Combined Use of Fine Needle Aspiration Cytology and Polymerase Chain Reaction in the Diagnosis of Cervical Tuberculous Lymphadenitis†

BOONCHU MANITCHOTPISIT, M.D.*, SOMYOS KUNACHAK, M.D.*,
BOONCHU KULAPRADITHAROM, M.D.*, TUNYACHAI SURA, M.D.**

Abstract

Although fine needle aspiration cytology (FNAC) is an effective mean for the diagnosis of cervical tuberculous lymphadenitis (CTL), it still poses a certain degree of false negative and false positive. The objective of this study was to determine the efficiency of polymerase chain reaction (PCR) in combination with fine needle aspiration cytology in the diagnosis of CTL. Thirty three patients who presented with enlarged cervical lymph nodes, and were clinically suggestive of CTL were included in the study. Fine needle aspiration or surgical biopsy of lymph nodes was performed, the specimens were studied for cytology, acid fast bacilli stain, culture for mycobacteria and PCR technique. The sensitivity and specificity of FNAC was 48 per cent and 87.5 per cent respectively, while that of PCR was 84 per cent and 75 per cent respectively. When FNAC and PCR were combined, the sensitivity and specificity increased to 84 per cent and 100 per cent respectively. We concluded that FNAC in combination with the PCR technique is a fast and effective clinical diagnostic approach for CTL.

Key word : Fine Needle Aspiration, Polymerase Chain Reaction, Tuberculous Lymphadenitis

Cervical tuberculous lymphadenitis is a common disease in this region.

From a 9 year retrospective study conducted at the ENT Department Ramathibodi Hospital, Bangkok the incidence of cervical tuberculous lymphadenopathy (CTL) was 23.8 per cent representing 301 cases out of 1263 cases who presented with cervical masses[1].

Fine needle aspiration cytology (FNAC) is useful in the diagnosis of cervical swellings. The reported sensitivity and specificity in the diagnosis of cervical tuberculous lymphadenitis ranged from

* Department of Otolaryngology - Head - Neck Surgery,
** Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Bangkok 10400, Thailand.
† Presented in part at the Annual Scientific Meeting, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, 1 May 1998.

71-90 per cent[1,2] and 93 per cent[2] respectively. There are, however, some inherent limitations in the use of FNAC to diagnose CTL; first, there is a certain percentage of false negative, second, the FNAC findings of CTL are not distinguishable from other forms of granulomatous lymphadenitis, e.g., sarcoidosis. Culture of *Mycobacterium tuberculosis* is currently the best method to confirm the diagnosis, but it takes a minimum of 6-8 weeks. Pathological study of excised tissue is another sensitive method to diagnose tuberculous lymphadenitis. However, surgical procedure may cause complications such as spreading of the disease and chronic draining sinus.

Many tests have been developed primarily for the diagnosis of tuberculous lymphadenitis, i.e.,

1. BACTEC (Becton Dickinson Diagnostic Instrument System, U.S.A), this method analyses Co_2 production from the metabolism of *Mycobacterium tuberculosis* after inoculation in the medium containing $C^{(14)}$ labeled palmitic acid.

2. Determination of antibody titers to *Mycobacterium tuberculosis* by using the ELISA technique.

3. DNA probe technique, using complementary base pair to identify *Mycobacterium tuberculosis*.

4. Molecular approaches to the diagnosis of tuberculosis[3].

a) SDA, an isothermal amplification process developed by Becton Dickinson Diagnostic System (Research Triangle Park, U.S.A.).

b) TMA, an isothermal target - based amplification system developed by Gen - Probe Incorporated (San Diego, U.S.A.), this has been combined with a homogeneous detection method to detect *Mycobacterium tuberculosis* in a clinical specimen.

c) PCR technique, uses oligonucleotide primers to direct the amplification of target nucleic acid sequence *via* repeated rounds of denaturation, primer annealing and primerextention. The specificity of the amplication process lies in the choice of primers. Most of the published PCR - based tests have been shown to work well only in the research laboratory. These research - oriented were transferred to the clinical laboratory. After PCR reaction, the amplification products were analyzed by agarose gel electrophoresis. The advantages of PCR[3] were (i) PCR - based assay easily fitted into the daily routine of the clinical laboratory with the turn around time of only 24-36 hours from the time of specimen collection (ii) do not necessarily require highly specialized personnel to perform the assay, and (iii) false positive product contamination was relatively rare with the use of appropriate precaution.

In this paper we studied the efficiency of the PCR technique in the diagnosis of CTL from fine needle aspirated specimens, and attempted to compare the efficiency of the FNAC technique to the PCR technique, also combining the use of PCR and FNAC in the diagnosis of CTL.

MATERIAL AND METHOD

Between June 1996 and March 1997, all patients presenting with enlarged lateral cervical lymph nodes at the Department of Otolaryngology, Ramathibodi Hospital were examined. A detailed medical history and physical examination, including indirect nasopharyngoscopy and indirect laryngoscopy were performed. The inclusion criteria were those patients who initially presented with clinical signs and symptoms suggestive of tuberculous lymphadenitis, i.e., a nontender, enlarged lymph node in the posterior triangle of the neck in a young and otherwise asymptomatic patient. At a later stage, if further investigations proved that the cervical lymph node was a metastatic node, the patient was finally excluded from the study. This study included 33 patients, aged 13 to 60 years (mean 33.2 years), men: women ratio was 9:24. Further investigations included : fine needle aspiration of the lymph node for cytologic examination, culture for mycobacteria and staining for acid fast bacilli, PCR technique for *Mycobacterium tuberculosis* ; tuberculin test ; chest - radiogram ; serum anti HIV. Subsequently, the patients underwent surgical removal of the cervical lymph nodes under local or general anesthesia. The excised lymph nodes were sent for histologic study and culture for mycobacteria.

Prior to surgery, fine needle aspiration was performed at the same lymph node using a 10 ml disposable syringe with a 24 or 25 gauge needle. The procedure was repeated 4 times at the same area in different directions. From each patient the specimens obtained were then divided into 6 parts. Two parts were transferred into 2 separate microtubes containing 500 microliters of HPV buffer for PCR study, two parts were smeared on slides and fixed in 95 per cent ethyl alcohol for cytologic examination, one part was sent for acid fast bacilli staining and one specimen was sent for culture of mycobacteria.

The PCR technique began with DNA extraction. The specimen from each microtube was subjected to DNA extraction by the following steps.,

1. Add 20 µl of 5 mg/ml Proteinase K, incubate at 55°C for at least 4 hours.
2. Add 500 µl of saturated Phenol, mix well gently (> 70 strokes) spin at 12000 g for 10 minutes.
3. Transfer aqueous phase (top) to a fresh tube, add 500 µl of Isoamyl - Chloroform, mix well gently (> 70 strokes) and spin at 12000 g for 10 minutes.
4. Transfer aqueous phase (top) to a fresh tube, add 50 µl of 3 M Na - Acetate, pH 5.6, mix then add 500 µl of Isopropanol, mix gently and spin at 12000 g for 4 minutes.
5. Discard supernatant, rinse roughly with 1 ml 70 per cent Ethanol, spin at 12000 g for 2 minutes.
6. Discard supernatant, dehydrate with 1 ml absolute Ethanol, spin at 12000 g for 2 minutes.
7. Discard supernatant, air dry for approximately 15 minutes.

The extracted DNA then underwent amplification of a target sequence of *Mycobacterium tuberculosis* at the MPB 64 area in the PCR cycle using a thermocycler and a thermostable DNA polymerase. The technique included the known positive and negative controls. In general, the amplification process could be completed within 2 - 4 hours. The amplification products were subsequently analyzed by agarose gel electrophoresis.

RESULTS

Final diagnosis of CTL was obtained from 25 cases using the criteria of positive culture of *Mycobacterium tuberculosis*, positive acid fast bacilli staining, histopathologic finding of caseous granuloma and response to antituberculous drugs.

From this study we had positive cultures for *Mycobacterium tuberculosis* in 6 cases, positive acid fast bacilli stain in 7 cases. Antituberculous drug response in 19 cases, loss to follow-up 5 cases, antituberculous drug was not given in 6 cases and antituberculous drugs were non - responsive in 3 cases.

FNAC demonstrated granulomatous lymphadenitis with or without caseous material in 13 cases, necrotizing lymphadenitis in 9 cases, nonspecific lymphadenitis in 10 cases and adult fatty tissue in 1 case. In this study we based the diagnosis of CTL only on the finding of caseous material or granulomatous inflammation. We did not include special stain and details of cytologic patterns.

The results of FNAC in this study were true positive 12 cases, false positive 1 case, true

Table 1. The results of FNAC examination.

FNAC	CTL (N=25)	NON CTL (N=8)
Positive	12	1
Negative	13	7

Table 2. The results of PCR technique.

PCR	CTL (N=25)	NON CTL (N=8)
Positive	21	2
Negative	4	6

Table 3. The results of combine usage of FNAC & PCR.

FNAC + PCR	CTL (N=25)	NON CTL (N=8)
Positive	21	0
Negative	4	8

negative 7 cases, false negative 13 cases (Table 1). This resulted in the sensitivity of 48 per cent, specificity of 87.5 per cent, positive predictive value of 92.3 per cent, accuracy of 57.6 per cent and relative liability of 4.63 per cent.

The PCR technique yielded 23 positive cases, 10 negative cases, which consisted of true positive 21 cases, false positive 2 cases, true negative 6 cases and false negative 4 case (Table 2). From this data we arrived at the sensitivity of 84 per cent, specificity of 75 per cent, positive predictive value of 91.3 per cent, accuracy of 81.8 per cent and relative liability of 12.42 per cent. When FNAC and PCR were combined, true positive was 21 cases, true negative was 8 cases, false negative was 4 cases and there was no false positive (Table 3). The sensitivity was 84 per cent, specificity was 100 per cent, positive predictive value was 100 per cent, accuracy was 88 per cent and relative liability was 91.37 per cent. The efficiency of the tests are summarized in Table 4. There was one case of HIV positive, whose result of both PCR and FNAC was positive. From this study, we obtained a marked positive tuberculin test ($\geq$ 1.5 cm wheal and bleb or pseudopod formation) in 17 cases.

DISCUSSION

Tuberculous lymphadenitis is a common problem in under developed and developing countries, including Thailand. Typically, CTL presents as a painless neck mass. The diagnosis and treatment are usually delayed. In children, 76 per cent have lymphadenopathy for more than 3 weeks and 14 per cent for more that 3 months[4].

The best diagnosis of tuberculous lymphadenopathy is either discovery of mycobacteria from a cultured specimen or positive acid fast bacilli staining, however, these two techniques give low yield. In this study, we had only 28 per cent (7 of 25 cases) with positive acid fast bacilli staining and 24 per cent (6 of 25 cases) positive culture for *Mycobacterium tuberculosis*. Other positive results were obtained by histopathological study of excisional tissue biopsy demonstrating caseous granuloma. The diagnosis may be confirmed by response to antituberculous drugs.

In this study, the sensitivity of FNAC was as low as 48 per cent, this was probably because we did not include the pathology reports of necrotizing lymphadenitis in our diagnostic criteria as one study did[5]. Caseous material was the most sensitive and specific criterion[6]. Granulomas were often present in patients with mycobacterial infection but were also occasionally present in other diseases. Some studies used cytomorphologic feature as a criteria in the diagnosis which depends on the demonstration of epitheloid cells and Langhans' giant cell in the smears[7,8]. In this study, the diagnosis of tuberculosis was based on routine cytologic examination, we did not use special acid fast bacilli stain such as auramine - rhodamine and Ziehl - Neelsen staining.

There were 23 positive PCR in this series, including 2 cases of false positive. The first false positive case was a malignant non Hodgkins lymphoma, who presented with and area of caseous necrosis. The possibility of coexisting tuberculous infection cannot be totally ruled out. However, in this case the result of culture was negative. The second PCR false positive case was necrosis, focal fibrosis and vascular proliferation. Ten cases gave a negative result, including 4 false negative cases. One false negative case had taken an antituberculous drug 1 month prior to the test. The rest of the false negative results may be due to absence of *Mycobacterium tuberculosis* organism in the specimens received. Moreover, the specificity and accuracy of PCR depend on the choice of primers. In this study, we used MPB64[9] which had a 241 base - pair specific sequence of *Mycobacterium tuberculosis*. The other factors that may influence the accuracy of the PCR technique are technical errors.

When we combined the FNAC and PCR technique, the results had a higher value of all test parameters including specificity, sensitivity, positive predictive value, accuracy and relative liability. Based on this less invasive, out patient procedure

Table 4. Efficiency of FNAC, PCR, FNAC + PCR.

	FNAC	PCR	FNAC + PCR
Sensitivity %	48	84	84
Specificity %	87.5	75	100
Relative liability	4.63	12.42	81.3

the total time required for diagnosis was significantly shortened. The true invasive procedure of surgical excision or incision can be avoided. For the surgeon's sake, the chance of exposure to an HIV infected patient is minimized. Rapid identification of *Mycobacterium tuberculosis* in the specimens allows the physician to promptly initiate proper infection control procedures and therapeutic regimens. This could limit the possibility of nosocomial transmission of *Mycobacterium tuberculosis* and may bring the resurgence of tuberculosis under control. Surgical excision or incision biopsy of CTL had additional adherent disadvantages i.e., risk of prolonged cutaneous sinus, slow healing and required hospitalization in some cases.

The rate of positive mycobacterial culture in this study was low when compared to other series, which may due to the difference in lymph node status, and tuberculous abscesses were fewer in our series.

SUMMARY

FNAC or PCR is individually a relatively sensitive test for the diagnosis of *Mycobacterium tuberculosis* infection. The combination of these 2 techniques could significantly enhance the efficacy of this diagnostic approach as it is a fast and less invasive measure. We recommend combined FNAC and PCR as part of the diagnostic investigation of tuberculous cervical lymphadenopathies.

(Received for publication on October 21, 1998)

REFERENCES

1. Augkara J, Kunachak S, Prakunhangsit S, Kulapaditharom B. T.B. Lymph Node. In : The handbook of the 3rd annual meeting of CDC's allied hospital. Bangkok, 1996: 115-32.
2. Lau Sk, Wei WI, Hsu C, Engzell UCG. Efficacy of fine needle aspiration cytology in the diagnosis of tuberculous cervical lymphadenopathy. J Laryngol Otol 1990; 104: 24-7.
3. Shinnick TM, Jonas V. Molecular approaches to the diagnosis of tuberculosis. In : Barry R. Bloom ed. Tuberculosis Pathogenesis, Protection, and Con-trol. Washington. DC : American Society for Microbiology, 1994: 517-30.
4. Donald BH, Maisie LS, Emily JK, Eithne FM. Mycobacterial cervical adenitis in children : Medical and surgical management. Ear Nose Throat J 1993;72:733-42.
5. Bailey TM, Akhtar M, Ali MA. Fine needle aspiration biopsy diagnosis of tuberculosis. Acta Cytol 1985; 29: 732-6.
6. Finfer M, Perchick A, Burstein D, Fine needle aspiration biopsy diagnosis of tuberculous lymphadenitis in patients with and without the acquired immune dificiency syndrome. Acta Cytologica 1991; 35: 325-32.
7. Arora B, Arora DR. Fine needle aspiration cytology in diagnosis of tuberculous lymphadenitis. Indian J Med Res [A] 1990: 189-92.
8. Cleary KR, Batsadis JG. Mycobacterial disease of the head and neck : current perspective. Ann Otol Rhinol Laryngol 1995; 104: 830-3.
9. Sura T, Tunlayadechanont S, Boongird P, Phuapradit P. Diagnosis of tuberculous meningitis by polymerase chain reaction (PCR). Rama Med J 1993; July - September : 208-12.

การวินิจฉัยโรควัณโรคต่อมน้ำเหลืองที่คอ โดยวิธีดูดตรวจด้วยเข็มขนาดเล็ก ร่วมกับปฏิกิริยาลูกโซ่โพลีเมอเรส

บุญชู มานิตโชติพิศิษฐ์, พ.บ., สมยศ คุณจักร, พ.บ.*, บุญชู กุลประดิษฐ์สารมย์, พ.บ.*, ธันยชัย สุระ, พ.บ.***

ในการวินิจฉัยโรควัณโรคต่อมน้ำเหลืองที่คอ ถึงแม้ว่าวิธีการใช้เข็มขนาดเล็กดูดเนื้อตรวจ (fine needle aspiration cytology, FNAC) จะเป็นวิธีที่ได้ผลค่อนข้างดี แต่ก็ยังมีปัญหาเรื่องให้ผลลบลวงและผลบวกลวงมากพอสมควร จุดมุ่งหมายของรายงานนี้คือ ศึกษาถึงประสิทธิภาพของการใช้ปฏิกิริยาลูกโซ่โพลีเมอเรส (polymerase chain reaction, PCR) ร่วมกับการใช้เข็มเล็กดูดเนื้อตรวจ ในการวินิจฉัยดังกล่าว โดยทำการศึกษาในผู้ป่วยที่มาด้วยก้อนที่คอ ที่มีอาการหรืออาการแสดงของวัณโรคต่อมน้ำเหลือง จำนวน 33 ราย ตัวอย่างเนื้อเยื่อที่ใช้ในการตรวจได้จากการใช้เข็มเล็กดูดเนื้อ หรือการผ่าตัดก้อนและนำเนื้อเยื่อไปศึกษาดูเซลล์ (cytology) ตรวจหาและเพาะเชื้อวัณโรค รวมทั้งปฏิกิริยา PCR ผลปรากฏว่าความไวและความจำเพาะของวิธี FNAC เท่ากับ 48% และ 87.5% ตามลำดับ ความไวและความจำเพาะของวิธี PCR เท่ากับ 84% และ 75% ตามลำดับ และเมื่อใช้ PCR ร่วมกับ FNAC ความไวและความจำเพาะจะเพิ่มขึ้นเป็น 84% และ 100% ตามลำดับ จากการศึกษานี้อาจสรุปได้ว่าการใช้วิธี PCR ร่วมกับวิธี FNAC ในการวินิจฉัยโรควัณโรคต่อมน้ำเหลืองที่คอเป็นวิธีที่ให้ผลรวดเร็วและแม่นยำ

คำสำคัญ : วิธีดูดตรวจด้วยเข็มเล็ก, ปฏิกิริยาลูกโซ่โพลีเมอเรส, วัณโรคต่อมน้ำเหลือง

* ภาควิชาโสตนาสิกลาริงซ์วิทยา,

** ภาควิชาอายุรศาสตร์, คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี, มหาวิทยาลัยมหิดล, กรุงเทพ ฯ 10400