Opc gene as a specific genetic marker for human bacterial meningitis

Ashok Kumar¹*, D. Asha¹, Simrita Singh², Shashi Khare²

1. Institute of Genomics and Integrative Biology, Mall Road, Delhi-110007, India. 2. National Centre for Disease Control, Sham Nath Marg, Delhi-110054, India.

*Corresponding Author: Ashok Kumar (ashokigib@rediffmail.com)

Received: 15 June 2011, Accepted: 11 September 2011, Published: 28 October 2011

ABSTRACT: The usual diagnosis of bacterial meningitis is expensive, time consuming and non-confirmatory. We report a rapid PCR based diagnosis of bacterial meningitis using specific primers of virulence opc gene of Neisseria meningitidis. Our method is an improvement of the existing methods and the overall analysis completes in 80 min which is the least time reported so far for the confirmation of the disease. Amplicon of 304 bp of opc gene does not show homology with other organisms and can be used as a specific genetic marker for detection of N. meningitidis causing bacterial meningitis in human.

KEYWORDS: bacterial meningitis, DNA marker, Neisseria meningitidis, opc gene

Neisseria meningitidis is a major cause of life-threatening meningitis in human. N. meningitidis (meningococci) is a gram-negative diplococci human pathogen. Bacterial meningitis is a serious infection of the fluid in spinal cord and fluid surrounding the brain. Meningitis can also be caused by virus which is aseptic, less severe and resolves without specific treatment within 1-2 weeks while bacterial meningitis can be very serious and results in disability or death if not treated promptly. Infection in the meninges (outer membrane covering of the brain and spinal cord) cause dangerous inflammation which increases intracranial pressure and results brain damage, stroke, seizures, or even death. Meningococci colonize the nasopharyngeal mucosa [1] of healthy individuals and can cross epithelial and endothelial cell barriers and enter the blood stream. The commonly used diagnostic tests for meningitis are CSF culture, X-ray and CT scanning, immunological test, biochemical test and PCR [2-4]. The advent of PCR technology has positive impact on biomedical research by providing the most sensitive and rapid method to detect microbial pathogens in clinical samples [5-7].

N. meningitidis can be detected by PCR using universal primers of 16S-23S rRNA genes [8, 9] but this method was not sufficiently sensitive and specific for detection directly from clinical specimens. Filippis et al (2005) describes rapid detection of N. meningitidis in CSF by PCR of nspA gene which takes 2-3 h for confirmation of the disease and they observed about 48% negative samples, after culture and latex agglutination, were found positive by nspA PCR [10]. Fraiser et al (2009) used single multiplex PCR based assay for direct simultaneous characterization of six N. meningitidis serogroups using serogroup X-specific primers in Africa [11]. This method also takes more than 2h and is not very specific. Multiplex quantitative PCR was also developed for detection of lower respiratory tract infection and meningitis caused by Streptococcus pneumoniae, Haemophilus influenzae and Neisseria meningitidis which take more time and have certain limitations [12]. All reported methods of PCR are either time consuming or have some limitations such as two step PCR reactions [13,14], nested PCR [15] or PCR in combination with other methods. Diggle et al (2003) developed dual-labeled end-point fluorescence PCR (DEF-PCR) with a reporter dye carboxyfluorescein at 5’ end and a quencher dye carboxytetramethylrhodamine at 3’ end for detection of meningococcal meningitis [16]. Fluoresence based detection is time consuming and expensive method.

N. meningitidis strains express opacity-associated proteins opa and opc. opa protein is closely related to N. gonorrhoeae, while opc is unique to meningococci. In a later stage of the infection, the outer membrane proteins opa and opc are thought to strengthen the adherence and promote entry of the bacteria into the host cell. opc has a β-barrel architecture with 10 strands and five loops protruding into the extracellular space [9, 17]. opc interact with the serum glycoprotein vitronectin and attached to the integrons that are present on the apical surfaces of the endothelial cells, whereas on epithelial cells, it interacts with cell surface proteoglycans [18-20]. Recently, microarray based diagnosis of meningitis [21] and DNA based biosensor for detection of N. meningitidis causing bac-
terial meningitis was also initiated in our lab [22-24] and work on these are in progress. The aim of the present study was to develop a rapid PCR using opc gene (opacity associated protein) as a genetic marker during outbreak of the disease and save life of people in early stage of infection.

MATERIALS AND METHODS

Bacterial culture and chemicals

The bacterial cultures from patients CSF samples were obtained from National Centre for Disease Control, Delhi. Brain Heart Infusion broth and Chocolate agar were purchased from Hi Media. Tris (Trizma base) and EDTA were obtained from Sigma-Aldrich. dNTP, *Taq* polymerase, PCR reaction mixture, RNase, MgCl₂ and agarose were obtained from Bangalore Genei. Primers were synthesized from The Centre of Genome Application (TCGA), India. All other chemicals were analytical reagent grade and purchased from local market.

Genomic DNA isolation

The bacteria from patient CSF was cultured in Brain Heart Infusion (BHI) broth at 37 °C for 12-18 h and the genomic DNA was isolated from chloroform and phenol method [24]. The purity (A₂₆₀/A₂₈₀) and concentration of genomic DNA was determined by Nanodrop spectrophotometer.

PCR amplification

The sequence of opc gene was retrieved from NCBI. The specific opc gene based forward primer (5'-CACAAAGCTGCCAAACACAT-3') and reverse primer (5'-GAAGCGTATGCTTGGTGGTT-3') were synthesized and PCR was carried out with the following steps: Initial heating at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 5 sec, annealing at 55 °C for 10 sec, extension at 72 °C for 2 sec and final extension for 4 min after the last cycle. The PCR was performed with 25 µl of reaction mixture containing 1x assay buffer, 0.4 mM of dNTP (0.1 mM of each dATP, dGTP, dCTP and dTTP), 0.4 µM each of forward and reverse primers, 50 ng genomic DNA, 0.75 units *Taq* polymerase and Milli Q water. The PCR product was purified using GFX column and electrophoresis of the PCR product was carried out in 1.5% agarose gel. The PCR product was sequenced using ABI 3130XL genetic analyzer and homology of the sequence was confirmed by BLAST.

PCR with patient samples

The suspected patient samples (20) were obtained from National Centre for Disease Control (NCDC). The patient CSF samples (0.5 ml) were centrifuged at 6000xg for 2 min and supernatant was discarded to remove CSF. The pellet (bacteria) was washed and suspended in 25 µl of PCR reaction mixture. PCR was carried out as described above.

RESULT AND DISCUSSION

The amplicon of 304bp was viewed in 1.5% agarose gel in UV light. The DNA sequence of amplicon with BLAST shows similarity which confirmed that the right fragment within opc gene had been correctly amplified (Figure1). opc is a unique virulence gene of meningococci and therefore, PCR based detection of *N. meningitidis* has higher specificity. It is a rapid method (80 min including electrophoresis) for the amplification of DNA and has been demonstrated to be effective in early diagnosis of meningitis caused by *N. meningitidis* even in the case where the patient has received prior antibiotic therapy. Primers used in this experiment are specific and amplify only the specific region under specific conditions. Since the opc is virulence gene and does not have homology with other organisms, it can be used as genetic marker (304bp) for the detection of *Neisseria meningitidis* causing bacterial meningitis disease. The diagnosis of suspected bacterial meningitis patients (20 samples) were carried out using different available methods (Table 1) as well as PCR using opc gene primers (Figure2). Sample No.13, 15 and 18 were confirmed negative by PCR whereas it was showed positive by other methods. The other tests have some limitations and also sometimes give false results. Sample No. 3-12, 14, 16,17,19 and 20 were positive and their band correspond to 304 bp of DNA marker. The results suggest that opc gene can be used as genetic marker for detection of bacterial meningitis.
Table 1. Diagnosis of suspected bacterial meningitis patients using different available methods and PCR using opc gene as specific genetic marker.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Microscopic (Gram +/−)</th>
<th>Immunological (Latex agglutination)</th>
<th>Enzymatic (Oxidase/Catalase)</th>
<th>Biochemical Test Glucose/Maltose/Sucrose/Lactose</th>
<th>PCR with marker Opc gene (304 bp)</th>
<th>Normal/Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control</td>
<td>Nil</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>2 Control</td>
<td>Nil</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>3.</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>12.</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>13.</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>14.</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>15.</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>16.</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>17.</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>18.</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>N</td>
</tr>
<tr>
<td>19.</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>M</td>
</tr>
<tr>
<td>20.</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>M</td>
</tr>
</tbody>
</table>

Normal healthy individuals (control) = N
N. meningitidis infected patients = M

ACKNOWLEDGEMENTS

The authors thank to Prof. Rajesh Gokhale, Director IGIB for their encouragement and scientific suggestions. This work was supported by Department of Science and Technology, Govt. of India and CSIR, New Delhi.

REFERENCES


