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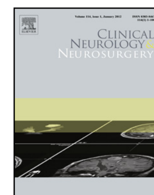

Detection of enteroviruses in pediatric patients with aseptic meningitis

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ABSTRACT

Aseptic meningitis is an acute viral infection of the central nervous system that occurs most frequently in infants and young children. This study was conducted on 100 pediatric patients with ages range from 1.5 months to 6 years. Cerebrospinal fluid (CSF) specimens were obtained with criteria of aseptic CNS infections as documented by pleocytosis, negative Gram stain and negative bacterial culture. Clinical and CSF findings of the affected children were analyzed and CSF specimens were submitted to viral culture and polymerase chain reaction (PCR) techniques to determine the enteroviral etiology. Fifty six percent patients had positive PCR results for the enteroviral genome, compared with 20% by virus culture. We can conclude that PCR is a rapid, reliable and sensitive diagnostic tool for the detection of enteroviral infections. A positive EV-PCR result may affect clinical decision making and may significantly alter the medical care offered to infected patients.

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1. Introduction

Aseptic meningitis is an acute viral infection of the central nervous system that occurs most frequently in infants and young children [1]. Enteroviruses were the most frequent cause of the aseptic meningitis and infant febrile syndromes [2]. With the control of poliovirus and mumps virus infections in the developed countries, the nonpolio enteroviruses (EVs) are recognized as the principal causes of aseptic meningitis. Nonpolio enteroviruses, include echoviruses and coxsackie A and B viruses [3]. Diagnosis of the enteroviral etiology is particularly difficult in children because of the frequently indistinguishable clinical and the cerebrospinal fluid (CSF) findings of aseptic and bacterial meningitis and because of the absence of a rapid assay for EVs [4]. During summer outbreaks of meningitis, children are frequently infected with EVs and in this age group, clinical features can overlap with those of bacterial origin [1,4]. To distinguish between viral and bacterial origin of meningitis, hospitalization, empiric prescription of antimicrobial agents, and use of diagnostic testing are common.

Aseptic meningitis is often reported to be characterized by a mononuclear cell predominance in the CSF, whereas bacterial meningitis is characterized by a polymorphonuclear (PMN) cell predominance. In contrast, other studies suggest that PMNs can be the most prevalent cell in early aseptic meningitis followed by a shift to mononuclear cells within 24 h. These contradictory reports may lead to uncertainty in the diagnosis and treatment of meningitis. The PMN predominance is not limited to the first 24 h of illness so a PMN predominance does not discriminate between aseptic and bacterial meningitis [5]. Several studies noted that CSF lactate may be a better single indicator for predicting bacterial meningitis. However, the CSF lactate level is likely to be affected by prior administration of antimicrobials [6].

Laboratory diagnosis of EV infection relies on virus isolation in cell cultures that provide the gold standard for direct diagnosis. However, this technique is limited by its relatively low sensitivity (65–75%) [7] as well as the poor growth of some EV serotypes [8,9]

The rapid availability of results may improve patient's care [10] and there can be substantial cost savings and avoidance of unnecessary treatment of aseptic meningitis with antibiotics [11]. Thus, the technique of nucleic acid amplification using EV-specific reverse transcriptase polymerase chain reaction (EV-PT-PCR) can provide prompt results. In comparison to viral culture, EV-PCR is more accurate with a sensitivity and a specificity of virtually 100% [9,10,12].

Abbreviations: CPE, cytopathic changes; EVs, enteroviruses; RT-PCR, reverse transcriptase polymerase chain reaction.

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Table 1
Clinical characteristics of aseptic meningitis in studied cases.

Clinical characteristics	No.	%
Age group		
<2 years	48	48
2–6 years	20	20
>6 years	32	32
Sex		
Male	56	56
Female	44	44
Signs and symptoms		
Fever	100	100
Convulsions	64	
Altered level of consciousness	56	56
Manifestation of ↑ ICT	32	32
Signs of meningeal irritation	60	60
Cranial nerve affection	12	12
Motor affection	8	8
Extra CNS manifestation	56	56

Also, PCR offers the potential benefits of rapidity and cross reactivity with most, or all, EV serotypes and can be applicable for the rapid diagnosis of EV infections [13,14]. In this study, we aimed to find a reliable method for diagnosis of aseptic meningitis due to EVs and to compare between the most common 2 methods for EV diagnosis (cell culture and PCR).

2. Subjects and methods

2.1. Subjects

Subjects of the present work were selected from patients attending Pediatrics Outpatients Clinics and Emergency Unit of Abou Elreesh Hospital, Cairo University in the period from June 2010 to May 2012, with acute symptoms and/or signs suggestive to be meningitis as fever, convulsions, altered consciousness, focal neurological manifestations or signs of meningeal irritation. They underwent lumber puncture and according to the result, patients with aseptic meningitis were recruited in the present study. Aseptic meningitis is defined as patients with pleocytosis in the CSF of at least 20 WBC/mm^3 with absence of any bacterial growth on culture of CSF. Subjects of the present study included 100 patients beyond the neonatal period. Their age ranged from 1.5 months to 12 years (48 infants and 52 children). They were 56 males and 44 females. Their clinical characteristics were represented in Table 1. The study was performed with the approval of the local ethics committee and carried out in compliance with the Helsinki Declaration (2008). Parents provided consent for the children's participation in this study.

2.2. Virus culture

Virus isolation and typing was performed from CSF using Vero cell line (African green monkey kidney) obtained from the Egyptian Organization for Biological Products and Vaccines (VACSERA). The cells were grown in Eagle's minimal essential medium (MEM, Flow Laboratories), supplemented by 10% fetal calf serum and antibiotics (Sigma). One hundred microliters of CSF were inoculated into two tubes of confluent monolayer cells under standard conditions. Cultures were regularly observed for cytopathic effects (CPE) up to two weeks after inoculation. Cultures demonstrating CPE were passaged once for confirmation. Viruses isolates were titered by end-point dilution and serotyped by antisera were kindly provided by Dr. M.A. Aly, Environmental Virology Lab., Water Pollution Research Dept., National Research Center, Egypt. These antisera were echovirus types 5, 9; coxsackievirus A3 and coxsackievirus B2, 4, and 6.

2.3. RNA extraction

RNA was extracted from 200 μl of CSF using pure viral RNA kit (Boeringer Mannheim, Germany) as instructed by the manufacturer.

2.4. Reverse transcription polymerase chain reaction (RT-PCR)

Two primers selected from the conserved regions of the 5′ end of the genome described by Thoren and Widell [15]. The downstream primer R1 (ATTGTC ACC ATA AGC AGC CA) and upstream primer R2 (CCT CCG GCC CCT GAA TGC GGC TAAT) were used. RT reaction wad performed as followed, 10 μl of RNA added to the mixture consisted of 20 units of ribonuclease inhibitor, 2 μl of 5× RT buffer, 10 mM of each dNTPs (dATP, dCTP, dGTP and dTTP), 5 units of murine moloney leukemia virus reverse transcriptase (MMLV-RT) and 10 pmol of primer R1 and 1 μl of diethylpyrocarbonate (DEPC)-treated water, the mixture was incubated for 90 min at 37 °C then 95 °C for 5 min for enzyme inactivation.

2.5. Polymerase chain reaction (PCR)

The following PCR reagents were added to RT mixture: 10 μl DEPC-treated water, 4 μl of 10× PCR buffer, 0.1% gelatin, 10 mM of each deoxynucleotides (dNTPs), 40 pmol of each primer R1, R2 and 2.5U of Taq polymerase. The cycling condition was 95 °C for 5 min then 35 cycles consisted of 95 °C for 1 min, 50 °C for 1 min and 72 °C for 1 min followed by finally extension at 72 °C for 9 min in thermocycler (Perkin-Elmer 4800). The PCR products were analyzed by 2% agarose gel electrophoresis with ethidium bromide stain. A reference sizing ladder of known fragment length of DNA was included with each electrophoresis run. A band of 154 base pairs (bp) was considered positive for enterovirus nucleic acid. Precautions against contamination were adhered to criteria specified by Kwok and Higuchi [16]. The different major steps in the PCR were performed in different rooms completely isolated from each other. Filter pipette tips were used in all stages.

2.6. Statistical analysis

Data were statistically described in terms of mean ± standard deviation (±SD), or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student t test for independent samples assuming unequal variance. Comparison of numerical variables between more than two groups was done using one way analysis of variance (ANOVA) test. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. A p-value of less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows (2006).

3. Results

Clinical characteristics of the studied cases were represented in Table 1.

3.1. Virus isolation in cell culture

20% CSF samples of the patients demonstrated cytopathic changes (CPE) characterized by nuclear pyknosis, rounding, refractivity, degeneration and eventually complete or partial detachment of infected cells from the glass (Table 2).

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