SHORT REPORT

Application of the polymerase chain reaction to monitor *Mycobacterium tuberculosis* DNA in the CSF of patients with tuberculous meningitis after antibiotic treatment

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Abstract

The polymerase chain reaction (PCR) was used to detect *Mycobacterium tuberculosis* DNA in 29 CSF specimens from seven patients with tuberculous meningitis after the start of antituberculous chemotherapy. Ten of the 13 CSF specimens taken from these patients with an initial treatment of three weeks were positive for the PCR study. By contrast, only one of the other 16 CSF specimens taken from patients treated for more than three weeks was positive. This study shows that *M tuberculosis* DNA can exist in the CSF of a patient with tuberculous meningitis for three weeks after treatment and that PCR can still be a sensitive method to detect *M tuberculosis* DNA in the CSF after the start of treatment in patients with tuberculous meningitis.

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Early accurate diagnosis of tuberculous meningitis remains a challenge with conventional methods. A method of polymerase chain reaction (PCR) based on DNA amplification has the sensitivity to detect small numbers of mycobacteria present in clinical specimens[1 2] and has proved to be the most sensitive method for an accurate diagnosis of patients with tuberculous meningitis.[3–8] Although studies have successfully applied PCR for the diagnosis of patients with tuberculous meningitis, using this method to monitor *Mycobacterium tuberculosis* DNA in the CSF of patients with tuberculous meningitis after the start of antituberculous treatment has not yet been established.

Table 1 CSF studies and clinical outcome of patients with tuberculous meningitis

Patient No	Age (y)/ sex	Time (days)†	CSF study: WCC*	Protein (mg/dl)	Glucose (mg/dl)	PCR	Culture	Clinical outcome	Duration of follow up
1	76/F	0	20	60	125/255‡	+	−	Complete recovery	8 m
		6	36	84	118/306	+	−		
		25	12	56	124/212	−	−		
		83	2	48	96/156	−	−		
2	20/M	0	80	186	24	+	−	1 Complete recovery except mild slowness of movement	1 y
		4	101	279	33	+	−		
		10	54	164	48	+	−	2 Received VP shunt§	
		20	16	86	59	−	−		
		28	18	80	56	−	−		
		40	2	97	64	−	−		
		74	6	58	70	−	−		
3	16/F	0	386	312	20	+	−	Mild weakness in right limbs¶	18 m
		13	450	306	38	+	−		
		20	280	214	34	+	−		
		28	120	98	46	−	−		
		50	50	86	78	−	−		
		112	12	54	68	−	−		
4	24/M	0	100	86	25	+	+	1 Vegetative state	1 y
		7	93	109	42	+	−	2 Decorticate posture	
		13	119	166	46	+	−	3 Received VP shunt	
		28	80	138	76	+	−		
		40	7	83	77	−	−		
		106	16	60	80	−	−		
5	8/F	0	146	104	32	+	−	Complete recovery	10 m
		14	66	74	66	+	−		
		28	12	48	60	−	−		
6	35/M	0	28	76	40	+	−	1 Complete recovery	2 y
		5	40	70	33	+	−	2 Seizure attacks and well treated with antepileptic drugs	
		16	24	56	62	−	−		
		36	6	32	54	−	−		
		92	2	36	78	−	−		
7	22/M	0	245	324	24	+	+	1 Mild weakness in right extremities	2 y
		7	278	272	54	+	−		
		18	102	86	40	−	−	2 Focal dystonia in right foot	
		26	82	56	58	−	−	3 Received VP shunt	
		74	12	48	78	−	−		

*WCC = white cell count/mm³; †time CSF samples were taken from patients with tuberculous meningitis after the start of treatment. Day 0 means the CSF samples were taken from patients before treatment; ‡CSF glucose/blood glucose; §acute hydrocephalus after a ventriculoperitoneal shunt operation; ¶focal neurological deficit caused by cerebral infarction complicated by vasculitis.

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Table 2 Results of applying the polymerase chain reaction to monitor M tuberculosis DNA in the CSF of patients with tuberculous meningitis, after the start of treatment

Time of CSF sampling after treatment	PCR result		
	Positive	Negative	Total
<3 weeks	10 (78%)	3	13
3–6 weeks	1	8	9
>6 weeks	0	7	7
Total	11 (38%)	18	29

Materials and methods

In our previous report,[8] we prospectively applied PCR to detect *M tuberculosis* DNA in CSF specimens taken from 18 patients with clinically suspected tuberculous meningitis. Results showed that PCR was positive in 14. After the start of antituberculous treatment, we repeatedly sampled CSF specimens from these 14 patients with PCR positives at an irregular time to monitor the existence of *M tuberculosis* DNA. Four out of the 14 patients refused lumbar puncture again after the treatment, two were lost to follow up, and one died of sepsis. This study included seven patients with 29 CSF specimens (table 1).

Methods of extraction of DNA from CSF specimens, designation of primers, and procedures of PCR and Southern blot hybridisation were carried out as described in our previous publication.[8] A sample was regarded as positive when DNA with a molecular weight expected for the amplified product (240 base pairs) was seen on autoradiography. Each CSF specimen was tested at least twice in different batches with the same procedures to assure the reproducibility of the results.

Results

This study showed that PCR could detect *M tuberculosis* DNA in 11 of 29 CSF specimens taken from these seven patients after the start of treatment. Ten of the 11 PCR positive CSF specimens were taken from patients with an initial treatment of three weeks (13 CSF specimens, 78%; table 2). Another PCR positive CSF specimen was taken from one patient on the 28th day after treatment. Specimens taken from patients with treatment of more than six weeks were all negative.

Table 1 summarises the detailed data of CSF specimens and outcome. Mycobacterial cultures of the 29 CSF specimens were all negative despite the fact that two patients (4 and 7) had positive CSF cultures of their specimens before the start of treatment. None of the CSF samples was positive by direct smear (Ziehl-Neelsen's stain). Each patient had, however, received antituberculous chemotherapy consisting of at least a triple regimen of isoniazid, rifampicin, and pyrazinamide (or ethionamide). As well as the triple regimen, patients 6 and 7 had also received streptomycin. Outcomes of six patients were good after follow up for eight months to two years, four patients with complete resolution and two with residual mild neurological deficits. One was in a vegetative state.

Discussion

The PCR has been used to define novel diagnostic and epidemiological data for neurological diseases based on the fact that the method is rapid, accurate, and non-invasive.[9] For identification of mycobacterial DNA in CSF specimens, PCR has proved to be the most sensitive method for an accurate diagnosis of tuberculous meningitis.[3–8] Nevertheless, PCR assay has problems, especially when dealing with samples containing small amounts of mycobacterial bacilli. The study of Hance *et al* showed that when using hybridisation with a ^{32}P-labelled oligonucleotide, the sensitivity of the application of PCR for detection of *M tuberculosis* DNA in clinical specimens could be improved.[10 11] Some studies showed that a nested PCR protocol could also improve the sensitivity of detecting mycobacterial DNA in clinical specimens.[6 12 13] The study of Ostergaard *et al*, however, showed that Southern hybridisation on the nested PCR product did not give further PCR positive results.[14] Therefore, regarding sensitivity, the nested PCR is superior to Southern hybridisation for the detection of *M tuberculosis* DNA in CSF specimens, due to fact that nested PCR is simpler and less time consuming. Because the detection of mycobacterial DNA in our previous report was done by PCR after Southern hybridisation,[8] we therefore still used the same method in this study to have the advantage of a comparison between the sensitivity of PCR in detecting *M tuberculosis* DNA in the CSF of patients with tuberculous meningitis before and after antituberculous treatment.

After the start of treatment, mycobacterial cultures of CSF in patients with tuberculous meningitis rarely yield a positive result,[15] which was the same as found in our study. Yet our study showed that PCR could persistently detect *M tuberculosis* DNA in the CSF of patients with tuberculous meningitis within the initial three week treatment period. After treatment for more than three weeks, the PCR rarely yielded a positive result. Because treatment was effective in this study (as shown by radiology and the resolution of clinical symptoms), the persistent presence of *M tuberculosis* DNA in CSF may indicate that antituberculous treatment induces the release of mycobacterial DNA in CSF. This persistence of positive PCR results was also found in patients with neurosyphilis in whom *Treponema pallidum* DNA was detectable in the CSF several months after effective treatment[18] and patients with cerebral toxoplasmosis in whom *Toxoplasma gondii* in the blood could also be detected for up to 12 days after initiation of treatment.[19]

When compared with our previous report,[8] this study does not indicate a significant difference of PCR sensitivity for applying the method to detect *M tuberculosis* DNA in the

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CSF of patients with tuberculous meningitis before treatment (70% of sensitivity) and after an initial treatment of three weeks (78% of sensitivity). The sensitivity of applying PCR to detect *M tuberculosis* DNA in the CSF of patients with tuberculous meningitis having the initial treatment for three weeks is also similar to that of other studies.[3-8] Therefore, we advise that it is still valuable to apply PCR in patients with clinically suspected tuberculous meningitis who promptly receive a diagnostic antituberculous trial.

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