

POLYMERASE CHAIN REACTION (PCR) FOR THE DIAGNOSIS OF CERVICAL TUBERCULOUS LYMPHADENITIS

Sunarto Reksoprawiro¹, Tjahjo Winantyo¹, Indrayana NS²

ABSTRACT

Tuberculous lymphadenitis is a common form of extrapulmonary tuberculosis with multiple differential diagnoses. The diagnosis of tuberculosis requires the presence of Mycobacterium tuberculosis by acid-fast staining or bacterial growth in culture. However, these are often difficult in cervical tuberculous lymphadenitis. The objective of this prospective study was to investigate the value of the polymerase chain reaction (PCR) technique for detection of Mycobacterium tuberculosis in the aspirate from fine needle biopsy (FNB) of suspected cervical tuberculous lymphadenitis. The primer to amplify Mycobacterium tuberculosis-complex-specific 123-bp DNA was used. Among 22 cases of cervical tuberculous lymphadenitis diagnosed in clinical situation, Mycobacterium tuberculosis DNA was found by PCR in 21 cases (95.40%). This study concludes that PCR is a useful technique for the demonstration of Mycobacterium tuberculosis DNA fragments in patients with clinically suspected cervical tuberculous lymphadenitis, and its clinical application with FNB could reduce the necessity for open biopsy.

Keywords: Polymerase chain reaction, tuberculosis, lymphadenitis

INTRODUCTION

Tuberculosis remains a major public health problem worldwide, especially in the developing countries like Indonesia. About thirty percent of the tuberculous infections are extrapulmonary (van Altena R and Richter C, 2002). Peripheral tuberculous lymphadenitis is the most common form of extrapulmonary tuberculosis, mostly affects the cervical lymph nodes (van Altena R and Richter C, 2002; Thompson MM, 1992). A definitive and accurate diagnosis of tuberculosis is important because satisfactory results can be achieved with chemotherapy alone, obviating surgery (Huhti E et al, 1975)

Up to now, the gold standard for diagnosis of tuberculous lymphadenitis is the demonstration of mycobacteria from biopsy specimen by smear or culture. The sensitivity of these conventional methods is, however, low when the specimen contains only a small number of organisms. Some studies demonstrated the accuracy of these conventional bacteriologic methods is less than 50% (Gupta SK et al, 1993; Radhika S et al, 1993). Excisional biopsy has traditionally been the standard of diagnosing tuberculous lymphadenitis. However, it can be associated with significant morbidity as well as delay in diagnosis.

Over the past decades, fine needle biopsy (FNB) cytology, a less invasive procedure than open biopsy,

has played an important role in the diagnosis of peripheral lymphadenopathy. However, the amount of material obtained in FNB is usually so small that it is often inadequate for performance of acid-fast smear and culture examinations with reasonable sensitivity.

Recently, the amplification of specific DNA sequences by polymerase chain reaction (PCR) is a novel tool for the detection of different infectious organisms and has already been applied to detect mycobacterial DNA sequences in several materials (Kim SS, et al, 1996; Kidane D, et al, 2002). PCR is the most sensitive technique in the demonstration of *M. tuberculosis* in clinically suspected patients, when their acid fast bacillus (AFB) stain or culture are negative.

In this study, the authors performed *Mycobacterium tuberculosis* PCR for mycobacterial DNA sequences from the fine-needle aspirates and evaluated its diagnostic efficacy in clinical situations.

PATIENTS AND METHODS

Patients

The adult patients with clinical diagnosis of cervical tuberculous lymphadenitis treated at Department of Surgery Dr. Soetomo hospital Surabaya between February and April 2002 were included in this study. FNB of the suspected lymph node were performed using 22-gauge needles without aspiration; the aspirates were sent to the laboratory of Airlangga University Tropical Diseases Center for mycobacterial DNA detection by PCR.

¹Department of Surgery

Airlangga University School of Medicine

²Airlangga University Tropical Disease Center, Surabaya, Indonesia

Methods

Primer: The primers used for the amplification were originally designed by Eisenach et al. from sequences which are repeated 10-16 times in the chromosome of *Mycobacterium tuberculosis* (Eisenach et al, 1990). The sequences of the primer (synthesized by Genzet Singapore), which amplify a 123 base-pair fragment of the repetitive sequence (6) were 5-CCTGCGAGCGTAGGCGTCGG-3 and 5-CTCGTCCAGCGCCGCTTCGG-3.

DNA preparation: The specimen was placed in an Eppendorf tube, incubated with proteinase K at 56°C, and put it in a sonicator (35.000Hz) for 1 hour, the DNA was isolated with Chelex 10%, and then used directly for amplification without any further processing.

PCR procedure: Using disposable, positive-displacement pipettes, 5µl of the DNA extract from each sample was added to 50 µl of reaction mixture containing the primers (50pmol), 1 U Taq polymerase buffer (25mM Tris-HCl pH 9.5, 50mM KCl, 10mM

MgCl₂), 0.2mM each of dATP, Taq Bead polymerase (PROMEGA) 1.25 Units. The DNA was denaturated for five minutes at 94°C, 35 amplification cycles were performed using an automated thermal cycler (MJ research Thermal). Each cycle consisted of denaturation at 94°C for 1 minute, annealing of primers at 68°C for 2 minutes, and primers extension at 72°C. The presence of the 123 base-pairs amplification product was analyzed by electrophoresis of 10 µl of the amplified mixture on 2% agarose gel. The DNA was stained by silver staining and photographed using digital camera on a white light transilluminator.

RESULTS

In this study of 22 consecutive patients with a clinical diagnosis of cervical tuberculous lymphadenitis, 7 (31.8%) were male and 15 (68.2%) were female, The age ranged 15 to 45 years with the mean age was 24.6 years (table 1). The involved cervical lymph nodes was level I-II in 10 (45.5%), level III-V in 5 (22.7%), and level I to V in 7 (31.8%) patients (table 2)

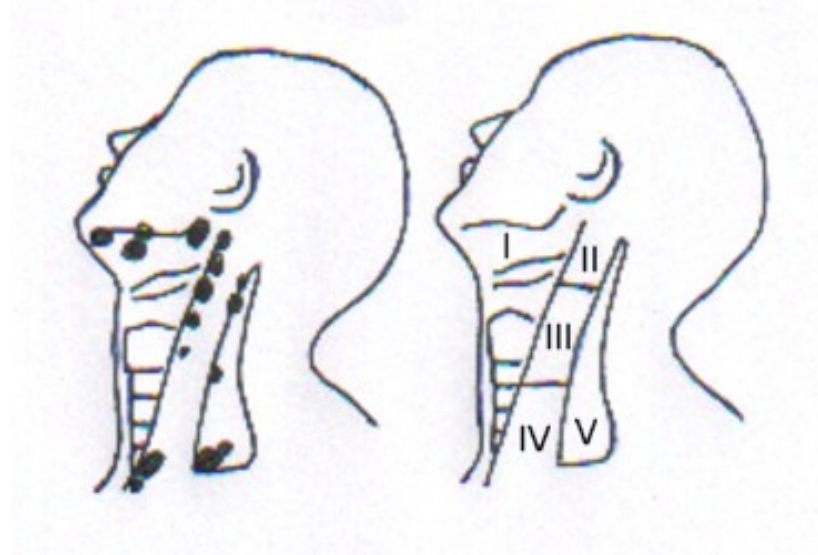


Figure 1. Levels of cervical lymph nodes

Table 1. Age and sex distribution

Age (year)	Sex		No. (%)
	Male	Female	
15-20	3	5	8 (36.4%)
21-25	2	5	7 (31.8%)
26-30	0	2	2 (9.1%)
31-35	0	1	1 (4.5%)
36-40	1	2	3 (13.6%)
41-45	1	0	1 (4.5%)
Total	7	15	22 (100%)

Table 2. Age and site of lymph nodes distribution

Age (year)	Site of lymph nodes			No. (%)
	Level I-II	Level III-V	Level I-V	
15-20	4	0	4	8 (36.4%)
21-25	2	3	2	7 (31.8%)
26-30	1	0	1	2 (9.1%)
31-35	1	0	0	1 (4.5%)
36-40	1	2	0	3 (13.6%)
41-45	1	0	0	1 (4.5%)
Total	10	5	7	22 (100%)

The amplification of the 123 base-pairs fragment of *Mycobacterium tuberculosis* DNA, after preparation of the template DNA is shown in Figure 1. Positive results

of the PCR examination were found in 21 out of 22 patients (95.4%)

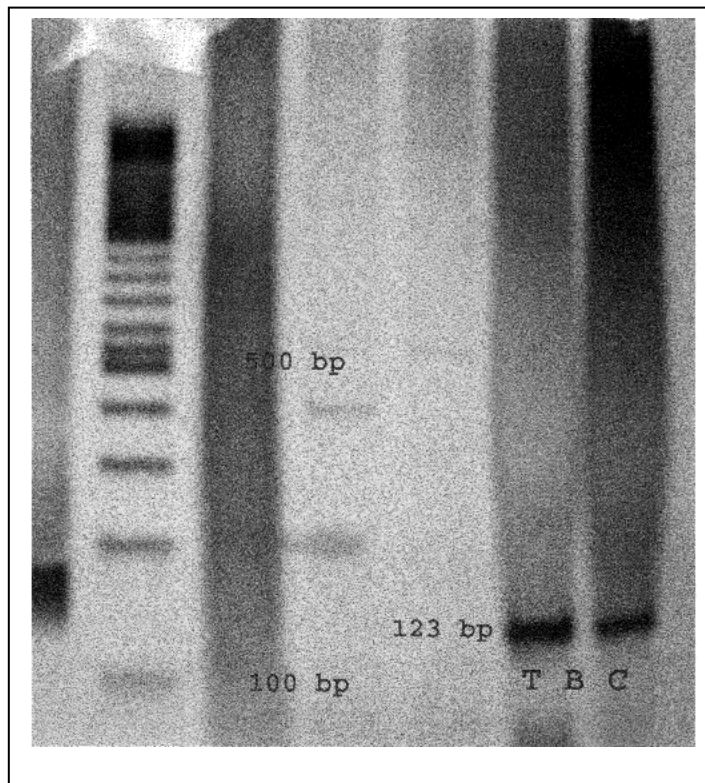


Figure 1. Result of PCR from the fine-needle biopsy of cervical tuberculous lymphadenitis patient showing an amplification of the 123 base-pairs fragment of *M. tuberculosis*

DISCUSSION

The tuberculous lymphadenitis is diagnosed certainly by mycobacterial culture or staining. Lowenstein-Jensen method is commonly used for culture, needs 2-4 weeks for growing of the mycobacterium. Direct microscopic

examination using auramine or Ziehl-Neelsen staining will shows the acid fast bacilli if the number of the mycobacterium in the sample is more than 10^4 /ml (van Altena R and Richter C, 2002). Some previous report demonstrated AFB detection was relatively low, 25 to 45% on AFB smear (Gupta SK et al, 1993, Radhika S et

al, 1993). The result of combined smear and culture was also low, only 23 to 32% (Kim SS et al, Aljafari AS et al, 2004).

Introduction of the PCR provided new possibilities for identification of mycobacteria tuberculosis in various types of clinical samples (Buck GE et al, 1992, Eisenach KD et al, 1991). PCR is a common method of creating copies of specific fragments of DNA, rapidly amplifies a single DNA molecule into many billions of molecules. PCR has been successfully applied to detect members of *M. tuberculosis* complex. Amplification the specific sequences of DNA by the PCR technique has been reported to provide rapid detection of *Mycobacterium tuberculosis* in the samples. The specimen for PCR examination can be taken from surgical biopsy or fine-needle aspiration (Manitchotpisit B, 1999; Goel MM et al, 2001; Kim SS, 1996), paraffin-embedded tissue (Popper HH, 1994; Osaki M, 1997), and peripheral blood mononuclear cells (Mirza S, 2003). Based on the amplification of common sequences, the identification time for detection of mycobacteria in clinical specimens was reduced (del Portilo P et al, 1991; Espinosa et al, 1998). PCR is not only a rapid and sensitive diagnostic method for tuberculous lymphadenitis, but also clinically significant in retrospective study for detecting *M. tuberculosis* even in some preserved lymph node tissue without evident of acid fast stained organisms (van Altena R and Richter C, 2002; Yang B et al, 1998; Mulder et al, 2002)

Theoretically only two organisms of AFB are enough to detect successfully with PCR amplification (Pao CC et al, 1990; Pilkatys et al 1991). So, the culture showed a little more sensitivity, and the PCR revealed far more sensitivity. In this study, PCR targeting IS6110 was used in detecting *Mycobacterium tuberculosis* in the specimens; among 22 cases of cervical tuberculous lymphadenitis diagnosed in clinical situation, *M. tuberculosis* DNA was found by PCR in 21 cases (95.4%). The high sensitivity of the PCR to detect *Mycobacterium tuberculosis* in patients with clinical diagnosis of tuberculous lymphadenitis was also reported in the literatures as 76.4-96% (Baek CH, 2000; Aljafari AS et al, 2004; Manitchotpisit B, 1999). Of the 10 samples that were negative by both smear and culture. Of the 10 samples obtained from patients clinically suspected of tuberculosis, that were negative by both smear and culture, 9 were positive with PCR, suggesting that the assay is probably more sensitive than the culture (Soehardjo IN, 2001)

False-positive reactions are a major problem with PCR methods for the detection of the *M. tuberculosis* (Eisenach KD et al 1990; del Portilo P, 1996; Eisenach 1991). A laboratory-associated contamination must be controlled during the DNA preparation, amplification and detection procedure. Prevention of amplicon

contamination should be accomplished by physical separation of different steps in the PCR procedure, using different pipettes, wearing separate gloves in each laboratory, and by using positive displacement pipette tips for preparing the PCR mixed and agarose gel electrophoresis step (Kim SS).

Correct diagnosis of tuberculosis could be made in 94.87% of cases by a combination of the fine needle aspiration cytology (FNAC), Zeihl-Neelsen staining of smears for acid-fast bacilli, culture for *Mycobacterium tuberculosis*, and lymph node biopsy (Goel MM et al, 2001). However, PCR proved to be the most valuable and a success rate of 100% was reported when PCR and fine needle aspiration cytology were combined (Aljafari AS et al, 2004). Manitchotpisit et al (1999) also reported the increasing of sensitivity and specificity as 84% and 100% respectively when PCR and FNAC were combined.

CONCLUSION

We conclude that PCR is the most sensitive single technique available to date for the demonstration of *M. tuberculosis* in specimens derived from clinically suspected of tuberculous lymphadenitis patients.

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