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Use of universal polymerase chain reaction assay and endonuclease digestion for rapid detection of *Neisseria meningitidis*

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Neisseria meningitidis is a major cause of bacterial meningitis worldwide, especially in children. Early diagnosis and empiric antibiotic treatment have led to a reduction in morbidity and mortality. The value of the traditional gold standard diagnostic tests, blood culture and cerebrospinal fluid (CSF) culture, has been adversely affected by preadmission use of parenteral penicillin and fewer lumbar punctures. We report a case of *N. meningitidis* in a 19-year-old male who was admitted after suffering from progressive severe headache, and intermittent high fever for 2 days. Gram stain and culture of CSF, and culture of throat swab were negative. However, *N. meningitidis* was detected by polymerase chain reaction (PCR) with a universal primer set and endonuclease digestion. This report indicated that the PCR method may be an alternative method for the rapid diagnosis of meningococcal meningitis.

Key words: DNA primers, DNA restriction enzymes, meningitis, *Neisseria meningitidis*, polymerase chain reaction

Meningitis is an infection within the subarachnoid space and an important health problem worldwide [1]. The classic clinical symptoms of meningitis are fever, headache, and nuchal rigidity [2]. Nausea, vomiting, and photophobia are also common complaints [2]. The organisms most commonly responsible for community-acquired bacterial meningitis are *Streptococcus pneumoniae*, *Neisseria meningitidis*, group B streptococci and *Listeria monocytogenes* [3]. *N. meningitidis*, a pathogen exclusive to humans, remains the leading worldwide cause of acute meningitis and fatal sepsis in healthy individuals [4]. *N. meningitidis* is the second most common cause of bacterial meningitis in North America, with an incidence of 0.6 per 100,000 population reported in the United States in 1995 [3]. In Taiwan, a recent study about bacterial meningitis demonstrated the microbiologic data identified for causative pathogens from 48 cases were as follows: *Klebsiella pneumoniae* (33%), *S. pneumoniae* (28%), *L. monocytogenes* (11%), *N. meningitidis* (6%), *Staphylococcus aureus* (6%), streptococci (6%), and *Pseudomonas aeruginosa* (6%) [5].

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Meningitis is a medical emergency. Rapid identification of bacterial pathogens in cases of suspected meningitis is important to the initiation of appropriate treatment and prophylaxis. This case suggests that a universal polymerase chain reaction (PCR) with endonuclease digestion method may be an alternative method for rapidly detecting a pathogen from cerebrospinal specimens.

Case Report

A 19-year-old male soldier, a heavy smoker, suffered from productive cough and sore throat for 2 weeks. He visited local medical clinics and was treated with medication but without response. Intermittent high fever and headache developed 2 days before this admission. He was admitted due to progressive severe headache, vomiting and conscious disturbance.

Physical and neurologic examination on admission showed stupor, neck rigidity and positive Bruzinski sign. Babinski sign was absent. Mild petechial rash was located on lower extremities.

Routine blood count revealed leukocytosis with predominant neutrophils (white blood cell count 17,600 cells/μL, neutrophils 89.2%, lymphocytes 4.9%). Erythrocyte sedimentation rate was elevated to

65 mm/h and C-reactive protein was increased. Blood chemistry variables, including sodium, potassium, aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen, creatine, fasting sugar, thyroid-stimulating hormone and free thyroxine were all within normal limits. Lumbar puncture showed high opening pressure (470 mm H_2O). Cerebrospinal fluid (CSF) sample was turbid in appearance and showed neutrophilic pleocytosis (white blood cell count 14,130 cells/μL, neutrophils 98%, lymphocytes 2%, red blood cell count 140/μL), with protein 318 mg/dL and glucose 15 mg/dL. Rapid test for *N. meningitidis* antigen and Gram stain were both negative. Cultures from CSF, throat swab and blood were all negative for aerobic and anaerobic bacteria. Brain computed tomography showed diffuse brain swelling whereas other findings were negative.

For detection of bacterial pathogens and confirmation of the diagnosis, PCR with universal primer and endonuclease digestion for the CSF specimen was performed as described previously [6]. Briefly, 500 μL of CSF was centrifuged at 13,000 × *g* for 5 min. The pellet was resuspended in 180 μL of sterile distilled water, and the DNA was purified with the QIAamp Tissue Kit (QIAGEN Inc., Chatsworth, CA, USA) according to the procedures provided by the manufacturer. A reaction mixture containing approximately 50 ng of template DNA in a total volume of 50 μL PCR mixture was prepared. The primers for PCR reaction were: U1, 5'-CCAGCAGCCGCGGTAATACG-3'; and U2, 5'-ATCGG(C/T)TACCTTGTTACGACTTC-3'. After a 10-min denaturation at 94°C, the reaction mixture was run through 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and extension for 2 min at 72°C, followed by an incubation for 10 min at 72°C. A reagent control consisted of all PCR components except for the template DNA. Five μL of each PCR product was digested with *Hae*III in appropriate restriction enzyme buffer in a total volume of 20 μL. After incubation for 2 h at 37°C, the digested DNA and previous PCR product were electrophoresed on a 6% polyacrylamide gel as shown in Fig. 1.

The resulting digestion patterns were identical to that previously described for *N. meningitidis* ATCC 10377 (Fig. 1) [6], indicating that the most likely pathogen was *N. meningitidis*. The diagnosis was also compatible with his clinical signs and symptoms.

During hospitalization, acute meningitis was well controlled under treatment with penicillin G 4 million units intravenously every 4 hours. After 3 weeks of treatment, he had recovered completely without any neurologic sequellae.

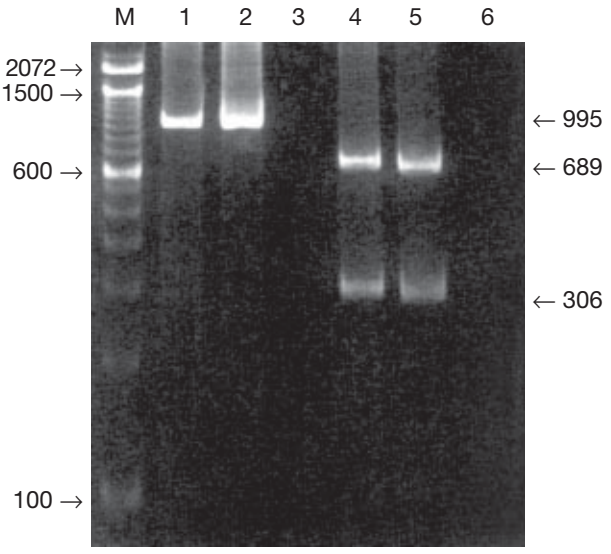

Fig. 1. Restriction digestion patterns of the universal polymerase chain reaction (PCR) products. Lanes 1 and 2, the PCR products (995 base pairs) of the cerebrospinal fluid (CSF) specimen and *Neiserria meningitidis* ATCC 10377 amplified with the universal primers, respectively; lanes 4 and 5, the patterns of *Hae*III-digested PCR products (689 and 306 base pairs) of the CSF specimen and *N. meningitidis* ATCC 10377, respectively; lanes 3 and 6, negative controls. Lane M contained molecular size standards (base pairs). The sizes of the molecular size standards are marked on the left of the gel.

Discussion

In this case, the patient was immediately isolated after meningococcal meningitis was suspected. All persons who had had close contact with the patient received chemoprophylaxis with a 2-day regimen of rifampin (600 mg every 12 h for 2 days). Isolation and early antibiotic treatment was given as soon as possible after admission. The results of empirical antibiotic treatment were satisfactory and the final diagnosis was made by universal PCR assay and endonuclease digestion when all cultures were negative for *N. meningitidis*.

N. meningitidis is a Gram-negative diplococcus with a polysaccharide capsule [7]. The capsule is an important virulence factor specifically acting as an attribute responsible for bloodstream survival and dissemination [2,7-9]. Factors predisposing to meningococcal colonization include institutional crowding, exposure to tobacco, and a recent viral infection. Overall mortality of meningococcal disease is between 5 and 10%. This has reduced recently, probably in part due to more

complete notification, earlier recognition and improved management [7,10]. The early diagnosis and treatment of bacterial meningitis is important, especially for *N. meningitidis* which is transmitted by direct contact via nasal or oral secretions or through inhalation of large droplet nuclei [4]. The infection is characteristically fulminant with rapid clinical deterioration in a few hours [11].

Bacterial cultures, Gram stain and latex particle agglutination tests are useful for making the diagnosis of meningitis. However, the value of blood culture and CSF culture, the traditional gold standard diagnostic tests, has been adversely affected by preadmission parenteral penicillin and fewer lumbar punctures [9,12, 13]. Molecular diagnostic tests have been developed for detecting DNA from bacteria in CSF and blood recently [6,13-17]. The sensitivity and speed of the PCR assay indicated that it could be used as a routine diagnostic test for meningococcal meningitis, enabling a diagnosis to be made within 2 hours of receipt of the specimen [14]. According to a survey in England and Wales of 103 cases with a clinical diagnosis of meningococcal meningitis by the Meningococcal Reference Unit of the Public Health Laboratory Service over a 5-month period in 1997, 92 were positive (89%) by PCR assay but only 46 (44%) were positive by traditional tests [18]. Investigators in the United Kingdom have routinely used PCR for the diagnosis of meningococcal disease since late 1996. PCR achieved a 56% increase in laboratory-confirmed cases of meningococcal disease when compared with culture [19,20].

Prior studies using the universal PCR method demonstrated the ability to detect 16S rDNA amplicons at the level of 10 colony-forming units (CFU) of Gram-negative bacteria [6]. This would be adequate for detecting most Gram-negative pathogens (including *N. meningitidis*) in CSF specimens, since 85% of CSF samples with bacterial infection contained more than 10^3 CFU of bacteria/mL [21]. Studies also demonstrated that the sensitivity of the universal PCR for detection and identification of bacteria directly from CSF specimens was 92.3% [6]. These results indicated the ability of universal PCR with restriction enzyme analysis to detect and identify bacterial pathogens in clinical specimens. Another study found that the assay also could be used effectively for testing all CSF samples from patients assessed in an emergency department for bacterial meningitis when there is pleocytosis but no sutainable organisms, and was particularly helpful in patients who had received antibiotics before the lumbar puncture was done [3]. In this study, we did not perform the sequence analysis for the amplicon and we could not rule out the possibility of infection by other bacteria closely related to *N. meningitidis* or the DNA level; however, we diagnosed the case to be meningococcal meningitis according to the symptoms and signs along with the positive result of PCR with restriction enzyme analysis.

In conclusion, meningococcal meningitis is a fulminant condition, with high mortality within a few hours. The management of patients with meningococcal meningitis and the public health implications of this disease require that the diagnosis be rapid and accurate. Cerebrospinal fluid with an absence of pleocytosis, normal chemistry, and a negative Gram stain is often presumed to exclude the diagnosis of bacterial meningitis and frequently contributes to difficulty in deciding whether to hospitalize a patient with possible meningitis [22]. PCR provides a more rapid and sensitive method than the conventional methods for detecting Gram-negative pathogens. It is regarded as the gold standard for the diagnosis of meningococcal disease. The assay is likely to be particularly helpful in patients who have received antibiotics before lumbar puncture was done. PCR is likely to see increasingly widespread use in the diagnosis of meningococcal disease in the future.

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References

1. World Health Report. Geneva: World Health Organization; 2002.
2. Memish ZA, Alrajhi AA. Meningococcal disease. Saudi Med J 2002;23:259-64.
3. Schuchat A, Robinson K, Wenger JD, Harrison LH, Farley M, Reingold AL, et al. Bacterial meningitis in the United States in 1995. N Engl J Med 1997;337:970-6.
4. Tzeng YL, Stephens DS. Epidemiology and pathogenesis of *Neisseria meningitidis*. Microbes Infect 2000;2:687-700.
5. Fang CT, Chang SC, Hsueh PR, Chen YC, Sau WY, Luh KT. Microbiologic features of adult community-acquired bacterial meningitis in Taiwan. J Formos Med Assoc 2000;99:300-4.
6. Lu JJ, Perng CL, Lee SY, Wan CC. Use of PCR with universal primers and restriction endonuclease digestions for detection and identification of common bacterial pathogens in cerebrospinal fluid. J Clin Microbiol 2000;38:2076-80.

7. Cartwright KA. Epidemiology of meningococcal disease. Hosp Med 2002;63:264-7.
8. Booy R, Kroll JS. Bacterial meningitis and meningococcal infection. Curr Opin Pediatr 1998;10:13-8.
9. Cartwright KA. Early management of meningococcal disease. Infect Dis Clin North Am 1999;13:661-84.
10. Jódar L, Feavers IM, Salisbury D, Granoff DM. Development of vaccines against meningococcal disease. Lancet 2002;359: 1499-508.
11. Hodgetts TJ, Brett A, Castle N. The early management of meningococcal disease. J Accid Emerg Med 1998;15:72-6.
12. Hackett SJ, Thomson AP, Hart CA. Cytokines, chemokines and other effector molecules involved in meningococcal disease. J Med Microbiol 2001;50:847-59.
13. Carrol ED, Thomson AP, Shears P, Gray SJ, Kaczmarski EB, Hart CA. Performance characteristics of the polymerase chain reaction assay to confirm clinical meningococcal disease. Arch Dis Child 2000;83:271-3.
14. Richardson DC, Louie L, Louie M, Simor AE. Evaluation of a rapid PCR assay for diagnosis of meningococcal meningitis. J Clin Microbiol 2003;41:3851-3.
15. Enright MC, Knox K, Griffiths D, Crook DW, Spratt BG. Molecular typing of bacteria directly from cerebrospinal fluid. Eur J Clin Microbiol Infect Dis 2000;19:627-30.
16. Corless CE, Guiver M, Borrow R, Edward-Jones V, Fox AJ, Kaczmarski EB. Simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicemia using real-time PCR. J Clin Microbiol 2001;39:1553-8.
17. Vogel U, Morelli G, Zurth K, Claus H, Kriener E, Achtman M, et al. Necessity of molecular techniques to distinguish between *Neisseria meningitidis* strains isolated from patients with meningococcal disease and from their healthy contacts. J Clin Microbiol 1998;36:2465-70.
18. Ragunathan L, Ramsay M, Borrow R, Guiver M, Gray S, Kaczmarski EB. Clinical features, laboratory findings and management of meningococcal meningitis in England and Wales: Report of 1997 survey. J Infect 2000;40:74-9.
19. Guiver M, Borrow R, Marsh J, Gray SJ, Kaczmarski EB, Howells D, et al. Evaluation of the applied biosystems automated Taqman polymerase chain reaction system for the detection meningococcal DNA. FEMS Immunol Med Microbiol 2000;28:173-9.
20. Kaczmarski EB, Ragunathan PL, Marsh J, Gray SJ, Guiver M. Creating a national service for the diagnosis of meningococcal disease by polymerase chain reaction. Commun Dis Public Health 1998;1:54-6.
21. La Scolea L, Dryja D. Quantitation of bacteria in cerebrospinal fluid and blood of children with meningitis and its diagnostic significance. J Clin Microbiol 1984;19:187-90.
22. Sivakmaran M. Meningococcal meningitis revised: normocellular CSF. Clin Pediatr 1997;36:351-5.