TURKISH JOURNAL OF MEDICAL SCIENCES

VOLUME 26 NUMBER 4 1996

TURKISH JOURNAL OF MEDICAL SCIENCES

ISSN 1300 - 0144

VOLUME **26** NUMBER **4** AUGUST **1996**

Turk J Med Sci
26 (1996) 329-332

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Rapid Diagnosis of Tuberculous Meningitis Using Polymerase Chain Reaction

Received: June 27, 1995

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Abstract: In order to determine the sensitivity and specificity of polymerase chain reaction (PCR) in diagnosis of tuberculous meningitis, cerebrospinal fluid samples were studied form 35 children admitted with diagnosis of meningitis and from 12 control patients with other neurological disorders. PCR was performed by using primers specific for the *IS 6110* region of Mycobacterium tuberculosis. Thirteen patients were PCR positive for Mycobacterium tuberculosis in their cerebrospinal fluid samples (CSFs). All but one had tuberculous meningitis; the exception had bacterial meningitis, which was proven clinically by the outcome of treatment and follow-up. The CSF samples of the other patients without tuberculous meningitis and of the control group were all PCR negative. It was concluded that, with 100% sensitivity and 95.7% specificity, cerebrospinal fluid analysis with PCR could be reliably used for the rapid diagnoses of tuberculous meningitis.

Key Words: Tuberculous meningitis, cerebrospinal fluid, polymerase chain reaction

Introduction

Tuberculosis is still a widespread infection at the end of the twentieth century. Although the disease is more common in its pulmonary form, tuberculosis of the central nervous system arouses greater concern due to the severe sequeale and mortality. The complications can be prevented if antituberculous treatment is started early enough. At the present time, however diagnosis of tuberculous meningitis with conventional methods is considerably delayed. The growth of mycobacteria in the culture medium is slow, and the detection of acid fast bacilli the cerebrospinal fluid (CSF) has a low sensitivity. Thus, other reliable methods for the rapid detection of Myocobacterium tuberculosis are needed (1).

Analysis of mycobacterial DNA in clinical samples by PCR is a fast and sensitive technique (2, 3). Various methodologies have been tried centering on amplification of different DNA regions in order to find those that would yield the best results. Some investigators performed amplification of genecoding myocobacterial antigens, such as 65 kD protein, MPB 64 protein and antigen b (4). Still others amplified some repetitive sequences (5, 6). Another group detected Mycobacterium tuberculosis by amplifying ribosomal RNA (7). The sensitivity of PCR is determined to be 51-85% and specificity, 53-98%. False negative and false positive results are reported to be high (16% and 6-21%, respectively) (12, 13). Therefore, new studies are needed that will establish the most suitable PCR methods for routine use in the diagnosis of tuberculous meningitis. This study was planned to investigate the sensitivity and specificity of PCR in the diagnosis of tuberculous meningitis by using specific primers for the *IS 6110* region to detect myocobacterial DNA in cerebrospinal fluid samples.

Materials and Methods

Thirty-five patients hospitalized with clinical findings of meningitis between January 1993 and September 1994 were included in the study. The control group was comprised of 12 children with febrile convulsion or other neurological diseases from whom CSF samples were obtained by lumbar puncture. Among the 35 patients with meningitis, the criteria for diagnosis of tuberculous meningitis were clinical manifestations, CSF findings, culture on Löwenstein-Jensen medium, acid-fast stain, chest X-ray, tuberculin skin testing, family history of tuberculosis, erythrocyte sedimentation rate (ESR), computed tomography (CT) and pathology (Table 1).

Table 1. Clinical and Laboratory Data of Patients with PCR Positive Results for Mycobacterium Tuberculosis in CSF.

Patient No	Age	Sex	Clinical Diagnosis	Culture (CSF)	Acid-fast Stain (CSF)	Family History	Chest X-Ray	PPD	ESR (mm/h)	CT Scan	Pathology	PCR
1	8.5/12	F	Tbc meningitis	-	-	-	Hilar adenopathy	-	84	Hydrocephalus	ND	+
2	3	F	Tbc meningitis	+	+	-	N	+	94	Mild ventricular dilatation	ND	+
3	6	M	Tbc meningitis	-	-	+	N	+	120	ND	ND	+
4	9	M	Purulent meningitis	-	-	+	N	-	46	ND	ND	+
5	5	M	Chronic pulmonary infection, cerebral palsy, chronic suppurated otitis	-	-	?	Broncho pneumonia	+	76	ND	ND	+
6	6/12	F	Multiple brain abscesses	-	-	-	N	-	5	Calcified nodular lesion	Tbc in nodular lesion in brain	+
7	4.5/12	M	Tbc meningitis, millary Tbc.	-	-	+	Miliary Tbc.	-	12	N	Tbc of lymph node	+
8	8/12	M	Tbc meningitis	-	-	?	N	-	34	ND	ND	+
9	7	m	Tbc meningitis	+	-	-	N	+	44	Normal	ND	+
10	1.5	M	Tbc meningitis	-	-	?	Hilar adenopathy	+	36	ND	ND	+
11	1.5	F	Tbc meningitis	+	-	-	N	-	33	ND	ND	+
12	4	F	Tbc meningitis	-	-	-	N	+	66	Hydrocephalus	ND	+
13	2	M	Tbc meningitis	-	-	+	Hilar adenopathy	+	12	Hydrocephalus	ND	+

Abbreviations:

CSF: Cerebrospinal fluid
N: Normal
ND: not done
PCR: Polymerase chain reaction
Tbc: Tuberculosis
ESR: Erythrocyte sedimentation rate
CT: Computed tomography

All CSF samples were delivered at 4°C to the laboratory of the Department of Medical Biology, Dokuz Eylül University Hospital, on the same day. The PCR study was carried out blinded as to the type of meningitis. Cerebrospinal fluid samples were centrifuged at 1500 g for 10 minutes. Cell pellets were suspended in 500 µl Tris EDTA buffer (TE) and transferred to boiling water bath, where they stayed 10 minutes in order to lyse mycobacteria. Afterwards, 3 M Sodium acetate was added and centrifuged at 2500 g for minutes. Following ethanol precipitation, DNA was dried and resuspended in 50 µl of TE buffer. A 10 microliter aliquot of the suspension was used for PCR. Each samples form a patient was amplified at least twice. Controls with Mycobacterium tuberculosis DNA obtained from clinical isolate of Mycobacterium tuberculosis strain (positive control), as well as those without DNA and those with human leucocyte DNA (negative control) were amplified for each reaction (8).

PCR Procedure: Primers specific for the *IS 6110* region, originally described by Eisenach et al. (9), were used. Amplification was performed on a total volume of 50 µl with the following materials and after taking standard precautinos to prevent contamination:

1 X PCR reaction buffer [50 mM Tris HCl (pH: 9.0), 20 mM $(NH_4)_2SO_2$, 1% TritonX 100]

1.5 mM $MgCl_2$

20µM each deoxynucleotide triphosphates (dATP, dCTP, dGTP and dTTP)

20 pM each primers (Integrated DNA Technologies, IA, USA)

2.5 U Taq DNA polymerase (Panozyme, Panorama Research Inc, CA, USA)

10µl DNA template

Amplification was performed in a PCR thermal cycler (Perkin-Elmer Cetus, Norwalk, CT). After an initial 5-minute denaturation step at 94°C, thirty five cycles were completed by repeating cycles of 1 minute at 94°C, 2 minutes at 68°C, and 2 minutes at 72°C. A final extension was done for 7 minutes at 72°C. For analysis of amplification products, 10 µl of reaction mixtures was electrophoresed on ethidium bromide-containing 2% agarose gels. Ultraviolet transillumination was used for visualization.

Results

Clinical and laboratory data for 13 patients with PCR positive results for Mycobacterium tuberculosis are in Table 1. Ten of the patients were considered to have tuberculous meningitis based on clinical and lobarotary findings. Their follow-up with antituberculous treatment later supported the diagnosis. One patient (Case 4) had clinical and laboratory findings not suggestive of tuberculous meningitis. This patient improved with nonspecific meningitis treatment, and the control CSF sample was PCR negative. Case 5 had motor mental retardation (cerebral palsy), chronic lung infection and chronic supported otitis media. He did not respond to nonspecific antibiotherapy and developed mastoiditis. After mastoidectomy and tympanoplasty, his CSF was found positive for Mycobacterium tuberculosis by PCR. Pulmonary tuberculous, otitis and tuberculous meningitis were highly probable, so antituberculous treatment was started.

Case 6, who was also PCR positive, was admitted with a diagnosis of brain abscess. Nonspecific antibotherapy failed; the calcified nodule in the brain was surgically draind, and pathologicl examination suggested tuberculosis.

Among the 22 patients with menigtis who had PCR negative results for Mycobacterium tuberculosis, 9 had purulent meningitis and 13 had aseptic meningtis. When 12 patinets with tuberculous meningitis and PCR positive results were evaluated for other diagnostic criteria, 3(25%) were culture positve, 1 (8%) had positive acid-fast staining of CSF, 4 (35%) had positive family histories for tuberculosis, 5 (41%) had chest X-rays suggesting tuberculoss, 7 (58%) were PPD positive and 9 (75%) had high erythroycte sedimentation rates. Cranial tomography was suggestive of tuberoulous meningitis in 5, normal in 2 patients, and was not performed in the remaning 5 patients.

Discussion

Tuberculous meningitis is still an important health problem in developing countries. Even though antibacterial chemotherapy is available, delays in diagnosis increase morbidty and mortaliy, and about 20-25% of patients have neurologic sequelae (10, 11). Conventional diagnostic procuders yield quite varable results (Table 2), even when the symptoms and CSF cytology and biochemistry are strongly suggestive of tuberculous meningitis. Acid-fast stain may be negative in a considerable percentage of specimens, and it takes weeks to obtain the results of mycobactrial culture. In contrast, PCR is considered a rapid diagnostic method in tuberculous meningitis.

Table 2. Percentage of Positive Findings in Various Diagnostic Criteria for Tuberculous Meningitis

	Positive in (%)
History of contact with a tuberculosis case	20-30
CSF acid-fast stain	10-90
CSF culture	10-90
High erythrocyte sedimentation rate	80
Tuberculin skin test	50-95
Chest X-ray	50-90
CT (Hydrocephalus)	75-100

In this PCR study, the primers specifically recognize the repetitive sequences in the Mycobacterium tuberculosis complex, and higher concentrations of thes sequences in the bacteria increased the sensitivity (e.g., 2-3 copies per bacterium in Mycobacterium bovis, 10-16 copies per bacterium in Mycobacterium tuberculosis). Sensitivity studies with this primer show that it would be possible to identify even 1 fg (10 fg corresponds to two bacteria) of DNA in 30-35 cycles with no false positive resuts (6, 9). Here the sensitivity was shown to be about 1-10 bacilli in a sample. Among the 35 patients who were PCR positive, only one did not have tuberculous meningitis. His repeat CSF analysis was PCR negative.

As a result, the high sensitivity (100%) and specificity (95.7%) of PCR when compared with other bacteriological methods suggest that it is a rapid and reliable method for the diagnosis of tuberculous meningitis.

References

1. Joklik WK, Willet HP, Amos DB, Wilfert CM. Zinsser Microbiology 20th ed. London: Prentice-Hall International Inc, 497-525, 1992.
2. Ross BC, Dwyer B. Rapid simple method for typing isolates of Mycobacterium tuberculosis by using the polymerase chain reaction. J Clin Microbiol. 31: 329-34, 1993.
3. Noel AB, Anar C, Chureau C, Nguyen S, Pierre C, Bartoli M, Bonte R, Pialoux G, Gicquel B, Garrigue G. Diagnosis of tuberculosis by DNA amplification in clinical practice evaluation. Lancet. 338: 364-6, 1991.
4. Brisson-Noel A, Gicquel B. Rapid diagnosis of tuberculosis by amplification of mycobacterial DNA in clinical samples. Lncet. 11: 1069-71, 1989.
5. Folgueria L, Delgado R, Palenque E, Noriega AR. Polymerase chain reaction for rapid diagnosis of tuberculous meningitis in AIDS patients. Neurolgy, 44: 1336-8, 1994.
6. Kocagöz T, Yılmaz E, Özkara Ş, Kocagöz S, Hayran M, Sachedeva M, Chambers HF. Detection of Mycobacterium tuburculosis in sputum samples by polymerase chain reaction using a simplified procedure. J Clin Micrbiol. 31: 1435-8, 8, 1993.
7. Böddinghaus B, Rogall T, Flohr T, Blocker H, Bottger EC. Detection and identification of mycobacteria by amplification of rRNA. J Clin Microbiol 28: 1751-9, 1990.
8. Kolk AHJ, Schuitemia ARJ, Kuijper S, van Leeuwen J, Hermans PWM, van Embden JDA, Hartskeeri RA. detection of Mycobacterium tuberculosis in clinical samples by using polymerase chain reaction and a nonradioactive detection system. J Clin Microbiol. 30: 2567-75, 1992.
9. Eisanach KD, Cau MD, Bates JH, Crawford JT. Polymerase Chain Reaction amplification of a repetitive DNA sequence specific for Mycobacterium tuberculosis. J Infent Dis. 161: 977-81, 1990.
10. Molavi A, LeFrock JL. Tuberculous meningitis. Med Clin North Am. 69: 315-31, 1985.
11. Synder RD. Bacterial infections of the nervous system. In: Swaiman KF, ed. Pediatric Neurology Principles and Practice. St. Lous, NY: The C.V. Mosby Company, 466-8, 1989.
12. Shankar P, Manjunanth N, Mohan KK, Prasad K, Behri Shriniwas M, Ahuja GK. Rapid diagnosis of tuberculous meningitis by polymerase chain raeaction. Lancet. 337: 5-7, 1991.
13. Pan YX. The diagnosis of tuberculous meningitis using polymerase chain reaction. Child 16: 77-8, 1993.