Accuracy of GeneXpert in Diagnosis of Smear Negative Tuberculosis; A Cross Sectional Study

Running Title:

Diagnostic Accuracy of GeneXpert in Smear Negative TB

Abstract

Objective: This study was aimed to compare the diagnostic accuracy of GeneXpert in smear negative as compared to smear positive pulmonary *tuberculosis* patients.

Methods: This comparative cross sectional study was undertaken at Health Research Institute (National Institute of Health) TB Research Centre, Pulmonology King Edward Medical University Lahore, Pakistan. A total of 101 patients in each smear negative and positive groups were included. After taking the informed consent a predesigned questionnaire was used to collect the data.

Results: A total of 202 patients consisting of 94 (46.5%) male and 108 (53.5%) females with mean age of patients as 39.83±18.14 years were included in this study. Highest proportion of 45% patients remained to be in age range of 18-35 years. Culture presented the highest accuracy of 96.1% with sensitivity, specificity and positive predictive value of 95.9%, 100%, and 100% respectively while GeneXpert remained to be 94.6%, 94.9%, 80%, and 99.5% respectively in this study.

Conclusion: Accuracy of GeneXpert is excellent in diagnosis of smear positive pulmonary *tuberculosis* patients and very well in diagnosing smear negative pulmonary TB patients and a great addition in early and definite diagnosis of TB and keeps the ability to confirm around 75% smear negative pulmonary tuberculosis.

Keywords: Tuberculosis, Acid Fast Bacilli, GeneXpert, Culture on Lowenstein Jensen, Accuracy, *Mycobacterium*.

1. Introduction

Tuberculosis (TB) is contagion in nature and easily transmittable disease instigated by a cluster of bacterial micro-organism said to be *Mycobacterium tuberculosis complex* (MTBC), where the disease is lies amongst ten topmost reasons of causalities and leading cause of transient with single infectious agent.[1] Typically MTBC cause lungs infection which leads to pulmonary TB however they may go through every tissue of body to cause extra-pulmonary TB.[2] According to a recent report of World Health Organization (WHO) around 9.9 to 10 million people were infected with TB with estimated incidence rate of 114-127 persons per 100,000 thousand population. Geographically, South East Asia contained the most 43% Global Burden of TB and Pakistan remained at 5th highest TB burden country in the world with an incidence of around 255/100,000 population. Adult men remained the hot target of TB with a proportion of 56% followed by women as 33% and 11% children were also infected with TB during 2020.[3]

Definite diagnosis and prompt treatment of TB patients are very crucial to control the disease in community. [4] Sputum smear microscopy to observe acid fast bacilli (AFB) is important in developing countries due to its low cost, rapidity, easy performance and non-requirement of fully furnished laboratory. [5] Anti-tubercular treatment of patients is immediately started among the cases of AFB positive sputum smears on the other hand sputum smear negative patients remain in dilemma of further investigations and empirical therapy. [4] It is also pertinent that smear microscopy lacks sensitivity. [6]

Diagnosis of TB is not possible without microbiological evidence while many patients remain with smear negative pulmonary TB (SNPT) for weeks and months further smear negative patients have been reported to spread TB among 20% cases. [7] Culture for MTB is considered as gold standard for sure diagnosis of TB which significantly delays the results due to number of reasons.[8] Similarly in cases with SNPT a complicated criterion is carried out for imperial treatment unless conclusive diagnosis is made which not only delays the standard treatment but also threatens in spread of disease to many healthy contacts. [7]

Molecular diagnostic techniques have shown their impact in diagnostics since three decades and definitely influenced the diagnosis of TB also. GeneXpert MTB/RIF assay is based on polymerase chain reaction (PCR) and endorsed by WHO for diagnosis of MTBC with

simultaneous provision of rifampicin susceptibility in only two hours. [9] Variable accuracy of GeneXpert MTB/Rif assay has been claimed in different studies, further a scarce work on its diagnostic accuracy among smear negative TB is done. Therefore this study was aimed to compare the diagnostic accuracy of GeneXpert in SNPT cases as compared to smear positive pulmonary TB patients.

2. Material and Methods

This comparative cross sectional study was undertaken at Health Research Institute, National Institute of Health, TB Research Centre in collaboration with Department of Pulmonology King Edward Medical University/Mayo Hospital Lahore, Pakistan from November 2020 to September 2021. A sample size of 202 patients (101 patients in each group) is calculated by taking confidence level of 90%, absolute precision as 10% and expected prevalence of smear positive pulmonary TB as 40.08% and smear negative as 15.63%. [10]

2.1 Inclusion & Exclusion Criteria

All the adults of both genders with pulmonary TB registered for treatment of TB in Mayo Hospital Lahore were included in this study. All the extra-pulmonary TB, co-infected with other chronic diseases like HCV, HBV, HIV, diabetes and cardiac diseases patients were excluded from present study. Patients already registered for TB treatment were also excluded from study.

2.2 Data Collection

After taking the informed consent data was collected from 202 patients. Using non probability convenient sampling technique patients were divided in two groups on the bases of smear microscopy result. Demographic data and clinical information of patients were collected on a pre-designed questionnaire and two fresh sputum specimens from each patient belonging to both smear positive group and smear negative group were obtained on the spot. One sample of each patient was subjected to GeneXpert test and other was used for TB culture isolation.

2.3 Smear preparation and Culture

Direct and concentrated smears were prepared from clinical specimens by using modified Petroff's method. Specimens were treated with 4 % NaOH (sodium hydroxide) for decontamination and digestion of clinical specimens. Sterile phosphate buffer, pH 6.8 was added to neutralize the effect of NaOH and the samples were concentrated by centrifugation at 3000 rpm for 15 minutes. Supernatants were discarded and sediments were re-suspended to be used

for inoculation on the slants of LJ medium and concentrated smear preparations. Each Culture was inoculated in three slants of LJ medium to find the results even in case of contamination in one or two slants. Each culture was considered positive if it contained only 1 colony, however actual colony counts were reported if there is less than 50 colonies, more than 50 and less than 100 colonies 1^+, 100 to 200 colonies 2^+ and more than 200 colonies were reported as 3^+. Known positive and known negative slides are included with each run and each batch of staining. An experienced microbiologist rechecked the smears for internal quality assurance. Smears are also sent to National TB Control program for external quality assurance. LJ media is tested by inoculation of known American Type Culture Control (ATCC) strain of $H37_{Rv}$. Random slants of LJ media inoculated with sterile distilled water are incubated from each batch as negative controls.

2.4 Staining & Microscopy

Auramine staining was used to place on the slides for 20 minutes. Acid alcohol is used for decolonization and methylene blue was used for secondary stain. Taking all the precautions stand the slide on edge on the drying rack and allow to air-dry after staining and kept in dark.

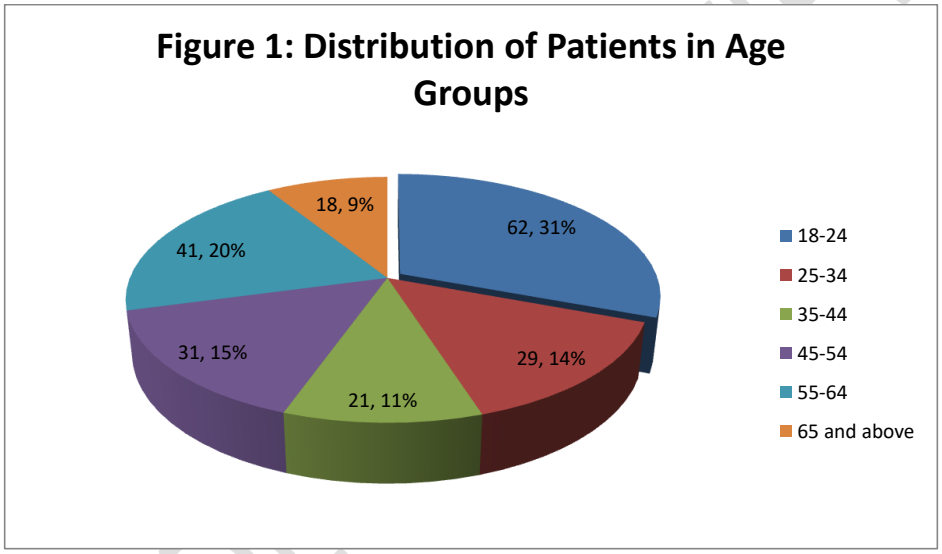
Bright greenish fluorescent AFB against dark background and are reported according to criterion lay down by WHO. [11]

2.5 Data Analysis

Data was entered and analyzed by using statistical package for social sciences (SPSS) version 26.0. Qualitative variables are presented as frequency and percentages while quantitative variables are presented as mean ± standard deviation (SD). Chi-square test was applied to compare qualitative variables and a p value <0.05 is considered as significant. Patients were followed for two months to observe the treatment response and good response was taken as gold standard to calculate the sensitivity and specificity of diagnostic outcomes.

RESULTS

A total of 202 patients were included in this study consisting of 94 (46.5%) male and 108 (53.5%) females. Mean age of patients remained to be 39.83±18.14 years where mean age of male patients was remained as 43.26±18.93 years remained high as compared to females i.e. 36.85±16.96 years. Distribution of patients in various age groups is presented in diagram 1. Highest number of 91 (45%) patients remained to be in age range of 18-35 years while lowest number of 18(9%) patients remained in age group of ≥65 years.



Mean family size of patients was remained to be 8.99±2.82 persons with family size of smear positive group as 8.91±2.88 and smear negative group as 9.06±2.78 persons with an insignificant difference (p value = 0.709) showing equal distribution of study subjects. Histories including history of smoking, history of TB treatment previously and history of TB contact were also explored and gender-wise distribution presented a significant difference (p >0.05) regarding history of smoking and history of previous TB treatment where male were more prone as compared to female patients while an insignificant difference was noted regarding history of TB contact among patients as depicted in Table I. However an insignificant difference (p>0.05) of histories was noted regarding smear negative and smear positive groups.

Table I: Gender-wise Distribution of Histories among Patients

Histories		Gender				p-value
		Male		Female		
		n	%	n	%	
History of smoking	Present	36	38.3	11	10.2	<0.0001
	Absent	58	61.7	97	89.8	
History of TB Treatment	Present	30	31.9	15	13.9	0.002
	Absent	64	68.1	93	86.1	
History of TB contact	Present	42	44.7	42	38.9	0.475
	Absent	52	55.3	66	61.1	

Important sign and symptoms of TB are also noted among patients and revealed that smear negative patients present lesser frequencies as compared to smear positive patients as depicted in Figure 2. Cough fever and asthenia were remained to be the most prevalent sing and symptoms in patients of both groups.

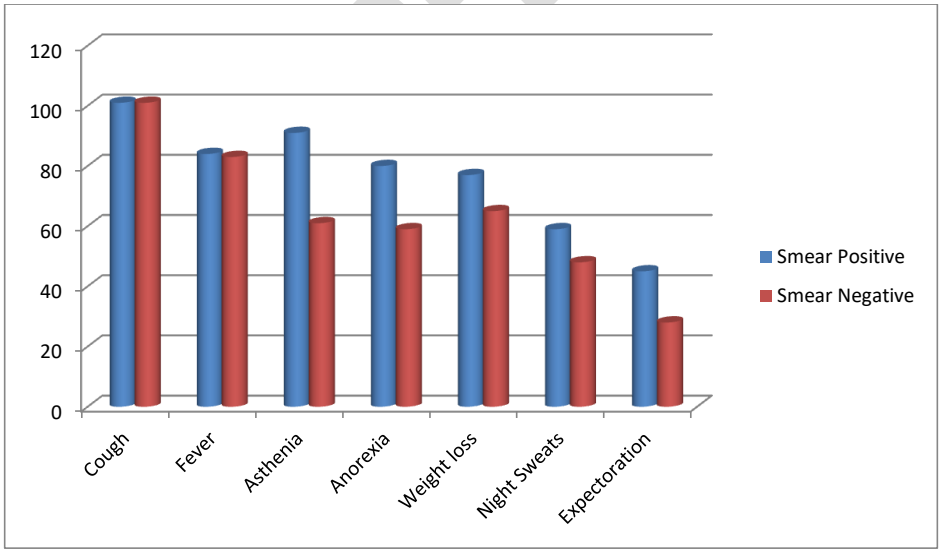


Figure 2: Sign and Symptoms among Smear Negative and Smear Positive Patients

Comparison of GeneXpert MTB Rif Assay and culture results among smear positive group showed a high level of agreement as 100/101 (99.01%) cases were also diagnosed by GeneXpert ~~and culture on LJ medium~~ while in smear negative group GeneXpert showed an agreement rate of 26/101 (25.7%) with smear results while culture showed even lower agreement of 12/101 (11.9%) with smear negative results. Semi quantitative results of culture and GeneXpert are compared to smear positive and negative groups as depicted in Table II.

Table II: Comparison of GeneXpert and Culture Techniques with Smear Groups

Diagnostic Techniques		Smear Group				Total	
		Positive		Negative			
		n	%	n	%	n	%
MTB Status By GeneXpert	Not Detected	1	1.0	25	24.8	26	12.9
	Detected Very Low	1	1.0	30	29.7	31	15.3
	Detected Low	19	18.8	46	45.5	65	32.2
	Detected Medium	53	52.5	0	0.0	53	26.2
	Detected High	27	26.7	0	0.0	27	13.4
Culture Result on LJ Medium	Scanty Growth	0	0.0	15	14.9	15	7.4
	1+	31	30.7	29	28.7	60	29.7
	2+	51	50.5	45	44.6	96	47.5
	3+	18	17.8	0	0.0	18	8.9
	No Growth	1	1.0	12	11.9	13	6.4

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of AFB smear, sputum culture on LJ and GeneXpert MTB Rif Assay were calculated by taking the response to ATT after two weeks follow up. Culture presented the best outcomes followed by GeneXpert with minute difference while smear presented the compromised findings as presented in Table III.

Table III: Accuracy parameters of AFB Smear, LJ Culture and GeneXprt

Parameters	AFB Smear	LJ Culture	GeneXpert
Sensitivity	50.51%	95.9%	94.9%
Specificity	66.67%	100.0%	80.0%
PPV	98.02%	100.0%	99.5%
NPV	3.96%	38.5%	28.6%
Accuracy	50.99%	96.1%	94.6%

Comment [nc1]: This statement is incorrect- needs modification as Genexpert is able to pick up 76 of those with smear negative rather than discussing it as shows agreement in 26

Same way for culture too

Comment [nc2]: Same way the discussion need to be re written for culture too

Comment [nc3]: This cannot be based on the response to therapy. we are discussing the accuracy of a diagnostic test here geneexpert vs culture. Needs to be re written

Discussion

Culture presented the highest accuracy of 96.1% with sensitivity, specificity, PPV and NPV of 95.9%, 100%, 100% and 38.5% respectively in this study. Results of present study are not comparable with study that presented a lower sensitivity of 85%, specificity 86.3%, PPV 75.6% and NPV 92% in their findings. [12] Another study has presented comparable findings with sensitivity, specificity, PPV and NPV as 92.7%, 99.4%, 92.1% and 99.4% respectively. [13]

Similarly sensitivity, specificity, PPV, NPV and accuracy of GeneXpert remained to be 94.9%, 80%, 99.5%, 28.6% and 94.6% respectively in this study. A study from same settings has already presented the results as sensitivity of 83.9%, specificity 87.9%, PPV 88.1% and accuracy of 85.1% are not comparable with present findings. [14] A study recently evaluated the performance of GeneXpert and presented lower outcomes of diagnostic accuracy by presenting sensitivity, specificity, PPV and NPV as 75%, 90.6%, 72% and 92% respectively in sputum samples. [15] Presently, NPV remained very low in all three parameters is only because of absence of control group in this study.

Comment [nc4]: This paragraph needs modification as the entire study results shows the superiority of genexpert TB in smear negative patients. No one is casting doubts on culture which is the gold standard

Delayed diagnosis is the main hindrance in effective control of TB further smear negative cases remain on lingering and go through various clinical processes and kept on empirical therapy until final diagnosis. Thus the patients remain on spreading the bacilli in the environment which effect the healthy contacts. It is also evident from decades that smear negative pulmonary TB patients also have a great tendency of spreading TB and pose a threat of 17.3% to 41% of healthy contacts. [16] Microbiological finding of TB bacilli is core evidence in definite diagnosis of TB therefore smear positive cases are started with ATT as soon as report is generated while smear negative remain as dilemma. Smear microscopy has been remained the cheapest and specific method in diagnosing TB in under developed and developing countries with a compromised sensitivity and requires a high number of bacilli per ml of sample. [14]

Under the circumstances of delayed diagnosis by LJ culture and lesser sensitive smear microscopy techniques, GeneXpert MTB Rif Assay has been considerably a new addition and ray of hope in definite diagnosing of smear negative pulmonary TB. It presented a 99% diagnostic agreement with smear positive pulmonary TB and remained able to diagnose 74.3% smear negative pulmonary TB patients which is the need of time. Further, besides sputum sample the efficacy of GeneXpert in diagnosis of TB by variety of specimens like bronchial wash,

cerebrospinal fluid, ganglion samples, pus, pleural effusions, urine, and various other extra-pulmonary samples has also been registered. [15]

Smear negative TB has become an important challenge now a days as these patients were considered less infectious in the past but after epidemics of Human-immunodeficient virus (HIV) smear negative TB has hiked the mortality rates. [17] A study from northwest Iran has presented a proportion of 22.8% smear negative patients and proposed factors included were smoking, asthma and extra-pulmonary TB. Further study claimed that the mortality rate among smear negative pulmonary TB is relatively high. [18] An older study explained the importance of untreated TB due to due to incorrect diagnosis and patients receive no or inadequate treatment while case fatality and duration of TB are key factors in epidemiological data. Thus the study reported duration of untreated case fatality as 2 years either in case of smear positive or negative TB. [19]

Presently proportion of male patients as 46.5% and female patients as 53.5%) in this study are not in agreement with previous studies of same settings and male gender remained dominant most of the times. [2, 4,14] Similarly a high proportion of 45% patients in the most productive age group of 18-35 years is also in agreement and factor contributes in economic burden of families of patients as well as for the country. [2, 4, 14]

Accuracy of GeneXpert is excellent in diagnosis of smear positive pulmonary TB patients and very well in diagnosing SNPT patients and a great addition in early and definite diagnosis of deadly TB in given settings thus keeps the ability to confirm around 75% SNPT patients as is evident by the current finding.

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