Detection of tuberculosis by Xpert MTB/RIF assay in exudative lymphocytic pleural effusion

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ABSTRACT

INTRODUCTION: Pleural tuberculosis is a common type of extrapulmonary tuberculosis and it is a frequent cause of pleural effusion. Xpert MTB/RIF assay has been used to detect tuberculosis in aspirated pleural fluid, with variable sensitivities. In Iraq, tuberculosis is still a common cause of exudative pleural effusion. Pleural biopsy and cytological examination of the pleural fluid are the most helpful diagnostic investigations.

OBJECTIVE: To evaluate the role of Xpert MTB/RIF assay for the diagnosis of tuberculosis in exudative lymphocytic pleural effusion.

METHODS: A retrospective study was conducted at the Specialized Centre for Chest and Respiratory Diseases in Baghdad from 1 January to 30 August of 2013. It is based on the records of 52 patients diagnosed as having exudative lymphocytic pleural effusion samples. Data were extracted from the records patients were grouped into TB and non-TB groups based on the results Bactec culture and histopathological examination. The results of Xpert MTB/RIF assay were compared to that of Bactec culture and histopathological examination in order to define the accuracy measures of it.

RESULTS: Mean age of study patients was 45.3±17.7 years with male to female ratio 1.73:1. Xpert MTB/RIF assay was positive for 36.5% of the patients and negative for 63.5% of them, while Bactec and Histopathological finding revealed that half of the patients had TB pleural effusion. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of Xpert MTB/RIF assay test were 58%, 85%, 79%, 67% and 71%, respectively. There was a significant difference regarding the validity characteristics of Xpert MTB/RIF assay between males and females ($p < 0.05$).

CONCLUSION: Xpert MTB/RIF assay of pleural fluid specimen is a useful test for diagnosis of pleural tuberculosis. It is highly specific, but low sensitive test in the diagnosis of TB pleural effusion. A negative Xpert MTB/RIF assay finding does not rule out the diagnosis of pleural TB.

Key words: Xpert MTB/RIF, Tuberculosis, Pleural effusion.

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INTRODUCTION

Tuberculosis remains an important cause of morbidity and mortality worldwide. The estimates show that 8 - 10 million new tuberculosis (TB) cases occur each year in the world and 2 - 3 million die.[1]

Tuberculosis is classified as pulmonary, or extra pulmonary.[2] The increase in tuberculosis in the world and the emergence of multidrug-resistant strains has prompted more rapid and sensitive methods in the laboratory diagnosis than previous conventional diagnostic techniques. Pleural Tuberculosis is a common manifestation of extra-pulmonary tuberculosis and is a frequent cause of pleural effusion.[3] Tuberculous pleural effusion (TPE) occurs in up to 30% of tuberculosis patients.[4] An increasing proportion of pleurisy with effusions occurs in older individuals with chronic pulmonary TB, often with complicating comorbid illnesses such as cirrhosis, malignancy and congestive heart failure to which the effusion is mistakenly attributed.[5]

In Iraq, whenever pleural effusion is seen in

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an adolescent or young adult the most likely cause is tuberculosis6. Malignancy, whether pulmonary, metastatic, or of lymphatic origin, may be becoming an increasingly common cause of Pleural effusion in Iraq. Pleural biopsy and cytological examination of the pleural fluid were the most helpful diagnostic investigations available.[6]

The diagnosis of tuberculous pleural effusion is made by identifying the existence of tubercle bacilli in the sputum, the pleural fluid, or the pleural biopsy specimen, or by demonstrating the existence of granulomas in the pleura.[7] However, there are several difficulties in the diagnosis of TB, such as specimen collection, low sensitivity of detection method and the time delay in waiting for culture results. Studies on the pathogenesis indicate that only a few organisms may gain access to the pleura in the early stage of tuberculous infection, and cause a hypersensitivity response in the presence of cell-mediated immunity.[8] Therefore, the number of organisms in pleural fluid for tuberculous pleural effusion is very small. In the absence of concurrent pulmonary TB, the diagnosis of pleural TB requires thoracentesis and even pleural biopsy.[9] Diagnosis of pleural tuberculosis (TB) remains a challenge due to its nonspecific clinical presentation and paucibacillary nature. Conventional methods, such as direct testing for acid-fast bacilli (AFB) and culture of pleural fluid, lack sensitivity (less than 5% and 40%, respectively).[10]

New molecular diagnostic tools have been developed to improve tuberculosis detection and decentralize drug resistance testing.[11] Over the past several years, there has been a significant increase in using the Xpert MTB/RIF assay (also referred to as Xpert; Cepheid Inc., USA), which is an automated, cartridge-based nucleic acid amplification test for TB. This assay has the ability to simultaneously detect M. tuberculosis nucleic acid and resistance to rifampin (RIF) in less than 2 h. Due to its excellent performance, this assay has been recommended by WHO for diagnosing TB and detecting rifampicin resistance in pulmonary and extra-pulmonary TB in adults and children as well as for initial screening of individuals suspected of having multiple drug resistant-Tuberculosis (MDR-TB).[12]

The Xpert MTB/RIF assay is an automated nucleic-acid amplification test. It consists of a single-semultichambered cartridge preloaded with liquid buffers and lyophilized reagent beads that are required for sample processing, DNA extraction, and hemi-nested real-time polymerase chain reaction. The assay can be used with sputum samples, and, with varying sensitivity, with other specimens including cerebrospinal fluid, lymph node tissue aspirates, pleural fluid, ascetic fluid, urine, dialysis fluid, and pus.[11] The assay can be performed at peripheral laboratories or health facilities without biosafety cabinets, and minimal training for laboratory staff is required. Xpert MTB/RIF can detect Mycobacterium tuberculosis complex (MTBC) and rifampicin resistance within two hours.[13]

Tuberculous pleural effusion presents a diagnostic problem due to the limitations of traditional diagnostic methods.[11] We conducted this study to assess the diagnostic accuracy of Xpert MTB/RIF as an initial diagnostic test for Tuberculous pleural effusion.

The aim of this study was to evaluate the role of Xpert MTB/RIF assay for the diagnosis of TB pleural effusion.

METHODS

Setting and Study Design: This retrospective study was carried out from first of January to end of August, 2013. The data were collected from the Specialized Centre for Chest and Respiratory Diseases in Baghdad.

Ethical Consideration: The study protocol was approved by the local committee of the department of medicine at the Medical college of Baghdad.

Definition of the cases, inclusion and exclusion criteria: We reviewed all the records at the Specialized Centre for Chest and Respiratory Diseases who were referred during the studied period. All Iraqi patients who have exudative lymphocytic pleural effusion patients as diagnosed by examination of pleural fluid aspirates were included in this study. Patients who were fulfilling the diagnosis and their records were incomplete or were having heart failure, renal failure and chronic liver diseases were excluded from the study.

The procedure: we prepared a data retrieving form to extract the data from the records. The form has included the following domains:

1. Demographic information: Age and gender.
2. The results of Xpert MTB/RIF.
3. Results of Bactec culture.
4. Results of histopathological examination.

The gold standard diagnostic tests in the present study were Bactec culture and Histopathological examination. Patients who were positive for TB on Bactec culture and or histopathology were considered as TB cases, those who did not were considered as non-TB cases.

The PCR test that used in the laboratory of Specialized Chest and Respiratory centre in Baghdad is the GeneXpert Dx System which integrates and automates sample processing, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR and reverse transcriptase PCR. The system consists of an instrument, personal computer, barcode scanner, and preloaded software for running tests on collected samples and viewing the results. Xpert MTB/RIF includes reagents for the detection of tuberculosis and RIF resistance as well as a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The Bactec culture is a liquid culture which is more sophisticated equipment, faster detection of growth, higher sensitivity than solid media and can also be used for drug susceptibility test. Histopathological examination was done by an expert consultant histopathologist at the lab of Early Detection of Tumour centre in Baghdad.

For pleural TB, culture of pleural fluid has low sensitivity (on average, 30–50%). Culture specificity is 100% if the presence of Mycobacterium tuberculosis complex is confirmed with antigen tests or nucleic acid amplification tests (NAATs). Often, biopsy with culture and histopathological examination is necessary to achieve a diagnosis.[14] studies evaluated Xpert in pleural fluid versus culture. The pooled sensitivity was 46.4% (95% CI 26.3–67.8%) and the pooled specificity was 99.1% (95% CI 95.2–99.8%).[11]

Statistic: Data were entered and analysed by using statistical package for social sciences (SPSS), version 20. Sensitivity, specificity, accuracy, and predictive values of positive and negative results were calculated for the Xpert MTB/RIF assay in comparison to Bactec and histopathology. Chi square test was used to assess the significance of association between variables in study. Level of significance of ≤ 0.05 was considered as significant.

Table 1: General Characteristics and findings of patients

Characteristics		No.	%
Gender	Male	33	63.5%
	Female	19	36.5%
Age(years) mean±SD (45.3±17.7)	≤30	13	25.0%
	31-40	11	21.2%
	41-50	8	15.4%
	51-60	10	19.2%
	>60	10	19.2%
PCR	Positive	19	36.5%
	Negative	33	63.5%
Final diagnosis	TB	26	50.0%
	Non-TB	26	50.0%

RESULTS

There were 52 patients enrolled in this study, they were 33 males (63.5%) and 19 females (36.5%) with a male to female ratio of 1.73: 1. The mean age of patients was (45.3 ± 17.7) years with a range of (11-84) years, further categorization of age into 5 age groups revealed that 13 (25%) patients were aged ≤ 30 years, 11 patients (21.2%) aged 31- 40 years, 8 patients (15.4%) aged 41-50 years, 10 patients (19.2%) aged 51-60 years and 10 patients (19.2%) aged > 60 years. Patients who were positive for TB on PCR were 19 vs. 33 were negative, on Bactec culture and histopathology, TB cases were 26 (50%) and the non-TB cases were 26 (50%). These findings were shown in table 1.

Table 2: Distribution of PCR results among studies group according to gender and age group

Demographic features		Positive(N=19)		Negative(N=33)		P
		No.	%	No.	%	
Gender	Male	12	63.2	21	63.6	0.97 NS
	Female	7	36.8	12	36.4	
Age group	≤30	3	15.8	10	30.3	0.614 NS
	31-40	6	31.6	5	15.2	
	41-50	3	15.8	5	15.2	
	51-60	4	21.1	6	18.2	
	>60	3	15.8	7	21.2	

Table 3: Distribution of findings of final diagnosis among studied group according to gender and age groups.

Variable		Final diagnosis				P value
		TB (N=26)		Non-TB (N=26)		
		No.	%	No.	%	
Gender	Male	17	65.4	16	61.5	0.77 NS
	Female	9	34.6	10	38.5	
Age groups	≤30	7	26.9	6	23.1	0.89 NS
(years)	31-40	5	19.2	6	23.1	
	41-50	5	19.2	3	11.5	
	51-60	5	19.2	5	19.2	
	>60	4	15.4	6	23.1	

Table 4: Validity of PCR versus Final diagnosis in detection of TB cases among studied groups

		Final diagnosis		Total
		TB	Non-TB	
PCR	Positive	15	4	19
	Negative	11	22	33
Total		26	26	52
Validity parameters of PCR versus final diagnosis				
	Sensitivity	58%		
	Specificity	85%		
	Accuracy	71%		
	PPV	79%		
	NPV	67%		

PPV: positive predictive value.
NPV: negative predictive value.

Table 2 shows the distribution of PCR findings according to age and gender, no statistically significant association between positive PCR results and both genders or age groups , P value > 0.05.

In table 3, the distribution of TB and non-TB cases, according to final diagnosis (histopathology and Bactec culture), revealed no significant association was found between TB cases and gender or age, P>0.05.

The comparison of PCR versus final diagnosis was demonstrated in table 4, it had been found that PCR was correctly identified as positive 15 of the 26 TB cases, giving a sensitivity of 58%, on the other hand, PCR correctly identify as negative 22 of the 26 non-TB cases giving a specificity of 85% and the accuracy was 71%.As the PCR erroneously labelled 4 cases of non-TB cases as positive (false positive) and 11 cases of TB cases as negative (False negative) so the PPV and NPV were 79% and 67% respectively.

Furthermore, the comparison of PCR versus final diagnosis according to gender, revealed significantly that PCR in males had higher sensitivity, specificity, and accuracy than females, 65% , 94% and 79% versus 44%, 70% and 58% respectively, in all comparison P<0.05, table 5.

CONCLUSIONS

TB is considered as a major cause of disability and death in most of the world, especially developing countries.[14] In recent times, attention has been devoted to new nucleic acid amplification diagnostic technologies, owing to their rapidity, sensitivity, and specificity.[15] Mycobacterial culture takes at least 2 weeks to perform, depending on the culture media that is used.[16] Previous studies have shown highly variable results regarding the usefulness of PCR because of the use of different PCR methods, small study populations, and diverse criteria for pleural tuberculosis.[17] In the present study, the demographic characteristics of the studied patients revealed the predominance of male gender (male to female ratio was 1.73:1) and most of the patients were middle aged. This demographic picture of our study is consistent with results of Brazil study (2003-2005).[18]

Table 5: Validity of PCR versus final diagnosis in detection of TB cases according to gender

Gender			Final Diagnosis		Total	Sensitivity	Specificity	Accuracy	PPV	NPV
			TB	Non-TB						
Male	PCR	Positive	11	1	12	65%	94 %	79 %	92 %	57 %
		Negative	6	15	21					
	Total		17	16	33					
Female	PCR	Positive	4	3	7	44 %	70%	58%	57 %	58 %
		Negative	5	7	12					
	Total		9	10	19					
					P value	0.004	0.001	0.002	0.001	0.5

PPV: positive predictive value.
NPV: negative predictive value.

The Xpert MTB/RIF assay was positive in about one-third of all the studied cases and negative in about two-thirds of them. However, the PCR test was positive in about 58% of those cases which were confirmed to had TB by Bactec culture or histopathological examination. This finding is higher than that of a Systematic Review and Meta-analysis study in which a pooled sensitivity of Xpert MTB/RIF was 51.4% with culture used as a reference standard.[19] Final diagnosis of studied patients revealed that about two-thirds of TB patients were males and about one-third of them were females with highest proportion of them in age group less than thirty years of age (active age group). This demographic picture of TB is like results reported in previous Iraqi literatures.[20] In this study, there was no significant association regarding age and gender with results of PCR test or the final diagnosis. This finding is inconsistent with results reported by Colombian study (2000).[21] This inconsistency might be attributed to small sample size of present study.

Our results demonstrated Xpert MTB/RIF assay with relatively low sensitivity of 58% when TB cases are defined based on laboratory confirmation using Bactec culture or histopathological examination. Although we have found higher PCR sensitivity than other studies evaluating similar clinical specimens and target nucleic acid sequences for amplification,[17,22,23] our results corroborate the suboptimal sensitivity as the main limitation of PCR testing for replacing conventional diagnostic tests for diagnosis of pleural TB.[17] Low sensitivity values can be explained by the low bacillary load and the presence of substances that inhibit amplification in pleural fluid.[24] The specificity of Xpert MTB/RIF assay in our study was high (85%) which is within acceptable results reported by other literatures as in South Korean study (2005).[17] In more other studies using pleural fluid, PCR has a sensitivity of 70% and specificity of 100% by Indian study (2001)[24] and 50% sensitivity and 61% specificity by Thailand study (2000).[25] The accuracy of PCR test reported by our study was 71% that is consistent with results of Switzerland study (2011).[15] The positive and negative predictive value (PPV) and (NPV) in this study were 79% and 67%, respectively, which is close to results of Indian study (2013).[26] Although the best sensitivity was achieved by combining the results of culture and histopathology, the prompt confirmation of tuberculous pleuritis in about 58% of the patients using the PCR test would allow early treatment initiation. Rapid diagnosis and initiation of chemotherapy are essential to prevent secondary fibrothorax and to avoid subsequent pulmonary or extrapulmonary TB development.[27] In the present study, there was a significant difference in accuracy, sensitivity and specificity of PCR test between male and female ($p < 0.05$).This finding is consistent with results of South Korean study (2002)[28] and it is not a barrier of PCR test use but it might be attributed to predominance of male gender among TB.

CONCLUSIONS

Xpert MTB/RIF assay from pleural fluid specimen is a useful and minimal-invasive additional test for fast diagnosis of pleural TB. This assay is highly specific but with less sensitivity for the diagnosis of TB pleural effusion. A negative PCR result does not exclude the diagnosis of pleural TB

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