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ORIGINAL ARTICLE

Diagnostic Accuracy of GeneXpert in Pulmonary and Extra-pulmonary Specimens in Tertiary Care Settings of South Punjab

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ABSTRACT

Aim: To determine the diagnostic accuracy of GeneXpert in pulmonary and extra-pulmonary specimens taking LJ media as the gold standard.
Study design and settings: This cross sectional study was undertaken in the Provincial Reference TB Laboratory collaboration with Pathology Department, Nishtar Medical University Multan from November 2020-November 2021.
Methodology: A total of 210 suspected TB cases of aged 18 years and above were included. Samples from suspects were collected. A pre-designed questionnaire was used to collect demographic information, history, and other required information and samples were processed for culture on LJ medium while second sample was processed for GeneXpert. Data was entered and analyzed in SPSS.
Results: A total of 210 patients were enrolled consisting of 113(53.8%) males and 97(46.2%) with overall mean age of patients as 36.81±16.22 years. Sensitivity, specificity, PPV, NPV and accuracy were calculated by taking LJ culture as gold standard remained to be 75.13%, 88.24%, 98.64%, 23.81% & 76.19% for smear and 99.48%, 83.33%, 98.45%, 93.75% and 98.10% respectively for GeneXpert.
Conclusion: GeneXpert has been found to be extremely efficient technique in certain diagnosis of TB along provision of rifampicin susceptibility thus, helpful in early indication of MDR TB.
Keywords: GeneXpert, Culture on LJ, Tuberculosis, Diagnosis.

INTRODUCTION

Tuberculosis is a contagious disease caused by various species of *Mycobacterium tuberculosis complex* (MTBC) and recognized as one of the top reasons of global death and foremost cause of death being single infectious agent which and ranked above *Human immunodeficiency virus* (HIV)[1]. Predominantly, MTBC infects the lungs to cause pulmonary TB however it may infect every part of the body outside the lungs to be stated as Extra-pulmonary Tuberculosis (EPTB)[2]. Although TB can affect anyone in any part of body, but etiology of disease is expressed in adults mostly and men are observed to be more prone for acquiring disease as compared women[1].

Almost 90% global TB cases are reported in 30 high burden countries and it is said to be the disease of economic stress & poverty, vulnerability, marginalization, discrimination and stigma are usually faced by the patients. Around 10 million (8.9-11.0 million) people are estimated to acquire TB infection during 2019[1]. Presently, Pakistan has been ranked 5th highest TB burden country bearing 5.7% global TB cases. [1] The spread of TB is through air-born droplets which are produced through coughing, sneezing, talking, and laughing of infectious smear-positive pulmonary TB patient[3].

Drug-resistant TB is a major challenge globally. Multidrug-resistant TB (MDR TB) is a type of TB which is resistant to isoniazid and rifampicin, the two major first-line anti-TB drugs.[4] There is a strong need for rapid diagnosis and early detection of MDR TB to reduce TB-induced morbidity and mortality. Early diagnosis of Tuberculosis is very crucial to prevent the spread of disease and also for better management. Detection of acid-fast bacilli by Ziehl- Nielsen (ZN) smear microscopy is widely used as it is a cheaper and easier method but its sensitivity is very low[5]. Light-emitting diode fluorescence microscopy (LED-FM) has replaced conventional ZN microscopy as it is more sensitive in detecting sputum smear-positive cases. Smear-negative culture-positive TB patients are although considered less infectious but have been reported to transmit disease in 13% and 17-24% patients in various studies[6].

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Culture on Lowenstein Jensen (LJ) media is considered a gold standard but it requires well-established infrastructure and takes a long time so it delays the early diagnosis[7]. It causes a delay in the early diagnosis of TB and delays initiation of treatment so considerably increases the possibility of transmission and development of drug-resistant TB[4]. Various serological tests introduced in the past for diagnosis of TB were not appreciated by clinicians due to number of reasons. On the other hand, polymerase chain reaction (PCR) test for diagnosis of TB has already started to influence the TB diagnostics from last three decades, therefore evaluated comprehensively as detection limit of PCR is as low as 10fg which makes only 2 bacilli per ml of specimen[8].

There are number of factors making diagnosis of EPTB difficult as compared to pulmonary TB including low yield of AFB in EPTB specimens, diversity in sites of TB infection, paucibacillary nature of specimen, and veracity of differential diagnosis. Besides this almost all sites need invasive procedure to obtain specimen for diagnosis which increase the chances to miss actual site of TB lesion which are consequently associated lower efficiency of TB culture and smear[9].

Various Molecular methods like line probe assay and GeneXpert are now frequently used due to their higher sensitivity and specificity. World Health Organization (WHO) has endorsed the use of GeneXpert for the rapid detection of MTB and the detection of rifampicin resistance. The results are obtained within 2 hours[10]. A minimal biosafety level is required and there is a less chance of cross-contamination. It is also very effective in the diagnosis of EPTB also. In view of above, present study was undertaken to determine the diagnostic accuracy of GeneXpert in pulmonary and extra-pulmonary specimens taking LJ media as the gold standard.

METHODOLOGY

This cross sectional study was undertaken in the Provincial Reference TB Laboratory (PRL) collaboration with Pathology Department, Nishtar Medical University Multan from November 2020-November 2021. A sample size of 210 is statistically calculated Keeping the confidence interval equal to 95%, the margin of error equal to 5% and anticipated Sensitivity of Gene

Xpert MTB/RIF assay as84%[11]. Suspected TB cases on clinical symptoms aged 18 years and above were included while patients with previous history of taking anti-TB drugs within 3 months before enrollment and patients with co-morbidities like HIV and renal failure were excluded.

Data Collection Procedure: After taking the informed consent duplicate sputum samples from pulmonary suspects and respective samples like fluids, pus, urine, etc. from extra-pulmonary suspects were collected individually in sterile containers. These patients were referred from outpatients or inpatients of Nishtar Hospital Multan. A pre-designed questionnaire was used to collect demographic information, history, and other required information. Direct and concentrated smears were prepared from one sample and processed for culture on LJ medium[12] while second sample was processed for GeneXpert[13].

Statistical Analysis: Data was entered and analyzed in SPSS. Qualitative variables like gender, age ranges and GeneXpert results were presented as frequency and percentage while quantitative variables were presented as men±standard deviation. Two by two tables were made to observe the accuracy of smear and GeneXpert taking Culture as Gold Standard.

RESULTS

A total of 210 patients were enrolled consisting of 113(53.8%) males and 97(46.2%) females with a female to male ratio of 1:1.17. An overall mean age of patients remained to be 36.81±16.22 years while mean ages of male and female patients were remained as 39.35±16.10 and 33.36±15.94 years respectively.

Segregation of patients in different age groups with reference to gender was also observed and highest frequency (30%) of patients was seen in youngest age group of 18-24 years and gradual decrease in frequency of patients was observed in higher age groups. It is also notable that frequency of female patients (39.2%) in youngest age group of 18-24 years remained remarkably higher as compared to male patients (22.1%) in same age group as shown in table 1.

Table 1: Distribution of Patients with Age Range (n=210)

Age range (years)	Male(n=113)	Female(n=97)	Overall
18-24	25(22.1%)	38(39.2%)	63(30%)
25-34	26(23%)	18(18.6%)	44(21%)
35-44	21(18,6%)	17(17.5%)	38(18/1%)
45-54	23(20.4%)	10(10.3%)	33(15.7%)
≥55	18(15.9%)	14(14.4%)	32(15/2%)

Most of the samples (95.2%) were from pulmonary source and sputum was provided for investigation while only 7 (3.4%) were pus samples and 3(1.4%) were from other sources. Sensitivity, specificity, PPV, NPV and accuracy of smear microscopy and GeneXpert were calculated by taking LJ culture as gold standard as presented in table 2 and 3 respectively. Presently, NPV of smear microscopy was remained very low (23.81%) in this study is the main reason of its poor sensitivity and specificity. On the contrary, an appreciable performance of GeneXpert in diagnosing TB was presented with a high accuracy of 98.10% in this study.

Table 2: Comparison of Smear Microscopy and Culture (2X2 Table)

Smear Microscopy	LJ Culture Result		Total
	Positive	Negative	
Positive	145	2	147
Negative	48	15	63
Total	193	17	210

Sensitivity=75.13%, Specificity=88.24%, PPV=98.64%, NPV=23.81%, Accuracy=76.19%

Table 3: Comparison of GeneXpert and Culture (2X2 Table)

GeneXpert	LJ Culture Result		Total
	Positive	Negative	
Detected	192	2	194
Not Detected	1	15	16
Total	193	17	210

Sensitivity=99.48%, Specificity=83.33%, PPV=98.45%, NPV=93.75%, Accuracy=98.10%

DISCUSSION

At present Sensitivity, specificity, PPV, NPV and accuracy were calculated by taking LJ culture as gold standard. For smear microscopy these were remained to be 75.13%, 88.24%, 98.64%, 23.81% & 76.19% while for GeneXpert these were 99.48%, 83.33%, 98.45%, 93.75% and 98.10% respectively. A clear difference may be seen where performance GeneXpert showing marvelous outcomes in terms of diagnostic parameters as compared to smear microscopy. Another study has reported lower values of sensitivity as 83.9%, PPV(88.1%), NPV(83.6%) and accuracy (85.8%) and higher specificity as 87.9% for GeneXpert and not in agreement with this study[4]. On the other hand findings of present study are very much comparable with studies carried out by WHO and presented a sensitivity of 92.1% and specificity of 93.5%[14].

A study evaluated the GeneXpert MTB/RIF Assay and presented a much lower sensitivity of 79.4% while comparable specificity of 96.5% however the study consisted a very low sample size of smear positive as 14 cases and presented GeneXpert positivity as 92.9% (13/14 cases) may be the only reason of reporting lower sensitivity. On the other hand study revealed that GeneXpert diagnosed as many as twice of the cases as compared to smear microscopy and detected MTB among (14/20) 70.0% of smear negative cases[15]. Present study also revealed concomitant results and showed efficiency of 76.19% in diagnosing smear negative TB cases. A study from Royal Free Hospital London also reported a lower sensitivity of GeneXpert as 86.1% and comparable specificity of 95% while a great agreement was shown in case of diagnosing 74.7% smear negative TB cases[16].

An Indian study claimed to be the first study in the country with a comprehensive sample size and evaluated the performance of GeneXpert in pulmonary TB cases only and presented a sensitivity and specificity of 95.7% and 99.3% respectively[17] and the results of present study are greatly in concomitant to the above findings.

The Accuracy of GeneXpert MTB/RIF assay in diagnosis of pulmonary TB as well as rifampicin resistance was assessed in a meta-analysis. Data was consisted of 9008 study subjects in total 22 studies. Purpose was to observe the value of GeneXpert test being used as primary diagnostic technique instead of smear microscopy and presented a pooled sensitivity of 88% and specificity of 99%. Afterwards 24 studies involving 2969 study subjects were assessed to observe the accuracy of GeneXpert test in detection of rifampicin resistance and presented a pooled sensitivity 95% and specificity of 98%. High quality studies were used to generate the evidence of the accuracy of GeneXpert MTB/RIF Assay[18].

Segregation of patients in different age groups and overall mean of patients as 36.81±16.22 years in this study are also in agreement with previous study which presented mean age of patients 36.9±14.99 years[4], while not in agreement with studies presented a lower mean age of 32.9±12.6[19] and higher age of 39.34±15.06 years[20]. Male gender is shown to be more exposed and presented a higher proportion as 53.8% as compared to females having 46.2% proportion in this study. A recent study has already presented a higher proportion of 62% males and 38% females [20].GeneXpert MTB/RIF Assay was designed to decrease the diagnostic postponement for drug resistant TB suspects which is necessary to manage patients promptly. It has helped not only in finding MDR TB cases but also revolutionized the diagnosis of MTB complex where only smear was present as rapid diagnostic technique with very poor sensitivity [21].

GeneXpert has been found to be extremely efficient technique in certain diagnosis of TB along provision of rifampicin susceptibility which is an important first line anti-TB drug thus, helpful in early indication of MDR TB. Its importance in diagnosing smear negative TB cases could not be denied anywhere.

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Conflict of Interest: Authors declare that they have no conflict of interest.

Ethics Approval: Ethical approval for this study was obtained from Institutional Ethical Review Board of Nishtar Medical University Multan on February 19, 2020 through letter No. 4000/NMU&H.

Author's contribution: All authors have significant contribution in this study. **RN and SMAN:** conceived the idea and designed the project, **RS, JS and GJ** collected the data, **RN, JS and SR** wrote the paper, **GJ and SS** observed the clinical relevance of data, **SMAN and SS** revised the manuscript, **RN and SR** did the data analysis.

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