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ORIGINAL RESEARCH

Rapid Detection of Mycobacterium Tuberculosis and Rifampicin Resistance in Clinically Suspected Cases of Extrapulmonary Tuberculosis Using Gene Xpert MTB/RIF Assay

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ABSTRACT:
Background: Mycobacterium tuberculosis (MTB) is the causative agent of tuberculosis (TB). TB is the second leading cause of death after HIV worldwide. GeneXpert MTB/RIF (Xpert; Cepheid, USA) is a fully automated real time hemi-nested PCR system which simultaneously detects Mycobacterium tuberculosis complex (MTB) genome as well as mutations that confer rifampicin resistance. Hence, the aim of the present study is to assess the sensitivity and specificity of GeneXpert MTB/RIF in the detection of M. tuberculosis in cases of Extrapulmonary tuberculosis and to detect the proportion of rifampicin resistance in samples of Extrapulmonary tuberculosis. **Materials & Methods:** A total of 120 patients were studied. Extrapulmonary samples were collected in a standard sterile container. Diagnosis of EPTB was considered on the basis of clinical and radiological examination; and biochemical and cytological testing. Each sample was divided into three parts: one for Acid Fast Bacilli (AFB) smear, one for LJ media culture and one for GeneXpert MTB/RIF assay. Data were analysed using IBM SPSS V. 20.0.0. **Results:** Positive result on L-J culture media were found to be present in 54.16 percent of the patients (65 patients), while negative results were found to be present in 45.8 percent of the patients (55 patients). Positive result on GeneXpert MTB/RIF were found to be present in 40.83 percent of the patients (49 patients), while negative results were found to be present in 59.16 percent of the patients (71 patients). Out of 49positive patients of GeneXpert MTB/RIF, Rifampicin sensitivity was found to be present in 46 patients (93.88 percent), while rifampicin resistance was found to be present in 3 patients (6.12 percent). **Conclusion:** The use of GeneXpert test is effective in routine for diagnosis of extrapulmonary TB, especially for pus and CSF samples where a very high detection rate was observed as compared to conventional techniques.
Key Words: Extrapulmoanry Tuberculosis, GeneXpert, Mycobacterium tuberculosis

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INTRODUCTION

Mycobacterium tuberculosis (MTB) is the causative agent of tuberculosis (TB). TB is the second leading cause of death after HIV worldwide.[1]

The diagnosis of tuberculosis (TB) still offers major diagnostic challenges related to the detection limit of smear microscopy, long time to AFB culture-confirmation and variable sensitivity of molecular tests. In majority of the TB cases, diagnosing active smear-negative pulmonary TB (PTB) is a major concern.[2, 3]

The big challenge in the diagnosis of extrapulmoanry TB (EPTB) is mostly the atypical clinical presentation simulating other inflammatory and neoplastic conditions, which results in delay of the diagnosisand deprivation of treatment.[2]

The conventional Mycobacterium tuberculosis detection techniques based on microscopic examination of acid fast stained specimens (by Ziehl-Neelsen method) are still widely used for diagnostic purpose especially in TB endemic countries, although they fail to provide the required sensitivity and specificity and are unable to differentiate between M. tuberculosis and non-tuberculous mycobacteria (NTM).

Improving the diagnostic accuracy and reducing diagnostic delay in both smear-negative PTB and EPTB is therefore paramount. GeneXpert MTB/RIF (Xpert; Cepheid, USA) is a fully automated real time hemi-nested PCR system which simultaneously detects Mycobacterium tuberculosis complex (MTB) genome as well as mutations that confer rifampicin resistance.[3]

The GeneXpert test has been evaluated for testing genomic DNA from M. tuberculosis isolates with 23 different commonly occurring rpoB mutations with 100% sensitivity.[4-6]

Hence, the aim of the present study is to assess the sensitivity and specificity of GeneXpert MTB/RIF in the detection of M. tuberculosis in cases of Extrapulmonary tuberculosis and to detect the proportion of rifampicin resistance in samples of Extrapulmonary tuberculosis.

MATERIALS & METHODS

A prospective study was conducted on patients of suspected Extrapulmonary TB (EPTB) from various departments reporting at Guru Gobind Singh Medical College and Hospital, Faridkot, Punjab. The study was conducted on cases of suspected extrapulmonary tuberculosis patients. A total of 120 patients were studied. Extrapulmonary samples were collected in a standard sterile container. Diagnosis of EPTB was considered on the basis of clinical and radiological examination; and biochemical and cytological testing. Each sample was divided into three parts: one for Acid Fast Bacilli (AFB) smear, one for LJ media culture and one for GeneXpert MTB/RIF assay. Samples were collected in a leak proof sterile container and transported to the laboratory immediately after proper collection with proper labelling. Samples collected were broadly divided into two groups which were processed in different ways to improve the yield of tests.

Procedure of GeneXpert MTB/RIF assay:

- Transfer all thesample to a conical centrifuge tube, and concentrate the specimen at 3000 g for 15 minutes.
- Carefully pour off the supernatant through a funnel into a discard container containing 5% phenol or other mycobacterial disinfectant.
- Re-suspend the deposit and make a final volume of 2 ml by adding the Xpert MTB/RIF sample reagent.
- Label the GeneXpert/MTB/RIF cartridge with the specimen's identification number.
- Using a fresh transfer pipette, transfer 2 ml of the concentrated CSF specimen to the Xpert MTB/RIF cartridge.
- Load the cartridge into the GeneXpert machine following the manufacturer's manual instructions.

Data were analysed using IBM SPSS V. 20.0.0. Comparison of continuous variables among 3 investigation modalities was done using analysis of variance (ANNOVA). Categorical variables were analysed using Chi-square test/ Fischer exact test. Correlation among the continuous variables was assessed using Pearson's product moment correlation. The p value of <0.05 were considered statistically significant for the purpose of this study.

RESULTS

53.3 percent of the patients (64 patients) belonged to the age group of 20 to 40 years. 27.5 percent of the patients (33 patients) belonged to the age group of 41 to 60 years. 10 percent of the patients (12 patients) belonged to the age group of less than 20 years. Mean age of the patients was 37.73 years. Positive result on L-J culture media were found to be present in 54.16 percent of the patients (65 patients), while negative results were found to be present in 45.8 percent of the patients (55 patients).

TABLE 1: Distribution of patients according to results of L-J culture media

Parameter	Number of patients	Percentage
Positive	65	54.16
Negative	55	45.84

Positive result on GeneXpert MTB/RIF were found to be present in 40.83 percent of the patients (49 patients), while negative results were found to be present in 59.16 percent of the patients (71 patients). Out of 49positive patients of GeneXpert MTB/RIF, Rifampicin sensitivity was found to be present in 46 patients (93.88 percent), while rifampicin resistance was found to be present in 3 patients (6.12 percent).

TABLE 2: Distribution of patients according to results of GENE XPERT MTB/RIF

Parameter	Number of patients	Percentage
Positive	49	40.84
Negative	71	59.16

TABLE 3: Distribution of L-J media culture results in relation to GENE XPERT MTB/RIF

Parameter		L-J Media culture		Total
		Negative	Positive	
GeneXpert	**Negative**	55	16	71
	Positive	0	49	49
Total		55	65	120

TABLE 4: Overall sensitivity and specificity of GENE XPERT MTB/RIF in the detection of m. Tuberculosis in cases of extrapulmonary tuberculosis

Parameter	Value (%)	90% CI (%)
Sensitivity	75.78	63.13 to 85.23
Specificity	100	93.51 to 100.00
Positive predictive value	100	-
Negative predictive value	78.57	69.20 to 84.03
Accuracy	86.67	79.25 to 92.18

GRAPH 1: Distribution of patients according to presence of rifampicin resistance in samples of extrapulmonary tuberculosis

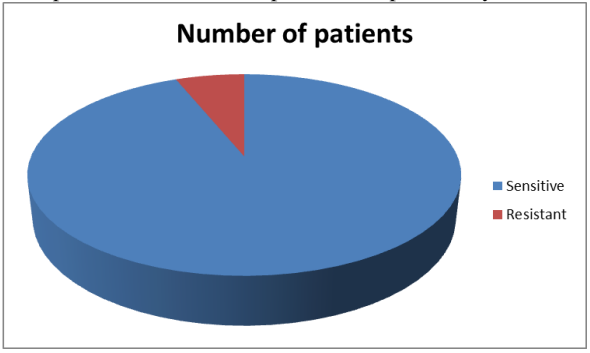

TABLE 5: Detection of M.TB by GENEXPERT and LJ media culture in EPTB samples

Sample type	Total no.	GeneXpert	LJ media	Sensitivity of GeneXpert
Pus/Cold Abscess	16	12 (75%)	14 (87.5%)	85.71%
Ascitic fluid	14	2 (14.2%)	6 (42.8%)	33.33%
Pleural fluid	64	23 (35.9%)	30 (46.8%)	76.67%
CSF	26	12 (46%)	15 (57.6%)	80%

DISCUSSION

The present study was conducted on patients from various departments at Guru Gobind Singh Medical College and Hospital, Faridkot. A total of 120 patients with potential symptoms of extrapulmonaryTB reporting to different departments at Guru Gobind Singh Medical College and Hospital, Faridkot were included. So, study was conducted on cases of suspected extrapulmonary tuberculosis patients by their clinical and radiological presentation; and was confirmed on the basis of biochemical and cytological investigations. Out of 120 specimens tested, 53.33 percent of the specimens (64 specimens) were obtained from pleural fluid, while 21.67 percent of the specimens (26 specimens) were obtained from CSF. 13.3 percent of the specimens (16 specimens) were obtained from pus, while 11.67 percent of the specimens (14 specimens) were obtained from Ascitic fluid. Our results were similar with the findings of the study done by Avashia S et al. Majority of the specimens in their study group (34.33%) were of pleural fluid, followed by pus and CSF samples. In another study, conducted by Sajed AN et al, authors reported that pus samples were obtained in 60 percent of the cases, while pleural fluid sample was obtained in 19 percent of the cases.[7, 8]

In the present study, we have used LJ media culture as gold standard for assessing the sensitivity and specificity of GeneXpert. All patients who were detected as M.TB positive on GeneXpert were later found to be LJ culture positive. Overall sensitivity and specificity of GeneXpert MTB/RIF in the detection of M. tuberculosis in cases of Extrapulmonary tuberculosis was found to be 75.78% and 100% respectively as compared with LJ media culture. Previous studies done by Zeka AN et al, Boehme CC et al, and Marlowe EM et al, of the GeneXpert assay have reported sensitivities of 57-76.9% in smear-negative, culture-positive respiratory specimens, and 98-100% in smear-positive, culture-positive respiratory specimens, while specificity remained at 99-100%.[9- 11]

A WHO meta-analysis indicated that suspected adult TB patients, when tested by GeneXpert against culture for samples of expectorated or induced sputum, the pooled sensitivity was 66% and specificity was 98%.[12]

Optimal performance of the assay was observed for various body fluids, with sensitivity approximately 76.92%. The present study also indicates exemplary performance of the assay in patients with pus and CSF samples; and these findings are similar to previously published literature. The difference in sensitivity of GeneXpert for various body fluid samples could be attributed to the fact that in aspirates from some specific areas, the bacteria are localised to the site of infection; whereas in body fluids, presence of PCR inhibitors and the paucibacillary nature of the specimens may result in lower sensitivity in previous studies. Also, results from the studies conducted in the past years have reported variable sensitivity of LJ media culture in extrapulmonary samples.[13- 16]

The study revealed that the GeneXpert test has true diagnostic potential with good sensitivity for specimens such as pus which is difficult to diagnose by other laboratory techniques. Our findings supported the use of GeneXpert test in routine for extra pulmonary TB diagnosis especially for pus samples where a very high detection rate was observed as compared to conventional techniques.

In the present study, out of 49 positive patients of GeneXpert MTB/RIF, Rifampicin sensitivity was found to be present in 46 patients (93.88percent), while rifampicin resistance was found to be present in 3 patients (6.12percent). Our results correlates with the results obtained by Avashia S et al, who reported that in 94.59 percent of the GeneXpert positive cases, Rifampicin sensitivity was present. In the another study conducted by Sajed An et al, none of the cases showed Rifampicin resistance which indicated that MDR TB is not common in extra pulmonary TB cases in our country.[7, 8]

In another previous study conducted by Metaferia Y et al, only two samples demonstrated rifampicin resistant Mycobacterium tuberculosis complex making the prevalence of rifampicin resistant-TB 6.5% (2/31). Report from Addis Ababa showed a 2.3% MDR-TB among newly diagnosed EPTB cases. In the North-western Ethiopia, the prevalence of MDR-TB among EPTB cases was found to be 3.7%. Even though the observed frequency of rifampicin resistant case is low, it highlights the need of screening of EPTB patient for MDR-TB.[17- 19]

However, GeneXpert does not eliminate the need of conventional microscopy, culture and anti-tubercular drug sensitivity that are required to monitor the progression of treatment and to detect resistance to drugs other than Rifampicin.

CONCLUSION

The use of GeneXpert test is effective in routine for diagnosis of extrapulmonary TB, especially for pus and CSF samples where a very high detection rate was observed as compared to conventional techniques.

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