WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH
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DIAGNOSTIC UTILITY OF XPERT MTB/RIF ASSAY FOR DETECTION OF MYCOBACTERIUM TUBERCULOSIS AMONG SUSPECTED TUBERCULOUS PLEURAL EFFUSION PATIENTS WHILE TAKING AFB CULTURE AS GOLD STANDARD

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ABSTRACT

Background and aim of study: Tuberculosis is deadliest communicable disease which infects populations globally and it is most common cause of pleural effusions. The aim of the study is to find diagnostic utility of Gene Xpert among suspected tuberculous pleural effusion patients. **Materials:** This study was conducted from January 2018 to October, 2019 in Tertiary Care Hospital of Lahore, Pakistan. 200 pleural fluid specimens were processed for AFB smear microscopy (ZN and Auramine stain), AFB cultures (LJ and MGIT), Xpert MTB/RIF assay and Drug Susceptibility Testing. **Results:** Among 200 pleural effusion patients, 3(1.5%) were positive on AFB smear microscopy, 61(30.5%) were positive on AFB cultures and 17(8.5%) were positive on Gene Xpert. Sensitivity of Gene Xpert and AFB smear was 5% and 20% respectively against AFB culture. 51/200 were positive on liquid culture while 61/200 were positive on solid culture. Contamination rate of liquid culture was more as compared to solid culture. DST was performed on culture positive specimens which was compared with Gene Xpert rifampicin resistance results. **Conclusion:** Due to pucibacilary nature of pleural effusion specimens, conventional methods like AFB smear and culture have low sensitivity. Gene Xpert can be useful for MDR tuberculosis cases for early, accurate diagnosis and it guide for proper treatment. Both conventional and rapid diagnostic modalities should be performed for best isolation of Mycobacterium tuberculosis.

KEYWORDS: TPE: Tuberculous Pleural Effusion, ATT: Anti-Tuberculosis Treatment, MGIT: Mycobacterium Growth Indicator Tube, ZN: Ziehl-Neelsen, LJ: Lewenstein-Jensen, AFB: Acid Fast Bacili.

INTRODUCTION

Tuberculosis is communicable disease caused by bacteria Mycobacterium tuberculosis which belongs to family Mycobacteriaceae. It is health issue globally, 10 million people infected by it and 1.5 million people died in 2016. Pulmonary tuberculosis is common type of tuberculosis but mycobacterium also infects other parts of the body (lymph nodes, abdominal, joints, bones, pleural fluid, meninges etc) that is known as Extra Pulmonary Tuberculosis (EPTB).[1] Among Extra pulmonary tuberculosis tuberculous lymphadenitis is common type, which is about 25% of EPTB cases. Tuberculous Pleural effusion is second most well-known type of EPTB after tuberculous lymphadenitis which accounts for about 1-8% of all EPTB cases. 30% of pulmonary tuberculosis have concomitant with tuberculous pleural effusion. Pleural fluid tuberculosis is 82% of all pleural effusions. Patients with pleural effusion presented in all aged groups with unilateral or bilateral with fever, weight loss, night sweats, chest pain, and fatigue among both male and female.[2]

Accurate and early diagnosis and early treatment reduces morbidity and mortality of patients. For tuberculous pleural effusion diagnosis, conventional methods like AFB smear microscopy and AFB cultures are used in low income countries. Low sensitivity of microscopy is due to pucibacilary nature of pleural fluid.[3] Sensitivities of AFB smear and AFB culture among pleural fluid patients are 10% and 20% respectively. AFB cultures are considered as gold standard but these techniques have

drawbacks like more expertise for performance of tests, turnaround time is 4-6 weeks and Biosafety level III lab. Due to all these drawbacks this is difficult to perform in routine laboratory. With advancements in molecular based tests tuberculosis is still a challenge for accurate diagnosis and treatment especially in EPTB cases.[4]

World Health Organization (WHO) endorsed Xpert MTB/RIF assay for diagnosis of tuberculosis in 2010. It is rapid semi-automated nested PCR based diagnostic test which give result within 2 hours. It detects rpoB gene mutation by different types of probes and also tells about rifampicin resistance. It does not require any expertise for performance of assay.[5] Gene Xpert has good diagnostic accuracy for pulmonary samples while in case of EPTB its accuracy is "Off-Label". Pulmonary tuberculosis diagnosis and treatment is improved after invention of Gene Xpert. In case of pleural fluid there is limited data available to evaluate diagnostic accuracy of Gene Xpert. Limitations from previous published includes pucibacilary nature of pleural fluid, less number of patients of pleural fluid, presence of inhibitors of PCR in specimens, analysis with other markers and less available data. Previous data showed sensitivity of gene xpert ranged 25-97%.[6]

Due to all above limitations this study was conducted to evaluate diagnostic accuracy of gene xpert for isolation of Mycobacterium tuberculosis complex among suspected tuberculous pleural effusion patients while taking AFB culture taking as gold standard in tertiary care hospital of Lahore, Pakistan.

MATERIAL AND METHODS

Study design and setting

A diagnostic accuracy study was conducted from January 2018 to October 2019. Patients were enrolled from indoor and outdoor department of tertiary care hospital of Lahore, Pakistan.

Study population
Inclusion criteria: 1) patient with age > 18 2) both male and female were included 3) suspected tuberculous pleural effusion on clinically, radiographically and exudative effusion 4) consent was taken from patients which were enrolled.

Exclusion criteria: 1) previously treated patients with ATT 2) procedural contraindication for pleural biopsy.

Screening and Recruitment of patients
After informed written consent of patients who have clinically and radiographically suspicion of tuberculosis underwent thoracentesis for removal of pleural fluid for further analysis. Exudative pleural fluid was enrolled in the study. Exudative pleural fluids in the present study was those fluids which had LDH level > 200 IU/L. At the time of enrollment of patients study specific demographic and physical examination was taken. After

procedure pleural fluid was sent quickly to microbiology and mycobacteriology department of the hospital for further processing.

Laboratory procedures
AFB microscopy
After centrifugation, slides for ZN staining and Auramine staining were prepared by deposit of the fluid. After staining with ZN stain, AFB were seen on 100 X lens of microscope while for Auramine staining florescent microscope was used to see AFB at 40X lens.

AFB culture
By using modified Petrof's method, specimens for culture were processed and inoculated on LJ media. Media was incubated at 37 degree for 6-8 weeks. After every week media was checked for growth till 8th week. For liquid culture, after processing sample was inoculated in MGIT tubes and incubated in MGIT machine. MGIT machine automatically detects growth and give results.

Drug Susceptibility testing
Positive cultures underwent phenotypic drug susceptibility testing which was performed by standard protocols.

Gene Xpert testing
Deposit after culture inoculations was mixed with gene Xpert buffer in 1:2 dilutions and put it for 15 minutes after proper mixing. After 15 minutes, cartridge was filled with 2 ml of solution and it is loaded in Gene Xpert machine. Machine gave result within 2 hours after loading sample cartridge into it. Its result was considered as positive if MTB was detected. Indeterminate results were repeated before final report.

Statistical analysis
Data was analyzed by using SPSS 20. Chi square test, sensitivity, specificity, positive predictive value, negative predictive value, p value and ROC curve was calculated by using this software. Overall diagnostic accuracy was calculated by using AUC curve who's Confidence Interval was 95%.

Ethical considerations
Study was approved by Institutional Ethical Review Committee of hospital. Informed written consent was taken from patients before enrollment in the study.

RESULTS

Patient's baseline investigations

Out of 250 patients of pleural effusion, 230 gave written consent. 200/230 was exudative fluids therefore included in the study. Different characters of 200 patients are summarized in table # 1. 70% were male and 30% were female. Mean age (±SD) of patients enrolled was 33 ± 10. Mostly patients with tuberculous pleural effusion have fever, weight loss, cough, night sweats and chest

pain as shown in table # 1. Majority of patients were non-smokers (85%) and non-alcoholic (66%). Radiological examination showed 120 patients with right sided effusion, 70 left sided and 10 patients with both sided effusion (Table # 1). Hematological examination showed 72% cases with lymphocytes predominant as compared to neutrophils and other cells (Table # 1).

Table # 1: Different characters of enrolled patients.

Sr #	Characteristics	N (%)
General Characters		
1	Male	140 (70%)
2	Female	60 (30%)
3	Age (SD)	33 ± 10
Symptoms of TPE patient		
4	Fever	140 (70%)
5	Weight loss	150 (75%)
6	Cough	170 (85%)
7	Night sweats	130 (65%)
8	Chest pain	160 (80%)
9	Mean weight (SD)	55 (±7)
History of patients		
10	Alcohol	68 (34%)
11	Smoking	30 (15%)
Hematological Examination of TPE patients		
12	Lymphocytes	148 (74%)
13	Neutrophil	44 (22%)
14	Other cells	8 (04%)
Radiological Examination		
15	Right side	120 (60%)
16	Left side	70 (35%)
17	Both sides	10 (5%)

TPE: tuberculous pleural effusion, N: number of patients, SD: Standard deviation

AFB smears and Culture results
Sputum was collected from all enrolled patients. 1/200 was positive on sputum for AFB smear microscopy examination. Giemsa staining was performed on slides prepared after centrifugation. Giemsa staining results predominantly showed lymphocytes 92% of cases while RBCs were seen in 40% of cases (Table # 2). 2 patients were positive on ZN staining while on fluorescent staining 3 were positive. Solid culture has less positivity as compared to liquid culture for isolation of mycobacterium tuberculosis. 51/200 was positive on LJ media while 61/200 was positive on MGIT media. There was more contamination (8/200) on liquid media as compared to solid media. Liquid media give results earlier than solid media(Table # 6). Drug susceptibility testing was performed on culture positive isolates which showed MDR 2 cases, Poly-R 2 cases, Pre-XDR 2 cases, Pan-S 57 cases (Table).

Sensitivity and specificity of AFB smear against AFB culture was 4.9% and 100% respectively while PPV and NPV was 100% and 70.6% respectively (Table #7).

Gene Xpert Results
17/200 was MTB detected on Xpert MTB/RIF assay. Mostly 172/200 was MTB not detected (Table # 2). There was high rate of errors in pleural effusion cases which was seen in 8 cases while 3 cases were invalid on assay. Out of 17 MTB detected cases 14/17 was VL (very low), 2/17 was M (medium) and 1/17 was L (low) on quantitative analysis by assay(Table # 3). Rifampicin resistance was detected in 2 cases while 1 case was indeterminate for detection of RIF resistance. 12/17 cases were rifampicin resistance not detected on Xpert MTB/RIF assay(Table # 4). We compared gene xpert rifampicin resistance results with DST results. 2 cases which were MDR on DST were also rifampicin resistant on Xpert result.

Sensitivity and specificity of Gene Xpert against AFB culture was 19.6% and 96.4% respectively while PPV and NPV was 70.6% and 73.2% respectively (Table # 7).

Table # 2: Xpert MTB/Rif Assay Results (Qualitative).

	Frequency	Percent	Valid Percent	Cumulative Percent
error	8	4.0	4.0	4.0
Invalid	3	1.5	1.5	5.5
MTB+	17	8.5	8.5	14.0
MTBND	172	86.0	86.0	100.0
Total	200	100.0	100.0	

Above table showing qualitative results of Gene Xpert.

Table # 3: Xpert MTB Rif Assay Results (Quantitative).

	Frequency	Percent	Valid Percent	Cumulative Percent
L	1	.5	.5	.5
M	2	1.0	1.0	1.5
NA	183	91.5	91.5	93.0
VL	14	7.0	7.0	100.0
Total	200	100.0	100.0	

This table showing quantitative results of Gene Xpert. L: low, M: medium, VL: very low, NA: not applicable.

Table # 4: Gene Xpert Rifampicin Resistance Results.

	Frequency	Percent	Valid Percent	Cumulative Percent
IND	1	.5	.5	.5
NA	183	91.5	91.5	92.0
ND	2	1.0	1.0	93.0
RR+	2	1.0	1.0	94.0
RRND	12	6.0	6.0	100.0
Total	200	100.0	100.0	

Above given table showing rifampicin resistance results by Gene Xpert. IND: indeterminate, NA: not applicable, ND: not detected, RR+: rifampicin resistance detected: RRND: rifampicin resistance not detected.

**Table # 5: Drug Susceptibility Testing Profile.**

	Frequency	Percent	Valid Percent	Cumulative Percent
MDR	2	1.0	1.0	1.0
Mono_R (INH)	2	1.0	1.0	2.0
Mono_R (OFX)	1	.5	.5	2.5
Mono_R (STREPT)	3	1.5	1.5	4.0
NA	130	65.0	65.0	69.0
NTM-DST_ND	1	.5	.5	69.5
Pan-S	57	28.5	28.5	98.0
Poly-R	2	1.0	1.0	99.0
Pre-XDR	2	1.0	1.0	100.0
Total	200	100.0	100.0	

This table showed DST findings of culture positive cases. MDR: multi drug resistant, INH: isoniazid, OFX: ofloxacin, STREPT: streptomycin, R: resistant: XDR: extended drug resistant.

Table # 6: Comparison of solid culture with liquid culture.

		culture L J		Total
		Negative	Positive	
culture MGIT	Contamination	7	1	8
	Negative	120	11	131
	Positive	22	39	61
Total		149	51	200

Liquid cultures positivity was higher than solid culture but its contamination rate was also high.
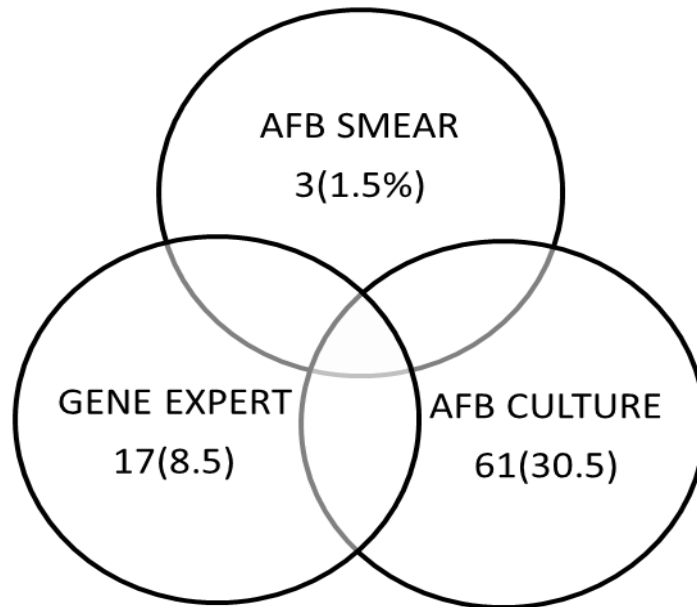
Figure # 1: Ven diagram of microbiological investigations showing positive results for tuberculosis.
This diagram shows positive cases for AFB smear, AFB culture and Gene Xpert of isolation of Mycobacterium tuberculosis.

Table # 7: Diagnostic accuracy of AFB smear and Gene Xpert against Mycobacterial culture taking as gold standard.

| | | AFB Culture | | Total | p-value | Sensitivity | PPV | Overall diagnostic |
		Negative	Positive			Specificity	NPV	Accuracy (95% CI)
AFB Smear	Smear negative	149	48	197	0.003	5.9	100	76
	Smear positive	0	3	3				
	Total	149	51	200		100	75.6	
Gene Xpert	MTB not detected	141	42	183	0.007	17.6	52.9	75
	MTB detected	8	9	17		94.6	77.0	
	Total	149	51	200				

P value < 0.05 is significant. Diagnostic accuracy was calculated by AUC (area under curve) while taking confidence interval 95%. PPV: positive predictive value, NPV: negative predictive value, CI: confidence interval.
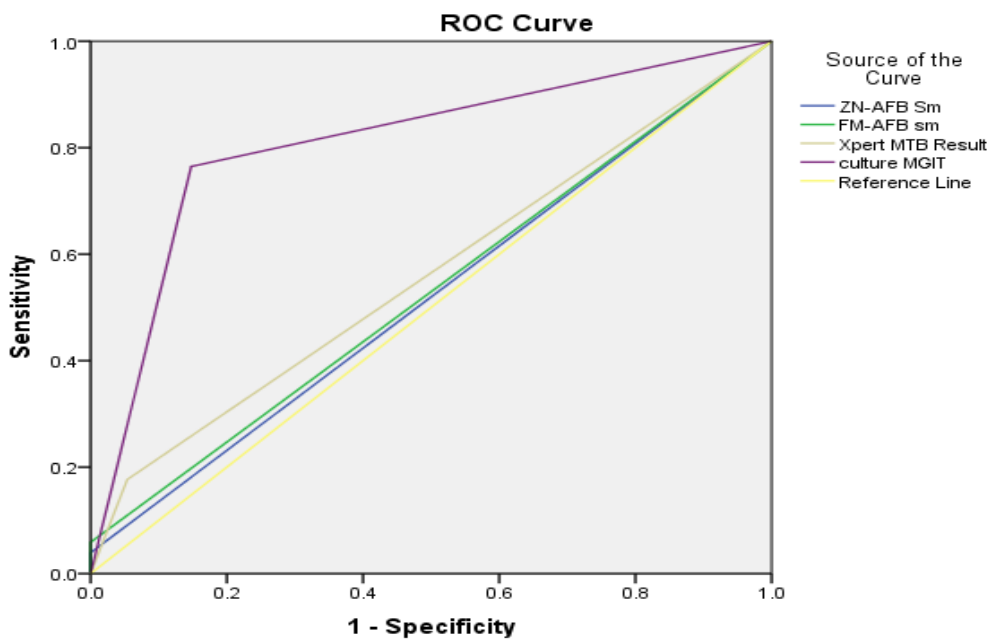
Diagonal segments are produced by ties.

Area Under the Curve					
Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
ZN-AFB Sm	.520	.048	.676	.426	.613
FM-AFB sm	.529	.048	.531	.435	.624
Xpert MTB Result	.561	.049	.191	.466	.657
culture MGIT	.809	.038	.000	.733	.884
The test result variable(s): ZN-AFB Sm, FM-AFB sm, Xpert MTB Result, culture MGIT has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.					
a. Under the nonparametric assumption					
b. Null hypothesis: true area = 0.5					

DISCUSSION

Tuberculosis is endemic in Pakistan because its diagnosis remains challenge due to number of factors. EPTB also have high morbidity and mortality so its early and accurate diagnosis is important for further treatment of patients. There is limited data available for tuberculous pleural effusion diagnostic validity. Tuberculous pleural effusion is difficult to diagnose due to standard criterion (presence of lymphocytes on exudative fluids, AFB smear positive of fluid, AFB culture positive and Xpert MTB detected). The present study highlighted the diagnostic utility of Gene Xpert of diagnosis of pleural effusion in terms of sensitivity, specificity, PPV, NPV and diagnostic accuracy against AFB culture taking as gold standard. AFB smear diagnostic accuracy was also calculated in this study.

Most common presenting symptoms in our study are non-productive cough (85%), chest pain (80%), fever (70%), weight loss (75%) which is also reported in other studies.[7,8] In the present study, radiological examination showed left sided effusion (35%), right sided effusion (60%) and both sides effusion (5%) which is also agrees with literature.[7] In our study, males are commonly infected as compared to females and mean age of patients is 33; these findings are concomitant with other study of India.[7]

ZN staining detects AFB in less than 10% of cases because its bacillary densities are 10,000 AFB/ml of fluid.[8] In this study AFB smear microscopy was positive in 3.1% of cases. Sensitivity of AFB culture rages from 12% to 70% because it detects 10-100 viable cells in fluid.[8] In our study, AFB culture was positive on 30.5% of cases which is also in acceptable rage of literature.[7,8] Solid culture has less positivity as compared to liquid culture for isolation of mycobacterium tuberculosis. 51/200 was positive on LJ media while 61/200 was positive on MGIT media. There was more contamination (8/200) on liquid media as compared to solid media. Liquid media give results earlier than solid media In case of AFB microscopy, sensitivity of AFB smear was 5% and specificity was 100% against AFB culture as gold standard which is same as in study of Spain.[9,10]

There is less data available for pleural effusion on Gene Xpert. In this study, specificity of Xpert MTB/RIF assay is higher than sensitivity when compared with culture taking as gold standard, this is similar to other studies in literature.[11] Pleural effusion commonly due to tuberculosis[12-17], this present study also showed this. In the present study exudative pleural effusion was seen in most of cases which also agrees with literature.[13,18,19] In this study, hematological examinations show mainly lymphocytes (74% of cases) and neutrophils (22% of cases). Literature showed sensitivity of gene xpert ranges 0-100% (16), while the present study has sensitivity of 19.2%. Specificity of gene xpert in this study was 96.4%, this is similar to literatures specificity.[10,20]

Table # 8: Sensitivity and specificity of Gene Xpert in previous studies.

year	TP	TN	FP	FN	Sensitivity	Specificity	Study Reference
2014, 2017	9	37	0	4	69%	100%	[10,21]
2015, 2017	25	94	0	7	78%	100%	[9,10]
2015	23	120	0	19	55%	100%	[10,18]
2014, 2012	0	30	0	2	33%	100%	[20,22]
2014, 2011	5	19	0	5	50%	100%	[20,23]
2014, 2012	5	312	3	10	0%	99%	[20,24]
2014, 2013	5	34	0	28	15%	100%	[20,25]
2014, 2013	4	61	0	26	13%	100%	[20,26]
2014, 2011	5	5	0	15	25%	100%	[20,27]

CONCLUSION

Due to pucibacilary nature of pleural effusion specimens, conventional methods like AFB smear and culture have low sensitivity. Gene Xpert can be useful for MDR tuberculosis cases for early, accurate diagnosis and it guide for proper treatment. Both conventional and rapid diagnostic modalities should be performed for best isolation of Mycobacterium tuberculosis.

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