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Original Article

Detection of Tuberculosis on Culture, Comparison of Findings with Fluorescence Microscopy and GeneXpert

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ABSTRACT

The GeneXpert MTB/RIF technique is used for the identification of tuberculosis and rifampicin (RIF) resistance. Xpert MTB / RIF provides patients with distinct advantages such as early diagnosis. **Objectives:** To compared the performance of fluorescence microscopy and GeneXpert with culture in TB samples from Narowal, Pakistan. **Methods:** A total of 299 TB positive specimens were obtained. Among these 54% (n = 160) were categorized to be obtained from male and 46% (n = 139) from female population. The sensitivity and specificity of fluorescence microscopy, GeneXpert and culture of TB samples were done. **Results:** The parameters including sensitivity and specificity calculated for GeneXpert were 73% and 100%, respectively, while the sensitivity and specificity calculated for culture was 100% and the sensitivity and specificity for FM microscope were 43% and 100%, respectively. **Conclusions:** We conclude that the GeneXpert is more sensitive than FM considering culture as a gold standard. Although the GeneXpert assay was also shown to be able to detect a limited number of bacillus from samples, the culture's sensitivity and specificity were both 100%.

INTRODUCTION

Lung is the main organ affected by *Mycobacterium tuberculosis*, but other organs of body may also be severely affected by it. In most cases, the ailment can reduce symptoms, in which case it is called drowsiness or inactive tuberculosis [1]. The identification of indolent tuberculosis depends on the special skin test called tuberculin skin test (TST) or by way of blood test [2]. Avoidance of tuberculosis includes vaccination against BCG, increased screening hazard, timely identification and correct management of the cases [3]. Mycobacteria are aerobic, non-motile, acidic alcohol or micro-curvature. These organisms have high molecular weight carbon atoms and mycolic acid in the cell wall, and after pyrolysis, they release C22-C26 linear saturated acidic chain are pathogenic bacteria of human tuberculosis [4]. Tuberculosis was reported nationwide in 1953, and incidence of tuberculosis declined steadily in 1984 [5]. *Mycobacterium tuberculosis* is transmitted mainly by inhalation of small infectious droplets (1-10 µm in diameter) of dry residue [6]. Based on the analysis of sequence of genome, *M. tuberculosis* does not recognize the characteristic virulence factors of bacteria [7]. A common host response to MTBC infection is cell-mediated activation of body's immune system. Infrequent infection

with intravesical instillation of *M. bovis* BCG is used to treat superficial bladder cancer [8]. AFB staining typically has a specificity of 99% or higher and a sensitivity of about 25% to about 75% [9]. Inoculation with solid and liquid media is suggested for optimum growth of *mycobacteria* from the samples [10]. AFB staining does occur in sputum in up to 75% of tuberculosis patients, less than 20% of *tuberculosis* children have significant AFB spread during sputum or stomach inhalation [11]. Traditionally, the identification of mycobacteria was based on the growth rate of solid media, biochemical test results and the morphology and coloration of colonies [12]. Recently Cepheid proposed GeneXpert MTB / RIF test [13]. GeneXpert assays, like real-time PCR assays, can simultaneously recognize MTB and specifically identify rifampicin resistance from sputum or other liquid samples [14].

METHODS

Cross sectional study was done in the tuberculosis department at DHQ Hospital in Narowal. The sample size of 299 was determined by formula as follows:

$$n = \frac{z^2_{1-a/b} * P * (1-p)}{d^2}$$

The patient's detailed clinical parameters were recorded and the patient was guided to collect the sputum sample in a defined container. Smears were prepared from samples after concentration and re-suspension of the pallet. Smear was covered with stain. After staining, the slides were examined by the microscopists. Lowenstein Jensen media was employed to detect the bacilli from samples. To prevent the growth of Gram-positive and Gram-negative bacteria as well as to restrict growth to Mycobacterium species only, low concentrations of penicillin and nalidixic acid are also added in LJ medium. Presence of malachite green in the medium inhibits most other bacteria. It is disinfected and solidified by a process of inspissation. Presence of glycerol enhances the growth of *M. tuberculosis*. For cultivation of *M. bovis,* glycerol is omitted and sodium pyruvate is added. Positive and negative results of samples as found by microscopy and GeneXpert were cultured on Lowenstein Jensen media. After inoculation, the plates were incubated for at least 6 weeks at 37°C. Any visible growth was observed and recorded as MTB and MOTT. For GeneXpert system. The sample reagent and the sputum collection container lids were opened. 02 volumes of sample reagent was added to 01 volume of sputum and lid was replaced. The mixture was thoroughly mixed over a vortex for at least 10 seconds. Then it was incubated for 10 minutes at room temperature and then mixed again. It was incubated for another 05 minutes. The sample was processed till it was perfectly liquid, if it was still viscous, a waiting time of 05-10 minutes was given. The side of the cartridge was labelled with the sample id before its lid was opened. Sample (2ml) was slowly transferred to the sample chamber of the cartridge taking care that care that bubbles don't form. The lid was firmly closed and the test was run on GeneXpert instrument. Using the 2×2 table in the SPSS-20 software and considering the sputum culture as gold standard. The sensitivity, specificity, PPV and NPV for each assay were calculated to diagnose TB in patients. The kappa(k) test was used to assess the consistency between the tests. Using the formula, the sensitivity was found as follows: Sensitivity % = true positive(TP)/(true positive(TP)+false negative(FN))X 100. Specificity was calculated using the formula given below: Specificity % = true negative (TN) / (true negative (TN) + false positive(FP))X 100.

RESULTS

The current study was conducted (in Narowal, Pakistan) to compare the diagnosis of tuberculosis using GeneXpert and fluorescence microscopy with culture. Total processed samples were 299 of which 54% (n = 160) were obtained from male and 46% (n = 139) from female population. Culture method declared 43% (n=128) samples as positive (Table 1).

n(%)	Methods		
	FM	GeneXpert	Culture
	55 (18%)	93 (31%)	128 (43%)

Table 1: Detection of tuberculosis on culture

The sensitivity and specificity recorded for culture were 100% and 100%, respectively. Furthermore, positive predictive value (PPV) and negative predictive value (NPV) estimated for culture methods were found to be 100% and 100%, respectively (figure 1). Also, no samples were found that had positive results on culture and negative on GeneXpert. Culture was taken as standard and had sensitivity of 100% as compared to FM technique that had 43% sensitivity. Sensitivity for culture was found to be half fold more than FM technique. It was noted that culture was more sensitive than FM in detecting tubercle bacilli. Culture was taken as standard which had a sensitivity of 100% as compared to GeneXpert technique that had 73% sensitivity. It was found that culture was more sensitive than GeneXpert (figure 1).

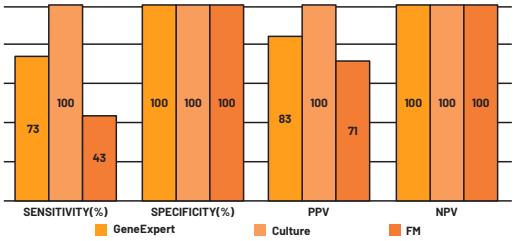

Figure 1: Comparison of sensitivity and specificity of FM,

GeneXpert and Culture

DISCUSSION

In this study total of 299 sputum samples were examined, with an FM detection rate of 18% (n = 55), GeneXpert of 31% (n = 93) and a standard culture technique of 43% (n = 128). The specificity and sensitivity of the GeneXpert assay were known to be 100% and 73%, respectively, in addition the sensitivity and specificity of the FM microscope were 43% and 100%, respectively. The findings showed culture as better than the two techniques used. This is in contrast with another study comparing the GeneXpert findings and stated GneXpert to be better [15]. As a reference standard, the culture revealed that the smear positive samples had a sensitivity of 98.4% (60/61) and the smear negative samples had a sensitivity of 93.7% (30/32)[16]. The result of this study are similar to our study which aimed to evaluate GeneXpert for culture and fluorescence microscopy, and GeneXpert analysis showed sensitivity and specificity of 73% and 100%, correspondingly. The specificity, sensitivity, positive predicted value and negative predicted value of XpertTB/RIF detection were 93%, 93.3%, 82.3% and 93.3%, respectively. Respectively. Xpert determination was significantly higher than the sensitivity of rapid smear of citric acid ($p < 0.001$). GeneXpert detected 50% additional positive cases compared to LJ culture and smear microscopy [17, 18]. Results of this study are quite comparable to our study which analyzed 299 samples. LJ culture cases are twice times higher than AFB smear cases, with sensitivity and specificity of 45.7% and 100%. In addition, from the clinically diagnosed 81 urinary tract tuberculosis cases, 51 were processed by the Xpert technique, showing the sensitivity of 63% that is considerably higher than AFB smear microscopy and LJ culture method. GeneXpert was only detected in 5 patients with RIF resistance, and all patients had a phenotypic sensitivity test with a sensitivity of 100% [19, 20]. This study is quiet similar to present study in which GeneXpert is more sensitive and specific as compared to FM.

CONCLUSIONS

We conclude that the GeneXpert is more sensitive than FM considering culture as a gold standard. The culture sensitivity and specificity was 100% but the GeneXpert assay was also found to detect small number of bacillus from samples. In addition, the estimated PPV and NPV values for the culture method were found to be 100% and 100%, respectively.

Conflicts of Interest

The authors declare no conflict of interest

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