Evaluation of Conventional and Molecular Techniques in Diagnosis of Clinical Samples of Mycobacterium Tuberculosis

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Abstract: In developing countries like Pakistan, rapid diagnosis and effective treatment in life threatening disease such as tuberculosis (TB) is very important. In the study 90 sputum samples from different hospitals were processed using auramine staining, Ziehl-Neelsen staining (ZN), culture on Löwenstein-Jensen (LJ) medium and Polymerase Chain Reaction (PCR). Out of all 90 samples, 44 (48.88%) and 56 (62.2%) showed positive sputum smear microscopy, tested by Zeil Neilson and auramine staining respectively. Culturing into Lowenstein Jensen-Medium revealed 64 (71.1%) and PCR yield highly significant 83 (92.2%) positive result. In conclusion, it was noted that PCR as a molecular technique is a very rapid, specific and sensitive method for diagnosis of TB as compared to other conventional methods.

Key words: Tuberculosis • Auramine Staining • Ziehl-Neelsen Staining • Löwenstein-Jensen Medium • PCR

INTRODUCTION

In a developing country where the incidence rate of tuberculosis is quite high, it’s the need to devise a somewhat faster method to diagnose the slowly progressing deadly disease. Tuberculosis is a curable but a major concern for global public health in the developing countries primarily. An estimated number shows 8.6 million new cases every year whereas 0.94 million die out of it. Every 360 people in 1 million people suffer from this disease in Eastern Mediterranean region [1]. In high incidence of tuberculosis globally, it is highly important for a clinician to take quick decision for patients in order to identify highly serious TB positive patients and start therapy [2]. There are several conventional methods like microscopy and culturing technique but molecular techniques like Polymerase Chain Reaction (PCR) was proved to be sensitive, specific and helped in early diagnosis of tuberculosis [3,4]. Present study was designed to compare different conventional methods with molecular techniques like PCR which could help in regarding to specificity and sensitivity during diagnosis of TB.

MATERIALS AND METHODS

Total ninety mycobacterium tuberculosis complex samples were used, of which 53 samples from the Institute of Public Health, Lahore, 20 Multi Drug Resistance (MDR) TB samples from the National Research Laboratory and 17 samples were taken from the Mayo Hospital, Lahore. Clinical samples were processed at local MTB designated laboratories for culturing, identification and drug susceptibility testing (DST). Smear slides were prepared from sputum samples using both ZN [5] and auramine stains [6]. The same samples were also cultured on LJ medium and subsequently DNA extraction through CTAB method [7]. Amplification with species specific primers (IS6110F 5’AC-CT-GA-AA-GA-CG-TT-AT-CC-AC-CA-T3’, IS6110R 5’ TC-CT-AT-GA-CA-AT-GC-AC-
TA-GC-CG3') to generate 157 bp product with the following amplification conditions: 95 °C for 5 min initial denaturation, 95°C for 1 min, 60°C for 1 min and 72 °C for 1 min for 35 cycles and final extension at 72 °C for 5 min. Reaction mixture of 25 µL was contained 0.75µL forward and reverse primers each; 2.5µL dNTPs (25mM), 2µl of PCR buffer, 0.3µl ml of Taq polymerase, 2.5µl MgCl₂, 1µl of sample DNA, 15.2µl nuclease free water. Amplified products were run on agaros gel (2%) with 50 bp ladder in presence of positive and negative control.

Statistical Analysis: All groups were compared using Chi square test and all values were expressed as percentage using SPSS version 16.0 and p value of compared results less than 0.05 was considered as significant.

RESULTS AND DISCUSSION

Results of present study depicted that Ziehl-Neelsen (ZN) stain showed the lowest positive percentage (48.88%) as compared with the other used methods, on the other hand, the fluorescent microscopy using auramine stain on the same samples showed a significantly higher positivity percentage (62.66%) as given in table 1. These results showed that fluorescent microscopy was more sensitive and gives 13.34% more positivity as compared to bright field microscopy during diagnosis of TB. Similar results were observed in previous studies [5, 8]. Overall in comparison to advanced techniques, microscopy is a rapid but insensitive method as described by Cohen et al. [2]. Total recovered positive samples by culturing the samples on Lowenstein Jensen medium was scrutinized 64 (71.1%) that showed significantly high sensitivity being gold standard test for detection of *Mycobacterium tuberculosis* (MTB) as compared to both bright field and fluorescent microscopy. It was noted that using the culture method, the number of positive samples was higher by 22.22% and 9% compared to ZN stain and auramine stain, respectively. Approximate results were achieved by Lima et al. [6] that described 67.6% positive samples as given in Table 1 but according to the previous study [9] culturing technique takes more than 3 to 4 week for detection of MTB and hence showed a big hurdle in need of rapid diagnosis of TB. In relative to other methods, PCR method revealed 83 (92.22%) positive samples and showed the highest sensitivity as given in Table 1 and the expected PCR product was shown in Figure 1. Preceding study showed that this sensitivity may range from 42 to 90.9 percent depending on quality of samples [2, 9, 10].

<table>
<thead>
<tr>
<th>Samples</th>
<th>Sample Number</th>
<th>Ziel Neelson Staining</th>
<th>Florescent Staining (Auramine)</th>
<th>Lowenstein Jenson</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>-ve</td>
<td>%+ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>PRL*</td>
<td>30</td>
<td>14</td>
<td>16</td>
<td>46.67%</td>
<td>19</td>
</tr>
<tr>
<td>Ganga Ram**</td>
<td>30</td>
<td>16</td>
<td>14</td>
<td>46.67%</td>
<td>20</td>
</tr>
<tr>
<td>Mayo***</td>
<td>30</td>
<td>14</td>
<td>16</td>
<td>53.33%</td>
<td>17</td>
</tr>
<tr>
<td>TOTAL</td>
<td>90</td>
<td>44</td>
<td>48.88%</td>
<td>56</td>
<td>62.22%</td>
</tr>
</tbody>
</table>

*PRL: (Provincial Reference Laboratory, Punjab, Pakistan)

**Ganga Ram Hospital, Lahore  ***Mayo Hospital, Lahore

+ve:Positive  -ve:Negative  -ve:Negative

Fig. 1: Gel electrophoresis of MTB hemolysine PCR amplified product (157 bp)
CONCLUSION

In conclusion, PCR is reliable, sensitive, rapid test and should be used for diagnosis of MTB as compared to conventional methods like microscopy which is insensitive and culturing techniques that are less sensitive and also time consuming.

REFERENCES