Comparative Evaluation between Detection of Mycobacterium Tuberculosis Complex in Samples of Extra Pulmonary Tuberculosis using Gene Xpert MTB/RIF Assay and Ziehl-Neelsen Staining in a Tertiary Care Hospital

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Abstract

➤ **Introduction:**
Tuberculosis is a global problem and incidence of extra pulmonary tuberculosis is increasing day by day. Diagnosis of extra pulmonary tuberculosis is a little bit difficult than pulmonary tuberculosis.

➤ **Aim and Objective:**
The aim of this study is to correlate between isolates of mycobacterium in GeneXpert 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. Seventy two samples from suspected patients of Extra pulmonary tuberculosis were taken. These samples were processed for test in GeneXpert assay, ZN staining and MGIT culture. Mycobacterium tuberculosis complex isolated from culture was taken as gold standard and compared with result of GeneXpert and ZN staining. Result: Out of 72 samples, detection rate of GeneXpert, MGIT and ZN staining were 27.77%, 26.38% and 18%. The sensitivity, specificity, PPV and NPV of ZN staining and GeneXpert were 63.15%, 98.11%, 92.3%, 88.13% and 94.73%, 96.22%, 90%, 98% respectively.

➤ **Conclusion:**
GeneXpert is a rapid and easy method for extra pulmonary tuberculosis diagnosis. It not only detect the bacilli but also diagnose rifampicin drug resistance. This method prompts in diagnosis and treatment.

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 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.

II. AIMS AND OBJECTIVES

➤ Importance of new diagnostic tool Gene Xpert assay for TB detection.
➤ To find out the sensitivity, specificity, PPV and NPV of Gene Xpert MTB/RIF assay over ZN method in diagnosis of extra pulmonary tuberculosis infection.

III. MATERIALS AND METHODS

This Descriptive and prospective study with sample size of seventy two was done in the Department of Microbiology, Rajendra Institute of Medical Sciences, Ranchi from April 2018 to March 2019. Each extra pulmonary sample was processed for Ziehl- Neelsen staining, MGIT 960 culture and GeneXpert assay. For GeneXpert test 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 and Microscopic detection of acid fast bacilli was done.

For MGIT 960 culture all the 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.

IV. 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 sample 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.

V. RSULTS

Samples	Total no. (72)	ZN staining positive	Gene Xpert positive	MGIT culture positive
FNAC Lymph node	24 (33.33%)	4 (16.66%)	6 (25%)	7 (29.16%)
Pus	18 (25%)	3 (16.66%)	5 (27.77%)	5 (27.77%)
Cerebrospinal fluid	4 (5.55%)	0 (0%)	1 (25%)	1 (25%)
Pleural fluid	6 (8.33%)	2 (33.33%)	2 (33.33%)	1 (16.66%)
Endometrial tissue	12 (16.66%)	3 (25%)	4 (33.33%)	3 (25%)
Gastric aspirate	4 (5.55%)	1 (25%0	1 (25%)	1 (25%)
Pericardial fluid	4 (5.55%)	0 (0%)	1 (25%)	1 (25%)
Total	72 (100%)	13 (18%)	20 (27.77%)	19 (26.38%)

Table 1

| ZN stain | Gene Xpert | | |
	Positive	Negative	Total
Positive	13	0	13
Negative	7	52	59
Total	20	52	72

Table 2:- Comparison between GeneXpert and ZN stain

Out of 72 samples 13 were AFB positive by ZN staining and 20 were positive by Gene Xpert assay. The case detection rate of Gene Xpert and ZN staining are 27.77% and 18% respectively.

| GeneXpert | MGIT Culture | | Total | ZN staining | MGIT Culture | | Total |
	Positive	Negative			Positive	Negative	
Positive	18	2	20	Positive	12	1	13
Negative	1	51	52	Negative	7	52	59
Total	19	53	72	Total	19	53	72

Table 3:- Comparison of MGIT culture with GeneXpert and ZN staining

Out of 72 samples MGIT culture positive and negative were 19 and 53 respectively. 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 63.15%, 98.11%, 92.3, 88.13% and 94.73, 96.22%, 90%, 98% respectively.

VI. 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[6] and shows Rifampicin resistance status. A confirmed positive culture of MTBC was used as reference for other methods of tests. In my study by MGIT culture, 26.38% were positive for MTBC. 27.77% were found positive by GeneXpert assay. 18% were positive by ZN staining. Case detection rate is more for the GeneXpert assay. In 2011, the WHO endorsed the Xpert MTB/RIF assay for the rapid diagnosis Extra pulmonary TB diagnosis[7].

The sensitivity, specificity, positive predictive value(PPV) and negative predictive value(NPV) of ZN staining and GeneXpert is 63.15%, 98.11%, 92.3, 88.13% and 94.73, 96.22%, 90%, 98% 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].

VII. CONCLUSION

Although ZN staining is a simple and not much technically sophisticated method of tuberculosis detection, but it has low sensitivity for extra pulmonary sample. Gene Xpert is useful for rapid detection of extra pulmonary 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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