A Comparative Study of Diagnostic Tests for Tuberculous Lymphadenitis : Polymerase Chain Reaction *vs* Histopathology and Clinical Diagnosis

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Abstract

To compare diagnostic tests for tuberculous lymphadenitis by polymerase chain reaction (PCR) with histopathology and clinical diagnosis in sensitivity, specificity and predictive value. This retrospective analytic, single blind study was done at King Chulalongkorn Memorial Hospital. Paraffin-embedded specimens were classified into 2 groups. The study group contained 30 proved AFB positive paraffin-embedded specimens from patients who also had clinical diagnosis of tuberculosis and improved by antituberculous treatment. The control group contained 30 formalin-fixed, paraffin-embedded specimens of lymph node hyperplasia proved by histopathological and clinical review. All 60 specimens were slided, and systematically labeled and sent to PCR lab. Polymerase Chain Reaction method had sensitivity = 43.33 per cent, specificity = 100 per cent, positive predictive value =100 per cent and negative predictive value = 63.83 per cent. The present findings revealed that the PCR results were related to the age of the paraffin-embedded tissues. No positive results were obtained from tissues kept since 1996. Positive results were obtained in 3/7 cases (42.86%), 2/3 (66.67%) and 8/10 cases (80%) from tissue of 1997, 1998 and 1999 respectively.

Conclusion : Polymerase chain reaction has sufficient reliability best as a confirmatory diagnostic test for tuberculous lymphadenitis; however, it is not appropriate as a screening test.

Key word : Tuberculous Lymphadenitis, Polymerase Chain Reaction, Histopathology, Clinical Diagnosis

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Tuberculosis is one of the most common communicable diseases in Thailand; its incidence has risen significantly due to widespread HIV infection. Although medical knowledge grows rapidly, the incidence of tuberculous lymphadenitis especially in the cervical region is still present and is more common than the past.

Cervical tuberculous lymphadenitis has a natural course of disease similar to other diseases such as malignant lymphoma, Kikushi (histiocytic necrotizing lymphadenitis), metastatic lymphadenopathy and other infectious or neoplastic lymph node diseases. Granulomatous lymphadenitis in particular has been known to be caused by *Mycobacterium tuberculosis* or atypical *Mycobacterium* (an infection which requires different treatment). *M. tuberculosis* infection is a medically curable disease, but atypical *Mycobacterium* infection needs surgical treatment(1).

Diagnosis of tuberculous lymphadenitis has long been problematic because the gold standard of diagnosis needs tissue culture (BACTEC technique), which is highly specific and sensitive. Normally, the culture technique also notifies the result of drug susceptibilities, the important information for an accurate anti-microbial treatment. Moreover, the culture technique is not only a time-consuming test (about 2 weeks), the BACTEC technique is also expensive and not available in most hospitals (except major university hospitals).

Most Thai physicians, who are primary care-giver for patients with tuberculous lymphadenitis, often ignore the culture test before starting antituberculous drugs. Usually, they merely rely upon clinical findings (history and physical examination) to diagnose and treat the patients. There is a high possibility that the diagnoses may be wrong. Some physicians diagnose TB lymph node by using fine needle aspiration cytologic examination which has some false positive and false negative results. Some physicians treat TB lymph node by using histopathological results compatible with TB such as granulomatous inflammation (caseating or non-caseating). Without identification of AFB positive organisms, the diagnosis of tuberculous lymphadenitis is not definite. In most cases of tuberculous lymphadenitis, the chance of finding AFB positive organisms is not common, because normally there are few organisms in the infected lymph node. Although positive AFB stained organisms are found in the specimen of lymph node, it can be other types of AFB stained organisms (such as atypical mycobacterium, Nocardia, etc). An accurate diagnosis of tuberculous lymphadenitis, therefore, needs reliable tests apart from compatible histopathologic findings(2).

Since the identification of *M. tuberculosis* by polymerase chain reaction (PCR) technique was developed to find out some traces of *M. tuberculosis* DNA in the specimen, the specificity of this test is very high. It detects a minor trace of DNA in the specimen that contains only a few *M. tuberculosis* cells (viable or nonviable cells). Moreover, the technique does not need a long time to give its result, so it is very beneficial for clinicians to give an accurate diagnosis and proper treatment.

The advantage of the technique can confirm the diagnosis of *M. tuberculosis* infection by other tests such as the histopathology test. There is no need for clinicians to wait for the BACTEC culture result, they can immediately give antituberculous drugs to the patients. The PCR technique can also be used in paraffin-embedded specimens(3-6); so it is very beneficial in cases of suspected *M. tuberculous* infected patients who were not set up for a PCR test at the time of excisional biopsy or in patients who were partially treated with anti-microbial drugs. The BACTEC culture technique cannot be tested in both situations.

Because the technique needs only a few organisms in a tiny specimen, fine-needle aspiration biopsy may be used for cytologic examination, in combination with the PCR test to diagnose TB accurately(7-10).

Goal of study

The aim of the study was to determine the efficiency of polymerase chain reaction (PCR) as the diagnostic tool for detection of *M. tuberculosis* in tissue specimens by comparing the results obtained from AFB-positive tissues of TB cases with those from tissues of lymph node hyperplasia.

MATERIAL AND METHOD

A retrospective analytic study was designed. Group 1 (study group) contained 30 proved AFB positive paraffin-embedded specimens from patients who also had a clinical diagnosis of tuberculosis and improved by antituberculous treatment. Group 2 (control group) contained 30 formalin-fixed, paraffin-embedded specimens of lymph node hyperplasia proved by histopathological and clinical review.

All specimens were collected from the

Department of Pathology, Faculty of Medicine, Chulalongkorn University. Every specimen was pathologically reviewed by a pathologist and clinically reviewed by clinicians, then all of them were labeled randomly (Number 1-60) and sent to a PCR technician. PCR test of every specimen was done at the Department of Microbiology, Faculty of Medicine, Chulalongkorn University under supervision of an authorized microbiologist. The PCR technician did not know the histopathology result of each specimen. The PCR result was reported as "positive" or "negative".

Preparation of formalin-fixed, paraffin-embedded tissues for PCR test

The paraffin-embedded cervical lymph node specimens with the diagnosis of granulomatous (caseating or non-caseating) lymphadenitis and acid-fast positivity by acid-fast stain of Kinyoun's method were retrieved randomly from 1996 to 1999. The H&E sections were reviewed to verify the diagnosis and a pathologist determined the amount of acid-fast bacilli. The control group comprised of the lymph nodes from patients with the diagnosis of lymphoid hyperplasia conventionally retrieved over the same period.

The paraffin-embedded tissue blocks of both groups were systematically labeled and sent for cutting to obtain two 10-micrometer-thick sections. The decontamination procedure was performed by application of xylene, 5 per cent sodium hypochlorite (diluted into 1:10 with water) and 100 per cent alcohol over the microtome overlay respectively before a fresh blade was used for each specimen. Only four or five consecutive blocks were sectioned in the same batch.

Polymerase chain reaction (PCR) technique

DNA extraction

Tissue specimens were extracted three times with xylene to remove paraffin, washed twice with 100 per cent ethanol to remove the solvent, and once with buffer (100 mM Tris-HCl, 25 mM EDTA, pH8). After centrifugation, the pellet was resuspended in 100 µl of digestion buffer (50 mM Tris-HCl, pH8; 1 mM EDTA, 0.5 per cent Tween 20 and 2 mg/ml proteinase K). Digestion was performed overnight at 55°C. DNA was purified by phenol-chloroform-isoamyl alcohol (25:24:1) extraction, precipitated by ethanol, and dissolved in 50 µl of TE buffer (10 mM Tris HCl, 1mM EDTA, pH8). Five microliters of the sample were used for the PCR assay. Each sample was spiked with *M. tuberculosis* DNA (100 fg) as a control to detect the presence of amplification inhibitors. A positive control containing 100 fg of *M. tuberculosis* DNA and a negative control containing no DNA were included in each run.

PCR analysis

PCR was performed by the amplification of *IS 6110* insertion sequence as described by Kolk et al[11], with modification. Primers INS1 (5'CGT GAGGGCATCGAGGTGGC) and INS2 (5'GCGT AGGCGTCGGTGACAAA) were used to amplify a 245-bp fragment, then nested primers Pt3 (5'GAAC GGCTGATGACCAAACT) and Pt6 (5'ACGTAGG CGAACCCTGCCCA) were used to amplify a 188-bp fragment situated within the 245-bp fragment.

The first PCR solution (final volume 50 µl) contained 50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 0.01 per cent (W/V) gelatin, 0.2 mM each of dATP, dCTP and dUTP (dUTP instead of dTTP), 0.2 U of uracil-N-glycosylase (BRL), 0.4 µM of primers INS1 and INS2 and 1 U of Taq polymerase (Perkin Elmer Cetus). The second PCR solution contained primers Pt3 and Pt6 and other reagents of the first PCR solution except uracil-N-glycosylase.

A volume of 5 µl was added to the treated sample and the reaction mixture was left at room temperature for 10 minutes. The reaction was carried out in a DNA thermal cycler (Perkin Elmer Cetus). The temperature of the sample was first raised to 94°C for 10 minutes to inactivate uracil-N-glycosylase and denature the DNA. Amplification of 40 cycles consisted of denaturing at 94°C for 90 seconds, annealing at 65°C for 90 seconds, and extension at 72°C for 90 seconds. For the second PCR, 10 µl of the reaction solution containing the amplified first PCR product was added to 40 µl of the second PCR solution and the reaction mixture was amplified for 40 cycles with the same PCR condition as the first PCR. The PCR products were electrophoresed in 2 per cent agarose gel containing 0.5 µg/ml of ethidium bromide and visualized by UV transillumination.

RESULTS

In this study the authors accept histopathology of granulomatous inflammation of lymph node with acid-fast stain positive combined with the cure results from treatment by antituberculous drug regimen as the gold standard. The histopathology, amount

Table 1. Histopathological data and PCR results in individual cases.

Patient no.	Yr	Amount of AFB	Histopathology with AFB stain	PCR for *M. tuberculosis*	Patient no.	Yr	Amount of AFB	Histopathology with AFB stain	PCR for *M. tuberculosis*
1	96	1+	Granuloma AFB+	Neg	31	96		LN hyperplasia	Neg
2	96		LN hyperplasia	Neg	32	97	1+	Granuloma AFB+	Neg
3	96	1+	Granuloma AFB+	Neg	33	96		LN hyperplasia	Neg
4	96		LN hyperplasia	Neg	34	98	3+	Granuloma AFB+	PCR+
5	96	1+	Granuloma AFB+	Neg	35	96		LN hyperplasia	Neg
6	96		LN hyperplasia	Neg	36	98	2+	Granuloma AFB+	PCR+
7	96	2+	Granuloma AFB+	Neg	37	96		LN hyperplasia	Neg
8	96		LN hyperplasia	Neg	38	98	2+	Granuloma AFB+	Neg
9	96	3+	Granuloma AFB+	Neg	39	96		LN hyperplasia	Neg
10	96	1+	Granuloma AFB+	Neg	40	98		LN hyperplasia	Neg
11	96		LN hyperplasia	Neg	41	99	2+	Granuloma AFB+	PCR+
12	96	2+	Granuloma AFB+	Neg	42	98		LN hyperplasia	Neg
13	96		LN hyperplasia	Neg	43	99	2+	Granuloma AFB+	PCR+
14	96	1+	Granuloma AFB+	Neg	44	98		LN hyperplasia	Neg
15	96		LN hyperplasia	Neg	45	99	3+	Granuloma AFB+	PCR+
16	96	2+	Granuloma AFB+	Neg	46	98		LN hyperplasia	Neg
17	96		LN hyperplasia	Neg	47	99	2+	Granuloma AFB+	PCR+
18	96	1+	Granuloma AFB+	Neg	48	98		LN hyperplasia	Neg
19	96		LN hyperplasia	Neg	49	99	1+	Granuloma AFB+	PCR+
20	96		LN hyperplasia	Neg	50	99	2+	Granuloma AFB+	PCR+
21	97	1+	Granuloma AFB+	Neg	51	98		LN hyperplasia	Neg
22	96		LN hyperplasia	Neg	52	99	1+	Granuloma AFB+	Neg
23	97	2+	Granuloma AFB+	PCR+	53	98		LN hyperplasia	Neg
24	96		LN hyperplasia	Neg	54	99	2+	Granuloma AFB+	PCR+
25	97	2+	Granuloma AFB+	Neg	55	98		LN hyperplasia	Neg
26	96		LN hyperplasia	Neg	56	99	2+	Granuloma AFB+	Neg
27	97	2+	Granuloma AFB+	PCR+	57	98		LN hyperplasia	Neg
28	96		LN hyperplasia	Neg	58	99	1+	Granuloma AFB+	PCR+
29	97	2+	Granuloma AFB+	Neg	59	98		LN hyperplasia	Neg
30	97	1+	Granuloma AFB+	PCR+	60	99		LN hyperplasia	Neg

Table 2. Histopathological data and PCR results.

	Granuloma with AFB+	Lymph node hyperplasia
PCR +	13	0
PCR -	17	30

Sensitivity = 43.33 per cent
Specificity = 100 per cent
Positive predictive value = 100 per cent
Negative predictive value = 63.83 per cent

of AFB, age of specimens and PCR results of individual cases are shown in Table 1. The PCR results of both groups were statistically analyzed using chi-square test. (Table 2)

The PCR results of both groups were analysed in subgroup classified by age of specimens. (Table 3, 4)

DISCUSSION

The study shows that the polymerase chain reaction (PCR) technique for detection of *M. tuberculosis* has a high specificity (100%), however, its sensitivity is not high (43.33%). The test is not as time-consuming as the culture technique. Because of its high specificity, it can be used as a confirmatory test for clinically suspected TB lymph nodes. Nevertheless, it may not be a good screening test for TB lymph nodes due to its low sensitivity.

The overall sensitivity of the current study was 43.33 per cent. The low sensitivity of this study was likely due to the age of the paraffin-embedded specimen. In the 30 cases of the acid-fast positive group, 10 cases were from 1996 and none of them was PCR positive (sensitivity = 0%). Three of 7 cases from 1997 were PCR positive (sensitivity = 42.86%). Two of three cases from 1998 were PCR positive (sensitivity = 66.67%). If specimens from 1997 to 1998 were combined, the sensitivity would be 50 per cent. 8 of 10 cases from 1999 were PCR positive (sensitivity = 80%). This result indicated that the paraffin-embedded specimens of 5 years of age greatly compromised the PCR result of lymph node with granulomatous lymphadenitis from *M. tuberculosis* infection. The newer the paraffin-embedded specimens, the more sensitive the PCR tests are. However, the specificity and positive predictive value

Table 3. PCR results and age of paraffin-embedded specimens.

Year	Group	PCR results	
		Positive	Negative
1996	Granuloma AFB+	0	10
	LN hyperplasia	0	19
1997	Granuloma AFB+	3	4
	LN hyperplasia	0	0
1998	Granuloma AFB+	2	1
	LN hyperplasia	0	10
1999	Granuloma AFB+	8	2
	LN hyperplasia	0	1

Table 4. Age of paraffin-embedded specimens and sensitivity, specificity and predictive values of the PCR test.

Age of specimens (year)	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %
5	0	100	-	65.52
4	42.86	-	100	0
3	66.67	100	100	90.91
2	80	100	100	33.33

of PCR were always 100 per cent in the present study. This issue will be answered by the authors' prospective study in which the specimens are new and *M. tuberculosis* DNA is better preserved.

The other known factors that could affect the PCR result included the number of organisms and the time elapsed before the formalin fixed tissue was processed into paraffin blocks. Using the hospital system, the majority of the formalin-fixed specimens was processed into paraffined block in 24-48 hours so DNA should be well preserved. Regarding the amount of organisms, in the present study if the cases from 1996 were ignored, there were only two cases which contained numerous organisms and their PCR results were positive. The negative PCR results were found among the remaining cases that contained few or scant organisms.

The low sensitivity of PCR was also due to the number of sections investigated. Sections should be cut at different levels to compensate for the uneven distribution of bacterial foci in tissues. It was demonstrated that a significant improvement of PCR assay was obtained by examinations of at least three paraffin sections from each specimen(12).

The cost of the PCR test (1,000 baht) and BACTEC culture for *M. tuberculosis* (1,000 baht) are twice as much as the histopathology examination (500 baht), so physicians who have to take care of patients suspected of cervical tuberculous lymphadenitis should consider the economic status, benefit and adverse effects from unnecessary antituberculous drug treatment.

The authors' recommended guideline for the management of patients suspected of TB lymphadenitis is lymph node biopsy with 3 tests (PCR, BACTEC culture for *M. tuberculosis* and histopathology). One week later if the result of PCR is positive and the histopathology result is compatible with TB lymphadenitis eg. granulomatous inflammation (caseating or non-caseating), a short course of antituberculous drug regimen should be started. The antituberculous drug regimen may have to be changed depending on the BACTEC culture and susceptibility result.

The authors will do a further prospective study comparing efficacy of histopathology, PCR technique, BACTEC culture and clinical manifestations in patients with suspected cervical tuberculous lymphadenitis.

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REFERENCES

1. Akhtar J, Howatson AG, Raine PA. Atypical mycobacterial infection in childhood: A "surgical disease". J R Coll Surg Edinb 1997; 42: 110-1.
2. Narita M, Shibata M, Togashi T, Kobayashi H. Polymerase chain reaction for detection of *Mycobacterium tuberculosis*. Acta Paediatr 1992; 81: 141-4.
3. Yang B, Koga H, Ohno H, et al. Detection of *Mycobacterium tuberculosis* in preservedtuberculous lymph nodes by polymerase chain reaction. Tohoku J Exp Med 1998; 184: 123-31.
4. Tarng DC, Su WJ, Huang TP. PCR diagnosis on formalin-fixed, paraffin-embedded tissues with acid- fast stain and culture negativity in chronic dialysis patients of cervico-mediastinal tuberculous lymphadenitis. Nephrol Dial Transplant 1998; 13: 1543-6.
5. Totsch M, Bocker W, Brommelkamp E, et al. Diagnostic value of different PCR assays for the detection of mycobacterial DNA in granulomatous lymphadenopathy. J Pathol 1996; 178: 221-6.
6. Sugita Y, Sasaki T, Okuda K, Nakajima H. Practical use of polymerase chain reaction for the diagnosis of steroid induced tuberculous lymphadenitis. J Trop Med Hyg 1994; 97: 65-8.
7. Ersoz C, Polat A, Serin MS, et al. Fine needle aspiration (FNA) cytology in tuberculous lymphadenitis. Cytopathology 1998; 9: 201-7.
8. Baek CH, Kim SI, Ko YH, Chu KC. Polymerase chain reaction detection of *Mycobacterium tuberculosis* from fine-needle aspirate for the diagnosis of cervical tuberculous lymphadenitis. Laryngoscope 2000; 110: 30-4.
9. Kim SS, Chung SM, Kim JN, et al. Application of PCR from the fine needle aspirates for the diagnosis of cervical tuberculous lymphadenitis. J Korean Med Sci 1996; 11: 127-32.
10. Manitchotpisit B, Kunachak S, Kulapraditharom B, Sura T. Combined use of fine needle aspiration

cytology and polymerase chain reaction in the diagnosis of cervical tuberculous lymphadenitis. J Med Assoc Thai 1999; 82: 363-8.
11. Kolk A, Schuitema A, Kuijper S, et al. Detection of *Mycobacterium tuberculosis* in clinical samples by using polymerase chain reaction and a non-radioactive detection system. J Clin Microbiol 1992; 30: 2567-75.
12. Totsch M, Bocker W, Brommelkamp E, et al. Diagnostic value of different PCR assays for the detection of mycobacterial DNA in granulomatous lymphadenopathy. J Pathol 1996; 178: 221-6.

การตรวจวินิจฉัยวัณโรคต่อมน้ำเหลืองโดยวิธีพีซีอาร์ เปรียบเทียบกับวิธีตรวจทางพยาธิวิทยาและการวินิจฉัยทางคลินิก

ประกอบเกียรติ หิรัญวิวัฒน์กุล, พ.บ., สมหญิง ธัมวาสร, วท.ม., ปร.ด.**, ชวิษฐ์ จันทรานุวัฒน์, พ.บ.***, อรรคพร ศิริชัย, พ.บ.**

เพื่อศึกษาเปรียบเทียบความไว ความจำเพาะ ความสามารถของการทำนาย ของการตรวจวินิจฉัยวัณโรคต่อมน้ำเหลืองโดยวิธี Polymerase Chain Reaction เปรียบเทียบกับ วิธีตรวจทางพยาธิวิทยาและ การวินิจฉัยทางคลินิก รูปแบบการวิจัยเป็นชนิด Retrospective analytic, single-blind study การศึกษานี้ทำที่โรงพยาบาลจุฬาลงกรณ์ วิธีการศึกษาทำโดยคัดเลือกชิ้นเนื้อของต่อมน้ำเหลืองในก้อนพาราฟิน (Paraffin-embedded specimen) ออกมาเป็น 2 กลุ่ม คือ กลุ่มศึกษา (Study group) เป็นชิ้นเนื้อจำนวน 30 ตัวอย่างที่ได้รับการวินิจฉัยว่าเป็นวัณโรคต่อมน้ำเหลือง โดยผลการตรวจทางพยาธิวิทยา พบเป็น Granuloma และย้อม Acid-fast พบมีเชื้อ ร่วมกับการวินิจฉัยทางคลินิกที่ยืนยัน และกลุ่มเปรียบเทียบ (Control group) เป็นชิ้นเนื้อจำนวน 30 ตัวอย่างที่ได้รับการวินิจฉัยว่าเป็นต่อมน้ำเหลืองอักเสบธรรมดา โดยผลการตรวจทางพยาธิวิทยา พบเป็น Lymph node hyperplasia ร่วมกับการวินิจฉัยทางคลินิกที่ยืนยัน ชิ้นเนื้อทั้งหมด 60 ตัวอย่าง จะถูกตัดออกเป็นแผ่นบาง ๆ แล้วได้รับการติดฉลาก เพื่อส่งไปตรวจหาเชื้อวัณโรคโดยวิธี Polymerase Chain Reaction ผลการศึกษาพบว่าการตรวจโดยวิธี Polymerase Chain Reaction มีความไวไม่มาก (43.33%) แต่มีความจำเพาะสูงมาก (100%) ความสามารถของการทำนาย ถ้าผลการตรวจให้ผลบวก ผู้ป่วยมีโอกาสที่จะเป็นโรค 100% ถ้าผลการตรวจให้ผลลบ ผู้ป่วยมีโอกาสที่จะไม่เป็นโรคได้ 63.83% การศึกษานี้พบว่า ผลการตรวจ PCR ยังเกี่ยวข้องกับอายุของชิ้นเนื้อ โดยพบว่าไม่พบผลบวกเลยในชิ้นเนื้อที่ตัดไว้เมื่อปี 2539 ส่วนชิ้นเนื้อของปี 2540, 2541 และ 2542 มีผลบวก 3 ใน 7 (42.86%), 2 ใน 3 (66.67%) และ 8 ใน 10 (80%) ตามลำดับ

สรุป : การตรวจโดยวิธี Polymerase Chain Reaction มีความจำเพาะสูงมาก เหมาะสำหรับใช้เป็นการทดสอบยืนยัน ในกรณีที่สงสัยว่าเป็นโรควัณโรคต่อมน้ำเหลือง แต่มีความไวต่ำ อาจไม่ดีสำหรับการใช้เป็นการทดสอบเพื่อคัดกรอง

คำสำคัญ : วัณโรคต่อมน้ำเหลือง, พีซีอาร์, พยาธิวิทยาของเนื้อเยื่อ, การวินิจฉัยทางคลินิก

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