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Role of Polymerase Chain Reaction (PCR) in Differential Diagnosis of Tuberculosis - A Comparative Study

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The present study was undertaken to evaluate the efficacy of Polymerase Chain Reaction (PCR) to detect *M. tuberculosis* in body fluids of patients with suspected Tuberculosis. Suspected cases were chosen for study and controls included patients free of clinical TB. PCR was done on the clinical specimens and compared with AFB staining, culture on Lowenstein Jensen (LJ) medium and qualitative analysis by Dot ELISA. The sensitivity of PCR was found to be 100% in all 3 types of body fluids namely ascitic fluid, pleural fluid and CSF. PCR technique clearly becomes a valuable diagnostic tool for extrapulmonary TB in detecting fewer undetectable viable or non-viable MTB microbes

Key words: PCR, Tuberculosis, *Mycobacterium tuberculosis,* CSF, Acid Fast Bacilli, ELISA.

World Health Organization has reported that over 3 million people die from TB and one third of the world population is infected with *Mycobacterium Tuberculosis*(Watterson and Drobniecoski, 2000). Patients spread the disease by producing aerosols containing the bacteria. Initial infection begins when the droplet nuclei are inhaled after which the bacteria multiplies virtually unrestricted within inactivated macrophages until it bursts. At this stage tubercle formation begins. It grows and may invade a bronchus or spread to other part of the lungs as well as the body. This haematogenous spread may result in extrapulmonary TB.

One essential factor for controlling the spread of this disease is the ability to diagnose the infection at its early stage. Conventional diagnostic test including acid fast stain and culture are frequently negative because of lower MTB count. Culture is considered to be a gold standard but the slow growth of pathogen is the main obstacle for laboratory diagnosis. ELISA measurement of IgG antibodies to mycobacterial antigen can be used in serological diagnosis but

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Table 1. Analysis of clinical samples for portable cases of TB

Samples	No. of samples	Staining + ve	Culture +ve	ELISA + ve	PCR + ve	Sensitivity of PCR
CSF	4	nil	nil	2	4	100
Ascitic fluid	4	nil	nil	2	4	100
Pleural fluid	2	nil	nil	1	2	100

DISCUSSION

TB continues to be a global health problem despite the technical advancements made in detection, isolation and identification methods (Prabhakara Reddi *et. al.,* 1993). The problem in diagnosing TB is that no symptom or sign is exactly typical of it. Presence of infection in the body does not necessarily mean disease. However, looking from the angle of disease presentation, recovering the bacilli from the patient specimen is specific but not sensitive. Any other indirect evidence for the presence of Mycobacteria in the body may be sensitive, but not specific (Ghosal and Roy, 2000).

In this study mycobacterial DNA was detected after amplification by PCR in all the suspected clinical samples. Pleural fluid, ascitic fluid and CSF were positive with PCR although none of the samples yielded MTB on staining and culture with LJ medium. Hence PCR was considered as 100% sensitive. Culture is still considered as gold standard for TB diagnosis. In case of TB meningitis it lacks sensitivity and positive results were obtained only in 10- 30 % of patients (Molavi and Le Frock, 1985).

Culture is able to detect as few as 10-100 bacteria/ml. MTB from CSF sample can be isolated very rarely (Shankar *et.al.,*1990 and Delacourt *et.al*., 1995). In a similar study conducted by Arnaud de Lassence *et.al.,* (1992) Pleural fluids from 15 patients with tuberculous effusion was examined for MTB DNA by PCR technique. Among them 9 were found to be PCR positive while only 3 were positive in culture. According to Patel *et.al.,* (1990) and Shankar *et.al.,* (1990) , PCR using Insertion sequence IS6110 as the target has the potential to overcome limitations of conventional methods and can be established as rapid, sensitive and specific technique for detecting MTB DNA in various clinical specimens. ELISA test produced a 50 % positive result indicating infection. Presence of IgG in the fluids indicate an infection either recent or remote (Ghosal and Roy, 2000). However isolation of MTB is imperative for definitive diagnosis of TB

PCR has been shown to be sensitive (88-100%) (Tan *et.al,* 1997 and Schluger *et.al*, 1994) and specific (>90%) (Tan *et.al*, 1997 and Eisenach *et.al*.,1991).

Various authors have shown a sensitivity ranging from 40- 100%. Delacourt *et.al*.,(1995) found 50% sensitivity in CSF sample, Smith *et.al*.,(1996), 40% while Monno *et.al*.,(1996) showed 100% sensitivity with PCR which coincides with this present study. 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 and/or fewer viable organisms (Arnaud de Lassence *et.al.,* 1992).

CONCLUSION

The summary of the findings of the present study revealed the following:

1. Though culture is considered as a gold standard for TB diagnosis, false negative results were obtained when the number of mycobacteria is low.
2. MTB was detected with PCR even in Culture negative cases.
3. Though ELISA gave a positive reaction for all 3 types of sample its sensitivity was only 50 %
4. The sensitivity of PCR was found to be 100%
5. PCR can be used as rapid, sensitive, specific and reliable tool for diagnosis of TB,

especially in cases where it is difficult to identify the bacteria by conventional methods which provides a definitive diagnosis for Tuberculosis

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