

PCR in the differential diagnosis of Tuberculosis: A comparative study

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Abstract

This study is to assess prospectively the performance of PCR in the diagnosis of Tuberculosis. The repetitive sequence IS6110 is used as a target for PCR. A total of five samples were analyzed all of which were found to be PCR positive. The results were compared with AFB staining, culture on Lowenstein Jensen (LJ) medium and Enzyme Linked Immunosorbant Assay (ELISA). PCR was found to be sensitive and specific and provides a definitive diagnosis of TB. Hence it is an effective tool for TB diagnosis.

Keywords: Tuberculosis, IS6110, AFB staining, ELISA and PCR

INTRODUCTION

On March 24th 1882, a German physician Robert Koch found that Tuberculosis, an infectious disease was caused by *Mycobacterium tuberculosis*. It is a disease of increasing importance. World Health Organization (WHO) has reported nearly three million deaths every year. In addition to this, there is an emergence of Multiple Drug Resistant (MDR) strains. Patients spread the disease by producing aerosols containing *M.tuberculosis* bacteria. Despite the availability of short term chemotherapy and the Bacilli-Calmette Guerin (BCG) vaccine, the tubercle bacilli continues to claim more lives than any other single infectious agent.¹ The rapid development and availability of a variety of new molecular genetic technologies present the clinicians with an array of options for the accurate diagnosis of infectious diseases. Nucleic acid amplification methods to detect MTB in clinical samples are increasingly used as a tool of diagnosing TB.² The emergence of PCR as a very powerful tool for the diagnosis of many infectious agents has been one of the momentous advances in the field of molecular biology.³ PCR based system have been useful for rapid detection of uncultivable or fastidious organisms.

MATERIALS AND METHODS

Clinical samples

The clinical samples were collected from suspected patients from various hospitals and nursing homes of Thanjavur. These include body fluids like pleural fluid, Cerebro spinal fluid and Ascitic fluid.

All the samples were stained for the presence of AFB by Ziehl-

Neelsen method and the cultures were inoculated on Lowenstein-Jensen medium. Immunological examination of the samples was made by using the Dot ELISA kit for human IgG.

Isolation of DNA

Isolation of DNA from various body fluids was performed using the rapid and simple method adopted by Buch et al.⁴ 1992 with minor modification⁴. 200 µl of lysis buffer (5.3 M Guanidine isothionate, 10 mM Diethyribiol, 1% Tween 20, 0.3 M Sodium acetate, 50 mM Sodium citrate) and the mixture was incubated at 65°C for 10 minutes. 50 µl of glass mixing powder was added to the solution and incubated at room temperature with occasional shaking. Thereafter it was centrifuged at 10,000 rpm for 1 min. Supernatant was discarded and the matrix was resuspended in wash buffer (50 % Ethanol, 10 mM Tris HCl, 100 mM NaCl). Bound DNA was eluted by periodic mixing in 10 µl of 10 mM Tris HCl.

Primers used

The primer sequences used for detection were

Primer	Primer Sequence
1	5' CCT GCG AGC GTA GGC GTC GG 3'
2	5' CTC GTC CAG CGC CGC TTC GG 3'

The target for PCR was the insertion sequence IS6110. The primers yielded a 123 bp fragment with an internal Sal I site.⁵ IS6110 has been said to be specific to MTB and most strains have between 8-15 copies.^{5, 6 & 7}

Amplification of DNA by PCR

Primers, enzymes, buffers and other components needed to carry out the reaction were obtained from Bangalore Genei Ltd. Reaction mixture contained Taq polymerase, assay buffer, dNTP's and primers with a total volume of 50 µl. Amplification was carried out on a thermal cycler (MJ-research) for about 35 cycles. The DNA was subjected to an initial denaturation at 94°C for 3 mins.

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Thereafter amplification was carried out at 90°C for 1 min, 60°C for 1 min and 72°C for 1 min. A final extension was carried out at 72°C for 5 mins. The amplification product i.e., the presence of 123 bp fragment was analyzed on 2 % agarose gel.

Positive and Negative controls

DNA extracted from MTB culture was used as a positive control. DNA extracted from normal patient was used as negative control.

RESULTS

In this study, the performance of PCR as a diagnostic tool for TB was assessed. A total of five samples were analysed. These include two samples of Ascitic fluid, two samples of Pleural fluid and one sample of CSF. DNA extracted from these samples was screened for the presence of 123 bp nucleotide sequence of IS6110, which is the target for PCR. All the five samples were found to be PCR positive when PCR products ran on gel with marker (Figure 1). The same samples were compared with other techniques namely, Microscopy, culture and ELISA. All the samples indicated smear negative and culture negative results. ELISA was found to be

positive for all samples. But it is necessary to establish the presence of MTB DNA in all the five samples. Amplification of the repetitive sequence of MTB was found to contribute to the sensitivity of PCR.

DISCUSSION

Tuberculosis continues to be a global health problem despite the technical advancements made in detection, isolation and identification methods^[8]. PCR has been acclaimed to be one of the sensitive tests for identifying the presence of an organism in question, in a clinical sample. PCR performed on five samples indicated the presence of MTB DNA in all the five samples. These five samples were found to be smear negative and culture negative as shown in table 1. Staining although rapid and inexpensive provides only a presumptive diagnosis of TB. A positive AFB smear result depends on the presence of atleast five thousand organisms/ml of the sample. Though it is straight forward, it does not allow differentiation between TB and non-TB bacterial^[9]. Validity of this technique and sensitivity of this method is truly reflected in the analysis.

Table 1: Analysis of clinical samples

S.No.	Samples	Staining	Culture	ELISA	PCR
1	Ascitic fluid 1	-	-	+	+
2	CSF	-	-	+	+
3	Pleural fluid 1	-	-	+	+
4	Ascitic fluid 2	-	-	+	+
5	Pleural fluid 2	-	-	+	+

Note: (-) Negative & (+) Positive

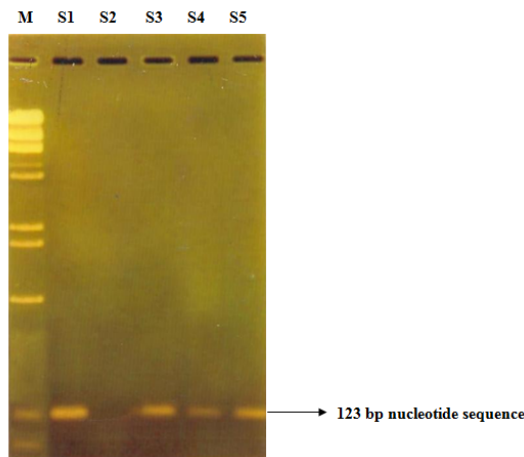


Figure 1: Confirmation of IS6110 sequence on AGE

On the other hand culture is still considered as a gold standard for TB diagnosis. It is able to detect as few as 10-100 bacteria/ml of digested concentrated material. In the case of TB-meningitis, it lacks sensitivity and positive results are obtained only in 10-30 % of patients^[10]. Further MTB can be isolated from CSF very rarely^[11 & 12]. Both culture and smear which are found to be the definitive diagnosis for TB have failed to produce positive results. In a study, there was a report that pleural fluids from fifteen patients with tuberculous effusion was examined for MTB DNA by PCR technique were found to be positive while none were positive in smears and only three samples were positive in culture^[8].

However, in the ELISA test all the five samples produced a positive result, thus indicating infection. Presence of IgG in the fluids

indicate an infection either recent or remote^[13]. However isolation of MTB is imperative for definitive diagnosis of tuberculosis^[14]. The problem of false positivity of PCR was neglected because the presence of infection was confirmed by the ELISA test, though the samples were culture and smear negative.

The detection of MTB by PCR in smear negative and culture negative cases assumes a lot of clinical significance, because such as these cases in the absence of a technique like PCR would have to be assumed to be tuberculosis and treated empirically or lose the benefit of life saving therapy if bacteriologic proof is insisted upon^[15].

PCR has been shown to be sensitive (88-100%)^[16 & 17] and specific (> 90 %). Various authors have shown the sensitivity of PCR^[18 & 19]. High sensitivity of PCR is due to the repetitive nature

of the target sequence amplified by PCR, suggesting that the assay is more sensitive than smear/culture in detecting non viable or fewer viable organisms[20].

In conclusion, it was suggested that early diagnosis is as crucial as treatment and prevention for the control of Tuberculosis. PCR can be used as a rapid, sensitive, specific and reliable tool for the diagnosis of TB, especially in cases where it is difficult to identify the bacteria by conventional methods. Thus it provides a bacteriological diagnosis. As per this study, it provides extraordinary results in extrapulmonary tuberculosis.

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