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ORIGINAL ARTICLE

The Diagnostic Performance of Fluorescent Microscopy and MTB/RIF Assay Gene Xpert in Pulmonary Tuberculosis at Tertiary Care Hospital of Lahore

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ABSTRACT

Objective: To find out the diagnostic precision of GeneXpert and AFB smear by fluorescent microscope for diagnosis of pulmonary tuberculosis keeping the culture as a gold standard.

Methodology: A survey performed in the Pulmonology and Pathology Department, Lahore General Hospital, Lahore. Patients of age 16-70 years, both genders presented with cough>2weeks, fever >99^{o}F, and sputum, with radiographic findings constant with pulmonary T.B were included in this study. Patients previously diagnosed with PTB and on antibiotics within 2 weeks at the time of inclusion and patients having extrapulmonary TB were excluded from this study. Patients were asked to submit sputum samples in 2 containers, one having 2-3 ml and the other having 4-5 ml of sputum. The samples were proceeded according to SOPs for FM sputum microscopy, MTB/RIF assay & sputum culture. The collected data was analysed statistically with the help of SPSS version 20.

Results: There were 100 patients included in this study with mean age of cases 42.66 ± 15.69 years. There were 59% male and 41% female, with a higher male to female ratio. 73% cases were positive on Gene Xpert, 68% on AFB smear, and 72% cases were positive on culture. The specificity, sensitivity, NPV, PPV & overall diagnostic accuracy of Gene Xpert was 71.43%, 90.28%, 74.07%, 89.04% and 85.00% and for AFB LED-FM microscopy was 86.11%, 78.57%, 91.18%, 68.75% and 84.00% respectively.

Conclusion: Simple modality like fluorescent microscopy gave better results and diagnostic yield as compared to Zn microscopy. Gene Xpert MTB/RIF assay detected TB in very short time, and it is sensitive and accurate method along with detection of rifampicin resistance. However, more studies like that should be recommended as Pakistan is still at 5th number among high load diseased countries. The culture is always a gold standard method among all these sensitive and specific methods.

Keywords: Pulmonary TB, Gene Xpert, Fluorescent microscopy, sensitivity, specificity.

INTRODUCTION

Among communicable diseases, the most common disease is of TB Tuberculosis. Its mortality rate is very high due to its communicable nature. Causative agent is mycobacterium tuberculosis. In 2017, World Health Organization has reported 1.8 millions of people died away due to this disease. In the same year, 0.5 million people were found having type of TB known as multi drug resistant.[1] this type of TB showing no response against the drugs like isoniazid & rifampicin.[2] Those which are Rifampicin resistant are also resistant to isoniazid , therefore named as MDR.[3]

Early diagnosis and prompt treatment of PTB is the main factor to reduce spread of T.B, morbidity, mortality, and drug resistance. Usually, acid-fast bacillus (AFB) smear microscopy is used as the first-line technique for diagnosis, as it is a simple method being time-saving and cost-effective but having low sensitivity (20- 60% of positive cultures).[4]Among the existing methods, a Fluorescent microscope (LED-FM) has comparatively higher sensitivity (75.5%) as equated to conventional i.e Zeil Nelson (ZN) microscopy (59.4%).[5] WHO also endorsed Fluorescent microscopy as a substitute of ZN- staining of sputum microscopy for TB diagnosis.[6]

The gold standard for the diagnosis of TB is Mycobacterial culture but this required 6-8 weeks for the growth of tuberculous microorganisms because of their slow growth. Newer methods and techniques are required to lessen the time for diagnosis of TB and drug resistance.[7] Recently reputable and tested real-time automatic integrated system, known as the GeneXpert(GX) system using the Xpert MTB/RIF assay is quicker than smear or culture & delivers results in less than 2 hrs. MTB/RIF assay is assimilated DNA abstraction, genomic amplification, a semi-quantitative acquaintance of MTBC (mycobacterium tuberculosis complex), and RIF (rifampicin) resistance resolve in a single cartridge, thus lessens the work time and cross-contamination risk.[8-10]

GeneXpert (GX) has added benefit of sensing rifampicin resistance; it permits early recognition of MDR T.B. So, it is strongly recommended as a preliminary diagnostic test in patients assumed of MDR T.B. Most of the local studies equate gene expert with conventional smear. Limited data is available equating FM with gene experts. This study aims to assess whether gene expert done following AFB smear by FM expands the diagnosis of T.B and to witness the additional diagnostic value of this technique i.e. rifampicin resistance as both these techniques are available in the hospital.

The objective of this study was to investigate the diagnostic precision of GeneXpert and Acid- Fast Bacilli (AFB) smear by fluorescent microscope for diagnosis of pulmonary tuberculosis keeping the culture and sensitivity as the gold standard.

METHODOLOGY

It was a cross-sectional study conducted in the Department of Pulmonology & department of Pathology, Lahore General Hospital, Lahore. The duration of the study was six months after getting synopsis approval [July 24, 2018, till Jan 24, 2019].

Patient's selection: A sample size of 100 was considered with a 95% confidence interval taking the expected frequency of TB as 63.9%[11] and sensitivity and specificity of Gene expert as 94% [12] (6% margin of error) and 92%[12] (8% margin of error) respectively. The sampling technique was non-probability, consecutive sampling.

Patients of age 16-70 years, both genders presented with cough>2weeks, fever >99^{o}F, and sputum which was suitable in quantity with radiographic findings constant with pulmonary T.B were included in this study. Patients previously diagnosed with

PTB and on antibiotics within 2 weeks at the time of inclusion (on medical record) and patients having extrapulmonary TB were excluded from this study.

100 patients who fulfilled the inclusion and exclusion criteria were registered from the indoor and outdoor Department of Pulmonology, Lahore General Hospital, Lahore. Informed consent was taken from each patient. Demographic data (name, age, sex, BMI, and duration of symptoms) was also noted. Radiographic findings were also noted.

Sample collection: Patients were asked to submit sputum samples in 2 containers, one having 2-3 ml and the other having 4-5 ml of sputum. The container with 4-5 ml sputum was divided in two, 2 ml was sent for gene expert and 3 ml for AFB smear by light-emitting diode FM in Pathology Laboratory of Lahore General Hospital. The other container was sent to Ghulab Devi hospital for AFB culture.

Sample processing: The samples were proceeded by following standard operating procedures (SOPs) for FM sputum microscopy, MTB/RIF assay, and sputum culture. Sputum samples were processed by N-acetyl -L-cysteine and Sodium hydroxide with a concentration of NaOH of 2%. The sample was centrifuged at 3000 for 15- 20 mins after which deposit was used for FM microscopy, GeneXpert, and culture and sensitivity.

Fluorescent staining: 2-3 drops of the sputum deposit were smeared on the glass slide and dried. They were stained with Auramine -phenol stain for 20 mins. Then the slides were washed with water and decolorized acid-alcohol and counterstained with methylene blue for 2 minutes, then washed and dried. The acid-fast bacilli were examined by fluorescent microscope 250X magnification. AFB is reported according to standard reporting protocol.

MTB/RIF assay Gene Xpert: For Gene Xpert, a centrifuged deposit of sputum samples was mixed with reagent at a 1:2 ratio, the mixture was then incubated for fifteen minutes, and when it completely liquifies transferred into Xpert MTB/Rif assay cartridge for DNA extraction. Results will be available within 2 hours and they were automatically interpreted.

Culture MGIT 960 TB system: For culture and sensitivity, all decontaminated samples were processed for MGIT 960 TB system with positive and negative controls. At positive signals, the bar code was scanned, and ZN staining was done for confirmation of AFB from culture. Reports were gathered and labeled as positive and negative. All this information was documented through pre-designed Performa. Other variables i.e. rifampicin resistance was also recorded.[13]

The data were and collected and then analysed with SPSS version 20. Quantitative variables like age and symptoms duration were calculated as mean and standard deviation. Qualitative variables like gender and PTB was calculated as frequency and percentage. 2x2 table was made to calculate specificity, sensitivity, NPV, PPV, and diagnostic accuracy of AFB Smear LED-FM and GeneXpert taking culture as the gold standard.

RESULTS

Total 100 samples were collected

- The mean age of cases 42.66 ± 15.69(N=100) years with minimum and maximum age as 17 and 70 years.
- There were 59 %(N=100) male and 41%(N=100) female cases in this study, with higher male to female ratio.
- 72 samples came positive on culture for mycobacterium tuberculosis.
- Rest of the tables shows specificity and sensitivity of fluorescent microscopy and MTB/RIF assay Gene Xpert.

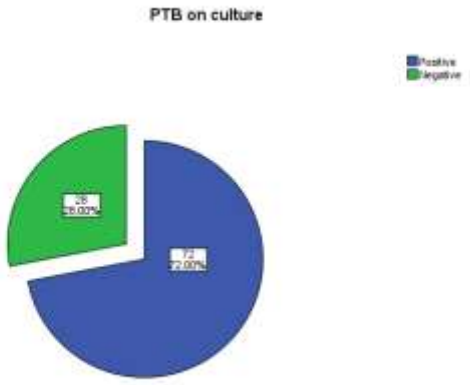

Fig-1: Diagnosis of Pulmonary TB on culture

Table-1: Diagnostic accuracy of Gene Xpert for diagnosis of Pulmonary TB taking culture as gold standard

		PTB on culture		Total
		Positive	Negative	
Pulmonary TB on GeneXpert	Positive	65(90.3%)	8(28.6%)	73(73.0%)
	Negative	7(9.7%)	20(71.4%)	27(27.0%)
Total		72(100.0%)	28(100.0%)	100(100.0%)

Table 2:

Sensitivity	90.28%
Specificity	71.43%
Positive Predictive Value	89.04%
Negative Predictive Value	74.07%
Diagnostic Accuracy	85.00%

Table-3: Diagnostic accuracy of AFB smear by FM microscopy for diagnosis of Pulmonary TB taking culture as gold standard

		PTB on culture		Total
		Positive	Negative	
Pulmonary TB on AFB Smear	Positive	62(86.1%)	6(21.4%)	68(68.0%)
	Negative	10(13.9%)	22(78.6%)	32(32.0%)
Total		72(100.0%)	28(100.0%)	100(100.0%)

Table 4:

Sensitivity	86.11%
Specificity	78.57%
Positive Predictive Value	91.18%
Negative Predictive Value	68.75%
Diagnostic Accuracy	84.00%

DISCUSSION

Early diagnosis of T.B is authoritative for early patient treatment & effective patient outcome.[14] False-negative results & misdiagnosis of TB suspects are common in developing countries, as most TB control programmes use Ziehl-Neelsen (ZN) smear microscopy, having poor sensitivity and multiple visits are also needed which causes increase rate of default. Mycobacterial culture, although considered as the gold standard but time consuming & requires proper infrastructure and technical expertise[15] recently, the WHO certified Fluorescent microscopy and GeneXpert (Xpert® MTB/Rif assay) for the early diagnosis of TB.[16]

A study was done on 403 patients which included 48.1% females & 51.9% males with mean age of 35.3±15.9. [17] current study we also found higher male ratio i.e. 59% male and 41% female cases, and the mean age was in 40's, i.e. 42.66 ± 15.69 years with minimum and maximum age as 17 and 70 years.

In this study, the diagnostic accuracy of LED-FM microscopy of sputum and GeneXpert MTB/Rif assay were equated in contrast to culture as gold standard. The sensitivity, specificity, PPV value and NPV of GeneXpert or our results were 90.28%, 71.43%, 89.04% and 74.07% and LED-fluorescent microscopy was 86.11%, 78.57%, 91, 18% and 68.75% respectively.

Similar study was conducted in Ethopia and their results were sensitivity, specificity, PPV and NPV for GeneXpert 88.55%, 92.90%, 91.34% and 90.57% and for LED-FM was 80.15%, 95.48%, 93.75% and 85.06%, respectively, which is almost like this study. They also gave reference of metanalysis on the same comparison methodology in which 22 studies were included and overall sensitivity of Gene Xpert in that was 88%, which is also like our study.[18] Meta analyses was also done on 45 studies which showed the sensitivity of LED-FM extended 52% to 97%, that comprehend sensitivity of FM microscopy of present study.[19]

In this study, sensitivity and overall accuracy of Gene Xpert was higher than FM smear microscopy. Similarly, a study was done by Pachpute et al, who estimate sensitivity, specificity, PPV & NPV of Nucleic acid amplification assay (GeneXpert) via respiratory samples in suspected pulmonary tuberculosis cases and compare their results of Gene Xpert with ZN smear microscopy and AFB culture. The sensitivity, specificity, PPV and NPV of GeneXpert and ZN microscopy of their study were calculated using Liquid culture of Mycobacterium tuberculosis as gold standard. The overall specificity, sensitivity, NPV and PPV of GeneXpert were 93.1%, 86.8%, 96% and 78.5%. The sensitivity and specificity of smear microscopy were 22.2%, and 78.5% respectivel.[20] The Auramine fluorescent microscopy showed better results and more sensitive as compared to Zn staining. This is also comparable with our study in which FM staining is more sensitive as compared to their ZN smears. FM staining is cost effective and making the diagnosis easy.[20] Countries like in Pakistan where Tuberculosis is endemic, the diagnostic accuracy of Gene Xpert MTB/RIF assay and FM microscopy can help to diagnose the disease in time.[21]

In this study one sample was positive for GeneXpert but negative on TB culture. The result of Gene Xpert was very low detected. When retrospective history was probed, history of Anti tuberculous treatment (ATT) was there, with very low bacterial load. This is the reason, for active TB diagnosis, physicians must be careful to choose Xpert as a sole method for diagnostic modality. History of ATT must be inquired to avoid false results.[20]

CONCLUSION

Simple modality like fluorescent microscopy gave better results and diagnostic yield as compared to Zn microscopy. Gene Xpert MTB/RIF assay detected TB in very short time, and it is sensitive and accurate method along with detection of rifampicin resistance. However, more studies like that should be recommended as Pakistan is still at 5th number among high load diseased countries. The culture is always a gold standard method among all these sensitive and specific methods.

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