Role of GeneXpert in extrapulmonary tuberculosis

Ahmed M. Azzouz[a], Raafat T.I. El-Sokkary[a], Lamiaa S. Wafi[b], Mohammad G.A. Khalaf[a]

[a]Department of Chest Diseases, Faculty of Medicine, Assiut University, [b]Department of Chest Diseases, Assiut Chest Hospital, Assiut, Egypt

Correspondence to Mohammad G.A. Khalaf, MD, Department of Chest Diseases, Faculty of Medicine, Assiut University, Assiut 71515, Egypt
Tel: +20 882 413 709/20 882 413 509/20 102 660 0071; fax: +20 882 333 327;
e-mail: dr.mga2011@aun.edu.eg

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Background
Tuberculosis (TB) commonly affects the lung (pulmonary TB). Extrapulmonary TB (EPTB) can involve many systems. Diagnosis is often difficult. EPTB may be misdiagnosed with many other inflammatory, granulomatous, and neoplastic disorders. GeneXpert mycobacterium tuberculosis /rifampicin is a valuable test in diagnosis of pulmonary TB. There is limited research about its diagnostic role in EPTB.
Patients and methods
The aim of this study was to assess the diagnostic value of GeneXpert in EPTB. Samples from EPTB cases were sent for standard mycobacterial culture and GeneXpert assay. The sensitivity and specificity of GeneXpert were calculated.
Results
A total of 100 patients were included. Overall, 61 cases were definitely diagnosed as EPTB, whereas 39 cases were negative according to culture. GeneXpert was positive in 40 cases, which represent 65.6% of EPTB cases, and negative in 21 (34.4%) cases. Sensitivity and specificity of GeneXpert was 65.6 and 97.4%, respectively. The positive predictive value and negative predictive value were 97.4 and 64.4%, respectively.
Conclusion
GeneXpert is a rapid, valuable tool in diagnosis of patients with EPTB. It is a highly specific test with fair sensitivity. A multicenter study with larger sample size is needed to evaluate the diagnostic role of GeneXpert in different sites of EPTB.

Keywords:
extrapulmonary, GeneXpert, tuberculosis

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Introduction

Tuberculosis (TB) remains a global health problem. Worldwide, approximately one-third of global population is infected with TB. TB represents a great challenge, especially in developing countries. Global incidence of TB was ~7.1 million in 2019. Case notifications were markedly decreased in 2020 to 5.8 million cases affected by COVID-19 pandemic. Mortality from TB increased in 2020 to 1.3 million deaths among HIV-negative patients [1].

Acid-fast bacilli microscopy has very low sensitivity in extrapulmonary TB (EPTB) as well as in HIV-positive patients. Approximately 2000–10 000 bacilli are needed for a positive result [2]. Histopathological diagnosis is commonly not feasible, invasive, and not accessible. This results in delayed diagnosis, disease progression, and complications, resulting in poor patient outcomes [3].

Xpert MTB/RIF is a real-time cartridge-based PCR test. It simultaneously detects mycobacterium tuberculosis (MTB) in clinical specimen as well as rifampicin resistance in the same time [4]. It has the advantage of rapid result (within 2 h). The Centers for Disease Control and Prevention recommends that Nucleic Acid Amplification Test should be performed on at least one specimen from patients who have a moderate or high suspicion of having TB [5]. The test has a high sensitivity and specificity in pulmonary TB (88 and 99%, respectively) [4]. However, in EPTB, its sensitivity and specificity are variable according to the source. It is higher for lymph node aspirates, gastric lavage, and cerebrospinal fluid [6] and lower in pleural fluid aspirate [7]. There are still limited number of studies that involved the use of Xpert MTB/RIF in EPTB specimens. Evidence for using GeneXpert assay for diagnosing EPTB is still relatively weak.

Patients and methods

We conducted this cross-sectional analytic study on 100 patients suspected to have EPTB with the aim to

evaluate the role of GeneXpert (Cepheid, Sunnyvale, California, USA) in diagnosis of EPTB, as well as to analyze the local pattern of EPTB. Patients referred to the hospital with suspected EPTB from June 2017 to May 2021 were included in the study. Patients with pulmonary tuberculosis were excluded from the study.

The study was approved by the ethical committee of the Ministry of Health (MOH). It was registered in ClinicalTrials.gov under the number: NCT03173261. After taking the informed consent, full history was taken from all patients participating in the study. Detailed clinical examination and routine laboratory investigations (complete blood count, liver function test, urea, creatinine, and ESR) were done. Samples collected from patients, either pus, pleural fluid, urine, or ascetic fluid, were sent for adenosine-d-aminase, cytology and culture for MTB. Cultures were done either on solid (egg base and agar base) or liquid media (broth media). Culture result were obtained after about 4 weeks to get a conclusive test result.

Prepared samples were inoculated in bottles that contained Lowenstein–Jensen media. Incubation was done in a horizontal position for eight weeks. Bottles were examined weekly. If more than 50 colonies appeared, the culture was considered positive.

GeneXpert assay was done for all samples. The GeneXpert diagnostic system with four modules used in this study is developed by Cepheid Inc., Sunnyvale, California, USA. The test was carried out within 2 h of sample collection. On sample arrival to the GeneXpert laboratory, the reagent was added in a 15-ml falcon tube. Clinical samples were treated with sample reagent (SR) that contained sodium hydroxide and isopropanol. The SR was added to the sample (2: 1 ratio for unprocessed samples). Incubation at room temperature for 15 min was done, during which the tube was manually agitated two times at least to minimize risk of infection [5].

Then, a sterile disposable pipette was used to transfer 2 ml of the inactivated material to the test cartridge. Finally, cartridges were loaded into the GeneXpert device. Subsequent processing was fully automated.

Data were collected and analyzed using SPSS (Statistical Package for the Social Sciences, version 20; IBM, Armonk, New York, USA). Data were expressed in the form of frequency (percentage). χ^2 test was used to compare the nominal data of patients with extrapulmonary TB in the study, whereas Student *t* test was used to compare mean of two studied groups. *P* value was significant if less than 0.05.

The sensitivity and specificity for the diagnosis of TB were calculated for GeneXpert as the following equations:

(1) Sensitivity=true positives/(true positive+false negative).
(2) Specificity=true negatives/(true negative+false positives).

Results

This study included 100 patients with suspicion of EPTB. The demographic characteristics of patients are illustrated in Table 1. Cases with EPTB were more

Table 1 Demographic data

	Groups				*P* value
	Positive GeneXpert (*N*=40)		Negative GeneXpert (*N*=21)		
	Count	%	Count	%	
Age group					
1st group (18–33)	4	10.0	4	19.0	
2nd group (34–48)	11	27.5	4	19.0	0.433
3rd group (49–64)	15	37.5	5	23.8	
4th group (>64)	10	25.0	8	38.1	
Sex					
Female	8	20.0	9	42.9	0.059
Male	32	80.0	12	57.1	
Marital status					
Married	32	80.0	16	76.2	0.751
UnMarried	8	20.0	5	23.8	
Occupation residence					
Housewife	6	15.0	7	33.3	
Farmer	23	57.5	9	42.9	0.275
Others	11	27.5	5	23.8	
Rural	36	90.0	14	66.7	
Urban	4	10.0	7	33.3	0.036*
Special habits					
Smoker	16	40.0	8	38.1	
Passive	8	20.0	8	38.1	
Non	2	5.0	1	4.8	
Addict	14	35.0	4	19.0	
DM					
Positive	19	47.5	11	52.4	0.717
Negative	21	52.5	10	47.6	
HTN					
Positive	24	60.0	15	71.4	0.377
Negative	16	40.0	6	28.6	
HIV					
Positive	2	5.0	0	0.0	0.541
Negative	38	95.0	21	100.0	
Previous TB					
Positive	15	37.5	9	42.9	0.684
Negative	25	62.5	12	57.1	

*, significant difference

common in elderly patients, far more common in males. Majority of cases were from rural areas. A total of 15 (37.5%) cases were recurrent.

Cases with definite diagnosis as EPTB were 61, whereas 39 cases were negative according to culture (the gold standard test in this study). GeneXpert was positive in 40 cases, which represented 65.6% of EPTB cases, and negative in 21 (34.4%) cases (Table 2). A total of 24 (40.3%) cases were pleural, 18 (29%) cases were genitourinary, 10 (16.1%) cases were peritoneal, and nine (14.5%) cases were TB lymphadenitis (Fig. 1). Most of cases with positive GeneXpert were those with TB pleural effusion. Negative GeneXpert was most associated with genitourinary TB (Table 3). Cytology was lymphocytic in most cases (96.7%). Adenosine D-aminase was positive in only 57.4% of cases, as illustrated in Table 4.

Table 5 illustrates the specificity of GeneXpert, which was 97.4%. Its sensitivity was only 65.6%. Positive predictive value and negative predictive value were 97.4 and 64.4%, respectively.

Discussion

This study was designed to evaluate the performance of GeneXpert assay in the diagnosis of EPTB and to identify the sensitivity and the specificity of GeneXpert assay for biopsies from extrapulmonary samples (urine, pus, and gastric aspirates). This study included 100 patients with clinical suspicion of EPTB, where 61% of them were definitely diagnosed as extrapulmonary TB cases. This percentage is close to what was found by the recent observational, analytical study of ElBouhy *et al*. [8], as they found that 64.14% of their patients were affected with EPTB.

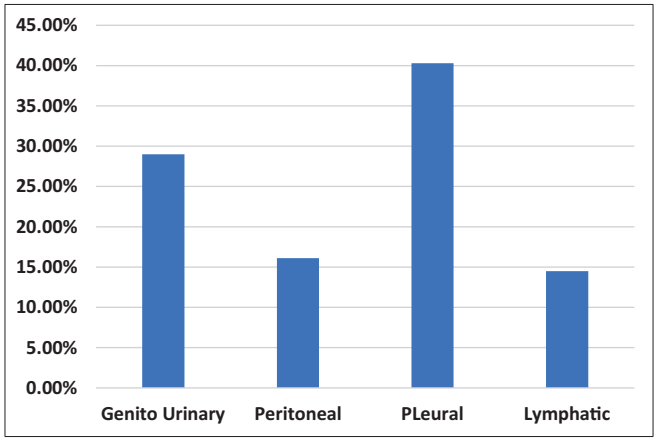
In the present study, 29% of the EPTB culture-proved cases were genitourinary, 16.1% were peritoneal, 40.3% were pleural, and 14.5% were TB Lymphadenitis. These findings go with a previous report, which indicated that the commonest EPTB sites were lymph node, pleura, and urogenital TB [9]. Vivar *et al*. [10] also reported that the pleural TB is the most common form of the EPTB. In the current study, more than three-quarters of the EPTB cases were males (76.3%). This is in harmony with what was documented by ElBouhy *et al*. [8], and explained this male predominance by that men are more exposed to life stress. This also addresses the sex differences explained by more community exposure and progression to overt disease owing to sex differences [11,12]. This study showed that most

Table 2 GeneXpert results in comparison with culture

	Culture				Total	*P* value
	Positive		Negative			
	Count	%	Count	%		
GeneXpert						
Positive	40	65.6	1	2.6	41	
Negative	21	34.4	38	97.4	59	<0.001*
Total	61	100	39	100	100	

*, significant difference

Figure 1



Sites of EPTB. EPTB, extrapulmonary tuberculosis.

Table 3 Different sites of extrapulmonary tuberculosis

	GeneXpert group				*P* value
	Positive gene Xpert		Negative gene Xpert		
	Count	%	Count	%	
Site TB					
Genitourinary (*N*=18)	7	17.5	11	52.4	
Peritoneal (*N*=10)	6	15.0	4	19.0	0.007*
Pleural (*N*=24)	18	45.0	6	28.6	
Lymphatic (*N*=9)	9	22.5	0	0.0	

*, significant difference

Table 4 Results of cytology and adenosine-d-aminase in patients with positive GeneXpert

	N (total=61)	Percentage	*P* value
Predominant cell type in cytology			
Lymphocyte	59	96.7	0.004
Others	2	3.3	
ADA			
High	35	57.4	0.006
Normal level	26	42.6	

ADA, adenosine-d-aminase.

Table 5 Validity of GeneXpert

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Total accuracy
GeneXpert	40/61 (65.6)	38/39 (97.4)	40/41 (97.4)	39/60 (64.4)	78/100 (78)

NPV, negative predictive value; PPV, positive predictive value.

patients with EPTB (82.3%) came from rural areas and more half (51.6%) were farmers. This is consistent with the studies of Pang *et al.* [13] and Sobh *et al*. [14], which revealed that residents of rural areas were more exposed to EPTB. Low socioeconomic status, poor sanitation, and exposure to diseased animals explain higher incidence of TB cases coming from rural districts.

In this study, GeneXpert assay was used to reassess the culture-positive EPTB cases. The GeneXpert test was positive in about two-thirds (65.6%) of cases and negative in about one-third (34.4%). The results of Mechal *et al*. [15] were partially comparable to this study findings; they found that the sensitivity of GeneXpert for EPTB diagnosis was 64.3% in the pus specimens and 78.2% in the total EPTB specimens. The study of Pandey *et al*. [16] revealed the sensitivity of GeneXpert assay was higher (83–100%) for CSF, lymph node aspirate, sterile fluid, pus, and respiratory samples and lower sensitivity (60–63%) for pleural fluid and lymph node samples. Regarding the high specificity found in this study (97.4%), this was comparable to the results of a study in Spain by Moure *et al*. [17], with specificity of 100%, a study in India by Vadwai *et al*. [18], with specificity of 99.6%, and a study in Spain by Causse *et al*. [19], with specificity of 100%. The main limitation in this study is the relatively small sample size. We call for a multicenter study that can collect larger number of patients with EPTB. Further research is needed to highlight the role of GeneXpert in different sites of EPTB and compare its sensitivity and specificity between these groups.

Conclusion

GeneXpert is a rapid, valuable tool in the diagnosis of patients with EPTB. It is a highly specific test with fair sensitivity. We recommend conducting a multicenter study with a large sample size to evaluate its diagnostic role in different sites of EPTB.

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Conflicts of interest

There are no conflicts of interest.

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