ORIGINAL ARTICLE

The Utility of Xpert MTB/RIF Assay in Diagnosis of Extra Pulmonary Tuberculosis in a Tertiary Care Centre

Supriya Emekar[1] Sanjaykumar More[1] and Vimal Rathod[1]
[1]Department of Microbiology, Dr. Shankarrao Chavan Government Medical College, Nanded- 431606, Maharashtra, India.

Abstract:
Introduction: It is estimated that India accounts for 25% of global tuberculosis (TB) burden of the world. Extra pulmonary tuberculosis (EPTB) constitute about 15-20% of all cases of TB. *Aim:* To study the use of Xpert MTB / RIF test to determine the prevalence of extra pulmonary tuberculosis. *Material and Methods:* The Xpert MTB/RIF assay was performed on all the non-respiratory samples as per protocol. The patient related data along with results was retrieved from record registers. *Results:* Among the total of 1333 samples, 135 samples were found to be MTB positive and nine as MDR EPTB. The numbers of patients were maximum in age group 15-60 years with male predominance. Among all the sample types, pleural fluid (n=628) contributed the most followed by CSF (n=300), ascetic fluid (n=206), pus (n= 106), FNAC of lymph node (n=56) and other body fluid (n=37). The percentage MTB positivity for lymph node was (20/56) highest as 35% followed by pus (35/106) 33%, other body fluid (n=4/37) 11%. *Conclusion:* The result of our study suggests that the Xpert MTB/RIF assay shows good potential for the diagnosis of EPTB including MDR status especially in our country where the TB is endemic.

Keywords: Gene Xpert, extra pulmonary tuberculosis.

Introduction:

It is estimated that India accounts for 25% of global tuberculosis (TB) burden of the world [1]. As per WHO policy update given for the diagnosis of pulmonary and extra pulmonary tuberculosis in 2013, extra pulmonary TB accounts for about 25% of all cases of TB and an even higher percentage of cases in children and in people who are immunocompromised [2]. Out of 5,80,000 new cases of Multi drug resistant tuberculosis (MDR TB) globally, 1.3 lakh MDR TB patients were in India [3]. The diagnosis of EPTB is challenging due to its varied clinical presentations, pauci-bacillary nature of specimen, difficulty in obtaining specimen from deep seated organs and inability to get an additional specimen [4]. Hence the timely diagnosis of EPTB and MDR TB become difficult which leads to continued disease transmission, causing significant morbidity and mortality. Thus making a rapid diagnosis is of utmost importance [5]. The laboratory confirmation of the diagnosis of active TB is pivotal for the management of disease and is an effective public health intervention [6]. The WHO has recommended a number of molecular diagnostic devices for rapid diagnosis of MTB. The Xpert MTB / RIF test is recommended as the initial diagnostic test for patient being evaluated for pulmonary and extra pulmonary TB [7]. TB is curable disease. The WHO issued updated policy guidance providing revised recommendations onusing Xpert MTB/ RIF to diagnose pulmonary TB, paediatric TB, Extra pulmonary TB and rifampicin resistance [2]. This provides a diagnosis of TB and RIF resistance (RR) within two hours with minimal biosafety facilities and minimal training. This assay has helped in bridging the gap between diagnosis and treatment.

EPTB has complex and often subclinical presentations that contribute to significant burden of mortality and morbidity. This may lead to delay in diagnosis [8]. The conventional culture DST methods are time consuming and require trained technicians. This is a practical difficulty in our resource limited country as observed in a study by Chaudhary A at al. [8]. Recent reports suggest that Xpert MTB/RIF assay increases the rate of detection of RIF resistance, decreases unnecessary

empiric treatment among smear negative EPTB and increases the early initiation of second line drug treatment [9]. Therefore this study was done to know the prevalence of EPTB and multidrug resistant extra pulmonary tuberculosis (MDR EPTB) with the use of Gene expert assay among the clinically suspected extra pulmonary tuberculosis patients.

Materials and methods:

This is a retrospective observational study from 1st January 2016 to 31st December 2018 conducted in department of microbiology at tertiary care centre. As per Institutional policy ethical approval was not required.

We have included all the adult patients presenting with suspected EPTB from district tuberculosis centre and tertiary care centre. The results from the patients with positive and negative interpretation were included. The inderminate results were excluded from study. A total of 1333 consecutive EPTB samples were analysed in the study. The EPTB specimen comprised of pleural fluid (n=628), ascetic fluid (n=206), CSF (n=300), pus (n=106), fine needle aspiration cytology fluid (FNAC) (n=56) and others (included endometrial fluid, tissue biopsy, synovial fluid and urine) (n=37). The detailed clinical history was taken. The gene xpert assay was performed according to manufacturer instructions (Cepheid, Sunnyvale, CA, USA). In this assay, sample reagent was added at 2:1 ratio to clinical sample. It was then incubated for 15 minutes at room temperature with intermittent shaking. Then the two ml of processed sample was transferred to the cartridge. The cartridge containing processed sample in the tray inserted into cartridge based nucleic acid amplification test (CBNAAT) machine and the result were then generated within two hours [10]. The statistical analysis was done with SPSS software.

Results:

A total of 1379 samples were studied. Out of 1379 samples, 46 were excluded from study. The reason for exclusion were noted as invalid (n=07), error (n=32), no result (n=7). This contributes to indeterminate results by Xpert MTB/RIF assay. The repeat testing was done on all of these 46 samples. Some of them (8/46) were found as negative. The remaining extra pulmonary samples (38/46) 2.75% have non interpretable results.

Out of 1333 samples from extra pulmonary specimen, 135 samples were found to be MTB positive. A total of nine samples were MDR among 135 EPTB MTB positive samples. The percentage positivity was 10.12% (135/1333) with the use of Xpert MTB/RIF assay among the various clinical specimens collected from all the suspected EPTB patients.

Prevalence and its significance - The prevalence of EPTB & MDR EPTB with the use of Gene Xpert as a rapid diagnostic test was found as 10.12% and 0.7% respectively in our geographical region.

The number of patients were maximum in age group 15-60 years (n=1144), followed by age more than 60 years (n= 189). The percentage positivity with the use of Xpert MTB/RIF assay as diagnostic test was found as (n= 129)11.25% and (n=6) 3.2% for the age group 15-60 years and above 60 years respectively. All the MDR EPTB cases were found in age group of 15-60 years. The number of male and female was found as 800 and 533 respectively. Hence the male to female ratio become1.5:1. Xpert MTB/RIF assay results among male and female patients have been mentioned in table 1.

The different sites of involvement among EPTB patients presenting with the different clinical manifestations is mentioned in table 2. Among all the sample types, pleural fluid (n=628) contributed the most followed by CSF (n=300), ascetic fluid (n=206), pus (n= 106), FNAC specimen of lymph node (n=56) and other body fluid (n=37). The percentage positivity varied markedly among different clinical samples. The percentage positivity for lymph node tuberculosis was (20/56) 35%, pus (35/106) 33%, followed by other body fluid (n=4/37) 11%, pleural fluid (59/628)9.4%, CSF (11/300)3.7%, ascetic fluid (6/206)3%. The positivity rate of EPTB and MDR EPTB among all the non-respiratory samples is mentioned in table 3.

The percentage positivity of CSF samples collected from suspected tubercular meningitis cases was found as 3.7%. The lower percentage positivity may be due to

the fact that tubercular meningitis is challenging diagnosis with high mortality. In our study, maximum samples had low and medium bacillary load, which was detectable by Gene Xpert assay. Even the very low (bacillary) level can be detected with Xpert MTB/RIF assay.

Detection of Rifampicin resistance- Nine samples (three lymph node aspirate, three pleural fluids, three pus samples) were rifampicin (RIF) resistance with Xpert MTB/RIF assay. The remaining 126 samples were RIF sensitive when used the Xpert MTB/RIF test.

Table No. 1: Proportional age distribution for EPTB and MDR-EPTB cases according to gender

	Xpert MTB/RIF Assay									
Age /sex	Male					Female				
	Positive	Negative	Total	Percentage (+)	MDR (+)	Positive	Negative	Total	Percentage (+)	MDR (+)
15-60 yrs.	73	605	678	10.76	04	56	410	466	12	05
>60 yrs.	04	118	122	3.27	0	04	63	67	6	00
	77	723	800	9.62		60	473	533	11.25	

Table No. 2: Different sites of involvement among EPTB patients

Sr. no	Clinical features	No. of samples from Males (%)	No. of samples from Female (%)	Total no. of samples (%)
1	Pleural TB	410 (65.3)	218(34.7)	628 (47.1)
2	TB meningitis	159 (53)	141(47)	300 (22.5)
3	Abdominal tuberculosis	132 (59.7)	74(36)	206 (15.5)
4	Tubercular abscess/cold abscess	63(59.4)	43(40.5)	106 (8)
5	Lymph node TB	23(41)	33(59)	56 (4.2)
6	OTHER types TB (knee joint, renal TB, tissue biopsy, Endometrial TB)	13(35)	24(64.8)	37 (2.8)
		800	533	1333

Table No. 3: Performance characteristics of Xpert MTB/RIF in diagnosis of extra pulmonary TB

Sr. no	Type of sample	Number of samples	Number of positive	RIF resistance	Percentage positive	Percentage positive of RIF resistance
1	Pleural fluid	628	59	03	9.39	0.47
2	CSF	300	11	0	3.66	0
3	Ascetic fluid	206	06	0	2.91	0
4	Pus	106	35	03	33	2.83
5	FNAC	56	20	03	35.71	5.35
6	Others (synovial fluid, urine, endometrial fluid)	37	04	0	10.81	0
		1333	135	09	10.12	0.7%

Discussion:

Gene Xpert is a semi quantitative cartridge based nucleic acid amplification test based on molecular detection of mutated gene (rpoB gene). It is an automated method which is cost effective and does not require technical expertise [11]. In a study by Chaudhary A at al. [8]. it was mentioned that Gene Xpert is extremely helpful for rapid diagnosis of MDR TB in resource limited settings. It also has a significant role to play in the diagnosis of EPTB and EP MDR TB. Its potential in EPTB MDR detection has been underutilized due to lack of awareness regarding the same [8].

It is the method of choice for drug sensitivity testing (DST) under Revised National Tuberculosis Control Program (RNTCP) [11]. The assay's sample reagent, used to liquefy sputum, is tuberculocidal, which largely eliminates concerns about biosafety during the test procedure. These features allow the technology to be taken out of a central laboratory or reference laboratory and to be used nearer to patients. However, Xpert MTB/ RIF requires an uninterrupted and stable electrical power supply, temperature control and yearly calibration of the instrument's modules6. The indeterminate results for Xpert was reported as 1.1% in study by Tortoli E et al [12].

The prevalence of EPTB-MTB positive by Gene Xpert was found as 10.12% in our geographical region. This is similar to the study by Lawn S D & Zumla A I [13] (14.7%). This is lower as compared to the various studies by Habous M et al [14] (25.59%), Scott L E et al [15] (22%), Nataraj G. et al [16] (20.9%) & Banker S et al [17] (18.42%). Xpert MTB/RIF assay offers some diagnostic advantages being able to provide bacillary load in the given sample [17]. Hakeem A et al [18] mentioned that the Gene Xpert is likely to revolutionize the diagnosis and treatment of drug resistant TB, as it is cost effective and rapid.

Among the total of 1379 extra pulmonary samples, (38/1379) 2.75% have non interpretable results. The high sensitivity (97.4% and 95%) of Xpert in smear positive samples across sample types and the low proportion of non-interpretable results (1.2%) support the use of the test in non-respiratory samples in principle [19].

The prevalence of EPTB MDR is showing rising trend. A study by Chaudhary A et al [8] suggests that Gene Xpert plays a pivotal role in MDR EPTB diagnosis in high burden areas. The prevalence of MDR EPTB with the use of Gene Xpert as diagnostic assay was found as 0.7% which is very low when compared to the other studies [14-17]. In a study by Scott L E et al [15], fewer (7.6%) rifampicin resistant cases were identified by traditional methods than by Xpert MTB/RIF (9.6%), which provided an early diagnosis of RIF resistance. The number of patients with EPTB-MTB positive were maximum in age group 15-60 years (n=1144), followed by age above 60 years (n= 189). Lawn S D & Zumla A I [13], Scott L E et al [15] also found the maximum number of cases with age more than 18 years. The male to female ratio was found as 1.5:1 among all the EPTB – MTB positive cases. Habous M et al [14] found the male to female ratio as 1.4:1. Scott L E et al [15] found 55% population as males (ratio 1.2:1).

The highest percentage MTB positivity for lymph node tuberculosis was (20/56) 35% which is similar to the study by Scott L E et al [15] (35.71%) but lower (23.8%) in one study [16]. The positivity of lymph node aspirate sample with the use of Gene Xpert assay was found higher in studies by Habous M et al [14] (43.47%), Banker S et al [17] (41%) & Armand S et al [20] (50%). The higher positivity might be due to small sample size in these studies. The total number of MDR EPTB among lymph node aspirate were found as (3/20) (15%). These findings were similar to the study by Banker S et al [17] (10%) and Nataraj G. et al [16] (4.76%).

The percentage MTB positivity of pus samples obtained from various sites was (35/106) 33%. This is similar to the study by Habous M et al [14] (32.14%). The positivity rate of pus sample with the use of Gene Xpert assay was found higher in various studies Banker S et al [17] (40%) & Scott L E et al [15] (52.1%) and highest as (100%) in two studies [8,20]. The highest percentage may be due to the low sample size.

We found 9.4% (59/628) pleural fluid samples as MTB positive by Gene Xpert assay. This is consistent with the study by Banker S et al [17] (9.87%). The diagnostic test accuracy review by Kohli M et al [21] has also mentioned the Gene Xpert positivity as 8.3% for pleural fluid samples. Gene Xpert results showed low pooled sensitivity (18%) among pleural fluid samples when compared with composite reference standard [19]. Tuberculous pleural effusions in most of the cases is due to hypersensitivity reaction to tuberculous antigens than the direct invasion of pleura. This gives a lower yield on Gene Xpert Assay which had been mentioned in study by Chaudhary A at al [8]. The pleural biopsy is the sample of choice for culture but not the pleural fluid [22]. The inhibition of polymerase chain reaction by inhibitors in pleural fluid

and by the presence of blood in fluid is also a possible explanation for low sensitivity by Xpert MTB/RIF assay [23]. Among the clear specimens, pleural fluid performed most poorly (sensitivity 47%). This may be due to the reduced numbers of M. tuberculosis in the specimen and further dilution by SR (sample reagent) buffer [6]. It had been suggested that Gene Xpert assay on pleural fluid can be done for the diagnosis of pleural TB if the resources are available. This is because Gene Xpert has higher sensitivity than smear and provides more rapid diagnosis than culture and histology [19]. The percentage positivity among pleural fluid samples were found higher in various studies [8,20,24] as 57.14%, 33.3%, 31.57% respectively but was lower as 2.65% in one study [25]. The higher positivity might be due to small sample size.

In our study, the MTB positivity of CSF sample by Gene Xpert assay from the tubercular meningitis cases was found as (11/300) 3.7% which is lower than in various studies [15,16,24]. The percentage MTB positivity among CSF samples, reaching near to the findings in the studies by Banker S et al [17] (10%) and Habous M et al [14] (8%). The diagnostic test accuracy review by Kohli M et al [21] has mentioned the Gene Xpert positivity among CSF samples as (8.9%). Xpert MTB/RIF assay has moderately diagnostic sensitivity for detection of TB meningitis [19]. The lower rate of percentage positivity in our study may be due to the reason that we had not done centrifugation step for CSF samples. The centrifugation of CSF increases the Xpert sensitivity (from 51.3% to 84.2% for concentrated samples) with unchanged specificity, probably by increasing the bacillary load in cartridge input volume [19]. While Gene Xpert assay does not reach the sensitivity of culture, it could improve the diagnosis of CSF in places where culture and other diagnostic tests are not available or where a rapid diagnosis of TB is necessary [19]. It has been mentioned in a review by Kohli M et al [21] that the treatment should be based on clinical judgement, not withheld solely on Xpert result for the people with presumed TB meningitis, as is common practice when culture results are negative. The percentage MTB positivity of ascetic fluid samples by Gene Xpert testing was (6/206)3%. This is very similar to the findings by Nataraj G [16] where it was 4.16%. The finding was quite higher (21.42%) in the study by Mittal M and Kumar R [24] An optimised processing might also need to be different for different sample types and might further improve Xpert performance. Therefore, optimization of sample preparation is needed [19].

A positive Xpert result should be interpreted in light of clinical, histopathological and radiological findings because Xpert detect mycobacterial DNA (of dead bacilli too) not the viable bacilli. A negative Xpert assay should be confirmed by culture. All these findings make the use of the Xpert assay as initial diagnostic test for EPTB [16]. Xpert MTB/RIF assay has high sensitivity in smear negative pulmonary tuberculosis [17]. This is particularly important and relevant in patient with HIV infection. A study by Banker S et al [17] showed that Xpert MTB/RIF assay has a better diagnostic potential when compared to culture with good sensitivity.

The limitations of our study - 1. The study is retrospective and primarily laboratory based 2. We couldn't calculate the specificity of Xpert MTB/RIF assay for non-respiratory samples which need comparison with culture as gold standard. 3. The immunocompromised status of patients and associated history of pulmonary tuberculosis (PTB) was not available which may have an impact on percentage positivity among the different types of samples.

In diagnostic accuracy studies, an imperfect reference standard may lead to a misclassification of samples [26, 27]. The culture is an imperfect reference standard for EPTB due to the pauci bacillary nature of the disease. Assuming that Gene Xpert assay correctly identifies TB in a sample with negative culture, the result would appear to be false positive, leading to an underestimation of Gene Xpert assays true specificity. A CRS (composite reference standard) that classifies TB based on positive result of one out of several tests or clinical components may sometimes reclassify false positives' of Xpert (identifies as non TB using culture) as true positives (TB cases) and thus lead to an increased (i.e. more accurate) estimate of Gene Xpert specificity. However, a CRS itself may have reduced

specificity that could result in apparent false negative Xpert results, leading to an underestimation of Gene Xpert assay's true sensitivity [28,29].

Therefore, a comparison of the accuracy estimates based on these two reference standards, culture and CRS, should provide a plausible range for sensitivity and specificity [19]. Hence further studies are required in this arena to know the sensitivity and specificity of Xpert MTB/RIF assay. The different percentage positivity was observed for different extra pulmonary specimen. This may be due to specimen collection, storage and preparation techniques 30 or to reduced numbers (below the 131 CFU/ml threshold) 6,31of M. tuberculosis in the specimen, a further dilution or too harsh a treatment by SR buffer.

The findings of our study suggest that Xpert MTB/RIF assay is a better diagnostic test especially lymph node tuberculosis and cold abscess. Its diagnostic utility for tubercular pleuritic, meningitis and abdominal tuberculosis seems to be lower but it still holds good diagnostic use in a view of good sensitivity in smear negative pulmonary tuberculosis and extra pulmonary samples [17,32]. Despite of these limitations, we can conclude that the Xpert MTB/RIF assay is a rapid and reliable diagnostic test for non-respiratory samples for confirmation including MDR status.

Conclusion:

The result of our study suggests that the Xpert MTB/RIF assay shows good potential for the diagnosis of EPTB including MDR status especially in our country where the TB is endemic.

Conflict of Interest - Nil
Sources of Support - Nil

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Address for correspondence: *Dr Supriya M. Emekar, Associate Professor, Department of Microbiology, Dr. Shankarrao Chavan Government Medical College, Nanded 431606. Maharashtra. Email: emekarrani@gmail.com, Mobile: +91 9881767847.*

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