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Original Article

Diagnosis of Tuberculous Lymphadenitis from Fine Needle Aspirate by PCR

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Abstract

Lymph node involvement is the commonest form of extra pulmonary tuberculosis (EPTB). This study was undertaken to evaluate the role of PCR using IS6110 as a target for Mycobacterium tuberculosis complex in the diagnosis of tuberculous lymphadenitis (TB-L) and was done in Microbiology department of Sir Salimullah Medical College from July 2009 to June 2010. Fine needle aspiration was performed on 107 patients with a clinical suspicion of tuberculous lymphadenitis from different hospitals of Dhaka City Corporation area. All the aspirated samples were subjected to culture and PCR for M.tuberculosis. Out of 107 patients with lymphadenopathy most of the patients (85.9%) had cervical lymph node enlargement followed by axillary groups (12.1%) and inguinal groups (1.9%). Out of 107 cases, 65 (60.7%) were PCR positive and 56 (52.3%) were culture positive. Among 51 (47.7%) culture negative cases 9 (17.65) were PCR positive. Sensitivity of PCR was 100% and specificity was 82.4%.The positive predictive value (PPV), negative predictive value (NPV) and accuracy of PCR were 86.2%, 100% and 91.6% respectively. PCR is the most sensitive in the detection of M.tuberculosis in fine needle aspirate and could be useful technique in establishing the diagnosis of tuberculous lymphadenitis.

Key words: Tuberculous lymphadenitis, Extrapulmonary TB, PCR.

Introduction:

Tuberculosis can involve any organ or system in the body. It is classified according to anatomical site of the disease into (i) Pulmonary and (ii) Extra Pulmonary Tuberculosis (EPTB). Lymph node involvement is the commonest form of EPTB. In developing countries where the incidence of TB is high, tuberculous lymphadenitis (TB-L) is one of the most frequent causes (30-52%) of lymphadenopathy[1]. In Bangladesh, lymph node tuberculosis was found to be common (36.2%) among the EPTB[2].

Mycobacterium tuberculosis and atypical or non-tubercular mycobacterium (NTM) both causes tuberculous lymphadenitis. Lymphadenitis caused by *Mycobacterium tuberculosis* is a local manifestation of systemic disease and anti-TB chemotherapy for 6 months is the main treatment. In contrast, lymphadenitis by NTM is a localized disease that requires different plan of treatment[3]. Therefore, for better management of TB-L, accurate diagnosis is required.

Traditionally, the diagnosis of TB-L is established by histopathology and smear microscopy or by mycobacterial culture. Several conditions including sarcoidosis, fungal infections and other inflammatory conditions can present the same cytology and/ or histopathology as tuberculous lymphadenopathy. The diagnosis of tuberculosis is then only confirmed by the presence of AFB and / or isolation of *Mycobacterium tuberculosis* on culture. The culture is the gold standard and requires only 10-100 live bacilli per ml, and is also more sensitive and specific. AFB cultures are positive in 35%-65% of patients in published studies[1,4] and culture takes four to eight weeks.

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Over the past decades, fine-needle aspiration (FNA) cytology has played an important role in the evaluation of peripheral lymphadenopathy as a possible non-invasive alternative procedure to excisiosnal biopsy. FNA is convenient for outpatients and relatively painless procedure to collect material[5]. The aspirate obtained from affected lymph node can be used for cytology, microscopic examination, culture and even PCR.

In developing countries, the detection of TB-L with traditional diagnostic tools is a major challenge. The detection rate for *M.tuberculosis* from fine needle aspirates is low by microbiological techniques. Therefore, there is a definite need for improving the sensitivity of tuberculous lymphadenitis diagnosis in fine needle aspirates.

PCR is the most sensitive technique for rapid identification of Mycobacterium tuberculosis in FNAC materials than either AFB smear or culture[6]. PCR is used to detect mycobacterial DNA in FNA with sensitivity of 100% and specificity of 94%[7]. This technique successfully detects the presence of extremely small quantities of organism or even in nonviable organism in clinical samples[8]. Most of the PCR studies have targeted 1S6110 sequence and this DNA sequence is present in multiple locations in the M .tuberculosis genome[9], and is found to have the highest sensitivity and specificity for the organism and does not amplify atypical mycobacteria[10].

The purpose of the present study was to validate the diagnosis of TB-L by PCR using IS6110 primer.

Materials and Methods:

This cross-sectional study was done in the Microbiology laboratory of Sir Salimullah Medical College (SSMC), Dhaka and National Tuberculosis Reference Laboratory (NTRL), National Institute of Disease of Chest and Hospital (NIDCH), Mohakhali, Dhaka during the period of July 2009 to June 2010. A total of 107 patients of both sexes and different age groups with peripheral lymphadenopathy clinically suspected to be tuberculous origin were included in the study. These patients attended the Department of pathology, E.N.T and medicine outpatient department of Sir Salimullah Medical College Mitford Hospital, Dhaka (SSMCMH), National Institute of Disease of chest and Hospital (NIDCH), Mohakhali, Dhaka, Bangladesh Medical College Hospital (BMCH), Dhaka Medical College Hospital (DMCH) and Bangabandhu Sheikh Mujib Medical University (BSMMU). Patients on anti-tubercular drugs were excluded from the study. The protocol was approved by ethical review committee of SSMC. Informed written consents were taken from the patients or legal guardian of the patients before collection of sample. Confidentiality of the data was maintained. Before collecting specimens, each patient was interviewed and relevant information was recorded systematically in a pre- designed data sheet. Clinically suspected patients of tuberculous lymphadenopathy were selected by purposive sampling and fine needle aspirate samples were collected from them.

Fine Needle Aspiration (FNA): With all aseptic precaution, fine needle aspiration was done using 10 ml sterile disposable plastic syringe. Sample was carried to the laboratory and was transferred into a falcon tube within the Bio-safety cabinet (BSC) level 3 and few sterile glass beads were added. It was then, vortexed until all the materials became homogenized. The homogenized sample was divided into two portions. One portion was used immediately for culture or stored at 4°C. For PCR one ml sample was collected into eppendorf tube containing normal saline and stored at -20°C.

Laboratory procedure:

Culture for *Mycobacterium tuberculosis*

Two drops of homogenized FNA sample was inoculated on two slants of Lowenstein Jensen media (L-J) using Pasteur pipettes and incubated at 37°C. The culture bottles were examined weekly up to 8 weeks for the evidence of growth. The growth were confirmed by Z-N stain method and evaluated by colour, morphology, rate of growth, pigment production and biochemical tests. If no growth appeared after 8 weeks of incubation then the culture was reported as "no growth"[11].

Identification of Mycobacterium by PCR:

DNA Extraction: About 90 µl homogenized FNA sample, (heat treated at 80°C for 15 minutes in water bath) was mixed with 10-µl digestion buffer. After mixing by vortexing, the content was incubated at 37°C over night. After incubation sample was boiled at 100°C for 15 minutes, centrifuged at 10,000 rpm for 10 minutes and the supernatant was taken and used for PCR.

Amplification of DNA: Template DNA was amplified using **PCR Primer IS6110 (Invitrogen, Hong Kong Limited).** (MTB 1: 5µ - CCT GCG AGC GTA GGC GTC GG-3µ

MTB 2: 5µ- CTC GTC CAG CGC CGC TTC GG-3µ). A 22 µl master mix and 3-µl template DNA were added to 10 PCR reaction tube with the exception of 1st and 2nd tubes where 3 µl positive and negative controls was given instead of template DNA. So finally, each tube contained 25 µl[12].

Initially denaturation of template DNA was done for 5 minutes and then final denaturation for 1 min, which continued for 35 cycles at 94°C. Following denaturation, annealing and extension of primer was done for 2 minutes at

68°C and 72°C respectively. The reaction was repeated for 35 cycles.

Gel electrophoresis: The amplified PCR products were detected by 2% agarose gel electrophoresis, and then staining was done with ethidium bromide. The electrophoretic band was visualized in a gel documentation system by using UV transilluminator.

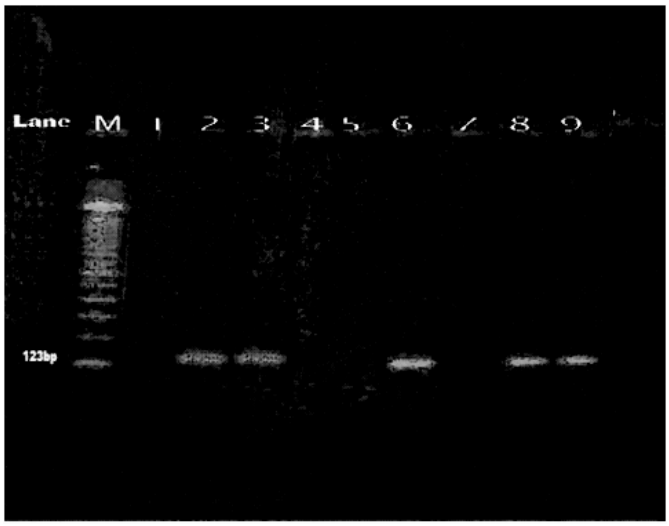

Fig 7: PCR band of the test sample with positive and negative control.

Photo - Amplification products 123 bp by PCR from M. tuberculosis in samples at

Sir Salimullah Medical College.

Lane M- Ladder

Lane 3,6,8,9 is positive.

Lane 2 positive control (H37Rv strain of Mycobacterium).

Lane 1 negative control (water).

Results:

A total number of 107 patients with peripheral lymphadenopathy with clinical symptoms suggestive of tuberculous origin were included in this study. Patients on anti-tubercular drugs were excluded from the study.

Out of 107 patients with lymphadenopathy, most of the patients (92, 85.9%) had cervical lymph node enlargement followed by 13 (12.1%) axillary groups and 2 (1.9%). Inguinal groups.

Out of 107 cases 56 (52.3%) were culture positive and 65(60.7%) were PCR positive. All culture positive cases were identified as *M.tuberculosis* and all of them were also PCR positive. Among 51(47.7%) culture negative cases, 9 (17.6%) were PCR positive (table I). The positivity rate of PCR is significantly higher than that of culture (P <0. 01).

Considering the culture as gold standard the sensitivity of PCR was 100% and specificity was 82.4%.The positive predictive value (PPV), negative predictive value (NPV) and accuracy of PCR were 86.2%, 100% and 91.6% respectively.

Table I: Comparison of PCR with culture of Mycobacterium tuberculosis among suspected tuberculous lymphadenitis cases

Culture	PCR		Total
	Positive	Negative	
Positive	56(100)	0(00)	56 (52.3)
Negative	9 (17.6)	42 (82.4)	51(47.7)
Total	65 (60.7)	42 (39.3)	107 (100.0)

Discussion:

The diagnosis of tuberculous lymphadenitis remains challenging in spite of the availability of various diagnostic tools. Traditionally, the diagnosis of TB-L is established by histopathology and smear microscopy or by mycobacterial culture from biopsy specimen. Fine needle aspiration cytology (FNAC) has been proved valuable in the diagnosis of tuberculous lymphadenitis and also provides an alternative and easy way for collecting materials for bacteriological examination[1,13]. However, FNAC has several limitations, especially in the absence of demonstrable AFB[14]. In the present study, PCR was used for identification of causative agent in FNA samples to find a suitable, rapid, less invasive and accurate method for diagnosis of tuberculous lymphadenitis.

Out of 107 patients with lymphadenopathy, 85.9% had cervical lymph node enlargement followed by axillary groups (12.1%) and inguinal groups (1.9%). This observation was comparable to finding of a study where 67.5% cervical, 9% axillary and 6.4% inguinal lymph node involvements were reported[15]. A study in Butajira, Ethiopia, revealed that 88(82.2%) of 105 tuberculous lymphadenitis cases had cervical involvement[16].

Three types of aspirates were obtained by FNAC from study population, which were pus or purulent (57%), blood mixed (26.2%), and caseous material (16.8%). Irrespective of types of aspirates, all 107 samples were tested for *Mycobacterium tuberculosis* by culture and PCR.

Culture is the gold standard, requires a minimum of 10 to 100 viable bacilli to grow in the culture medium and which is needed to do for drug susceptibility tests. In the present study, out of 107 cases 56 (52.3%) were culture positive. This result correlates with the different published studies where culture of *Mycobacterium tuberculosis* was positive in 35% to 65% of patients [1,5,17].

In this study, PCR positive cases were 65 (60.7%) and all the culture positive cases were found to be positive by PCR. This finding also correlates with study of Negi *et al(2007)*[18]. Where IS6110 based PCR showed the positivity of 100% in culture positive samples. Regarding EPTB cases efficient role of PCR is definitely noteworthy. Viable organisms are required for culture, where as PCR can detect *Mycobacterium tuberculosis* based on genomic DNA only or even dead bacilli. Theoretically, only two organisms of AFB are enough to detect successfully with PCR amplification[19,20].

Sensitivity of PCR was found to be 100% with 82.4% specificity in this study .Other studies demonstrated that PCR sensitivity was 100% and specificity was 94% for fine needle aspiration specimen[7]. Sekar *et al(2008)*[21] reported that sensitivity of PCR was 90% in their study. Different studies showed sensitivity ranged between 20% and 94% for PCR assay of extra-pulmonary specimens[22,23] .

Nucleic acid amplification techniques including PCR have a considerable impact on diagnosis of disease on account of their speed, specificity and enhanced sensitivity. The application of PCR to the diagnosis of tuberculous lymphadenitis has the potential to resolve one of the foremost challenges facing a diagnostic laboratory for diagnosis of extra- pulmonary tuberculosis. In this regard, it can be concluded from this study, that, PCR is the most sensitive and reliable method.

Conclusion:

In this study, it has been shown that PCR is 100% sensitive and 82.4% specific for the diagnosis of tuberculous lymphadenitis. This result concluded that PCR could be a sensitive technique to detect the presence of *Mycobacterium tuberculosis complex* derived from specimen, of a suspected tuberculous lymphadenitis patients.

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REFERENCES:

1. Gupta AK, Nayar M, Chandra M. Critical appraisal of fine needle aspiration cytology in tuberculous lymphadenitis. Acta Cytol 1992; 36: 391-394.
2. Karim MM, Chowdhury SA, Hussain MM, Faiz AM. A clinical study on extra spulmonary tuberculosi. Journal of Bangladesh College of Physicians and Surgeons 2006; 24: 19-28.
3. Muzaffer Kanlikama and Avni Gökalp. Management of Mycobacterial Cervical Lymphadenitis.World Journal of Surgery 1997; 21(5): 516-519.
4. Finch PJ, Millard FJ, Maxwell JD. Risk of tuberculosis in immigrant Asian: culturally acquired immunodeficiency. Thorax 1991; 46: 1-5.
5. Danapat MC, Mishra MB, Dash SP, Kar PK. Peripheral lymph node tuberculosis: a review of 80 cases. Br J Surg 1990; 77: 911- 917.
6. Kim SS, Chung SM, Kim JN, Lee MA, Ha EH. Application of PCR from fine needle aspirates for the diagnosis of cervical tuberculous lymphadenitis. Jour Korean Med Sci 1996; 11: 127-132.
7. Chantranuwat C, Assnasen T, Shuangoshti S. Polymerase chin reaction for detection of Mycobacterium tuberculosis in papanicolaou-stained fine needle aspirated smear for diagnosis of cervical tuberculous lymphadenitis. Southeast Asian J Trop Med Public Health 2006; 37: 940-947.
8. Giivne S, Esra Y. The diagnostic value of polymerase chain reaction in broncheo-alveolar leavage. Eastern Journal of sMedicine 2004; 9: 7-12.
9. Marchetti G, Gori A, Catozzi. Evaluation of PCR in detection Mycobacterium tuberculosis from formalin-fixed, paraffin-embeayrdded tissues comparison of four amplification assays. J Clin Microbiol 1998; 36: 1512-1517.
10. Li JY, Lo ST, Ng CS. Molecular detection of *Mycobacterium tuberculosis* in tissues showing granulomatous inflamation without demonstrable acid-fast bacilli. Diag Mol Path 2000; 9: 67-74.
11. World Health Organization. The Regional Report 2009: Tuberculosis Control in the South East Asia. WHO/SERO, 2009, New Delhi, India. (SEA-TB-315): 1.
12. Kocagoz,T and Yilmaz E. Detection of Mycobacterium tuberculosis in sputum samples by polymerase chain reaction using a simplified Procedure. J Clin. Microbiology 1993; 31(6):1435-1438.
13. Radhika S, Rajwanshi A, Kochhor. Abdominal tuberculosis: Diagnosis by fine needle aspiration cytology. Acta Cytol 1993; 37: 673-678.
14. Aljafari AS, Khalil EAG, Elsiddig KE, El Hag IA, Ibrahim ME, Elsafi EMO, et al. Diagnosis of tuberculous lymphadenitis by FNAC, microbiological methods and PCR: a comparative study. Cytopathol 2003; 15: 44-48.

15. Egea AS, Gonzalez MAM. Usefulness of Light microscopy in lymphnode needle aspiration biopsy. Acta Cytol 2002; 46: 368-369.

16. Yassin MA, Olobo JO, Kidane D, Negesse Y, Shimeles E, Tadesse A. Diagnosis of tuberculous lymphadenitis in Butajira, rural Ethiopia. Scan J Infect Dise 2003;35:1-4.

17. Radhika S, Gupta SK, Chakabarti A, Rajwanski A, Josi K. The role of culture of mycobacteria in fine needle aspiration diagnosis of tuberculous lymphadenitis. Diagn Cytopathol 1989; 5: 260-262.

18. Negi SS, Anand R, Pasha ST, Gupta S, Basir SF, Khare S, et al. Dignostic potential of IS6110, 38 KDA, 65KDA and 85B sequence based polymerase chain reaction in the diagnosis of mycobacterium tuberculosis in clinical samples. Indian J Med Micro 2007; 25: 43-49.

19. Pao CC, Yen TS, You JB, Maa JS, Fiss EH, Chang CH. Detection and identification of Mycobacterium tuberculosis by DNA amplification. J Clin Microbiol 1990; 28: 1877-1880.

20. Plikaytis BB, Eisenach KD, Crawfor JT, Shinnick TM. Differentiation of Mycobacterium tuberculosis and Mycobacterium bovis BCG by polymerase chain reaction assay. Mol Cell Probes 1991; 5: 215-219.

21. Sekar B, Selvaraj L, Alexis A, Ravi S, Arunagiri K, Rathinavel R. The utility of IS6110 sequence based polymerase chain reaction in comparison to conventional methods in the diagnosis of extra-pulmonary tuberculosis. Indian J Micro 2008; 26:352-355.

22. Bouakline SH, Vincensini JP, Giacuzzo V, Lagrange PH, Herrmann JL. Rapid diagnosis of extra pulmonary tuberculosis by PCR: Impact of sample collection and DNA extraction. J Clin Microbiol 2003; 41: 2323 -2329.

23. Singh KK, Muralidharn M, Kumar A,Chattopadhyaya TK, Kapalia K, Singh MK, et al.Comparison of in house polymerase chain reaction with conventional techniques for the detection of Mycobacterium tuberculosis DNA in granulomatous lymphadenopathy. J Clin Pathol 2000; 53:355-361.