagnostic test that is crucial for achieving control of TB.

Among the various diagnostic test available, PCR-based methods have shown promise to detect TB and resistance to RIF with good sensitivity and specificity from sputum samples.[2]

Conventionally, the diagnosis of pulmonary tuberculosis has solely rested on clinical features, chest X-ray findings and smear microscopy for acid fast bacillus, or bacterial isolation by the culture. In developing countries like India, diagnosis relies heavily on smear microscopy due to cost-factor but it has low sensitivity and specificity as compared to culture. Though, the microbiological identification of MTB by culture remains the gold standard for diagnosis of tuberculosis,it doesn't provide a rapid diagnosis, is cumbersome and requires strict biological safety lab level II/III which becomes its limiting factor.

Recently, WHO has endorsed the implementation of Gene-Xpert MTB/ RIF assay for national tuberculosis programs in developing countries, Owing to its rapidity, accuracy and user friendly technique.It is based on nested real time PCR assay and molecular beacon technology for MTB detection and RIF resistance. The results are automated and are obtained within period of 2.5 hours. The technique is said to be less prone to cross-contamination, has a high sensitivity and specificity in TB case smear negative TB.

The purpose of the study was to evaluate the sensitivity & specificity of the Xpert MTB/RIF assay for the detection of pulmonary TB along with additional advantage of rifampin resistance and compare that with conventional Ziehl-Neelsen techniques for TB diagnosis in sputum samples.[3]

Materials and Methods:
The sputum samples were studied for a period of 8 months from January 2015 to August 2015. This prospective study comprised of 205 samples of suspected pulmonary tuberculosis patients.

We included sputum samples from patients with clinical features suspicious of tuberculosis (more than two weeks of productive cough, mild-grade fever, night sweats and rapid loss of weight) and/or x-ray findings suggestive of tuberculous lesion.

The patients who have been suspected as tuberculosis but not confirmed by either sputum microscopy/ culture or genexpert method are excluded from the study.

From all the cases spot sputum samples were collected.

Steps for processing:
1. WHO protocol for Ziehl-Neelsen staining for smear preparation was followed.
2. Gene-Xpert MTB/ RIF assay: Protocol from manufacturer had been followed. Reagent with 2:1 ratio was added with sample in 15ml falcon tube. 2 ml from this sample was added with the help of disposable sterile pipette to cartridge. This cartridge was then loaded into the gene-xpert. Then test was performed; the interpretation was software based.
3. On Lowenstein-Jensen(LJ) media; Culture: Culture was performed after decontamination of the sputum samples on LJ media following the standard protocols.

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Culture was used as the standard reference in our study.

Sensitivity and specificity were calculated as followed:

$$\text{sensitivity} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}$$

$$= \frac{\text{number of true positives}}{\text{total number of sick individuals in population}}$$

$$= \text{probability of a positive test given that the patient has the disease}$$

$$\text{specificity} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}$$

$$= \frac{\text{number of true negatives}}{\text{total number of well individuals in population}}$$

$$= \text{probability of a negative test given that the patient is well}$$

$$\text{PPV} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false positives}} = \frac{\text{number of true positives}}{\text{number of positive calls}}$$

$$\text{NPV} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false negatives}} = \frac{\text{number of true negatives}}{\text{number of negative calls}}$$

Efficiency = True positives + True negatives/ True and false positives + True and False negatives

Result:

We studied a total of 205 sputum samples in suspected cases of tuberculosis. Out of 205 cases, 110 were positive for smear, Gene-Xpert & culture. 86 were negative for all. 2 of which showed positive results by smear but were negative for Gene-Xpert & culture. 7 of them were negative by smear method and positive by Gene-Xpert. (Table no.1)

Out of 205 spot samples studied mycobacterium tuberculosis (MTB) was detected by Gene-Xpert & culture in 117 (57.07%) and negative in 88 (42.93%). In comparison, ZN staining of the smears showed positivity in 112 (54.6%) and negative in 93 (45.37%). (Table no.2)

Out of the total 205 cases, suspected clinically or radiographically sensitivity of AFB smear with ZN stain was 94.02% and the specificity of the same was 97.73%. Sensitivity and specificity of the Gene-Xpert was 100% and 100% respectively when compared with gold standard culture method.

Positive predictive value (PPV) of AFB smear with ZN stain was 98.2% and Negative predictive value (NPV) was found to be 92.57%. PPV and NPV of the Gene-Xpertwas found to be 100% and 100% respectively. (Table no.3) Thus, Gene-Xpert has proved to be a better diagnostic modality as compared to traditional ZN staining.

Efficiency of our study was 95%.

Discussion:

In the present study, we evaluated the performance of Gene-Xpert assay in sputum samples of suspected pulmonary tuberculosis in comparison with standard AFB and culture methods.

Therefore, we evaluated the performance of Gene-Xpert and compared it with ZN- smear and culture techniques.

It has been stated that conventional laboratory method of ZN smear technique requires a bacillary load of 10^5/ml to show positivity, therefore making it an unreliable technique in the diagnosis of TB.

However, culture method, considered as Gold-standard for detection and to know the drug-sensitivity in TB is time-consuming and requires strict biosafety infrastructure and trained laboratory staff.

Compared to above techniques, Gene-Xpert assay has the advantages of less turn-around time (2.5 hours), high sensitivity of detection of TB with simultaneous assessment of Rifampicin resistance and thus has potential to replace standard culture method.

While in our study, for smear positive cases, the result was 100% sensitivity and specificity by Gene-Xpert, out of smear negative cases 7 cases showed positivity by Gene-Xpert, giving 94.02% sensitivity and 97.73% specificity.

In the study by Shaguftairam, there was 100% sensitivity and specificity similar to our study, but in smear negative cases, the sensitivity was 80% and specificity was 96%.[3]

Zeka et al in their study gave sensitivity of 43.5% and specificity of 99.5% for smear method and 82.3% sensitivity with 100% specificity for Gene-Xpert method. Their NPV and PPV were 84.4% and 96.4% for smear method while 94.6% and 100% was for Gene-Xpert. Except sensitivity of smear method all the other parameters are in concordance with our study. (Table no.3)

It has further been documented that Gene Xpert increases the detection rate by 10-15% compared to smear method in sputum samples.[3]

Eventhough, some reports claim high sensitivity and specificity only if three sputum samples were tested per patient[4], in the present study only one sample was tested by Gene-Xpert method due to cost factor for the patient. It is further reported that a single Gene-Xpert test report was independent of whether the sample was a spot, night or morning sputum.[4]

However, in our study these limitations were minimal and didn't negatively affect the study result.

Inspite of definite clear advantages of Gene-Xpert, some limitations are still reported. They include limited shelf-life of diagnostic cartridges, temperature and humidity restrictions, requirements for stable electricity supply and the need for regular maintenance and calibration of equipment.[4]

Eventhough some reports suggest false positive result by Gene-Xpert in comparison with smear and culture method, we didn't find any such discrepancy in our study.

The possible factors contributing to false positivity by Gene-Xpert include sub-clinical relapse and excretion of residual persistent DNA from dead bacilli, which can even be seen in treated patient, giving a false-atom of relapse. Further, it is stated that immunosuppression may result in confusing results due to the possible occurrence of atypical mycobacterial strains.[2]

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In our study, all the sputum smear negative patients who were positive on Gene-Xpert showed clinical features of TB.

Various studies have reported test sensitivity of 98-100% and specificity of 99-100%.

In our study, the sensitivity remained as 100% and specificity was 100% in concordance with other studies.

Conclusion:
Gene-Xpert MTB/RIF assay has proved to be a reliable method for the detection of mycobacterium TB and has high sensitivity, specificity and positive predictive value compared with standard ZN-smear technique for the detection of pulmonary TB.

Because of its feasibility, rapid turn-around time and minimal infrastructure requirement. It is recommended even in resource limited settings and especially valuable in areas with high risk of MDR-TB or HIV associated TB. Gene-Xpert will help in timely initiation of effective treatment in pulmonary TB patients and could play a significant role in controlling the epidemic of TB.

AFB smear, though cost effective, has low sensitivity as compared to Gene-xpert. Also we get information regarding rifampin resistance in the latter. Culture takes longer time compared to Gene-Xpert.

Tables: Table: 1: Showing the result of AFB smear, Gene-Xpert and Culture.

AFB	GeneXpert	Culture	No. of cases
+	+	+	110
-	-	-	86
+	-	-	2
-	+	+	7
			205 (Total)

Table: 2 : Showing the comparison of Smear, Gene-Xpert and culture.

	Smear (+)	Smear (-)	Total
Gene-Xpert & Culture(+)	110 (53.66%)	7 (0.03%)	117 (57.07%)
Gene-Xpert & Culture (-)	2 (0.01%)	86 (41.95%)	88 (42.93%)
Total	112 (54.6%)	93 (45.37%)	205

Table : 3 : Comparison of sensitivity, specificity, PPV and NPV for sputum and Gene-Xpert.

	Sensitivity (%)			Specificity (%)			PPV (%)		NPV (%)	
	Zeka et al	Iram et al	Our study	Zeka et al	Iram et al	Our study	Zeka et al	Our study	Zeka et al	Our study
Smears	43.5	80	94.02	99.5	96	97.73	96.4	98.2	84.4	92.57
Gen-eXpert	82.3	100	100	100	100	100	100	100	94.6	100

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