Diagnostic Accuracy of Xpert MTB Compared to Smear Microscopy in Pulmonary vs Extrapulmonary Tuberculosis

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ABSTRACT

Introduction: Mycobacterium tuberculosis is the most significant infectious disease causing death worldwide. Inability to rapidly diagnose and treat the affected patients leads to increased morbidity and mortality, development of secondary resistance. There is sparse data which show the accuracy of diagnosis of tuberculosis by Gene Xpert in patients. There is limited data on the usefulness of Xpert MTB in the diagnosis of extrapulmonary TB. Study aimed to evaluate the diagnostic accuracy of XpertMTB compared to smear microscopy in pulmonary and extra pulmonary tuberculosis.

Material and methods: Prospective observational study conducted from May 2017 to July 2017. The study was conducted in Department of medicine, Department of pulmonology at R.L.Jalappa hospital and research centre, Kolar. 139 tubercular suspects who were not human immune deficiency virus (HIV) positive were included in the study.

Result: Out of 139 patients 72 were males and 67 were females. 99 pulmonary samples and 40extrapulmonary samples were obtained. 93 pulmonary samples and 24 extrapulmonary samples were culture positive. Smear microscopy has lower sensitivity in diagnosing pulmonary TB (46.2%) as well as extrapulmonary TB(16.6%).

Conclusion: The Xpert MTB is a rapid, sensitive and a reliable diagnostic test for TB than smear microscopy in both pulmonary as well as extra pulmonary TB. The diagnosis of extrapulmonary TB should not solely depend on the results of Xpert MTB and should be subjected to culture of Mycobacterium.

Keywords: Smear Microscopy, Xpert MTB, Pulmonary Tuberculosis, Extra Pulmonary Tuberculosis

INTRODUCTION

Mycobacterium tuberculosis is the most significant infectious disease causing death worldwide.[1] The lifetime risk of progressing from latent to active TB(Tuberculosis) is estimated to be between5-10%. In 2017, new cases of TB were estimated around 10.0 million (9.0–11.1 million). 87% of all incident cases of TB occurred in the 30 high TB burden countries, India accounting for 27% of these cases.[1,2]

Patients with HIV have a higher incidence of active tuberculosis as HIV increases both the risk of reactivating latent *TB* infection, as well as the rapid TB progression after infection.[3]

Previously smear examination, culture were the main methods used in detecting tuberculosis. Sputum smear microscopy still remains the basis for diagnosis of TB in developing countries for its fast and relatively inexpensive results.[4] However, around 10,000 bacilli/ml of sputum needs to be present for detection. This results in poor and varying sensitivity ranging from 20% to 80%, especially in HIV infected people with higher rates of smear-negative disease.[4,5]

Conventionally the diagnosis of pulmonary tuberculosis has been based on clinical scenario, chest X ray finding, smear microscopy for AFB or bacterial isolation by culture. Culture of *Mycobacterium tuberculosis* still remains the gold standard for diagnosis and permits the diagnosis of drug resistance but it has a long turnover time (6-8 weeks for solid and 1-2 weeks for liquid media).[6,7]

Inability to rapidly diagnose and treat the affected patients leads to increased morbidity and mortality, development of secondary resistance. In this situation not only rapid TB case detection, but also the early determination of MDR (multidrug resistance) status is important.

Xpert MTB (RIF/Gene xpert) is a molecular cartridge-based nucleic acid amplification test which simultaneously detects both TB and resistance to rifampicin. The limit of detection is 5 genome copies of purified DNA per reaction or 131 colony forming units per mL of sputum.[8] Rifampicin resistance is reported on the basis of late or absent probe signals by the Xpert MTB software.[9] It is automated and provides results within 2 hrs.

In developing countries, out of all the lab investigations, diagnosis still relies heavily on the use of smear microscopy, which has a low sensitivity and specificity as compared to the culture. However, the conventional culture technique for Mycobacteria does not provide a rapid diagnosis, is a cumbersome procedure and requires sophisticated laboratory facilities of biological safety lab level II/III that cannot be

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How to cite this article: Niveditha S, Jagmohan S V, Abhishek K Verma, Minni Meka. Diagnostic accuracy of xpert MTB compared to smear microscopy in pulmonary vs extrapulmonary tuberculosis. International Journal of Contemporary Medical Research 2019;6(7):G1-G4.

DOI: http://dx.doi.org/10.21276/ijcmr.2019.6.7.1

afforded in most of resource limited settings.

The World Health Organization (WHO) has endorsed the implementation of GeneXpert MTB/RIF assay for national tuberculosis programs in developing countries. The Xpert MTB is an automated, user friendly and rapid test based on nested real-time PCR assay and molecular beacon technology for MTB detection and Rifampicin resistance. Further on, the technique is not prone to cross-contamination, requires minimal biosafety facilities, provides information about the drug resistance and has a high sensitivity in smear-negative pulmonary TB.[1]

However despite all data available about detection of TB by microscopy and XpertMTB, there is sparse data which show the accuracy of diagnosis of tuberculosis by Gene Xpert in patients. There is limited data on the usefulness of Xpert MTB in the diagnosis of extrapulmonary TB.So we take this potential scope to find out the accuracy of Gene Xpert over smear microscopy in early dectection of tuberculosis in individuals.

Study objectives were to evaluate the diagnostic accuracy of XpertMTB in pulmonary tuberculosis, to evaluate the diagnostic accuracy of XpertMTB in extra-pulmonary tuberculosis and to evaluate the accuracy of XpertMTB compared to smear microscopy in pulmonary and extrapulmonary tuberculosis

MATERIAL AND METHODS

Prospective observational study was done after approval from the Institutional Ethics Committee, for three months (From May 2017 to July 2017) on 139 subjects. The study was conducted in Department of medicine, Department of pulmonology at R.L.Jalappa hospital and research centre, Kolar. TB suspects were based on clinical symptoms (productive cough for more than two weeks, persistent low-grade fever, night sweat and weight loss) and radiological findings consistent with tuberculosis.

Inclusion criteria: Patients suspected for both pulmonary and extrapulmonary tuberculosis were taken for the study.

Exclusion criteria: HIV positive patients and patients who had been treated with anti-TB drugs previously.

Method of data collection

Processing of samples: Two sputum samples (1 spot and 1 morning) were collected and at the same time another sputum sample was collected in falcon's tube. Samples such as pleural fluid, ascitic fluid, cerebrospinal fluid, lymph node biopsy were collected as per symptoms (for extra pulmonary TB).

All samples were subjected to AFB smear, XpertMTB and culture inoculation at RNTCP certified lab

1. Smears were examines by ZN staining and visualization under LED microscope.[10]

2. GeneXpert: Sputum samples are processed directly from falcon tube. Sample reagent is added in a 2:1 ratio to unprocessed sputum in 15 ml falcon tube and the tube is manually agitated twice during a 15minute incubation period at room temperature. 2 ml of the inactivated material is transferred to the test cartridge by a sterile disposable pipette (provided with kits). The interpretation of data from MTB/RIF tests is software based and not user dependent.

3. Culture: Samples were inoculated in Mycobacteria Growth Indicator Tube (MGIT) which is a liquid culture. MTB was identified by colony morphology and growth rate. Culture was taken as the reference standard.

STATISTICAL ANALYSIS

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square test was used as test of significance for qualitative data. Continuous data represented as mean and standard deviation.

Graphical representation of data: MS Excel and MS word is used to obtain various types of graphs such as bar diagram, Pie diagram. p value (Probability that the result is true) of <0.05 is considered as statistically significant after assuming all the rules of statistical tests.

RESULTS

A total of 139 patients were included in the study.72 were males and 67 were females. 99 pulmonary samples and 40extrapulmonary samples were obtained. 93 pulmonary samples and 24 extrapulmonary samples were culture positive. The remaining patients were culture negative, but their clinical history and pathological and/or radiological evidence were indicative of tuberculosis.

Using culture as a reference standard, the sensitivity, specificity, NPV and PPV for smear microscopy and XpertMTB are calculated separately for pulmonary and extrapulmonary samples.

Majority 40.3% of the subjects were between 21-40yrs age group followed by 41-60yrs age group (table-1).

99/139 (71.2%) samples were from suspected pulmonary TB. The rest of the samples were pleural fluid, cerebro spinal fluid, synovial fluid, lymph node aspirate, gastric lavage fluid (table-2).

Smear microscopy has lower sensitivity in diagnosing pulmonary TB (46.2%) as well as extrapulmonary TB(16.6%) but is 100% specific as observed in our study (table-3,4).

Age Group	Frequency	Percent
1-20yrs	27	19.4
21-40yrs	56	40.3
41-60yrs	36	27.7
>60yrs	20	14.4
Total	139	100.0

Table-1: Distribution of subject according to age group

Specimens	Frequency	Percent
Pulmonary	99	71.2
Extra pulmonary	40	28.8
Total	139	100.0

Table-2: Distribution of subject according to Specimens

Smear microscopy		Culture		Total
		Negative	Positive	
Pulmonary Samples	Negative	6	50	56
	Positive	0	43	43
	Total	6	93	99
Extra pulmonary Samples	Negative	16	20	36
	Positive	0	4	4
	Total	16	24	40
Grand total		22	117	139

Table-3: Comparision between smear microscopy and culture

XpertMTB		Culture		Total
		Negative	Positive	
Pulmonary Samples	Negative	5	13	18
	Positive	1	80	81
	Total	6	93	99
Extra pulmonary Samples	Negative	1	6	7
	Positive	15	18	33
	Total	16	24	40
Grand total		22	117	139

Table-4:- Comparing XpertMTB with culture

	Pulmonary samples				Extra Pulmonary samples			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	specificity	PPV	NPV
Smear	46.2%	100%	100%	89.2%	16.6%	100%	100%	55.5%
XpertMTB	86.02%	83.3%	98.7%	27.7%	75%	4%	54.5%	14.2%

Table-5: Comparison between XpertMTB and smear microscopy in pulmonary vs extrapulmonary samples

	Overall			
	Sensitivity	Specificity	PPV	NPV
Smear	40.2%	100%	100%	76.1%
Gene Xpert (total)	83.7%	27.2%	85.9%	24%

Table-6: Overall comparison between XpertMTB and smear microscopy

XpertMTB has a higher sensitivity (86.02%) in pulmonary samples and it is 40% more than the sensitivity of smear microscopy and a specificity of 83.3% in diagnosing pulmonary TB. But it is highly non specific in diagnosing extrapulmonary TB (4% specific) (table-5,6).

DISCUSSION

Smear microscopy in pulmonary TB

The sensitivity of smear microscopy in the diagnosis of pulmonary tuberculosis is 46.2% in our study. Sputum smear microscopy is a simple and an inexpensive tool for diagnosing pulmonary TB but it has low and variable sensitivity. Study done by Bhalla M et al has shown a sensitivity and specificity of 83.1% and 82.4% for LED microscopy.[11] Other study done by Davis J et al has mentioned that a good-quality microscopy of two consecutive sputum specimens has been shown to identify the vast majority (95%-98%) of smear-positive TB patients.[12,13] But a study done by Jean Claude Semuto Ngabonziza et al which had 648 participants showed 51% sensitivity of smear microscopy which is comparable to our study result.[14] Lower sensitivity of smear microscopy in our study is probably due to evaluation of all samples by culture of MTB which is the gold standard test.

Xpert MTB in pulmonary TB

The same pulmonary samples which were subjected to acid fast smear microscopy were tested with Xpert MTB. The sensitivity of Xpert MTB in diagnosing pulmonary TB in our study is 86.02% with a positive predictive value (PPV) of 98.7%.The results Xpert MTB sensitivity of our study are comparable to the WHO statistics which mentions the sensitivity of Xpert MTB for detecting culture-positive samples was 77.7% (56/72).[12] Xpert MTB is reasonably specific (83.3%) in excluding the pulmonary tuberculosis but with a pure negative predictive value. Hence the samples with a strong suspicion of TB which are tested negative by the Xpert MTB must be subjected to culture of MTB.

The sensitivity of Xpert MTB in our study is 39.8% higher than the smear microscopy while in a study carried by Anwar sheed khan et al has shown a sensitivity 40-50% higher than microscopy technique.[15] Our study depicts the same results regarding sensitivity and specificity.

Smear microscopy in extrapulmonary TB

As known, in extra pulmonary TB the likelihood of negativity

of smear study is proved again. Only 4/24 extrapulmonary samples had smear positivity with a sensitivity of 16.6%. Study done by Soumitesh Chakravorty et al also has shown a lower sensitivity of 18.75% (3/16) when extrapulmonary TB samples were tested by smear microscopy.[16]

Xpert MTB in extrapulmonary TB

Sensitivity of XpertMTB in diagnosing extrapulmonary TB in our study is 75% which is in parity with the WHO published report from a pooled sample which has shown a sensitivity of Xpert MTB in diagnosis of extrapulmonary T B as 66.1%.[12] Sensitivity in diagnosing lymph node TB and gastric lavage aspirates were 84.9% and 83.8% respectively in the same meta analysis. Lowest detection rates were from pleural fluid and CSF samples (sensitivity of 43.7% ans 55.5% respectively).[12]

Contrary to the above mentioned sensitivity pattern, in our study the sensitivity rate of Xpert MTB in detecting the disease from the pleural fluid is 75% (9/12 samples), where as smear microscopy detected only3 out of 12 samples and the specificity of XpertMTB in diagnosing the pleural TB is 100%. The above number of 75% which is not in par with the WHO pooled report of 43.7% sensitivity suggests that all pleural fluid samples must be subjected to Xpert MTB for diagnosis of the disease. Xpert MTB has a detection rate of 40% (2/5 cases) of TB in ascitic fluid, 66.7% (2/3 cases) by lymph node aspiration and biopsy. Maximum sensitivity of Xpert MTB in extrapulmonary TB is in tubercular meningitis with a sensitivity of 100% in tested CSF samples. However studies on larger population of extrapulmonary TB are required to boost the evidence of existing data for rapid diagnosis and early treatment initiation by using Xpert MTB. Overall sensitivity of Xpert MTB in our study is 83.7% with a positive predictive value of 85.9%. When Xpert MTB/RIF was used as an initial diagnostic test, it achieved an overall pooled sensitivity of 88% and a pooled specificity of 99% (22 studies, 9008 participants).[12] But the specificity of Xpert MTB is 27.2% contrary to the WHO pooled result.

CONCLUSION

The Xpert MTB is a rapid, sensitive and a reliable diagnostic test for TB than smear microscopy in both pulmonary as well as extra pulmonary TB. Additional information of Rifampicin resistance given by Xpert MTB in short span helps clinicians to decide about the treatment.

All the TB suspect pleural fluids and CSF should be subjected to Xpert MTB as it has shown a significant sensitivity in diagnosing the disease. The diagnosis of extrapulmonary TB should not solely depend on the Xpert MTB and should be subjected to culture of Mycobacterium.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 01-06-2019; **Accepted:** 28-06-2019; **Published:** 07-07-2019