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Relative Frequency of Enteroviruses in Children With Aseptic Meningitis Referred to Aboozar Hospital in Ahvaz

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ABSTRACT

Background: Human Enteroviruses are members of the Picornaviridae family, they are nonenveloped,with icosahedral symmetry, positive RNA viruses and causes aseptic meningitis in both infants and adults.
Objectives: The aim of this study was to determine the relative frequency of Enteroviral meningitis among children referred to Ahvaz Aboozar hospital.
Patients and Methods: 57 Cerebrospinal fluid samples were collected from patients with aseptic meningitis and White Blood Cell (WBC) count of more than 5x106/mm3, Enterovirus RNA was extracted with Roch extraction kit and Enteroviral infection was detected by Revers Transcriptase polymerase chain reaction (RT-PCR) test.
Results: Enteroviral infection was detected in 34 cases out of 57 patient (59.6 %), 19 patients were younger than 1 year old (55.8 %), 8 cases were between 1 and 2 years (23.5 %) and 7 cases were older than 2 years (20.5 %). The relationship between gender and prevalence was not significant. Most cases were found in winter .
Conclusions: It was shown that human Enterovirus is the major cause of aseptic meningitis in Ahvaz, south west of Iran. The prevalence of Enterovirus infection was considerably high in this region. To decrease prevalence of Enteroviral infection, improved knowledge and personal hygiene is advised.

Implication for health policy/practice/research/medical education:
The data collected in this project assist paediatricians to manage infant gastroenteritis cases more effectively.

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1. Background

Aseptic meningitis (AM) is a mild disease localized in the central nervous system (CNS) and meningeal inflammation without common cause of meningitis such as bacteria and fungi in the cerebrospinal fluid is the symptom (1). Most patients show some indications such as fever, headache, stiff neck, lethargy, anorexia with or without vomiting, diarrahea, sore throat and rash. Viruses, specially Human Enteroviruses are major cause of aseptic meningitis. They are non- enveloped, positive single stranded RNA viruses and members of the Picornaviridae. Enteroviruses have been classified into 68 distinct serotypes and four species compromised of Human Enterovirus A (HEV A), Human Enterovirus B (HEV B), Human Enterovirus C (HEV C) and Human Enterovirus D (HEV D). Which mainly transmitted through

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fecal-oral rout and can cause sporadic cases, outbreaks and epidemics.

More than 10,000 cases of AM were reported to the Centers for Disease control and Prevention in USA. Children are more are more likely to become infected and develop illness from enteroviruses than adults (2-4). EVs also cause different kind of disease and infect possibly a billion or more people annually worldwide, causing significant short-term morbidity and economic impact. Most EV infections are inapparent, but EVs may cause a wide spectrum of clinical manifestations, including mild upper respiratory illness (common cold), febrile rash (hand, foot, and mouth disease and herpangina), aseptic meningitis (AM), encephalitis, acute flaccid paralysis, poliomyelitis, myocarditis, pericarditis, hemorrhagic conjunctivitis, insulin-dependent diabetes mellitus and neonatal sepsis-like disease. EVs are estimated to be the cause of 85 to 90 % of cases of viral meningitis for which an etiologic agent is identified. Children, neonates, and immunocompromised individuals are particularly susceptible to enterovirus infection of the central nervous system (5). Outbreaks of AM associated with a single serotype of enterovirus are often reported from different countries and represent a major public health problem (6, 7).

Research has shown that most of the infections happened in summer and fall in temperate area while infection can occur through the year specially in winter and fall in tropical area, where commonly have temperate weather in winter and fall (1, 4, 8).

2. Objectives

In this study we conducted a one year research to recognize the prevalence of Enteroviruses in patients with aseptic meningitis by Reverse Transcriptase Polymerase Chain Reaction test.

3. Patients and Methods

3.1. Patients

In this study 57 CSF samples were collected frompatients hospitalized in the pediatric infectious ward with the Signs of aseptic meningitis refered to Aboozar hospital in Ahvaz. Samples were collected with patients consent between May 2010 to May 2011. All patients had negative culture test for common bacterial pathogens. a patient is defined as an aseptic meningitis sufferer that have symptoms such as fever, Vomiting, headache ,signs of meningeal inflammation, CSF WBC count > 5×106/ mm3 and negative CSF culture (8)

3.2. Viral RNA Extraction

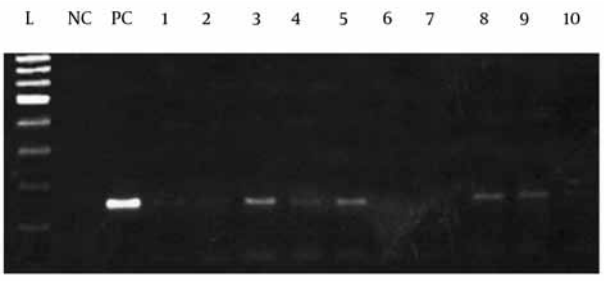
The viral RNA was extracted from CSF samples using the High Pure Viral Nucleic Acid kit (Roche company, USA) according to manufacturer's instructions.

3.3. Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Entero viral RNA was detected by using the RT-PCR kit (Vivantis, Malaysia). Isolated RNA was subjected to reverse transcription reaction according to the manufacturer's instruction. solution 1 was prepared in the first step as follows; 6μL template, 1μL random hexamer primer, 1μL dNTP mix (10mM) and filled with neuclease-free water to 10μL, the mixture incubated at 65°C for 5 minutes and chill on ice for 2 minutes then cDNA master mix was prepared separately as follow; 2μL 10x Buffer M-Mulv, 100 unit M-Mulv reverse transcriptase and filled with neuclease-free water up to 10μL,10μL of cDNA master mix was added into each microtube containing solution 1 and incubate at 42°C for 60 minutes. Terminate the reaction by incubating the tube at 85°C for 5 minutes. 5 μL of cDNA added into 25μL PCR reaction containing 2.5μL 10x viBuffer, 0.5 μL dNTP (10 mM), 0.125 μL (0.625 unit) taq polymerase , 1.5 mM $MgCl_2$, 0.75 μL (0.3 μM) each primer include; EVr (ATTGTCACCATAAGCAGCCA) and EVf (TCCTCCGGCCCCT-GAATGCG), these primers are design to amplify the 155 bp amplicon inserted in 5'UTR region of Enterovirus Genome (9).

PCR program was as follow; 1 cycle in 94°C for 5 minutes followed by 35 cycles at94°C for thirty seconds, 60°C for thirty seconds and 72°C for thirty seconds. Final extension was performed at 72°C for five minutes. 10μL of PCR product mixed with 6x loading bufferand loaded onto 2 % w/v agarose gel and then subjected to electrophoresis (100 volts for 50 minutes , 0.5x TBE buffer), stained with ethidium bromide (0.5 μg/mL) the amplicon was visualized and its size was determined under UV trans- illuminator Lambert (France) (*Figure 1*). DPEC water were used as negative control and Echo 30 used as control positive.

Figure 1. A155bp Specific Enterovirus Band



L, 100bp DNA ladder; NC, Negative control; PC, Positive control (Echo 30); Samples, 1 to 10

3.4. Statistical Analysis

Statistical analysis was performed using the χ2-test, SPSS software version 14.0. The level $P < 0.005$ was considered as the cut-off significance.

4. Results

In this study 57 CSF (Cerebrospinal fluid) samples from patients suspected of aseptic meningitis under 14 years-old with the average of 1.58 ± 2.25 years oldwere investigated by RT-PCR . Among these patients 25 of them were female (43.8 %) and 32 of them were male (56.1 %). virus genome were detected in 34 (59.6 %) samples, 14 cases were female (41.1 %) and 20 were male (58.8 %), there was no significance relationship between two genders ($P > 0.05$). The cases were subsequently devided into 3 groups, the first group consists of 20 patientsyounger than 1 year old (58.8 %),the second group consists of 9 patients who were between 1 to 2 years old (26.4 %) and 5 patients were older than 2 years old (14.7 %) (*Figure 2*).

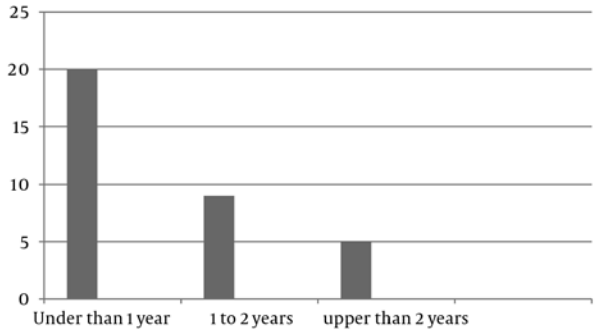


Figure 2. Distribution of Enterovial Infection Between Different Age Groups

There was significant difference between first age group with second and third age group ($P < 0.05$) . The youngest patients was 2 days old newborn and the oldest 10 years old, the average age of positive cases was about 1.5 years old. Seasonal distribution was extensive which comprises of every four season, however high prevalences were reported in Winter with 13 cases (38.2 %), spring with 9 cases (26.4 %), fall with 9 cases (26.4 %) and summer with 3 cases (8.8 %) (*Table*). There is no significant difference between Winter with spring and fall ($P > 0.05$).

Table. The Seasonal Distribution of Enteroviral Infections

	No. (%)
Spring	9 (26.4)
Summer	3 (8.8)
Fall	9 (26.4)
Winter	13 (38.2)

5. Discussion

Aseptic meningitis is one of the most common infectious diseases among children and adults, This presentation is characterized byclinical symptoms such as fever, headache, neck stiffness, photophobia, etc. (1, 4). Viruses are a major causes of aseptic meningitis, Enteroviruses are the most causative agent of Aseptic meningitis. Gold standard for diagnosis of Entroviral infection is virus isolation in cell culture, but currently most of researchers use sensitive molecular methods such as RT-PCR (10). Based on reports, prevalence of enteroviruses varies between 17 % to 52 % in Middle east (2, 8, 9, 11, 12). In present study Enteroviral prevalence were detected by RT-PCR and the result was about 59.6 %,which was close to the results of another study performed by Sohrabi *et al.* (52 %) in Ahvaz (8), and higher than other surveys reported by Sensoy *et al.* (17 %) (12) and Dalwai *et al.* (24 %) (3). poor personal and environmental hygiene and poor sanitation system in south and southwest of Iran, are major causes of this high prevalence of infection in this regions. The obtained results from first and second groups were similar to the Dalwai's *et al.* report in Kuwait (3). These observations indicate the important role of close relationship between children or newborns and some persons such as nursery nurses , siblings and parents, specially mothers during breastfeeding, which increases the possibility of infection among children and newborns. Congenital transmission specially in newborns that already mentioned is also probable. the infection could transmitted during pregnancy through blood transfusion (13, 14).

Enteroviral infectionsshow seasonal variation, enteroviral meningitis outbreaks in patients occure mostly in temperate region summer and fall and high year-round in tropical and subtropical region (1). Ahvaz is a tropical region with warm and humid climate with a temperature of 50 °C during summer time which is not optimum for virus survival, due to to a lower temperate in winter most cases were reported in winter and the lowest prevalence were observed in summer. These findings conform to those of previous studies (15, 16). According to the poor sanitation in southwestern parts of Iran andriver contaminated by urban and agricultural sewage, it could be concluded that the wide distribution pattern between seasons and high prevalence of virus is related the weather conditions, poor hygiene and lack of safe clean drinking water specially for inhabitants who live in poor area of the city. the best way to decrease the prevalence of infection is to improve the personal and environmental hygiene and to prevent water sources contamination by excreted sewage. The results indicate the importance of detection and diagnosis of viral and bacterial meningitis. Fast detection of viral meningitis, that is the main cause of meningitis in southwestern parts of Iran can also prevent ntibiotic consumption against viral meningitis and decrease treatment costs and in parrael would decrease bacterial drug resistance. The prevalence of enteroviral infection is considerably high in Ahvaz specially among children thus it is very important to improve environmental hygiene by preventing sewage contamination water sources and personal hygiene specially among persons that have close contact with children such as nursery nurses, parents and siblings in house, in order to decrease distribution of infection. Early diagnosis of viral meningitis can help us to reduce antibiotic consumptions , consequently will decrease treatment cost and the

drug resistance will decrease in parallel.

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