Comparative Study Of Gene XPERT MTB/RIF, ZIEHL NEELSEN Smear Microscopy And MGIT 960 Culture In Samples Of Suspected Pulmonary Tuberculosis In A Tertiary Care Hospital Of Jharkhand

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Abstract
Introduction: Tuberculosis is a major public health problem worldwide especially in developing countries like India. Now a days it becomes more deadly in the form of MDR TB and XDR TB. Aim and Objective: The aim of this study is to correlate between isolates of mycobacterium in GeneXpert, MGIT culture and Ziehl-Neelsen (ZN) staining. It also detects the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of GeneXpert assay and ZN staining. Methodology: This prospective study was carried out in the Department of Microbiology, RIMS, Ranchi, Jharkhand. One hundred fifty two sputum samples from suspected patients of pulmonary tuberculosis were taken. These samples were processed for test in GeneXpert assay, MGIT culture and ZN staining. Mycobacterium tuberculosis complex isolated from culture was taken as gold standard and compared with result of GeneXpert and ZN staining. Result: Out of 152 samples, detection rate of GeneXpert, MGIT and ZN staining were 28.95%, 26.31% and 23.68%. The sensitivity, specificity, PPV and NPV of ZN staining and GeneXpert were 80%, 96.43%, 88.89%, 93.10% and 100%, 96.43%, 90.91%, 100% respectively. Conclusion: GeneXpert is a rapid and easy method for tuberculosis diagnosis. It not only detect the bacilli but also diagnose rifampicin drug resistance. This method prompts in diagnosis and treatment where the sample load is high.
Keywords: *Pulmonary Tuberculosis, MGIT 960, GeneXpert MTB/RIF, ZN staining, Acid Fast Bacilli*

I. Introduction

Tuberculosis is one of the most common communicable diseases caused by *Mycobacterium tuberculosis* bacteria. According to global tuberculosis report, World Health Organization, 2018, TB causes 10 million cases and 1.3 million deaths annually and it is estimated that 3.6 million cases are either not detected or not notified to public health services each year[1]. After taking up residence in the lung, M. tuberculosis can disseminate to any part of the body[2]. Tuberculosis spread through inhalation of droplets produced by coughing, sneezing, singing, talking by infected person. For effective control and treatment of the disease, timely diagnosis and rational treatment is a must. Now a days, emergence of multi drug resistant TB(MDR-TB) is a threat to the society as well as global TB control programs, as it challenges treatment modality and prognosis. MDR-TB is defined as resistance to at least isoniazid and rifampin (RIF)[3,4]. The most easy and simple test, ZN staining has low sensitivity and specificity. The WHO has recommended different molecular methods for the rapid diagnosis of *Mycobacterium tuberculosis*. The Gene Xpert MTB/RIF test is recommended as the initial diagnostic test for patients being evaluated for pulmonary and extra pulmonary TB[5]. At present the gold standard of TB diagnosis is both solid and liquid culture method. The drawbacks of culture method is labour intensive and time consuming as it takes 6-8 weeks. For effective treatment of tuberculosis earlier diagnosis and appropriate medicine is a must.

AIMS AND OBJECTIVES
1. Importance of new diagnostic tool Gene Xpert assay for TB detection.

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2. To find out the sensitivity, specificity, PPV and NPV of Gene Xpert MTB/RIF assay over ZN method in diagnosis of tuberculosis infection.

II. Materials And Methods

This Descriptive and prospective study with sample size of one hundred fifty two was done in the Department of Microbiology, Rajendra Institute of Medical Sciences, Ranchi from November 2018 to October 2019. Each sputum sample was processed for Ziehl- Neelsen staining, MGIT 960 culture and GeneXpert assay. For GeneXpert test sputum sample was processed as per the manufacturer's instructions. Test results were available within two hours. Acid fast staining was done by ziehl-Neelsen technique. Microscopic detection of acid fast bacilli was done by ZN technique.

For MGIT 960 culture all the sputum samples were digested and decontaminated by standard N-acetyl-L-cysteine and sodium hydroxide (NaOH-NALC). NALC act as a mucolytic agent and NaOH as a decontaminating agent. After processing sample was put into culture tube and loaded in MGIT machine for the test. Positive growth were identified with the help of ZN smear microscopy. Mycobacterium tuberculosis complex(MTBC) were differentiated from Non tuberculous mycobacteria(NTM) with the help of Tuberculosis complex(TBC) identification kit.

ANALYSIS

The data was tabulated in a Microsoft excel sheet and it was studied for correlation. Stastical analysis of the data was conducted with the help of SPSS software version 20. Sensitivity, specificity, PPV and NPV were calculated, using culture of *Mycobacterium tuberculosis* from sputum as gold standard. By taking culture method as reference, samples that were positive and negative in culture were considered as true positive and true negative. Culture negative and GeneXpert positive samples were taken as false positive samples. GeneXpert negative and culture positive samples were considered as false negative likewise for ZN smear also.

III. Results

Table 1: Comparison between GeneXpert and ZN stain

ZN stain	GeneXpert			Total
	Positive and Rifampicin Sensitive	Negative	Positive Rif Resistant	
Positive	28	4	4	36
Negative	10	104	2	116
Total	38	108	6	152

Out of 152 samples 36 were AFB positive by ZN staining. In 44 and 40 samples, MTB detected by GeneXpert and MGIT culture respectively. In 12 samples which were AFB negative by ZN but MTB detected by GeneXpert in which 10 were rifampicin sensitive and 2 were rifampicin resistant. Out of 44 GeneXpert positive cases, 6 (13.64%) were rifampicin resistant.

Table 2: Comparison of MGIT culture with GeneXpert and ZN staining

GeneXpert	MGIT Culture		Total	ZN staining	MGIT Culture		Total
	Positive	Negative			Positive	Negative	
Positive	40	4	44	Positive	32	4	36
Negative	0	108	108	Negative	8	108	116
Total	40	112	152	Total	40	112	152

Out of 152 samples MGIT culture positive and negative were 40 and 112 respectively. In four GeneXpert positive and AFB positive samples, MGIT showed no growth.

While considering MGIT culture as gold standard, the sensitivity, specificity, positive predictive value(PPV) and negative predictive value(NPV) of ZN staining and GeneXpert is 80%, 96.43%, 88.89%, 93.10% and 100%, 96.43%, 90.91%, 100% respectively.

IV. Discussion

Conventional methods of MTBC detection have not only low sensitivity and specificity but also more time consuming. Molecular methods like GeneXpert have changed the scenario. It has more sensitivity and specificity and gives result within two hours and shows Rifampicin resistance status[6]. A confirmed positive culture of MTBC was used as reference for other methods of tests. In my study by MGIT culture, 26.31% were

positive for MTBC. 28.94% were found positive by GeneXpert assay. 23.68% were positive by ZN staining. Case detection rate is more for the GeneXpert assay. In December 2010, the WHO endorsed the Xpert MTB/RIF assay for the rapid diagnosis of TB and MDR-TB[7].

The sensitivity, specificity, positive predictive value(PPV) and negative predictive value(NPV) of ZN staining and GeneXpert is 80%, 96.43%, 88.89%, 93.10% and 100%, 96.43%, 90.91%, 100% respectively. Study by Bajrami et. al. showed Sensitivity, specificity, PPV and NPV for GeneXpert and ZN staining as 82.3%, 97.6%, 93.3%, 93% and 94.1%, 85.7%, 53.3%,98.8% respectively[8]. Study of Agrawal M et. al. showed the result of Sensitivity, specificity, PPV and NPV for GeneXpert and ZN staining as 72.7%, 100%, 100%, 76.9% and 100%, 90%, 91.6%, 100% respectively[9]. Other studies also have the comparable data[10,11]. Four specimen which were GeneXpert positive but did not showed growth on culture as dead bacilli also gives positive result with Xpert assay. The ZN staining not only have low sensitivity and specificity but also it cannot differentiate between MTBC and NTM.

V. Conclusion

Although ZN staining is a simple and not much technically sophisticated method of tuberculosis detection, but it has certain limitations. Gene Xpert is useful for rapid detection of TB along with identification of RIF resistance in a country like India where prevalence of TB is high. The results are superior to smear microscopy and comparable to culture with shorter turn-around time.

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