ORIGINAL REPORT

Detection of Acute Childhood Meningitis by PCR, Culture and Agglutination Tests in Tabriz, Iran

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Abstract- Meningitis is one of the hazardous and life threatening infections and is associated with mortality and morbidity. The aim of this study was to determine etiological agents of childhood bacterial meningitis. The culture, Gram staining, agglutination and PCR assays were used to examine CSF specimens from 277 patients with presumed bacterial meningitis for the occurrence of 4 most common infectious agents consist of *N. meningitis*, *H. influnsae*, *S. pneumoniae* and *S. agalactiae* between 2008 and 2009 at different wards of the Children Hospital of Tabriz. The mean age of patients was 35±2 (Mean±SEM) month, (minimum 11 days maximum14 years), of all cases 59.6% male and 40.4% female. Overall the diagnosis was confirmed with a CSF culture in 11/277 (3.97%), by agglutination test in 14/277 (5.05%). The isolated bacteria included *S. pneumoniae* 5 cases, *H. influnsae* 2 cases, *N. meningitis* 3 cases and *P. aeroginusae* 1 case. A positive PCR assay allowed us to diagnose bacterial meningitis in 19 patients (6.8%). In the present study, we found PCR to be a useful and sensitive method for the detection of bacterial DNA in the CSF samples from suspected meningitis patients. Furthermore, to maximize management of meningitis cases, a combination of culture and PCR is necessary.

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Keywords: Bacterial meningitis; Children; PCR

Introduction

Bacterial meningitis is the most common infection of the central nervous system. Bacterial meningitis is more common in developing countries and morbidity and mortality rates are even higher in this region (1). Bacterial meningitis is a grave and sometimes lethal infection affecting the central nervous system (2). Rapid diagnosis and treatment of meningitis are important, because stable neurological sequel such as hearing loss, mental retardation, seizures, and behavioral changes may occur in up to one-half of survivors. Antibiotic treatment is empirically initiated on the basis of clinical findings (3).

Traditional laboratory technique of culture for the detection of bacterial meningitis takes up to 36 h or more. Furthermore, it has been observed that antimicrobial therapy before to sample collection; decrease the sensitivity of culture assay to approximately 30% (2-4). The current standard for the identification of bacterial meningitis is microscopic examination and consequent culture of cerebrospinal fluid (CSF). However, this approach might have some disadvantages with regard to the desired rapidity and sensitivity (4).

Perfect therapy is guided by the results of culture, which may take 24-28 h to obtain; antibiotic susceptibility testing may need an additional 24 h (3-5). In recent years, PCR-based assays have become accessible to provide an early and accurate diagnosis of bacterial meningitis. Recent evidence suggests that some of these tests are aimed at specific pathogens of bacterial meningitis, such as *Neisseria meningitidis (N. meningitidis)*, *Streptococcus pneumoniae (S. pneumoniae)* and *Haemophilus influenzae (H influenzae),* whereas others use broad-range bacterial PCR.

The aim of this research was to determine etiological agents of childhood bacterial meningitis by three methods.

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Table 1. The results of culture, agglutination and PCR tests in 277 children.

	Culture	Agglutination	PCR
N. meningitides	3	4	5
H. influnsae	2	4	6
S. pneumoniae	5	6	8
Total	10	14	19

Materials and Methods

This research was carried out in a 250-bed pediatric university hospital, with highly specialized wards and which is unique in East Azerbaijan province of Iran. The study involved 277 patients with suspicious meningitis, between 2008 and 2009 at the different wards of the Tabriz Children Hospital. The culture, Gram staining, agglutination and PCR assays were used to prospectively test CSF specimens from 277 patients with presumed bacterial meningitis for the presence of 4 most common infectious agents included *N.meningitis*, *H. influnsae*, *S. pneumoniae* and *S. agalactiae*. All patients had meningitis as part of their differential diagnosis. Upon arrival at the laboratory, 0.2 to 0.4 ml of each CSF sample was removed under sterile and standard conditions and transported to microbiology laboratory of hospital. The CSF was used for standard bacterial culture, agglutination and Gram staining. The remaining sample stored at -70°C until further processing for DNA extraction and PCR. The CSF was centrifuged for 15 min at 1,500 g, the supernatant was used for agglutination test, and the sediment was re-suspended in the remaining liquid by vortexing. From this suspension, Gram stain was made and bacterial culture was inoculated. The bacterial culture was inoculated into 5% sheep blood and chocolate agar plates and then incubated in a CO_2 5% at 35°C for 48-72 h. DNA was extracted from CSF samples by a modification of the procedure described previously (4). The PCR was carried out as previously described method (4,6).

Results

A total of 277 children hospitalized with presumptive meningitis in Tabriz Children Hospital, included in the study. The mean age of patients was 35±2 (Mean±SEM) month, (minimum 11 days maximum14 years), of all cases 59.6% were male and 40.4% female and the male to female ratio was 1.87. Only 99/277 (36%) of patients were less than 1 month, 66/277 (24%) were between 1-5 years of age and 110/277 (40%) between 6-14 years old. The incidence of bacterial meningitis was high in age 1-5 years of age. The range of CSF WBC was 0-3500, mean 160±537, and 24/277 (8.7%) had a CSF WBC count above 500 cells. Seventy-five of 277 patients (27.3%) had been treated with antibiotics before admission. Only 13 sample of CSF were turbid, 13 bloody, 9 semi-turbid and others had clearness appearance. Twenty-eight of 277 (10%) CSF had abnormality according to protein and glucose measurement. Overall the diagnosis was confirmed with a CSF culture in 11/277 (3.97%), by agglutination in 14/277 (5.05%). Blood culture was obtained from 258 patients and 5 cases (1.93%) were positive. Gram positive bacteria was isolated from 5/11 (45.45%) of culture positive cases and Gram negative in 6/11(54.54%). The isolated bacteria included *S. pneumoniae* 5 cases, *H. influnsae* 2 cases, *N. meningitis* 3 cases and *P. aeroginusae* 1 case. A positive PCR assay enabled us to diagnose bacterial meningitis in 19 patients (6.85%) and no false positive cases were observed. In addition, PCR identified 4 cases of clinically suspected meningitis diseases that were not reported and confirmed by various combinations of conventional laboratory diagnostic techniques. In this research, no etiological agent was identified by PCR in one suspected case with culture confirmed *Pseudomonas aeroginusae* meningitis. Table 1 presents the results of culture, agglutination and PCR tests in 277 children. There was no cross-reactivity with any of the other bacterial extracts tested, and no cross-reaction with human genomic DNA. A comparison of results of three methods reveals that PCR was the most sensitive method.

Discussion

A differential diagnosis of meningitis requires the differentiation of viral, tuberculosis and acute bacterial meningitis (1). Bacterial meningitis is an emergency condition and affecting the central nervous system. Thus, the information needed for specific antimicrobial therapy is unavailable for the first 48 h. This has been noted, information in Iran which shows a growing inconsistency between the numbers of clinically

suspected and culture-confirmed cases of bacterial meningitis and septicemia (5). The widening gap between notified and culture confirmed cases have led to the expansion of non-culture based PCR assays to detect microbial DNA directly from clinical specimens, thereby enhancing the confirmation of bacterial disease. In 1996, a national PCR based service was established at the Public Health Laboratory Service Meningococcal Reference Unit in Manchester (8), which resulted in a 35% increase of laboratory confirmed cases in the first year (6). With the advent of PCR methods, examination of CSF has become the single most key laboratory test for the diagnosis of many infectious diseases of central nervous system. PCR amplification of bacterial DNA is a potential method for rapid detection of bacterial meningitis (7). This technique has been used to identify such organisms as penicillin-resistant *S. pneumoniae*, *N. meningitidis*, group B streptococci, *Listeria monocytogenes*, and *Mycobacterium tuberculosis* with use of species-specific primers (3).

In the present study, we found PCR to be a helpful technique for the detection of bacterial DNA in the CSF samples from suspected meningitis. The PCR showed a sensitivity of 100% compared to the usual culture method. Thus, the sensitivity of the PCR was better to that of culture and requires a short period of time compared to culture. However, determining the antibiotic susceptibility of isolates is a trouble in the PCR technique. The PCR assay is presently used in many countries of Europe and in Australia and New Zealand for the diagnosis of meningococcal disease but has not been introduced for routine diagnosis or surveillance in the world (9). In PCR method according to high sensitivity, the low number of microorganism was detected. Moreover, many patients with meningitis have contraindications to lumbar puncture procedure, reducing the accessibility of CSF for conventional culture-based diagnosis, Gram staining, or antigen testing. Molecular diagnoses have the would-be to get better the sensitivity and specificity of the clinical diagnosis of meningitis from blood samples (6,8-9).

Etiology of bacterial meningitis varies in the world (9-10). Prior to the introduction of *H. influnsae* conjugate vaccination, more than 95% of invasive *H. influenzae* disease was caused by *H. influnsae*. In countries where vaccination has been implemented, the incidence of invasive *H. influnsae* disease has decreased by upwards of 90%. Furthermore, the reemergence of invasive *H. influnsae* disease in a well-vaccinated population has been noted (8), emphasizing the necessity for permanent post vaccine surveillance. A recent study by Marandi *et al*. showed that microbiology laboratories in Iran are not qualified for identification of *H. influnsae* (5). In another major study, by *et al.*, ten hundred and sixty two CSF specimens collected from a group of children who were under investigation for suspected bacterial meningitis at university affiliated and other city hospitals (10). Definitive diagnosis of bacterial meningitis was recognized in 67 patients. Despite of clinical diagnosis of 31 patients with bacterial meningitis they were not proved by laboratory tests. The etiological agents of 67 proved cases of bacterial meningitis were *H. influnsae* (24 cases), pneumococci (23 cases), meningococci (17 cases), *Salmonella* (one case), *Pseudomonas* (one case) and *Citrobacter* (one case) respectively. Alborzi *et al.* reported that that incidence of bacterial meningitis especially caused by *H. influnsae* among the children in Shiraz, Iran is less common than other parts of the world prior to vaccination (10). In Tehran, Nakhjavani *et al.* noted that 100 CSF samples studied by culture and PCR. The PCR technique could detect *H. influnsae* in all 5 cultures positive and in 2 of 295 culture negative cases, showing sensitivity, specificity, and an accuracy index of 100%, 99% and 99%, respectively (7).

N. meningitidis is a cause of dangerous meningitis and sepsis in adults and children (11) and now is the major cause of bacterial meningitis in England and Wales (12). However, surveillance data based on standard laboratory evidence may considerably underestimate the exact burden of the disease. For example, in the United Kingdom, it has been estimated that as many as 44% of clinically suspected cases may be culture negative (9). In Turkish children, *N. meningitis* was the most common of cause and the authors highlighted the emergence of serogroup W-135 diseases in Turkey, and conclude that meningococcal vaccine in this region must provided reliable defense (13). In other ways, in India a low incidence of meningococcal meningitis has been reported (14). However in Iran, the researchers supported that the incidence rate of meningococcal meningitis is low, about 1.22 and 10.6 in 100000 in non-military and military population, in that order (11).

In this research, the most common isolated agents were *S. pneumoniae.* It have been the chief cause of bacterial meningitis in many countries where *H. influnsae* disease has been eliminated by vaccination and is the second most frequently reported cause of septic meningitis. Pneumococci were responsible for 19.1% of meningitis and or encephalitis cases and for 9.6% of laboratory reports of bacteremia in England and Wales

(8). An example of this is the study carried out by Bahador in Iran, of 114 bacterial cases, 102 had a negative CSF smear and culture (89.5%), 12 cases had a positive CSF smear and culture (10.5%) with 6 cases of *H. influenza* b, 3 cases of pneumococci and 3 cases of meningococcus (15). An interesting study by Yousefi Mashouf *et al*. illustrates this point, he reported that out of 582 children suspected to meningitis, 146 patients (25.1%) had positive bacterial culture that 58.9% was Gram positive cocci and 41.1% was Gram negative bacteria and the most common isolates were *S. pneumoniae* (16).

In this study we did not isolated *S. agalactiae* from childhood meningitis. Among United States of America neonates, group B streptococci are the most commonly identified organisms, implicated in roughly 50% of all cases of bacterial meningitis, and *E coli* accounts for another 20% (17). Studies from underdeveloped countries propose that Gram-negative bacilli, specifically *Klebsiella* and *E.coli*, may be more common than group B streptococci (18). Tiskumara *et al*. noted that 75% of cases of late-onset meningitis were due to gram-negative bacilli (19). In a review of studies from Asia, Africa, and Latin America, Zaidi *et al.* reported that the most common organisms were *Klebsiella* species, *E coli,* and *Staphylococcus aureus* (20). Aletayeb *et al.* from Ahvaz, south of Iran, based on CSF culture, 31 infants identified as having bacterial meningitis, and 11 (35.5%) of these cases were caused by *K. pneumoniae,* 9 (29%) by *Enterobacter* spp., 3 (9.6%) by *Escherichia coli*, 3 (9.6%) by *Enterococcus* spp., 2 (6.4%) by *Acinetobacter,* and one case each (3.2%) was caused by *S. aureus, P. aeruginosa* and non typeable *H. influenzae* (21).

In this research, the rate of bacterial meningitis that was detected by PCR, agglutination and culture were 6.85%, 5.05 and 3.97%, respectively. This can be due to the inappropriate state of hospitals microbiology laboratory, also the preceding antibiotic therapy of the patients had taken before admission (27.3% of the cases) and meningitis due to fastidious or slow-growing microorganisms. However, the above information mentions that smear and culture cannot be a method for early and exact diagnosis of meningitis. Perhaps the most serious disadvantage of the culture is low sensitivity. Bacterial concentration in the CSF has a thoughtful effect on the results of microscopy and culture. The use of broad-range bacterial PCR has a huge advantage in that it also detects microorganisms that are found less frequently or even unknown causative agents of bacterial origin. The PCR method is a rapid, sensitive, and specific diagnostic test for bacterial meningitis. Unfortunately, in Iran, most clinical laboratories have not yet put into practice this technique because its use is hampered by many problems. Furthermore, to maximize management of meningitis cases to reduce the morbidity, mortality, and complications of invasive infection, a combination of culture and PCR is essential for the accurate detection of infection.

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References

1. Balganesh M, Lalitha MK, Nathaniel R.Rapid diagnosis of acute pyogenic meningitis by a combined PCR dot-blot assay.Mol Cell Probes 2000;14(2):61-9.
2. Corless CE, Guiver M, Borrow R, Edwards-Jones V, Fox AJ, Kaczmarski EB.Simultaneous detection of Neisseria meningitidis, Haemophilusinfluenzae, and Streptococcus pneumoniae in suspected cases of meningitis and septicemia using real-time PCR.J ClinMicrobiol2001;39(4):1553-8.
3. Saravolatz LD, Manzor O, VanderVelde N, Pawlak J, Belian B.Broad-range bacterial polymerase chain reaction for early detection of bacterial meningitis.Clin Infect Dis 2003;36(1):40-5.
4. Schuurman T, de Boer RF, Kooistra-Smid AM, van Zwet AA.Prospective study of use of PCR amplification and sequencing of 16S ribosomal DNA from cerebrospinal fluid for diagnosis of bacterial meningitis in a clinical setting. J ClinMicrobiol2004;42(2):734-40.
5. Marandi FR, Rahbar M, Sabourian R, Saremi M.Evaluation of Iranian microbiology laboratories for identification of etiologic agents of bacterial meningitidis. Survey results of an external quality assessment scheme (EQAS) programme. J Pak Med Assoc2010;60(1):48-51.
6. 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(3):271-3.
7. Nakhjavani FA, BonakdarHashemi F, Kalani MT, Kazemi B, Nouri K, Azadi N, HaghiAshtiani M. Detection of Haemophilusinflunsae type b in CSF of suspected children with meningitis by PCR. Med J Islamic Rep Iran 2005;19(2):181-183.

8. Davison KL, Ramsay ME.The epidemiology of acute meningitis in children in England and Wales. Arch Dis Child 2003;88(8):662-4.
9. Pollard AJ, Probe G, Trombley C, Castell A, Whitehead S, Bigham JM, Champagne S, Isaac-Renton J, Tan R, Guiver M, Borrow R, Speert DP, Thomas E.Evaluation of a diagnostic polymerase chain reaction assay for Neisseria meningitidis in North America and field experience during an outbreak. Arch Pathol Lab Med 2002;126(10):1209-15.
10. Alborzi A, Vahedi F, Karimi A, Azmoodeh M, Labaf R, Kadivar MR, Aboodi B. Bacterial etiology of acute childhood meningitis in Shiraz Iran: incidence clinical pictures, complication and antibiotic sensitivity pattern.Iran J Infect Dis Trop Med 2002;7(18):26-31.
11. Mehrabi-Tavana A, Ataee RA. Meningococcal meningitis control in Iran: five year comparative study 2000-2004. J Med Sci 2009;9(1):51-4.
12. Tsolia MN, Theodoridou M, Tzanakaki G, Kalabalikis P, Urani E, Mostrou G, Pangalis A, Zafiropoulou A, Kassiou C, Kafetzis DA, Blackwell CC, Kremastinou J, Karpathios TE.The evolving epidemiology of invasive meningococcal disease: a two-year prospective, population-based study in children in the area of Athens. FEMS Immunol Med Microbiol2003;36(1-2):87-94.
13. Ceyhan M, Yildirim I, Balmer P, Borrow R, Dikici B, Turgut M, Kurt N, Aydogan A, Ecevit C, Anlar Y, Gulumser O, Tanir G, Salman N, Gurler N, Hatipoglu N, et al. A prospective study of etiology of childhood acute bacterial meningitis, Turkey.Emerg Infect Dis 2008;14(7):1089-96.
14. Grimwood K, Anderson P, Anderson V, Tan L, Nolan T.Twelve year outcomes following bacterial meningitis: further evidence for persisting effects. Arch Dis Child 2000;83(2):111-6.
15. Bahador MO, Amini M, Bahador MA. Common cause and cerebrospinal fluid changes of acute bacterial meningitis. Iran J Pathol 2009;4(2):75-9.
16. YousefiMashouf R, Hashemi SH, Bijarchi M. Bacterial agents of meningitis in children and detection of their antibiotic resistance patterns in Hamadan, Western Iran. Pak J Bio Sci 2006;9(7):1293-8.
17. Klinger G, Chin CN, Beyene J, Perlman M.Predicting the outcome of neonatal bacterial meningitis. Pediatrics 2000;106(3):477-82.
18. Ghotaslou R, Ghorashi Z, Nahaei MR.Klebsiellapneumoniae in neonatal sepsis: a 3-year-study in the pediatric hospital of Tabriz, Iran.Jpn J Infect Dis. 2007;60(2-3):126-8.
19. Tiskumara R, Fakharee SH, Liu CQ, Nuntnarumit P, Lui KM, Hammoud M, Lee JK, Chow CB, Shenoi A, Halliday R, Isaacs D; Asia-Pacific Neonatal Infections Study.Neonatal infections in Asia.Arch Dis Child Fetal Neonatal Ed 2009;94(2):F144-8.
20. Zaidi AK, Thaver D, Ali SA, Khan TA.Pathogens associated with sepsis in newborns and young infants in developing countries.Pediatr Infect Dis J 2009;28(1 Suppl):S10-8.
21. Aletayeb MH, Ahmad FS, Masood D.Eleven-year study of causes of neonatal bacterial meningitis in Ahvaz, Iran.PediatrInt2010;52(3):463-6.