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Research Article

Comparison of ZN Microscopy, Culture on LJ Media and Gene Xpert MTB/RIF Assay in Diagnosis of Extra-Pulmonary Tuberculosis

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Abstract

Tuberculosis is caused by *Mycobacterium tuberculosis* and classified as pulmonary tuberculosis (PTB) and Extra Pulmonary tuberculosis (EPTB). EPTB account for 15-20 % cases of tuberculosis. EPTB diagnosis is challenging due to inadequate sample volume, pauci-bacillary nature and unusual clinical presentation. This study determined the role of Gene Xpert MTB/RIF assay in diagnosis of EPTB and compared results with conventional smear microscopy and culture on LJ media.

All the samples coming to our laboratory during January 2015 to June 2016 were processed with Gene Xpert MTB/RIF assay, Smear microscopy and LJ media culture. Total samples examined were 514. 90(17.51%) samples were positive by Gene Xpert MTB/RIF assay of these 15 samples shows Rifampicin resistance. Samples which were positive by culture and smear microscopy were 58(11.28%) and 28(5.45%) respectively.

To conclude, we can say that Gene Xpert MTB/RIF assay is the novel test for rapid diagnosis of Extra Pulmonary Tuberculosis.

Introduction

Tuberculosis is an ancient disease and it is a global public health problem till date. The disease is caused by *Mycobacterium tuberculosis.* Tuberculosis generally affects lungs, but it can also affect any other organ of body such as bones, lymph nodes, central nervous system, kidneys etc. When tubercle bacilli affects any other part of body, other than the lungs, it is called as Extra Pulmonary Tuberculosis (EPTB). The last decade has witnessed shifting trends in TB infection, with extra pulmonary tuberculosis (EPTB) emerging as an important entity [1]. Extra-pulmonary tuberculosis most commonly seen in immunosuppressed persons and young children. The consequences of some forms of extra pulmonary tuberculosis (EPTB)such as TB meningitis, myocardial TB, may be life threatening, and thus timely diagnosis and initiation of appropriate therapy are crucial [2].

The burden of extra-pulmonary tuberculosis (EPTB) is high, ranging from 15-20 % of all tuberculosis (TB) cases in HIV negative patients, while in HIV positive it is than 50% of all new TB cases [4,5]. The diagnosis of EPTB is challenging due to inadequate clinical sample volumes available and paucibacillary nature of the biological samples and unusual clinical presentation [2,6]. Diagnosis of extra pulmonary tuberculosis(EPTB) is not always possible with conventional methods, due to the long time required and the paucibacillary nature of samples; hence the need of rapid molecular methods [1].

Gene Xpert MTB/RIF assay is a type of CB-NAAT based novel test for the diagnosis of EPTB and simultaneous detection of rifampicin resistance with very high sensitivity. Since its introduction to research settings in 2010, several investigators have tested the accuracy of this test in Non-respiratory samples for the diagnosis of various forms of EPTB. WHO in the year 2013 recommended use of Xpert MTB/RIF assay in the diagnosis of extra pulmonary tuberculosis.9 Isolation of Mycobacterium tuberculosis on LJ media requires 6-8 weeks' time and for drug resistance study it requires 8-12 weeks' time but in case of Gene Xpert the results are obtained in nearly two hours.

In this study we determined the role of Gene Xpert MTB/RIF assay in diagnosis of all forms of EPTB and compared the results with the conventional smear microscopy and culture on LJ media.

Material and Methods

The main aim of the study was to diagnose Extra Pulmonary Tuberculosis cases with simultaneous detection of MDR cases. A study was conducted in a tertiary care hospital on clinical specimens collected over a period of 1 and ½ year, January 2015 to June 2016. All the samples which were sent by the clinician to our laboratory were accepted with fully filled requisition form. The samples was opened in the biosafety cabinet.

Sample collection

Different samples depending upon the site of involvement were collected by physicians as per the WHO guidelines and was accepted at the laboratory at room temperature.

Specimen processing

All the samples received in the laboratory were divided into two parts. One part is used for microscopy and culture and another part for Gene Xpert assay. The Xpert MTB/RIF assay was used directly on CSF specimens and homogenized extra pulmonary specimens (from biopsies of lymph nodes or other tissues) or on decontaminated specimens. Whenever possible, specimens was processed as early as possible and if delay was unavoidable it was stored at 2–8 °C (the maximum time for storage and processing was 7 days).

Microscopy

For microscopy smear were prepared in the biosafety cabinet. Let it air dry and then shifted to the room where ZN staining was to be done. Procedure of ZN staining was done according to the RNTCP guidelines. All the smears were scanned and grading was done according to the guidelines provided by the RNTCP.

Culture

The samples were inoculated on the LJ media in the biosafety cabinet. After inoculation the LJ slopes were incubated at 37, 45 and at room temperature. All slopes were observed for the presence of growth for every day, till first week and then every week till 8 weeks.

Gene Xpert MTB/RIF assay

This test is done for the various samples according to the guidelines available as described below

Lymph nodes and other tissues: For tissue sample processing first of all tissue was cut into small pieces in a sterile homogeniser. Then approximately 2 ml of sterile phosphate buffer (PBS) was added to it and again the solution of tissue and PBS was grinded using homogeniser, until a homogeneous suspension was obtained. Approximately 0.7 ml of the homogenized tissue specimen was transferred with the help of transfer pipette, to a sterile, conical screw-capped tube. (if clumps were still there transferring them would be strictly avoided). To 0.7 ml of homogenized tissue, a double volume of the Xpert MTB/RIF Sample Reagent (1.4 ml) was added by using pipette. The tube was shaken Vigorously 10 to 20 times or vortex for at least 10 seconds. Incubated for 10 minutes at room temperature, and then again specimen was shaken properly. The specimen was incubated at room temperature for an additional 5 minutes. Using a fresh transfer pipette, 2 ml of the processed sample was transferred to the Xpert MTB/RIF cartridge. Following the manufacturer's instructions. The cartridge was loaded into the GeneXpert instrument.

Body fluids: For the body fluids there were no need of decontamination procedure. The sample was directly processed according to the Xpert MTB/RIF instruction manual.

Cerebrospinal fluid (CSF): The preferred processing method for CSF in Xpert MTB/RIF depends on the volume of specimen available for testing. Blood-stained and xanthochromic CSF specimens may cause false-negative results from Xpert MTB/RIF so before processing specimens were carefully observed for gross and if it was blood stained or xanthochromic, specimen was recalled by contacting the treating clinician

Results

All the samples received to the laboratory were processed by all the three methods i.e. ZN Microscopy, Culture on LJ media and Gene Xpert MTB/RIF assay. Total samples received were 514, of which samples positive by Gene Xpert MTB/RIF assay were 90, samples positive by Culture were 58 and samples positive by ZN Smear microscopy were 25 as shown in chart 1.

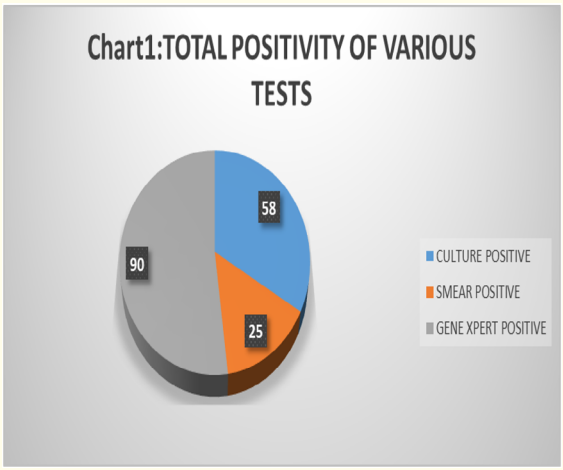

Chart 1

Number of samples positive by gene xpert are highest followed by the culture and then smear. Positivity of Gene Xpert, LJ culture and Microscopy is demonstrated in the table 1.

	Total specimens received in laboratory	Gene xpert positive		Zn smear positive		Culture positive	
		Number	%	Number	%	Number	%
Ascitic fluid	30	2	7%	1	3%	2	7%
Cold abscess	3	3	100%	1	33%	3	100%
CSF	78	9	12%	2	3%	4	5%
Gastric lavage	149	8	5%	2	1%	6	4%
Knee joint fluid	3	1	33%		0%	1	33%
Paracolic abscess	1		0%		0%		0%
Pericardial fluid	9	1	11%	1	11%	1	11%
Pleural fluid	160	35	22%	14	9%	23	14%
PUS	34	21	62%	4	12%	13	38%
Tissue	33	9	27%		0%	4	12%
Urine	14	1	7%		0%	1	7%
Grand total	514	90	18%	25	5%	58	11%

Table 1: Positivity of gene Xpert, LJ culture and ZN smear microscopy in various extra-pulmonary samples.

	Culture result		Grand Total
	Positive	NO growth (NG)	
Gene Xpert positive	58	32	90
Gene Xpert negative	0	424	424
Grand Total	58	456	514

Table 2: Comparison of gene Xpert MTB/RIF assay with LJ culture.

As described in the values of table 2: The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of Gene Xpert as compared to LJ culture were as follows-

(Where A= Gene Xpert as well as culture positive, B = Culture negative, Gene Xpert positive, C = Culture positive, Gene Xpert negative, D = Culture negative, Gene Xpert negative)

Sensitivity: A/(A+B) X 100= 58/58+0 X 100 = 100%

Specificity= D/(D+C) x 100= 424/456 x 100 = 0.929 x 100= 92.9%

Positive predicyive value (PPV) = A (A+B) x 100= 58/90 x 100= 0.644 x 100= 64.4%

Negative predictive value=D/(D+C) x 100= 424/424 x 100= 100%.

	ZN smear microscopy		
	Positive	Negative	Grand Total
Gene Xpert positive	24	66	90
Gene Xpert negative	1	423	424
Grand Total	25	489	514

Table 3: Comparison of gene xpert with ZN smear microscopy

(Where A= Gene Xpert as well as smear positive, B = Smear Negative, Gene Xpert positive, C = smear positive, Gene Xpert negative,D = smear negative, Gene Xpert negative)

Sensitivity = a/(a+b) x 100= 24/25 x 100=0.96 x 100= 96%

Specificity= d/(d+c) x 100= 423/489 x 100 = 0.865 x 100= 86.5%

PPV= a/(a+b) x 100= 24/66 x 100= 0.363 x 100= 36.3%

NPV= d/(d+c) x 100= 423/424 x 100= 0.997= 99.7%

	ZN smear negative		Grand Total
	Culture positive	Culture negative	
Gene xpert positive	34	32	66
Gene xpert negative	0	423	423
Grand Total	34	455	489

Table 4: Comparison of gene xpert with LJ culture in smear negative cases.

Where A= Culture as well as gene Xpert positive, B= Culture negative, Gene Xpert positive, C= Culture positive, Gene Xpert negative, D= Culture negative, Gene Xpert negative)

SENSITIVITY 100%, SPECIFICITY was 92.9%, PPV= 51.5%, NPV= 100%.

	Smear positive		Grand total
	LJ culture positive	LJ culture negative	
Gene xpert positive	24	0	24
Gene xpert negative	0	1	1
Grand Total	24	1	25

Table 5: Comparison of gene xpert with culture in smear positive cases.

Sensitivity = 100%, Specificity = 100%, PPV = 100%, NPV = 100%.

Specimen type	Sensitivity	Specificity
Ascitic fluid	100%	100%
Cold abscess	100%	Not estimable
CSF	100%	93.3%
Gastric lavage	100%	98.6%
Joint fluid	100%	100%
Pericardial fluid	100%	100%
Pleural fluid	100%	91.2%
Pus	100%	61.9%
Tissue	100%	82.7%
Urine	100%	100%

Table 6: Sensitivity and specificities of all the sample types.

Discussion

Extra-pulmonary tuberculosis accounts for 15- 20% of burden of TB globally. Low yield of smear and culture attributed to paucibacillary nature of specimen [7]. Conventional culture methods were very time consuming and require Biosafety setup and trained laboratory personnel. Therefore there was a need for newer and faster diagnostic methods and recent attention has been given to nucleic acid amplification techniques like Gene Xpert (CBNAAT) [8]. The Gene xpert technique enables diagnosis of TB and simultaneous assessment of rifampicin resistance to be completed within 2 hour. The extra advantage is the convenience of sample processing where unprocessed sputum samples as well as clinical specimens from extra pulmonary sites can be directly assayed [9].

In this study we compared the results of Gene Xpert MTB/RIF assay with the LJ culture and ZN smear microscopy both, for diagnosis of Extra-pulmonary tuberculosis. We used LJ culture as reference standard for diagnosis of TB [10-31].

	Our study	Ahmed., *et al.*	Vadwai., *et al.*	Zeka., *et al.*	E.tortoli., *et al.*
Sensitivity	100	77.3	73	100	79
Specificity	92.9	98.2	86	97	97.3

Table a

There were various studies showing different type of positivity among various extrapulmonary samples. Some studies are comparable to our study with slight variations were described below.

In most of the cases the sensitivity ranges from about 52-100% and specificity ranges from 73-100% [11,13,16,22,29-31] the present study results comparable with all these studies.

Overall sensitivity of Gene Xpert MTB/RIF Assay in various studies was as follows

The following table shows the percent positivity by various methods

	Ahemad., *et al.*	Avshia., *et al.*	Imran., *et al.*	Our study
Gene Xpert	37	37	22.5	17.5
ZN Stain	12	36	7.5	4.8
LJ Culture	17	-	10	11.2

Table b

In our study we tested 30 samples of ascitic fluid, 3 samples of cold abscess, and 78 of CSF, 149 gastric lavage, 3 joint fluids, 1 paracolic abscess, 160 pleural fluids, 34 pus, 33 tissue and 14 urine samples. positivity of culture was 7%(2/30), 100%(3/3), 5%(4/74), 4%(6/149), 33%(1/3), 0%, 11%(1/9), 14%(23/160), 38%(13/33), 12%(4/33), 7%(1/14) for ascitic fluid, cold abscess, CSF, gastric lavage, joint fluid, paracolic abscess, pleural fluid, pus, tissue and urine respectively.

Positivity of Gene Xpert was highest in case of aspirate from cold abscess 100% (3/3) followed by pus 62%(21/34), 33%(1/3) joint fluid, 27% (9/33) for tissue, 22%(35/160) pleural fluids, 12%(9/78) CSF, 11% for pericardial fluid, and 5% for gastric lavage. In case of pus samples we found slightly higher sensitivity (62%) as compared to the study by Avashia., *et al*. (56.7%) [10].

A systematic review and meta-analysis of commercial nucleic acid amplification tests for the diagnosis of tuberculous meningitis showed a combined average sensitivity of 56% and specificity of 98% [35]. Lower sensitivity and higher specificity was observed as compared to our study for CSF. The sensitivity and specificity in smear positive and smear negative samples were also analysed in our study and found to be 100% sensitive as well as specific in case of smear positive sample and in smear negative sensitivity is 100% but specificity was 92.9%. In a similar study by Vadwai, the sensitivity of expert assay was 64% for smear negative cases and 96% for smear positive cases with a specificity of 99.6% [30]. In present study we got the higher sensitivity (100%) in smear negative samples than in a study by Vadwai (64%), but slightly lower specificity (92.9%) as compared to (99.6%) Vadwai study.

Sensitivity in smear negative cases varies from 58 to 92 % in most of the studied [22,29,31,36,37]. Overall we observed higher sensitivity.

In present study sensitivity was 100% in all the cases, but specificity varies. In most of the samples overall specificity was above 90% except for two samples pus and tissue in which it was 61.9% and 82.7%. In a another study sensitivity in pus samples was 85.7% and specificity was 94.6% [29] which was higher than in the present study. In case of CSF samples we got the results of specificity comparable to a meta-analysis by Denkinger., *et al*. but higher sensitivity was observed in our study [16]. For pleural fluid results in present study observed sensitivity of 100% and specificity of 91.2% which shows higher sensitivity and slightly lower specificity as compared to other studies.

For reporting of MDR we had done Gene Xpert test and samples positive for rifampicin resistance are reported as MDR. From the total 90 positive by gene Xpert 14 were detected as rifampicin resistant.

Conclusion

We can conclude from the comparison done in this study that Gene Xpert assay is efficient in detecting all types of specimens in extra pulmonary tuberculosis as compared to conventional culture on LJ media and Smear microscopy. From the discussion we can say that we got the higher sensitivity compared to most of the studies, but slightly lower specificity was observed in our study.

The simplicity, sensitivity, speed and automation make this technique an attractive tool for diagnosis of extra-pulmonary specimens and Rifampicin resistance especially in smear negative cases of clinically suspected TB. As diagnosis is compromised in these cases due to low bacterial load, paucibacillary nature and less quantity of specimen. We observed that Gene Xpert gives higher positivity rates as compared to LJ culture and ZN smear with higher sensitivity and specificity. So from this observation we can conclude than Gene Xpert can be used as initial test to diagnose all forms of EPTB specimens. High sensitivity in smear positive cases supports its use in non-respiratory samples in principle [16].

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