COMPARISON OF CONVENTIONAL DIAGNOSTIC MODALITIES AND POLYMERASE CHAIN REACTION FOR DETECTION OF MYCOBACTERIUM TUBERCULOSIS


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Received: October 5, 2009; Accepted: October 30, 2009

Abstract: Tuberculosis continues to be an important cause of morbidity and mortality throughout the world. Early recognition of disease and beginning the quick antituberculosis treatment are essential to control tuberculosis. So the present study was done to compare conventional diagnostic methods and Polymerase Chain Reaction for rapid detection of Mycobacterium tuberculosis from sputum. One hundred and twenty one sputum samples were collected from suspected patients and processed to detect Mycobacterium tuberculosis by AFB staining, culture and PCR. Infection detection rate of PCR was found to be 66.11%, that is higher than that of AFB staining and culture. Sensitivity of PCR was found to be 88.88% followed by AFB staining with 77.77% and culture with 72.22%. PCR was found more accurate with 91.73% accuracy followed by AFB staining (81.81%) and culture (79.33%). PCR is the most rapid, sensitive, specific and accurate method than AFB staining and culture for detection of mycobacterium tuberculosis.

Key words: Mycobacterium tuberculosis

INTRODUCTION

Mycobacterium tuberculosis is the pathogenic bacterial species in the genus Mycobacterium and the causative agent of most cases of Tuberculosis in mammals. More than one species of Mycobacterium are responsible for Tuberculosis, so they are assembled as Mycobacterium tuberculosis complex (MTBC). Mycobacteria are aerobic, non motile, slender rod shaped, acid-fast bacteria.

Early recognition of cases and beginning the antituberculosis treatment quickly are essential in tuberculosis control [1]. In diagnosis of pulmonary tuberculosis, direct microscopy by Ziehl-Neelsen staining to identify acid – fast bacilli (AFB) is the leading bacteriological examination because it is easily done, has low cost and gives the results quickly but has low sensitivity [2,3]. Detection of acid fast bacilli by culture is comparatively more specific and sensitive than staining, but it takes 4 to 8 weeks to culture pathogenic mycobacteria because of its slow growing nature [3,4]. Serology and other new techniques (such as tuberculostearic acid) are not widely used due to high cost, low sensitivity and specificity or both [5].

More sensitive test is required to detect samples with a low bacterial load [6]. Nucleic acid of few mycobacteria can be amplified by nucleic acid amplification method like Polymerase chain Reaction (PCR) [7]. Since the advent of PCR, there has been exploitation in its use for TB diagnosis owing to its speed and sensitivity [6]. With the Use of PCR, nucleic acid sequences unique to Mycobacterium tuberculosis complex can be detected directly in clinical
specimens, offering better accuracy than AFB smear and greater speed than culture [7-10]. One of the common PCR targets is the repetitive sequence IS6110, the mobile insertion sequence, involved in genomic deletions, insertion mutations and modulation of the gene expression. The IS6110 can be responsible for an increased capacity for transmission and replication and for the virulent phenotype of outbreak strains [11]. In present communication PCR assay of IS6110 sequence is compared with conventional diagnostic methods for detection of *Mycobacterium tuberculosis* in sputum samples.

**MATERIALS AND METHODS**

A prospective study was undertaken at Shree P.M Patel institute of post graduate studies and research in science, Anand, Gujarat from 18th of December, 2008 to 15th April, 2009. Total 121 symptomatic cases of Tuberculosis were processed for detection of *Mycobacterium tuberculosis* infection by three methods viz., acid fast staining, culture, and polymerase chain reaction.

Samples for processing were collected from Government Tuberculosis Hospital, Anand. About 5-10 ml of early morning sputum was collected from each patient in sterile screw capped plastic containers. These specimens were decontaminated and concentrated by Petroff’s method using 4% NaOH. The specimens were further analysed by

i). **AFB staining method**: Smears were prepared from sediment and stained by Ziehl Neelsen method.

ii). **Culture method**: Sediment suspensions were inoculated onto the L J medium (HIMEDIA). Remaining suspensions were refrigerated for PCR assay. Cultures on L J medium were confirmed by catalase-peroxidase test.

iii). **PCR assay**: DNA Extraction and DNA amplification were performed using Banglore GENEI KIT - GeNeiTM amplification reagent set for *Mycobacterium tuberculosis* (Cat # 105935). Thermal cycler (2720) of applied biosystem was used for amplification.

**RESULTS**

Study shows that out of 121 total samples, Ziehl Neelsen staining for AFB was positive in 72 samples (59.50%) and culture on LJ media was positive in 65 samples (53.72%). Contrary to these, PCR detected 80 (66.11%) samples positive for *Mycobacterium tuberculosis* (Table 1).

<table>
<thead>
<tr>
<th>Total Number</th>
<th>AFB Staining (After concentration method)</th>
<th>Culture</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>121</td>
<td>72</td>
<td>49</td>
<td>65</td>
</tr>
</tbody>
</table>

Table 1: Distribution of result according to diagnostic methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Predictive value %</th>
<th>Accuracy %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>AFB Staining</td>
<td>77.77 %</td>
<td>97.54 %</td>
<td>97.22 %</td>
<td>59.18 %</td>
</tr>
<tr>
<td>Culture</td>
<td>72.22 %</td>
<td>100 %</td>
<td>100 %</td>
<td>55.35 %</td>
</tr>
<tr>
<td>PCR</td>
<td>88.88 %</td>
<td>100 %</td>
<td>100 %</td>
<td>75.60 %</td>
</tr>
</tbody>
</table>

Table 2: Sensitivity, specificity, positive and negative predictive values and accuracy of methods used for diagnosis of pulmonary tuberculosis

<table>
<thead>
<tr>
<th>Author</th>
<th>Sensitivity of PCR</th>
<th>Specificity of PCR</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wite et al (1992) [17]</td>
<td>81%</td>
<td>-</td>
<td>Pleural effusion</td>
</tr>
<tr>
<td>Claridge et al(1993) [16]</td>
<td>83.5%</td>
<td>99%</td>
<td>Sputum</td>
</tr>
<tr>
<td>Takagi et al (1998) [18]</td>
<td>89%</td>
<td>100%</td>
<td>Pleural biopsy</td>
</tr>
<tr>
<td>Babu et al (2001) [19]</td>
<td>100%</td>
<td>70%</td>
<td>Pleural effusion</td>
</tr>
<tr>
<td>Present study</td>
<td>88.88%</td>
<td>100%</td>
<td>Sputum</td>
</tr>
</tbody>
</table>
Sensitivity of Ziehl Neelsen staining, culture and PCR was 77.77%, 72.22% and 88.88% respectively. ZN staining was 97.54% specific and culture and PCR were 100% specific. Positive predictive value for ZN staining was 97.22% and for culture and PCR it was 100%. Negative Predictive Value of PCR was found highest with 75.60% followed by acid fast staining with 59.18% and culture with 55.35%. PCR was found more accurate with 91.73% accuracy than acid fast staining with 81.81% and culture with 79.33% accuracy (Table 2).

**DISCUSSION**

A rapid and accurate diagnosis of tuberculosis is a cornerstone of tuberculosis control strategies [12]. A rapid initial diagnosis of *Mycobacterium tuberculosis* is problematic if the techniques of direct visualization (AFB staining) are negative. The definitive diagnosis depends on the culture of the mycobacteria, a technique that is time consuming and not always sensitive enough [13]. Because of high sensitivity of the PCR to detect *Mycobacterium tuberculosis* DNA from clinical samples, potential use of this technique has been investigated as rapid diagnostic procedure for detection of *Mycobacterium tuberculosis* in sputum sample [13]. The test is rapid and can detect fewer than 10 microorganisms in the samples [14]. In the present study, the sensitivity and specificity of PCR from sputum samples were found to be 88.88% and 100% respectively. In their study with sputum, Prasad et al. [5] got 83% and 100% sensitivity and specificity of PCR respectively which is comparable to present reading.

Khan et al. [15] found only 14.50% sensitivity of PCR in blood samples in patients with pulmonary tuberculosis which is very low. These results indicate that for detection of pulmonary tuberculosis by PCR, sputum is sample of choice rather than blood. The sensitivity and specificity of PCR from sputum was found to be comparable with the report of Claridge et al. [16]. In present study the sensitivity and specificity of acid fast staining were found to be 77.77% and 93.54% respectively which are lesser than those of PCR. Acid fast staining is cheap and simple but less sensitive because a large number of bacilli (5000-10,000 bacilli/ml of sputum) must be present in a specimen for the smear to be positive.

In present study, PCR detected 16.32% of smear negative specimens while in study of Prasad et al. [5], PCR detected 54% of smear negative specimens. Further, no false positive results were obtained by PCR which is comparable with the study done by Prasad et al. [5]. In present study, sensitivity and specificity of culture were found to be 72.22% and 100% respectively. As mycobacteria are slow growing organisms, it requires 4-6 weeks for visible growth. Polymerase chain reaction can provide diagnosis of pulmonary tuberculosis within 8-10 hours after routine specimen decontamination. Here PCR detected 42.86% of culture negative specimens which is comparable with 42.10% found in study made by Prasad et al. [5].

Positive Predictive Value (PPV) of PCR was found, similar to that of Prasad et al. [5], 100%. This is because of its highest specificity to detect *Mycobacterium tuberculosis* complex. The relatively high PPV in smear negative patients makes interpretation of positive test more certain. Negative Predictive Value (NPV) of PCR, in present study, was found to be 75.6% which is lesser than that of Prasad et al. [5] report.

Interestingly, PCR was found to be the most accurate test for the diagnosis of pulmonary tuberculosis with 91.73% accuracy while the accuracy of acid fast staining and culture was found to be 81.81% and 79.33% respectively. We also evaluated the utility of polymerase chain reaction test by comparing smear microscopy, culture and PCR examination in a large number of specimens obtained from clinically diagnosed patients.

It is concluded from overall study that the use of PCR in a clinical mycobacteriology laboratory can provide diagnosis of pulmonary tuberculosis within 8-10 hours. PCR was found to be the most rapid, sensitive, specific and accurate method and has opened a new era of rapid mycobacteriological laboratory diagnosis, though it is recommended to use PCR as a complimentary test rather than substituting the standard microbiological analysis. Nevertheless, PCR has disadvantages like high cost, complexity and failure in discrimination between the living and dead bacilli.

**REFERENCES**