Quick Diagnosis of Human Brain Meningitis Using *Omp85* Gene Amplicon as a Genetic Marker

Sandip K. Dash · Minakshi Sharma ·
Shashi Khare · Ashok Kumar

Received: 18 January 2013 / Accepted: 1 February 2013 / Published online: 12 February 2013
© Association of Microbiologists of India 2013

Abstract The usual diagnosis of life-threatening human brain bacterial meningitis are expensive, time consuming or non-confirmatory. A quick PCR based diagnosis of meningitis in cerebrospinal fluids (CSF) using specific primers of virulent *Omp85* gene of *Neisseria meningitidis* can detect as low as 1.0 ng of genomic DNA (G-DNA) in 80 min for confirmation of bacterial meningitis caused by *N. meningitidis* infection. The 257 bp amplicon of *Omp85* gene does not show homology with other suspected pathogens in CSF and can be used as a specific genetic marker for diagnosis of the disease.

Keywords Genetic marker · Meningitis ·
N. meningitidis · *Omp85* gene

Meningitis causes inflammation of the meninges (outer membrane covering) in the brain and spinal cord of the patients [1]. Meningitis is usually detected by biochemical tests, microscopy, latex agglutination, PCR, microarray and biosensors [2–5]. Recently, blot microarray and SPR

biosensor were reported for detection of biological warfare agents [6, 7]. All these methods are time consuming, expensive and cumbersome. Boving et al. [8] reported an eight-plex PCR for simultaneous detection of *Neisseria meningitidis*, *S. pneumoniae*, *E. coli*, *S. aureus*, *L. monocytogenes*, *S. agalactiae*, herpes simplex virus and varicella-zoster virus which was time consuming. *Omp85* (outer membrane protein) gene which is highly conserved in all the strains of *N. meningitidis* and assists for effective insertion of lipids and integral proteins into the outer membrane [9]. *Omp85* gene can be used to develop genetic marker due to its virulence.

The patient 0.5 ml CSF samples (from National Centre for Disease Control) was centrifuged at $16,000\times g$ for 1 min to remove the CSF and the pellet was used in 25 μ l of PCR mix containing $1\times$ PCR buffer (1.5 mM $MgCl_2$, 0.01 M Tris–HCl, 0.05 M KCl, 0.01 % gelatin, pH 8.3), 0.4 mM dNTP mix (0.1 mM of each nucleotide), 0.4 μ M of each forward (5′-CGTTTCCCAAGCAACCTGT-3′) and reverse primers (5′-AAATTACGCCGTCGTATTC-3′) of *Omp85* gene of *N. meningitidis*, 0.75U of Taq polymerase and de-ionized water. The PCR was carried out in a MJ Mini thermal cycler at an initial denaturation: 95 °C; 5 min followed by 30 cycles of denaturation: 95 °C; 4 s, annealing: 54 °C; 5 s, extension: 72 °C; 1 s and final extension: 3 min; 72 °C. The amplicons were separated at 80 V in 1.5 % agarose gel electrophoresis and analyzed in UV light.

For quantification of bacterial G-DNA, the pellet obtained from CSF containing bacteria was suspended in 0.5 ml of TE (10 mM Tris and 1 mM EDTA) buffer, pH 8.0 and heated at 95 °C for 5 min for cell lysis. The heated solution was further centrifuged at $16,000\times g$ for 1 min to remove cell debris and the supernatant containing G-DNA was quantified by Nanodrop spectrophotometer. The sensitivity of the PCR method was performed using different concentrations of *N. meningitidis* G-DNA (0.5–5.0 ng) in

S. K. Dash · A. Kumar (✉)
CSIR-Institute of Genomics and Integrative Biology,
Mall Road, Delhi 110007, India
e-mail: ashokigib@rediffmail.com

M. Sharma
Department of Zoology, M. D. University, Rohtak 124001,
Haryana, India

S. Khare
National Centre for Disease Control, Sham Nath Marg,
Delhi 110054, India

Fig. 1 **a** Agarose gel electrophoresis (1.5 %) of PCR product in UV light, lane 1: 100 bp DNA ladder, lane 2: 257 bp PCR amplicon of *Omp85*. **b** Gene sequence of 257 bp *Omp85* gene using reverse primer; **c** 1.5 % Agarose gel electrophoresis of PCR product, lane 1:

257 bp *Omp85* gene marker, lane 2, 3, 4 and 5 are PCR product with 5, 3, 2 and 1 ng G-DNA, respectively. Lane 6: PCR product was not formed with 0.5 ng of G-DNA

Table 1 Diagnosis of suspected meningitis patients using present available methods and PCR with *Omp85* amplicon as genetic marker

Results	Microscopic	Enzyme test		Latex agglutination	Biochemical test		<i>Omp85</i> genetic marker (257 bp)
	Gram (–ve)	Oxidase	Catalase		Glucose	Sucrose	
Positive	04	02	01	04	05	04	03
Negative	26	28	29	26	25	26	27
False positive	01	00	00	01	02	01	00
False negative	00	01	02	00	00	00	00

Gram staining, oxidase and catalase test, latex agglutination and biochemical test were performed as reported earlier [2]

Control: Sample containing *N. meningitidis* in CSF

Fig. 2 Agarose gel electrophoresis (1.5 %) of suspected patients of bacterial meningitis in UV light. Lane C- control (*N.meningitidis* in CSF), lane 9, 21 and 28 single band (infected patients) corresponding to 257 bp *Omp85* genetic marker, lane 1–8, 10–20, 22–27 and 29–30 showed no bands (non infected)

25 µl of PCR mix as described above. The amplicons were detected in a 1.5 % agarose gel using UV light.

The PCR product 257 bp *Omp85* gene of *N. meningitidis* (Fig. 1a) was purified using GFX column and sequenced at The Centre for Genomics and Applications (TCGA), New Delhi (Fig. 1b) and *N. meningitidis* *Omp85* gene, partial cds was submitted to NCBI [Accession No.HQ712171]. The PCR method can detect as low as

1.0 ng of G-DNA of *N. meningitidis* directly from CSF of meningitis patients in 80 min (Fig. 1c). The diagnosis of suspected bacterial meningitis patients (30 samples) were carried out using present available methods (Table 1) as well as PCR using 257 bp *Omp85* gene amplicon (in 1.5 % agarose gel electrophoresis) as a genetic marker (Fig. 2). The data shows some false positive and negative results which are due to certain limitations of the test. For validation of the results, PCR was also carried out with 20 samples of *N. meningitidis* (MC58) and 06 samples each of *E. coli* (DH5α), *S. typhi* (CT 18), *S. pyogenes* (M 140) and *M. tuberculosis* (Smegmatis). Although, the biochemical tests showed false positive and false negative results with some of the samples but results with PCR using *Omp85* genetic markers of 257 bp was found specific only to *N. meningitidis*, not with other possible pathogens (Table 2). Therefore, PCR amplicon of *Omp85* gene can be used as a specific genetic marker for quick (80 min including electrophoresis) diagnosis of bacterial meningitis. Our method can save life of several patients during an outbreak of the bacterial meningitis disease.

Table 2 Testing the cross reactivity of *Omp85* gene amplicon (257 bp) as genetic marker with *N. meningitidis*, *E. coli*, *S. typhi*, *S. pyogenes* and *M. tuberculosis*

Samples	Total No.	Present methods of diagnosis [2]												<i>Omp85</i> genetic marker	
		Microscopic		Gram stain		Enzyme test		LA test		Biochemical test					
										Glucose		Sucrose			
		P	N	P	N	P	N	P	N	P	N	P	N	P	N
<i>N. meningitidis</i>	20	–	20	18	02	17	03	19	01	15	05	01	19	20	–
<i>E. coli</i>	06	–	06	01	05	05	01	06	–	04	02	01	05	–	06
<i>S. typhi</i>	06	–	06	06	–	01	05	–	06	04	02	–	06	–	06
<i>S. pyogenes</i>	06	06	–	01	05	02	04	06	–	–	06	01	05	–	06
<i>M. tuberculosis</i>	06	06	–	NA	–	NA	–	NA	–	NA	–	NA	–	–	06

P Positive, N Negative, NA Not applicable

Acknowledgments Financial support received under the DST project DST/TSG/ME/2008/37 is highly acknowledged. SKD thanks University Grant Commission, New Delhi for providing Senior Research Fellowship.

References

1. Dash SK, Sharma M, Kumar A (2012) Diagnostic techniques and treatment of meningitis. In: Houllis G, Karachalios M (eds) Meningitis: causes, diagnosis and treatment. Nova Science Publishers Inc, New York, pp 203–223
2. Negi SS, Grover SS, Rautela SS, Rawat DS, Gupta S, Khare S, Lal S, Rai A (2010) Direct detection and serogroup characterization of *Neisseria meningitidis* from outbreak of meningococcal meningitis in Delhi. Iran J Microbiol 2:73–79
3. Dash SK, Sharma M, Khare S, Kumar A (2012) *rmpM* gene as a genetic marker for human brain bacterial meningitis. Cell Mol Biol 58:24–27
4. Mahajan S, Sethi D, Seth S, Kumar A, Kumar P, Gupta KC (2009) Construction of oligonucleotide microarrays (biochips) via thio-ether linkage for the detection of bacterial meningitis. Bioconjug Chem 20:1703–1710
5. Patel MK, Solanki PR, Khare S, Gupta S, Malhotra BD, Kumar A (2010) Electrochemical DNA sensor for *Neisseria meningitidis* detection. Biosens Bioelectron 25:2586–2591
6. Vanlahmuaka, Thavachelvam K, Tuteja U, Sarika K, Nagendra S, Kumar S (2012) Reverse line blot macroarray for simultaneous detection and characterization of four biological warfare agents. Indian J Microbiol. doi:10.1007/s12088-012-0330-7
7. Ghosh N, Gupta G, Boopathi M, Pal V, Singh AK, Gopalan N, Goel AK (2012) Surface plasmon resonance biosensor for detection of *Bacillus anthracis*, the causative agent of anthrax from soil samples targeting protective antigen. Indian J Microbiol. doi: 10.1007/s12088-012-0334-3
8. Boving MK, Pedersen LN, Møller JK (2009) Eight-Plex PCR and liquid-array detection of bacterial and viral pathogens in cerebrospinal fluid from patients with suspected meningitis. J Clin Microbiol 47:908–913
9. Fitzpatrick DA, McInerney JO (2005) Evidence of positive Darwinian selection in *Omp85*, a highly conserved bacterial outer membrane protein essential for cell viability. J Mol Evol 60:268–273