

Comparative Analysis of Sputum Microscopy and Gene xpert Technique for Pulmonary Tuberculosis Along with Rifampicin Resistant in Tertiary Health Facility, Udaipur

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Abstract

Introduction: Mycobacterium tuberculosis (MTB) rapid identification, differentiation of MTB from nontuberculous mycobacteria (NTM) along with its resistance to rifampicin is necessary in mycobacterial diseases management. Culture being the “gold standard” for the detection of MTB, is time consuming. Smear microscopy, in spite of its rapidity and low cost, has poor sensitivity for the detection of acid-fast bacilli (AFB). GeneXpert MTB/RIF assay, a real-time PCR based rapid diagnostic method can simultaneously detect M. tuberculosis and its resistance or sensitivity to Rifampicin. Hence, we aim to compare the performance of GeneXpert assay with ZN smear along with rifampicin resistant pattern in tertiary health facility. **Aim:** The Study aims to analyze patients’ respiratory samples. **Objectives:** 1. Comparative analysis of GeneXpert and ZN Smear for detection of Mycobacterium tuberculosis. 2. To find out the sensitivity of GeneXpert Assay method along with Ziehl-Neelsen method in Tuberculosis infection diagnosis. 3. To determine the prevalence of Rifampicin resistance. **Materials and Methods:** This study included 1857 suspected tuberculosis patients, who had their sputum samples tested for ZN smear microscopy and GeneXpert during the period January to December 2019. The results of ZN smear microscopy and Gene Xpert was collected for data analysis. **Results:** A total of 1857 of patients were evaluated in final analysis. The Gene xpert performance was compared with Ziehl Neelsen technique. Out of the 1857 persons, 709 were smear Positive (38.2%) and 1148 were smear negative (61.8%), while for the Genexpert 873 (47.01%) were MTB detected, 984 (52.98%) MTB not detected. Out of 873 were 816 RIF (82.92%) Sensitive and 57 (5.79%) RIF resistance. 667 (35.91%) were TB Positive with both techniques, 206 (14%) were GeneXpert positive but ZN staining technique negative, 942 (50.72%) were negative with both methods and 42 (2.26%) was ZN stain Positive but GeneXpert negative. **Conclusions:** The GeneXpert is a cartridge-based nucleic acid amplification test (NAAT) is best available for simultaneous rapid tuberculosis diagnosis and antibiotic sensitivity test. Accuracy and negative predictive value of GeneXpert was found to be better than AFB staining. Thus, a negative GeneXpert test can rule out TB. Further, the test may be helpful in diagnosis of TB in patients likely to be missed by traditional tests. Also, positive ZN Stain and negative GeneXpert results indicate the presence of NTM. However, when compared to ZN smear, GeneXpert is expensive and needs sophisticated instrument.

Keywords: GeneXpert, ZN Stain, Tuberculosis.

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Introduction

Tuberculosis (TB) is a potentially serious infectious disease mainly affecting the lungs. The acid-fast bacilli causing tuberculosis spread from person to person through tiny droplets released into the air via coughs and sneezes. Every year, 10 million people are diagnosed with tuberculosis (TB)[1]. Despite being a preventable and curable disease. The disease is prevalent in countries where poverty, malnutrition and poor housing prevails. It kills about 3 million people and infects 9 million others every year taking it the world’s top infectious killer. TB is the leading cause of death of people with HIV and also a major contributor to antimicrobial resistance[2].

Acid fast bacilli (AFB) smear is the least expensive and widely used diagnostic tool for pulmonary tuberculosis. However, it has low sensitivity and needs a concentration of 10000 colony forming units/mL to be seen as positive under a microscope. Hence a sample with low bacterial count results in a negative report[3]. Accurate and timely diagnosis of TB will reduce the transmission of the disease and unnecessary antibiotic use[4]. Therefore the present study is undertaken to compare the performance of GeneXpert and smear microscopy with Mycobacterial growth indicator (MGIT) culture to choose the best available test for the diagnosis of TB.

Aim

The Study aims to analyse patients’ respiratory samples.

Objectives

1. Comparative analysis of GeneXpert and ZN Smear for detection of *Mycobacterium tuberculosis*
2. To find out the sensitivity of GeneXpert Assay method along with Ziehl-Neelsen method in Tuberculosis infection diagnosis
3. To determine the prevalence of Rifampicin resistance

Materials and methods

This cross-sectional study was done at Pacific Institute of Medical Sciences and Hospital, Udaipur after Institutional Ethics Committee approval during the period January to December 2019. Total 1857

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sputum samples of suspected pulmonary tuberculosis were included for the study. At least two sputum samples were taken from patient for tests analysis.

Inclusion Criteria

Patients with clinical suspicion of Pulmonary tuberculosis including cough for a minimum period of two weeks and above with or without blood-tinged sputum, night sweat, weight loss, fatigue.

Exclusion Criteria

Samples received without request of both tests
 Samples received without clinical history
 Patients with Lung malignancy or fungal etiology history respectively.

ZN staining

Heat Fixed smear slides of samples were stained with Ziehl-Neelsen (ZN) method and examined with a light microscope for the presence of AFB bacilli, in the Microbiology department, Pacific Institute of Medical sciences, Udaipur.

GeneXpert Samples were collected in containers provided and treated with sample reagent in a proportion of 2:1 and incubated for 15 minutes. Using the provided transfer pipette sample reagent treated sample was transferred into the sample chamber of the GeneXpert cartridge and put into the GeneXpert instrument system and the automatically generated results were read after 90 min.

Analysis

Statistical analysis of the data was conducted with SPSS 17.0. The specificity, sensitivity, PPV and NPV was calculated for ZN smear using GeneXpert. By taking GeneXpert as reference, samples

that were positive and negative in GeneXpert were considered true positive and true negative. ZN stain negative and GeneXpert positive were taken as false negative samples. GeneXpert negative and ZN stain positive were considered false positive. Receiver operating characteristic (ROC) curve analysis was performed and the area under curve (AUC) was determined.

Results

In this study, a total of 1857 sputum samples were tested by Z-N staining as well as by GeneXpert. Out of total samples, 709 (38.2%) samples were tested positive for acid fast bacilli (AFB) by Z-N staining while 1148 (61.8%) were negative for AFB. Table 1 shows the results of Z-N staining in total sputum samples.

When the Positive 709 ZN Smear sputum samples were subjected to GeneXpert assay, 667 (94.1%) were positive for *M. Tuberculosis* whereas 42 (5.9%) sputum samples gave negative result. In case of 873 positive sputum samples in GeneXpert assay, only 667 samples showed presence of acid-fast bacilli in Z-N staining while 206 samples were negative. The combined results of ZN Stain and GeneXpert (Table 1, Fig 1).

Out of total 873 positive GeneXpert samples, 57 samples were Rifampicin Resistant, and rest 816 samples showed Rifampicin sensitivity (Table 2).

Performance of the diagnostic test, Receiver Operating Characteristic (ROC) curve and measures of diagnostic accuracy for ZN smear microscopy taking GeneXpert as the reference test are summarized in Table 3, Table 4 and Figure 2.

Table 1: Combined Result of GeneXpert testing & ZN smear Examination

	GeneXpert Positive	GeneXpert Negative	Total
Smear Positive	667	42	709
Smear Negative	206	942	1148
Total	873	984	1857

Table 2: Rifampicin Resistance and Sensitivity

ZN Stain	Rifampicin Sensitive	Negative	Rifampicin Resistance	Total
Positive	627	42	40	709
Negative	189	942	17	1148
Total	816	984	57	1857

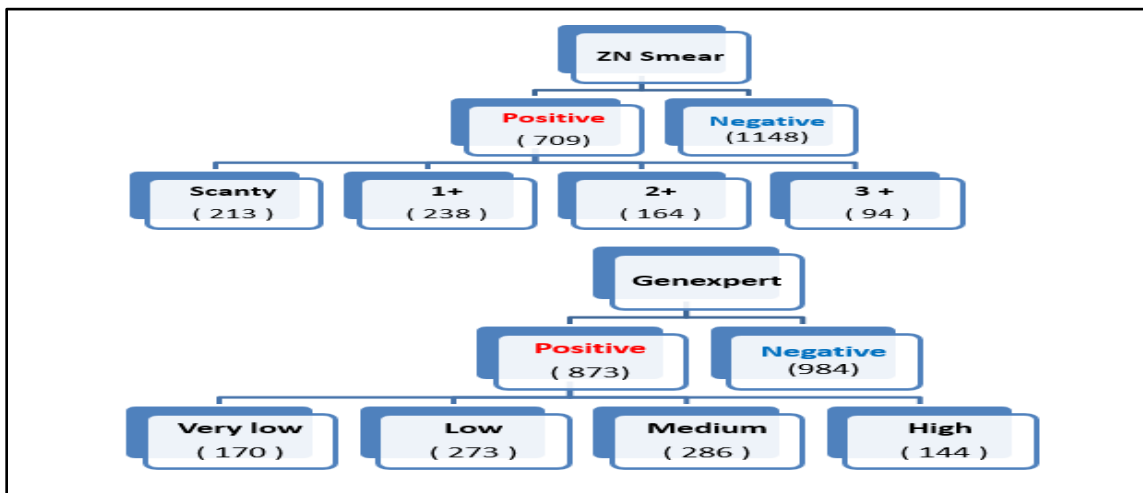


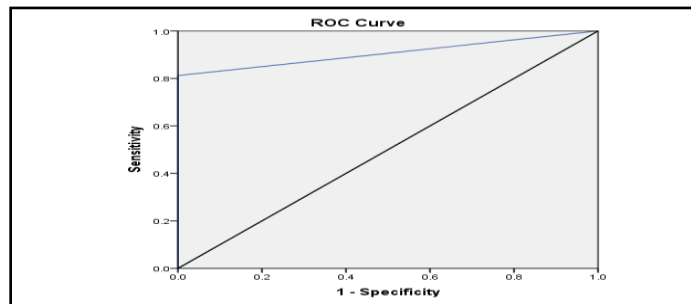
Fig 1: Summary of ZN smear and GeneXpert test results

Table 3: Showing performance of ZN smear taking GeneXpert as reference

ZN Smear	Genexpert	
	Positive	Negative
Positive	TP : 873	FP : 42
Negative	FN: 206	TN : 984

Table 4: Showing accuracy of ZN smear taking Genexpert as reference

Diagnostic Accuracy of ZN Smear	
Sensitivity	80.9%
Specificity	95.9%
Positive-Predictive Value	95.4%
Negative-Predictive Value	82.69%
Accuracy	88.22%
Positive Likelihood Ratio	Infinite
Negative Likelihood Ratio	0.16
Area Under Curve	0.906

**Fig 2: Receiver Operating characteristic curve for ZN smear, GeneXpert being taken as reference test.****Discussion**

Tuberculosis (TB) is a major health problem [6]. Although Culture is the gold standard test for TB diagnosis but our study relies mainly on ZN smear microscopy and GeneXpert assay. ZN staining and GeneXpert was available at the Pacific Institute of Medical Sciences (PIMS), Udaipur where this study was conducted. This study has evaluated the diagnostic accuracy of sputum ZN stain in comparison with GeneXpert as the reference test.

In this retrospective study we have evaluated the diagnostic yield of ZN stain to detect MTB in Pulmonary samples and compared it with GeneXpert which was taken as golden standard. In comparison with GeneXpert used as gold standard, sensitivity, Specificity, PPV and NPV for Smear microscopy for Pulmonary sample were recorded as 80.9 %, 95.9%, 95.4% and 82.69% respectively, which is in line with other studies as depicted in [Table 5][9,10,11].

Table 5: Comparison with Previous studies

Study	Sen	Spec	PPV	NPV
Kanwal et al .,[9]	39.53	100	100	11.86
Pierrae et al.,[10]	25	95.8	45.5	90.1
Dewald et al.,[11]	41	98.6	94.1	75.8
Monika et al .,[12]	22.2	100	100	85.3
Our study	80.9	95.9	95.4	82.69

GeneXpert assay had sensitivity of 47.01% which is superior to that of ZN Stain being 38.18%. In a study carried out by Chinedum OK et al [13] GeneXpert was positive in 65.7% cases as compared to ZN stain being positive in 38.6% cases. In another study by Mavengwa R et al [14] 32.20% samples were found to be positive by GeneXpert assay and only 24.05% were found to be positive by ZN stain. One more such study conducted by Bajrami R et al [15]. GeneXpert could detect 29.3% cases as compared to Z-N staining showing 14.6% positive cases only. All these studies including our study indicate that the GeneXpert assay is more sensitive for diagnosis of tuberculosis as compared to ZN smear microscopy. In present study we observed that prevalence of Rifampicin resistance was 5.79 % and rest studies almost share the same study (Table 6)

Table 6: Comparison with Previous studies (Rifampicin resistance)

Study	Rifampicin resistance
Urvashi et al .,[16]	21.49
Kishore ingole et al.,[18]	24.09
Raghuprakash Reddy et al .,[17]	9.2
Thomas et al.,[20]	9
Santosh kumar et al.,[19]	4.1
Our study	5.79

In our study we found the prevalence of MTB was 47.01% by using GeneXpert, similar observation noted in other study by Kumar M et al. [21]. Other studies by Chakroborty et al [22] and Khalil et al [23] observed similar result in their studies. Similar results were found in

Dravid MN et al. study, [24] with 40.63% detection, 82.90%, 17.10% rifampicin sensitivity and resistant respectively. In our study we observed that 42 sputum samples showed presence of AFB by ZN Stain but were negative in GeneXpert assay. These acid fast bacilli

may be considered as Nontuberculous mycobacteria (NTM) because the GeneXpert assay detects *M. tuberculosis* complex and not cases of NTM infection. Overall our study supports the fact that GeneXpert can be considered as an effective tool for early tuberculosis diagnosis along with possibility of its prevention and treatment. In countries like India where TB is quite prevalent, GeneXpert has made a huge impact.

Our study has some limitations, we have not performed Mycobacterial culture to rule out false positive results. However, both these tests have shown specificities above 95% in studies by Vadwai, Parsons and Tortoli. et al. [25-27]. Thus there could be a possibility of bias due to false positive results but it can be considered insignificant.

Conclusion

GeneXpert system has shown a much higher sensitivity for the detection of MTB in suspected pulmonary samples than ZN smear microscopy. Nevertheless, the GeneXpert system is considered a valuable diagnostic tool for the rapid detection of MTB along with MDR-TB. This early detection facilitates not only in controlling the disease transmission but also in initiation of earlier treatment. At the same time, the introduction of GeneXpert system results in a significant reduction of MDR-TB cases with faster reporting to TB programs.

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