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Original Research Article

Comparison of diagnostic yield of GeneXpert MTB/RIF assay and ZN (Ziehl-Neelsen) staining in serosal fluids from HIV and non-HIV patients with extra-pulmonary tuberculosis

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ABSTRACT

Background: India accounts for 23% of the global TB burden and of these 15-20% are extrapulmonary tuberculosis(EPTB). EPTB has diverse clinical presentation and often is over diagnosed because of lack of standardised diagnostic means. Yield of acid fast bacilli (AFB) from EPTB settings is very low and alternate facilities are nearly non-existent routinely. GeneXpert MTB/RIF has been recommended as a diagnostic tool for sputum samples. It has been also recommended for serosal fluids from HIV patients only. This study was done to compare the yield of GeneXpert MTB/RIF assay and ZN (Ziehl Neelsen) staining among HIV and non-HIV patients with suspected serosal TB.
Methods: GeneXpert MTB/RIF assay and ZN staining was done in all serosal fluid samples in which a clinical diagnosis of serosal TB had been made by conventional methods.
Results: A total of 81 extra pulmonary samples (21 from HIV and 60 from non-HIV patients) were processed in this study, which included 48 CSF, 19 pleural fluids and 14 ascitic fluid. Out of these, 34.5% (28/81) patients were both GeneXpert MTB/RIF as well as ZN stain positive whereas only 13.58%(11/81) patients were ZN stain positive. 4/21 (19.05%) samples from HIV patients were ZN stain positive and 9/21(42.85%) were GeneXpert positive. 7/60 (11.66%) samples from non-HIV patients were ZN stain positive and 19/60 (31.66%) were GeneXpert positive.
Conclusions: GeneXpert MTB/RIF is more sensitive than ZN staining in EPTB. The yield in both HIV and non-HIV patients is the same.

Keywords: Extra pulmonary tuberculosis, Gene Xpert MTB/RIF assay, Mycobacterium tuberculosis

INTRODUCTION

The annual incidence of tuberculosis in India is 1.96 million, out of which 20% constitutes extrapulmonary tuberculosis. Co-existence of HIV infection further increases the incidence of both pulmonary and extrapulmonary tuberculosis.[1]

The diagnosis of extrapulmonary TB is often difficult. It is both because the signs and symptoms of disease are either subtle or non-specific and also due to the difficulty in definitive diagnosis. Histology, if available, may be suggestive but demonstration of TB bacilli either by suitable staining or culture is difficult.[2]

Serosal TB (tubercular meningitis/pleuritis/peritonitis) is an easily diagnosed form of EPTB due to the ease of examination of the serosal fluid. Various biochemical criteria along with the clinical picture is used for the diagnosis. Demonstration of TB bacilli is seldom done.

The various biochemical and clinical criteria lack specificity and often TB is over diagnosed, leading to unnecessary and incomplete antitubercular treatment, factor that can lead to development of drug resistance.

GeneXpert (CBNAAT) and other nucleic acid amplification tests are latest in the armamentarium for the diagnosis of tuberculosis.[3] The role of GeneXpert is firmly established in detecting TB bacilli in sputum samples, even those which are negative for AFB.[4] The role of GeneXpert in extra-pulmonary tuberculosis is still investigational. This study was conducted to evaluate the diagnostic utility of GeneXpert and ZN staining in serosal TB in a high TB and HIV prevalence area.

METHODS

This study was conducted in Department of Medicine, BRD Medical College, Gorakhpur, Uttar Pradesh, India, on extrapulmonary serosal tuberculosis patients, to evaluate the clinical utility of GeneXpert assay in patients with serosal tuberculosis in comparison with ZN staining. All consenting patients in whom a clinical diagnosis of extrapulmonary serosal TB had been made by their respective clinics were studied. Ethical clearance was taken from institutional ethical committee. Clinical details and investigations of the patients were noted on a predefined proforma. AFB staining and GeneXpert were also done on the serosal fluid samples.

ZN staining

Prepared smear slides of each serosal samples (CSF, pleural fluid and ascitic fluid) were stained with Ziehl Neelsen (ZN) method and examined with a light microscope for the presence of AFB bacilli, in the Pathology department, BRD Medical College, Gorakhpur.

GeneXpert

Samples were collected in containers provided and treated with sample reagent in a proportion of 2:1 and incubated for 15 minutes at 20-300C.Using the provided transfer pipette sample reagent treated sample was transferred into the sample chamber of the Xpert cartridge and put into the GeneXpert instrument system and the automatically generated results were read after 90 min.

RESULTS

A total of 81 patients were studied in which 21 were HIV positive and 60 were HIV negative. Serosal samples included were CSF, pleural fluid and ascitic fluid (Table 1). The mean age of the patients was 40±22 years.

The AFB smear microscopy (ZN staining) positivity was found to be statistically not significant (p=0.4633) between HIV and non-HIV patients (Table 2).

Table 1: Distribution of extra pulmonary tuberculosis cases included in the study.

		Types of specimen			Total
		CSF	Pleural fluid	Ascitic fluid	
HIV	Positive	16	3	2	21
	Negative	32	16	12	60
Total		48	19	14	81

Table 2: Results of samples analysed by AFB staining (ZN staining).

HIV			Non-HIV		
	Positive (%)	Negative (%)		Positive (%)	Negative (%)
CSF (n=16)	3 (18.7)	16 (81.3)	CSF (n=32)	5 (15.6)	27 (84.4)
Pleural fluid (n=3)	1 (33.4)	2 (66.6)	Pleural fluid (n=16)	2 (12.5)	14 (87.5)
Ascitic fluid (n=2)	0 (0%)	2 (100%)	Ascitic fluid (n=12)	0 (0%)	12 (100%)

Table 3: Results of samples analysed by GeneXpert assay.

HIV			Non-HIV		
	Positive (%)	Negative (%)		Positive (%)	Negative (%)
CSF (n=16)	7 (43.7)	9 (56.3)	CSF (n=32)	12 (37.5)	20 (62.5)
Pleural fluid (n=3)	1 (33.4)	2 (66.6)	Pleural fluid (n=16)	5 (31.3)	11 (68.7)
Ascitic fluid (n=2)	1 (50%)	1 (50%)	Ascitic fluid (n=12)	2 (16.7%)	10 (83.3%)

The GeneXpert positivity was found to be statistically not significant (p=0.4268) between HIV and non-HIV patients (Table 3).

TB bacilli were detected in 11 patients by ZN staining and in 28 patients by GeneXpert (Table 4).

Table 4: Comparison of GeneXpert with AFB staining.

		AFB staining		
		Positive	**Negative**	**Total**
GeneXpert Assay	Positive	11 (13.58%)	17 (20.98%)	28 (34.56%)
	Negative	0	53 (65.44%)	53 (65.44%)
Total		11 (13.58%)	70 (86.42%)	81 (100%)

DISCUSSION

EPTB is an important cause of morbidity and mortality. Due to the high prevalence of TB in India, there is need of rapid diagnostic method to initiate early treatment and cure of the diseased individuals thereby to reduce the TB burden. GeneXpert MTB/RIF has been found to be more sensitive than AFB staining in confirmatory diagnosis of tuberculosis.[5,6] The positivity of GeneXpert in our study in various serosal fluids (39.58% CSF samples, 31.58% pleural fluid samples, 21.43% ascitic fluid samples) was similar to a study done by Ahmed et al.[7] This included a total of 100 extra pulmonary samples of which there were 19 pleural fluids, 16 ascitic fluids and 5 CSF. Others were pus and synovial fluid. MTB (Mycobacterium tuberculosis) by GeneXpert in that study was detected in 3 out of 19 (15.8%) pleural fluid samples, 1 out of 16 (6.3%) ascitic fluid samples and 2 out of 5 (40.0%) cerebro-spinal (CSF) samples. They concluded that GeneXpert assay has more diagnostic efficacy in extrapulmonary tuberculosis than other conventional methods like AFB smear microscopy (Ziehl-Neelsen staining). In our study tubercular meningitis was the most common EPTB, probably a bias, as we included only patients who were admitted in the ward.

Another study done by Avashia S et al had almost same results as in our study, with a total of 300 extra pulmonary samples which included 103 pleural fluids, 45 CSF, 20 ascitic fluids.[8] Others were pus, lymph node and synovial fluid. Out of these 37% (111) patients were Gene Xpert MTB/RIF Assay positive and of these only 36% (40 out of 111) were ZN smear positive. M. Tuberculosis by GeneXpert was detected in 23.3% pleural fluid samples, 33.3% CSF samples, 20% ascitic fluid samples. They concluded Gene Xpert MTB/RIF assay can be a rapid method for diagnosis of EPTB as compared to conventional methods along with advantage of detecting Rifampicin resistance.

GeneXpert has been recommended to be used as a diagnostic test in all samples in HIV patients 1.

In present study, the yield of GeneXpert in HIV patients was 42.8% (9/21) and 31.7% (19/60) in non-HIV patients. Although there was a higher yield in HIV, this difference was statistically not significant. This was probably because of a small sample size. This finding was in contrast to a study done by Alvarez-Uria et al9 in 2012 in which 253 extrapulmonary samples (142 CSF, 75 pleural fluid, 18 ascitic fluid and others) from HIV patients were included, out of them 74 (29.3%) were GeneXpert positive. Patel V B et al, included 235 cases of suspected TBM in which 167 were HIV infected out of which only 27% (45/167) were detected GeneXpert assay positive.[10]

The yield of GeneXpert assay in smear negative cases in our study was 24.3% (17 out of 70 patients) only and 100% in smear positive cases (11 out of 11 patients). The low yield in smear negative samples could represent a true low sensitivity of GeneXpert or could have been due to inclusion of patients which may not have been tubercular, as the study did not have predefined criteria for diagnosis of tuberculosis.

Study compared the clinical profile and other investigations of patients who were GeneXpert positive versus those who were negative. 4 out of 19 GeneXpert positive CSF patients had previous history of pulmonary tuberculosis and they had history of fever and headache of more than 1 month duration with vomiting, seizure and altered sensorium without any focal neurological deficit. Neck stiffness was present. CT head showed hydrocephalus.

3 patients presented only with headache of more than 2 months duration without fever, seizure and any focal neurological deficit. CT scan head had no intracranial abnormality.

Rest 12 patients had fever and headache of more than 1 month duration, vomiting and altered sensorium without seizure and any focal neurological deficit. Neck stiffness was present and CT Head were normal in these patients.

Those patients who were negative with GeneXpert also had a history of fever and headache of approximately 15 days followed by altered sensorium. This along with mild rise in CSF protein and pleocytosis was used as criteria for making the diagnosis of tubercular meningitis. Those patients who were GeneXpert negative in CSF had significantly low cell count and protein.

Although a history of fever more than 6 days, moderate pleocytosis should make tubercular meningitis probable, our findings suggested that a history of a month of fever having total count >13 cells/cumm with lymphocyte dominance, protein >112 mg/dl and glucose <41mg/dl in CSF, with or without headache may increase the specificity of clinical diagnosis of tubercular meningitis in most instances.

All the pleural fluid samples were classified as exudatives based on a high protein content and ADA levels >30 units/litre. All patients had a history of cough and breathlessness of more than 3 weeks duration. GeneXpert was positive in 31.57% (6/19) pleural fluid samples. GeneXpert positive pleural fluid samples had elevated total count with predominantly lymphocytes and those who were negative had paucity of cells in their pleural fluid. The positivity of GeneXpert in patient with high cell count but not in those with low cell count could be due to a high bacteriological load giving rise to a higher inflammatory response. Friedrich SO et al, investigated the diagnostic utility of the Xpert MTB/RIF assay in 20 cases with culture confirmed tuberculous pleural effusion.[11]

Percentage of detection by Xpert assay was 25% (5/20) in pleural fluid samples showing low positivity of GeneXpert as was in our study. They concluded that use of the Xpert assay on pleural fluid samples is feasible but has low detection rate and larger studies including more patients with pleural effusion are needed. As for ascitic fluid samples positive with GeneXpert had elevated total count with predominantly lymphocytes in their asctic fluid, those negative had paucity of cells in ascitic fluid. Rufai BS et al, included 67 ascitic fluid samples that all were negative with ZN staining of these only 17.9% (12/67) were positive with GeneXpert assay.[12]

Although study results are comparable to the reputed sensitivity of GeneXpert the yield in both HIV and Non-HIV individuals is equal. GeneXpert can also be used even in Non-HIV individuals as it increases the diagnostic yield. The limitations of our study are that we could not compare GeneXpert with culture which is considered as the goldstandard. Further studies with culture or predefined CRS (certified reference standard) with follow up are required to define the exact sensitivity and specificity of GeneXpert.

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