ABSTRACT

Work done for the Degree of Doctor of Philosophy

On Thesis Entitled

“DETECTION OF MYCOBACTERIAL SPECIES CAUSING TUBERCULOUS LYMPHADENITIS FROM FINE NEEDLE ASPIRATES, BIOPSY AND BLOOD SAMPLES USING POLYMERASE CHAIN REACTION (PCR).”

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INTRODUCTION

One Indian dies of tuberculosis every minute and over a thousand Indians dies of tuberculosis every day. In 2010, there were 8.8 million incident cases of TB, 1.1 million deaths from TB among HIV-negative people and an additional 0.35 million deaths from HIV-associated TB.

The incidence of mycobacterial lymphadenitis has increased in parallel with the increase in the incidence of mycobacterial infection worldwide. Tuberculous lymphadenitis is seen in nearly 35% of extrapulmonary TB which constituted about 15-20% of all cases of TB. Definitive diagnosis of tuberculous lymphadenitis depends upon the direct demonstration of tubercle bacilli in specimens. Culture of mycobacterium is diagnostic for tubercular lymphadenitis. However, a negative culture result should not exclude the diagnosis of tubercular lymphadenitis.

Introduction of Polymerase Chain Reaction (PCR) has enhanced the diagnostic predictability of the disease especially in the extrapulmonary, paucibacillary samples. In the recent years, peripheral blood mononuclear cells-PCR (PBMC-PCR) is being employed for the detection of Mycobacterium tuberculosis in clinically suspected tuberculous lymphadenitis cases.

Restriction fragment length polymorphism (RFLP) is being used for the detection of mycobacterial species.

In the present study, we have used various diagnostic techniques i.e. cytology, culture and Polymerase chain reaction (PCR) to diagnose clinically suspected cases of tuberculous lymphadenitis. We have also used the blood specimen and evaluated its importance in the diagnosis of tuberculosis.

AIMS AND OBJECTIVES

- To correlate the diagnostic efficacy of Polymerase chain reaction (PCR) with clinical, radiological, cytological, histological and other
microbiological techniques for the diagnosis of tuberculous lymphadenitis.

- To evaluate the utility of PCR using different molecular markers in these cases.

- To establish PCR assays to differentiate between *Mycobacterium tuberculosis* complex and Non-tuberculous mycobacterium (NTM).

- To evaluate the usefulness of PCR performance in blood samples of patients with tuberculous lymphadenitis.

**MATERIALS & METHODS**

The study included a total of one hundred (n=100) clinically suspected cases, which were suggestive of tuberculous lymphadenitis by cytopathology were considered for the study. Patients of age group 2 - 45 years were included in the study. Processing of FNA/pus samples were done for, staining, culture and DNA extraction. Extracted DNA was used for the PRA analysis. Processed Blood samples were used for the staining, culture and DNA extraction. Extracted DNA was used for the PCR amplification.

Processed samples were inoculated on to LJ medium and BACTEC 12B medium for conventional mycobacterial culture and radiometric (BACTEC) culture respectively. The biochemical tests were performed to identify the isolate to species level. MPB64, Hup B, Hsp 65, 16S-23Sr RNA (ITS) and rpoB gene primers were used for the diagnosis and identification of mycobacterial species.

Restriction fragment length polymorphism (RFLP) analysis was performed with the amplicons, using different restriction enzymes like *BstII, HaeIII, MspI, CfoI, TaqI, DdeI, AvaII* and *Hinfl*. Sequencing of few amplicons was also done to indentify and confirm the mycobacterial species. The findings of the PCR were
correlated with the findings of other diagnostic techniques. AFB culture was considered as a Gold Standard for the Statistical Analysis. The results were analyzed with SPSS version 16. The p values <0.05 were considered significant.

RESULTS

All the patients in the study group ranged from 2 years to 45 years. Age group 21-30 years contained the maximum number of patients (36%). Out of 100 patients 73 patients had systemic symptom of weight loss, 72 of them had fever, 66 patients had anorexia and 58 patients had complaint of night sweats. Out of 100 patients, the most common region of lymph node swelling was cervical region. On Cytopathology (i) 23 (23%) were suggestive of tuberculous lymphadenitis with AFB positivity and (ii) 77 (77%) were suggestive of tuberculous lymphadenitis and AFB negative.

Out of 100 cases, 24 (24%) were found to be AFB positive after ZN staining. On examination of culture on Lowenstein Jensen (LJ) medium, 34 (34%) samples were found AFB positive. On examination of BACTEC radiometric culture, 45 (45%) samples were found AFB positive. The biochemical tests like niacin production, nitrate reduction, catalase production test, Arylsulphatase test, urease test, pyrazinamidase test and tween 80 hydrolysis tests confirmed the presence of Mycobacterium tuberculosis in the isolates.

The maximum PCR positive results (48/100) were obtained by Hsp 65 gene followed by Hup B gene (47/100), MPB 64 (45/100), 16S-23Sr RNA (45/100) and rpo B gene (44/100). Overall 48% cases were PCR positive for tuberculous lymphadenitis in the study population (48/100). The sensitivity and specificity of PCR when compared with cytopathology was found to be 66.67% and 80% respectively in these cases. The PPV and NPV were found to be 83.3% and 61.5% respectively.
The sensitivity of PCR with five different primers namely Hsp 65, Hup B, MPB 64, 16S-23Sr RNA and rpoB when compared with BACTEC culture was found to be 100%, 100%, 100%, 100% and 95.55% respectively and the specificity was 94.45%, 96.36%, 98.18%, 98.18% and 100% respectively. The PPV were found to be 93.75%, 95.74%, 97.78%, 97.78% and 100% respectively and NPV 100%, 100%, 100%, 100% and 96.49% respectively.

A total of 45 culture positive samples were used for PRA analysis. Few samples were also sent for sequencing to confirm the species. The PRA patterns were observed to be similar for *M. tuberculosis*. Analysis revealed the presence of *M. tuberculosis* in the isolates.

Out of 100 cases, 4 (4%) were found to be AFB positive after ZN staining and 10 (10%) samples were found to be positive on Lowenstein Jensen (LJ) medium. On examination of BACTEC radiometric culture, 19 (19%) samples were found AFB positive on blood samples. After the blood PCR amplification for Hsp 65 gene 16 (16%) samples were found to be positive and 84 (84%) were negative. As the sensitivity of blood based PCR was found to be 63.16% when compared with BACTEC culture, it may said that blood based PCR, might be used as an additional diagnostic technique in diagnosis of tuberculous lymphadenitis. When Blood PCR was compared with FNA culture positive case the sensitivity and specificity of PCR was found to be 35.5% and 100% respectively in these cases. The PPV and NPV were found to be 100% and 65.47%, the p value was observed to be significant (p value = 0.001).

**CONCLUSIONS**

In our study we observed that FNA PCR was able to diagnose the cases, which were direct smear negative, culture negative and were suggestive of tuberculous lymphadenitis along with other cases that had no systemic symptoms. 100% PCR positivity was observed in AFB smear and culture positive
specimens’ proving itself again that PCR is rapid, sensitive, and useful technique in diagnosis of tuberculous lymphadenitis

The specificity of blood PCR when compared with BACTEC culture was found to be 95 %. When culture and PCR results were combined, diagnosis of tuberculous lymphadenitis was observed to increase.

It may be said that, blood based PCR but might be used as an additional diagnostic technique in diagnosis of tuberculous lymphadenitis. There is a need of new and rapid methods for diagnosis of tuberculous lymphadenitis, especially in paucibacillary and low sample amount cases.