Detection of Enterovirus Genome in Cerebrospinal Fluid of Children with Aseptic Meningitis; the Relationship with Interferon – Gamma

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Aseptic meningitis is one of the most common inflammatory disorders of the meninges. The most common viruses causing aseptic meningitis are the enteroviruses, which account for more than half the cases. We analyzed the combined diagnostic utility of reverse transcriptase polymerase chain reaction (RT-PCR) and the level of interferon gamma (IFN-γ) in cerebrospinal fluid (CSF) specimens obtained from children with aseptic meningitis, for the diagnosis of enterovirus. Out of 33 CSF samples, 9 (27.2%) gave positive results for enterovirus RNA by RT-PCR and 15 (45.4%) gave detectable level of IFN-γ, ranging from 15.6 up to more than 1000 picogram/ml. The mean CSF IFN-γ concentration was 243.73 picogram/ml, which was significantly higher in CSF samples gave positive results for enterovirus genome by RT-PCR (384.3 picogram/ml) when compared with CSF samples gave negative results for enterovirus genome (56.6 picogram/ml), the results was statistically significant (p=0.0001).

Overall, data from previous reports and from the present study indicated that RT-PCR for enteroviral meningitis is an important tool in the diagnosis of children with aseptic meningitis and IFN-γ is produced in the CSF in response to viral infection, and about half of the patients with aseptic meningitis contain IFN-γ in the cerebrospinal fluids.

INTRODUCTION

Acute aseptic meningitis, a common and significant disease in children, it is one of the most common inflammatory disorders of the meninges. It occurs at all ages, although more common in children. The most common viruses causing aseptic meningitis are the enteroviruses, which account for more than half the cases.¹

Enteroviruses are small non enveloped RNA viruses of the picorna virus family. They are subdivided into the ECHO viruses, Coxsackie and Polio viruses, each with several serotypes. More than 50 serotypes have been linked with meningitis. They are spread by hand to mouth contact and to a lesser extent by respiratory and fecal routes.²

The production of proinflammatory cytokines; interleukin [IL]-1, tumor necrosis factor - alpha, IL-6, and IFN-γ stimulated by infection induce the migration of leucocytes into the infected area and that inflammation is terminated by anti-inflammatory cytokines;IL-10, IL-4, and transforming growth factor (TGF)-1 that are produced after elimination of the microorganisms.³

Data from previous reports indicated that IFN-γ is produced in the CSF in response to viral meningitis and that the majority (65 to 70%) of patients with aseptic meningitis contain IFN-γ in the cerebrospinal fluids.⁴

The polymerase chain reaction (PCR) is an important advance in diagnosis of infectious meningitis. In recent years, it has become available for more and more agents.⁵,⁶

Enterovirus PCR is a sensitive diagnostic modality for Enterovirus CNS disease in the pediatric population. Up to 50% of children less than 1 month of age with clinical illness compatible with Enterovirus infection but without CSF pleocytosis have been noted to have detectable Enterovirus genome in their CSF by RT-PCR.⁵,⁷

The availability of Enterovirus RT-PCR in cases of aseptic meningitis has had an impact on hospital costs and reduced unnecessary use of antimicrobials and imaging studies. Enterovirus PCR has also allowed the rapid identification of cases of Enterovirus CNS infection, allowing for initiation of specific antiviral therapy.⁵,⁷,⁸,⁹

AIM OF THE WORK

The present work aimed at:
1. Detection of Enterovirus genome in cerebrospinal fluid of children with aseptic meningitis.
2. Determined the concentrations of IFN-γ in the CSF derived from children with aseptic meningitis and evaluated its relationship with the presence of enterovirus genome.
MATERIALS AND METHODS

The material of the present study consisted of 33 CSF samples collected from pediatric patients admitted to Al Shatpy Pediatric Hospital-Alexandria, From February 2006 through November 2006. The age range was between 6 days and 7 years. Upon admission to the hospital, all patients had clinical signs and symptoms consistent with clinical diagnosis of meningitis and all had CSF examinations and cultures for bacteria. The diagnosis was based on pleocytosis (>10/ mm³), negative CSF bacteriologic studies (CSF cultures and gram-stained smears). The CSF specimens were analyzed for white blood cell count with differential cell count and for glucose and protein concentrations. The remaining CSF samples were stored at -70°C for later assays of IFN-γ and RT-PCR.

Gamma interferon Assay

The IFN-γ concentration in the CSF samples was determined using monoclonal antibody immunoassay kit (Biosource International, Camarillo, CA) which is a solid phase sandwich Enzyme Linked immunosorbent assay. A monoclonal antibody specific for Human IFN-γ has been coated onto the wells of the microtiter strips. Samples, including standards of known Human IFN-γ content, and unknown samples were pipetted into these wells, followed by addition of a biotinylated polyclonal second antibody.

During the first incubation, Human IFN-γ antigen binds simultaneously to the immobilized (capture) antibody on one site, and to the solution phase biotinylated antibody on a second site.

After removal of excess second antibody, Streptavidin- Peroxidase (enzyme) was added. This binds to the biotinylated antibody to complete the four – member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution was added, which was acted upon by the bound enzyme to produce color. The intensity of this color was directly proportional to the concentration of Human IFN-γ present in the original specimen.

The strips were read by ELISA reader (Behring) at a wavelength of 450 nm. The IFN-γ concentration of each sample was determined by extrapolating OD values to IFN-γ concentration using the standard curve (ranging from 0 to 1000 picogram/ml).

Reverse transcriptase PCR (RT-PCR) (3)

Nested PCR was performed for detection of the enterovirus in the CSF as described previously. (3) Primer sequences used in this study were F1 (CAAGCACTTCTGTTTCCCCGG), F2 (TCCTCCGGCCCCCTGAAATCCG), and R1 (ATTGTCCACCATAAGCAGCCA) for the enterovirus. RNA was extracted from 250 µL of whole CSF using an RNA extraction kit (QIA amp for RNA isolation -QUIAGEN). After RNA extraction, cDNA was synthesized (42°C, 30 minutes) from the resuspended RNA using 2.5 U of Moloney murine leukemia virus reverse transcriptase (Toyobo, Osaka, Japan) and reaction mixture containing 50 mM KCl, 10 mM TRIS-HCl (pH 8.3), 5 mM MgCl₂, 0.2 mM each dNTP, 1 µmol each of primer F1 and primer R1, and 20 U of RNase inhibitor (Toyobo). The cDNA product was amplified in 50 µL of reaction mixture containing 50 mM KCl, 10 mM TRIS-HCl (pH 8.3), 5 mM MgCl₂, 0.2 mM each dNTP, 0.2 µmol each of primer F1 and primer R1, and 1.25 U of Taq DNA polymerase (Perkin-Elmer, Norwalk, CT). Thirty cycles were performed in a thermal cycler (Perkin-Elmer) as follows: denaturation for 1 minute at 93°C, annealing for 1 minute at 55°C, and extension for 2 minutes at 72°C. The second PCR was performed as above, using the second primer pair (F2 and R1) and 2 µL of the first PCR product. The nested PCR product was run on a 2% agarose gel containing ethidium bromide and photographed under ultraviolet light. The bands of predicted size 439 bp.

RESULTS

Out of 33 CSF samples, 9 (27.2%) gave positive results for enterovirus RNA by RT-PCR. and 15 (45.4%) gave detectable level of IFN-γ, ranging from 15.6 up to more than 1000 picogram/ ml. The mean CSF IFN-γ concentration was 243.73 picogram/ml. The correlation between RT-PCR results and detectable IFN-γ concentration shown in table I.
Table I. Correlation between RT-PCR results and detectable IFN-γ concentration.

<table>
<thead>
<tr>
<th>Serial</th>
<th>RT-PCR results</th>
<th>IFN-γ level (picogram /ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative</td>
<td>15.6</td>
</tr>
<tr>
<td>2</td>
<td>Positive</td>
<td>1072</td>
</tr>
<tr>
<td>3</td>
<td>Negative</td>
<td>16.9</td>
</tr>
<tr>
<td>4</td>
<td>Negative</td>
<td>25.2</td>
</tr>
<tr>
<td>5</td>
<td>positive</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>Negative</td>
<td>18.8</td>
</tr>
<tr>
<td>7</td>
<td>Negative</td>
<td>20.5</td>
</tr>
<tr>
<td>8</td>
<td>Positive</td>
<td>56</td>
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<td>14</td>
<td>Positive</td>
<td>567</td>
</tr>
<tr>
<td>15</td>
<td>Negative</td>
<td>20.1</td>
</tr>
</tbody>
</table>

The mean CSF IFN-γ concentration was significantly higher in CSF samples gave positive results for enterovirus genome by RT-PCR (384.3 picogram /ml) when compared with CSF samples gave negative results for enterovirus genome (56.6 picogram /ml), the results was statistically significant (p=0.0001).

DISCUSSION

The term aseptic meningitis encompasses all types of inflammations of the brain meninges other than that caused by pus producing organisms. It is usually a benign illness. Etiology of aseptic meningitis is very wide and includes many infections-both viral and non viral, drugs, malignancy and systemic illness. The most common viruses causing aseptic meningitis are the enteroviruses, which account for more than half the cases.\(^{1,10}\)

The early diagnosis of enteroviral meningitis is important for early patient discharge and discontinuation of inappropriate antibiotic therapy. The rapid diagnosis of this disease may result in elimination of at least 72 hours of unnecessary antibiotic treatment and hospitalization.\(^{11,12}\)

PCR is an important advance in diagnosis of infectious meningitis.\(^{13,14}\) In recent years, it has become available for more and more agents.\(^1\) It is especially suitable for clinical specimens likely to contain a low viral burden.\(^{15}\)

In our study we evaluated the detection of enterovirus genome by RT-PCR in cerebrospinal fluid of children with aseptic meningitis and we correlate its relationship with IFN-γ. Out of 33 CSF samples, 9 (27.2%) gave positive results for enterovirus RNA by RT-PCR.

In a study done by Schlesinger Y et al (1994)\(^9\) to evaluate the performance characteristics and potential clinical utility of a PCR assay for enteroviral RNA in comparison to viral culture in infants under 3 months of age with meningitis, PCR was positive for enteroviral RNA on CSF specimens from 11 of 12 patients with definite or probable enteroviral meningitis, as well as on 6 of 13 with possible enteroviral meningitis, and they concluded that enterovirus PCR performed on CSF is a sensitive and specific method for the diagnosis of enteroviral meningitis. This method has the potential for improving the accuracy of diagnosis in young infants and for saving costs by allowing earlier diagnosis and discharge from the hospital when clinically appropriate.

Stellrecht KA et al (2002)\(^{16}\) studied the impact of enteroviral RT-PCR assay in the diagnosis and management of enteroviral meningitis in 1056 hospitalized patients. They concluded that RT-PCR for enteroviral meningitis is an important tool in the diagnosis of children with meningitis and...
nonspecific febrile illness which translated into shortened hospital stay and significant health care savings.

In another study done by Thoren A, and Widell A. (1994) for the diagnosis of enteroviral meningitis, 2-step 'semi-nested' enterovirus PCR was developed and applied to CSF and serum specimens from 27 consecutive patients with aseptic meningitis. CSF Enterovirus RNA was detected in CSF by PCR in 15 (55.5%) of the patients with aseptic meningitis, compared with 6 by virus culture. The correlation of a positive or negative PCR result in CSF and/or serum versus combined conventional virology (serology and isolation from 1-3 sites, i.e. CSF, stool and throat) was 78%. All negative controls were negative by PCR. PCR is a reliable and sensitive diagnostic tool for the detection of enteroviral infections. Both CSF and acute-phase serum should be considered for testing.

Interferon response during acute bacterial or viral meningitis has been extensively studied. A literature review indicated that 68% of 196 patients with aseptic meningitis contained interferon (alpha, beta, and gamma types) in their cerebrospinal fluids. It has been suggested that IFN-γ, acting as one of the endogenous pyrogens, is produced in substantial quantities in response to acute viral infection . Two studies have reported elevated levels of IFN-γ in the CSF from patients with aseptic meningitis. Abbott et al. (1985) have observed 7 patients with viral meningitis having concentrations of IFN-γ in the CSF ranging from 2 to 8 micron/ml. Minamishima et al. (1991) have reported high levels of IFN-γ in the CSF from 19 (70%) of 27 patients in acute phase of aseptic meningitis.

In the present study, 15 (45.4%) CSF samples gave detectable level of IFN-γ, ranging from 15.6 up to more than 1000 pg/ml.

Overall, data from previous reports and from our present study indicated that IFN-γ is produced in the CSF in response to viral meningitis and about half of the patients with aseptic meningitis contain IFN-γ in the CSF. The factors affecting the production of IFN-γ and the role of IFN-γ in the pathogenesis of acute viral meningitis remain to be defined and deserve further investigation.

REFERENCES


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