Journal of Pharmaceutical Research International

33(50A): 6-12, 2021; Article no.JPRI.76312
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Efficacy of GeneXpert in Diagnosing Smear Negative Pulmonary Tuberculosis and Comparison to Culture

Muhammad Kashif Munir [a,b*], Iftikhar Ali [a], Ahsan Sattar Sheikh [a], Arif Malik [a], Asif Hanif [b], Sana Rehman [b], Samina Khaliq Munir [c] and Qurban Ali [a]

[a] Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan.
[b] PHRC TB Research Centre, Department of Pulmonology, King Edward Medical University, Lahore, Pakistan.
[c] Department of Compute Science, The University of Lahore, Lahore, Pakistan.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i50A33376
Editor(s):
(1) Dr. Dharmesh Chandra Sharma, G. R. Medical College & J. A. Hospital, India.
Reviewers:
(1) T.V.S.R. Raghu, DR. N T R University of Health Sciences, India.
(2) Sunil Kumar, Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences University, India.
(3) Ige Olusoji Mayowa, University Of Ibadan and University College Hospital, Nigeria.
Complete Peer review History: https://www.sdiarticle4.com/review-history/76312

Original Research Article

Received 30 August 2021
Accepted 10 November 2021
Published 15 November 2021

ABSTRACT

Objectives: Aim of present study is to observe the efficiency of GeneXpert MTB/RIF Assay in comparison to MTB culture on Lowenstein Jensen media in diagnosis of smear negative pulmonary tuberculosis cases.
Methods: This descriptive study was carried out in The University of Lahore in collaboration with King Edward Medical University/Mayo Hospital Lahore 11th September, 2020 to 10th April 2021. Smear negative for acid fast bacilli patients enrolled for anti TB treatment were the target population. After taking informed consent, patients were asked to submit first morning sputum sample for culture on Lowenstein Jensen Medium and GeneXpert.
Results: A total of 345 smear negative TB patients were diagnosed clinically and/or on the basis of radiological findings with mean age of 38.28±17.93,consisting of 47.5% male and 52.5% females recruited in this study. History of TB contact was present among 41.4% patients whereas history of smoking and diabetes remained to be 27.2% and 17.4% respectively. Culture showed significantly higher rate (35.1%) (p-value <0.05) of detection of MTB as compared to GeneXpert (21.5%). A

**Corresponding author: E-mail: munir_gemini81@yahoo.com, saim1692@gmail.com;*

sensitivity of 56.01% was calculated for GeneXpert whereas for culture on LJ medium it was 60.63%.

Conclusion: Sensitivity of GeneXpert MTB/RIF Assay is a bit low in diagnosing the SNPT patients as compared to the culture but still registers itself as a handsome tool in terms of promptness and definite detection of MTB complex. Further provision of rifampicin susceptibility is bonus in same time.

Keywords: GeneXpert; smear negative; tuberculosis; lowenstein jensen medium.

1. INTRODUCTION

Tuberculosis (TB) is a contagious communicable disease caused by a group of bacteria known as *Mycobacterium tuberculosis complex* (MTBC) and one of the 10 topmost reasons of global demises and the foremost cause of mortality due to solo infective agent [1]. It typically causes lung infection that leads to pulmonary TB though and may affect each and every part of body causing extra-pulmonary TB. Pakistan shares the 4th position with Philippines comprising 6% of total global load. There were 562000 new TB cases in Pakistan during 2018 with an incidence of 265/100000 population where an estimated portion of 4.2% new cases and 16% previously treated cases became rifampicin resistant (RR) or multidrug resistant (MDR) [1].

Prompt diagnosis and timely treatment of TB patients are the only ways to curtail further transmission of infection [2]. Presence of acid fast bacilli (AFB) in sputum smear is still a crucial test in diagnosis of pulmonary tuberculosis in developing countries [3]. Sputum smear positive patients are immediately put on anti-tubercular treatment (ATT) while smear negative patients remain dilemma even in the present era of science and technology. Although, smear microscopy is an efficient, fast and specific test in diagnosis of MTB however it lacks sensitivity [2,4].

Microbiological evidence is often used for definitive diagnosis of MTB yet there are high numbers of smear negative pulmonary TB (SNPT) patients being enrolled for treatment of TB [5,6]. It was reported that smear negative TB patients spread disease among 20% of total TB cases in the Republic of Congo [6]. Culture for MTB still stands as the most standardized test for definitive diagnosis but it considerably delays the results [7]. In high TB prevalent settings, suspected cases with at least two negative reports of sputum samples are given unspecified chemotherapeutic agents for 15 days and then advised for another sputum sample followed by a chest x-ray. Thus on the basis of supportive findings such patients are followed with standard ATT [6].

Improvements in investigative accuracy and a decrease in diagnostic delay of SNPT cases are necessary especially in high burden countries. A polymerase chain reaction (PCR) based automated system such as GeneXpert MTB/RIF was introduced few years back which detected MTBC and RR simultaneously [8]. Further WHO has endorsed it for diagnosis of drug resistant TB which requires only two hours performing the whole assay [8]. Since various studies have claimed variable sensitivity and specificity of this assay [2,6,8] therefore it needs to be evaluate further. Present study is aimed to observe the efficiency of Xpert MTB/RIF Assay in comparison to MTB culture on Lowenstein Jensen (LJ) media in diagnosis of smear negative pulmonary TB cases.

2. MATERIALS AND METHODS

The present study was undertaken at the Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan in collaboration with Pakistan Health Research Council TB Research Centre King Edward Medical University Lahore from 11th September, 2020 to 10th April 2021. A sample size of 345 SNPT patients was calculated by taking confidence level of 95%, level of precision as 5% and an expected prevalence of SNPT as 42% [6].

2.1 Data Collection

After taking an informed consent a pre-designed Performa was used to collect the data that comprising demographic information, presenting complaints, histories, radiological findings and socio-economic statuses of patients. Subjects were asked to provide first morning sputum specimens in two sterile containers. One of which was used for smear and culture while the other was used for GeneXpert MTB/RIF Assay.

Samples were then processed for Xpert MTB/RIF Assay according to the manufacturer's guidelines [2]. Permission for this work was granted by institutional review board via letter no. 646/RC/KEMU/2020.

2.2 Smear Preparation and Culture

Direct and concentrated smears were prepared from clinical specimens by using modified Petroff's method [7]. Specimens were treated with 4 % NaOH for decontamination and digestion of clinical specimens. Sterile phosphate buffer at pH 6.8 was added to neutralize the effect of NaOH and the samples were concentrated by centrifugation at 1500*g* for 15 minutes. Supernatants were discarded and sediments were re-suspended to be used for inoculation on the slants of LJ medium and for concentrated smear preparation. Cultures were considered positive if they contained only 1 colony. Smears were stained using Auramine staining technique and observed under fluorescent microscope. Data was analyzed by using IBM- Statistical Package for Social Sciences (SPSS) software (Version 26.0).

3. RESULTS

A total of 345 smear negative TB patients which were diagnosed clinically and/or on the basis of radiological findings consisted of 164 (47.5%) male and 181 females (52.5%) with a male to female ratio of 1:1.1 were recruited in this study. Mean age of respondents remained to 38.28±17.93 whereas mean age of males was found to be 41.00±18.44 and females as 35.82±17.15. Cough, the most prominent of all signs and symptoms was present among all patients followed by asthenia 89.6%, fever 82.6%, anorexia 79.4%, weight loss 75.9%, night sweats 58.6% and expectoration among only 27.8% patients. There was no much difference of signs & symptoms recorded.

History of TB contact remained at the top as 41.4% patients had definite known contact in their blood relations while others could not establish the contact history. Similarly, history of smoking was present among 27.2% patients and a significant difference was observed (p-value <0.05) among male and female gender as males remained more vulnerable due to smoking as compared to females. History of diabetes (17.4%) remained the major comorbidity among smear negative TB patients out of which females (19.9%) was more affected as compared to males (14.6%) though the difference was not significant (p-value >0.05). X-ray findings of all the patients were also observed and a total of 147 (42.6%) patients showed changes as depicted in Table 1.

Seventy four patients (21.5%) were detected MTB by GeneXpert while 121 (35.1%) showed growth on LJ culture and hence the rate of detection of MTB by culture remained significantly (p=0.0001) high as compared to detection rate of GeneXpert. An agreement of

Table 1. Histories, Comorbidities and x-ray findings among SNPT Patients

Variables		Gender				Total (n=345)	
		Male (n=164)		Female (n=181)			
		n	%	N	%	n	%
History of TB contact		67	40.9	76	42.0	143	41.4
History of TB Treatment		44	26.8	35	19.3	79	22.9
History of Smoking		86	52.5	8	4.4	94	27.2
Diabetes		24	14.6	36	19.9	60	17.4
Hepatitis B Virus (HBV)		9	5.5	14	7.7	23	6.7
Hepatitis C Virus (HCV)		20	12.2	16	8.8	36	10.4
X-ray Findings	*Mild Infiltration	4	5.1	32	12.0	36	10.4
	*Moderate Infiltration	1	1.3	35	13.2	36	10.4
	Calcification	64	81.0	11	4.1	75	21.7
	No Definitive Change	10	12.7	188	70.7	198	57.4
		n=69		n=78		n=147	
Lungs Involved	Right	24	34.8	38	48.7	62	42.2
	Left	19	27.5	29	37.2	48	32.6
	Bilateral	26	37.7	11	14.1	37	25.2

Table 2. Outcome of GeneXpert and MTB Culture Results among SNPT Patients

Parameter	Result	History of TB Treatment				Total (n=345)	
		Present (n=79)		Absent (266)			
		n	%	n	%	n	%
MTB Status	Not Detected	60	75.9	211	79.3	271	78.6
	Detected Very Low	8	10.1	25	9.4	33	9.6
	Detected Low	11	13.9	30	11.3	41	11.9
Rif Susceptibility	Resistant	0	0.0	1	0.4	1	0.3
	Sensitive	79	100.0	265	99.6	344	99.7
Culture Result On LJ Medium	Scanty Growth (>50 colonies) Colonies)	21	26.6	69	25.9	90	26.1
	1+ (50-100 colonies)	9	11.4	22	8.3	31	9.0
	No Growth	49	62.0	175	65.8	224	64.9

Table 3. Cross tabulation of GeneXpert with Culture Results

GeneXpert Results		Culture Result on LJ Medium					
		Scanty Growth (>50 Colonies)		1+ (50-100 colonies)		No Growth	
		n	%	n	%	n	%
MTB Status	Not Detected	46	51.1	1	3.2	224	100.0
	Very Low	27	30.0	6	19.4	0	0.0
	Low	17	18.9	24	77.4	0	0.0
Rif	Resistant	1	1.1	0	0.0	0	0.0
Susceptibility	Sensitive	89	98.9	31	100.0	224	100.0

bacterial load was presented amongst the samples that revealed detected very low (9.6%) and detected low (11.9%) by GeneXpert and showed low colony forming units as scanty growth (26.1%) or 1+ growth (9.0%) on LJ medium. Only 1(0.3%) patient showed resistance to rifampicin among SNPT patients on GeneXpert as shown in Table 2.

Cross tabulation was drawn between GeneXpert and LJ culture results and it was observed that higher colony forming units are associated with higher positivity rates of MTB detection by GeneXpert. ATT was taken as gold standard and all the patients were followed for first two months to observe treatment response in terms of improvement in symptoms and a sensitivity of 56.01% (95% C. I. 51.98%-59.97%) was calculated for GeneXpert MTB/RIF Assay as compared to Culture on LJ medium 60.63% (95% C. I. 56.48%-64.67%). Positive predictive value of both the tests remained 100% while negative predictive value and accuracy could not be calculated due to the absence of control group in this study as shown in Table 3.

4. DISCUSSION

Smear positive pulmonary TB cases are enrolled at the time of their first report but a consistent dilemma is carried out by SNPT patients as well as the physicians Whether to start an ATT or not. In this situation some of the SNPT patients may be missed however a large number of patients (30-65%) are enrolled every year especially in resource limited and high prevalence countries [9]. A positivity rate of 21.5% SNPT patients was confirmed by GeneXpert and sensitivity of the technique remained to be 56.01% whereas culture on LJ presented a significantly (p-value <0.05) higher positivity rate of 35.1% with higher sensitivity of 60.63% in this study.

An Egyptian study monitored the role of GeneXpert in diagnosis of SNPT and revealed much higher positivity rate of SNPT by GeneXpert as 36.7% and also suggested the GeneXpert as gold standard for definitive diagnosis infection with MTB complex [10] whilst, present results are not in agreement with this study. Definite diagnosis of TB is based on presence of TB bacilli in the given specimen and microbiology is still considered the standard to decide the cases of active tuberculosis or not however various factors in developing countries prohibit adopting this technique at mass level [11]. Even lower sensitivity of 38.1% as compared to present study with specificity of 74.5%, positive predictive value 52% and

negative predictive value of 62.6% was revealed in a study from Tanzania [3].

Despite the importance of bacteriological confirmation of TB bacilli radiological suspicion with the presence of clinical sign and symptoms has its role in promptness of starting ATT to the TB patients [12]. A total of 42.6% SNPT patients showed changes on their X-rays with a breakup of 20.8% patients showing infiltration while 21.7% had calcification of lungs since calcification was concomitant with presence of history of previous ATT in this study. A recent study evaluating the value of X-rays in similar settings presented a high proportion of mild to moderate infiltration among SNPT patients while moderate to severe infiltration leading to cavitation were characteristics of smear positive pulmonary TB patients [13]. It revealed a good treatment response of 92.91% with sensitivity of chest X-rays in diagnosing pulmonary TB as 96.87% [13]. suggesting an agreement with present study. End TB strategy imposed by WHO has also recommended Chest X-ray in diagnosis of SNPT cases and proved it as a sensitive and good diagnostic tool [14].

Signs and symptoms and relevant histories are also important in diagnosing and treating SNPT patients. Another study showed cough 83.3%, fever 66.6%, weight loss 73.3%, and anorexia 70.0% comparable with presenting complaints in this study [10]. A study from similar settings aimed to compare the conventional MTB culture with GeneXpert also presented fever, fatigue, cough, weight loss and anorexia as 100%, 98.4%, 96%, 91.2% and 77.6% respectively are in agreement with present findings [15].

Presently history of TB contact remains at the top as 41.4% patients showed definite contacts is not in agreement with a study shown higher rate of history of contact among TB patients (53.0%) was presented in a study aimed to observe the factors related to sputum smear conversion among smear positive TB patients [16] History of smoking has a great value and was found to be present among 27.2% patients in this study however males were more prone to smoking as compared to females with a significant difference (p-value <0.05). Results are in agreement with a study that presented 25% smokers among TB cases however contradiction persisted, as later study showed higher frequency of females as compared to male smokers [17]. This difference may be due to the various cultural norms in different demographic regions of the world.

Although only one SNPT presented rifampicin resistance in this study though the importance of GeneXpert could not be denied in diagnosing MTB as well rifampicin resistance in a single run within two hours. Diabetes was found to be the major comorbidity in this study where females were more affected with an insignificant difference in this study. Similarly female gender remained to be more prone of suffering from SNPT in lower mean age as compared to male gender and all such findings are in-concomitant with other studies [2,15-18].

5. CONCLUSION

Although the sensitivity of GeneXpert MTB/RIF Assay is a bit low in diagnosing the SNPT patients as compared to culture but still registers itself as a handsome tool in terms of promptness and definite detection of MTB complex further provision of rifampicin susceptibility is bonus in same time. It is suggested that symptomatic smear negative MTB suspects must be screened through GeneXpert before putting them on non-specific chemotherapy for 15 days may rule out around 15-20% cases which may prevent wastage of money, time, resources, efforts, and further spread of disease.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

ETHICS APPROVAL

Ethical approval for this study was obtained from Institutional Review Board of King Edward Medical University through letter No. 646/RC/KEMU dated 10th September, 2020 and it is certified that study was performed in accordance with ethical standards.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/76312