

# Enteroviral Meningitis in Neonates and Children of Mashhad, Iran

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## Abstract

**Background:** The highest incidence of meningitis occurs during the neonatal period and (then) infancy. Although Bacterial agents are the most dangerous cause of neonatal and childhood meningitis yet viruses especially, enteroviruses (EV), are by far the most common cause of meningitis in this age group.

**Objectives:** The aim of the current study was to evaluate the role of EVs in neonatal and childhood meningitis in the Mashhad city of Iran.

**Materials and Methods:** This was a descriptive study that was performed at Imam Reza hospital in a period of six months (March to September 2007), during which all of the cerebral spinal fluid (CSF) samples from the neonatal intensive care unit (NICU) and pediatrics ward were collected and real time-polymerase chain reaction (RT-PCR) for EVs was done on these samples. Clinical data were collected retrospectively from hospital files.

**Results:** We collected 58 CSF samples (35 neonates and 23 children) during six months. Pleocytosis of CSF was seen in 51.1% of the subjects (28% of neonates, and 66.6% of infants and children). Enteroviruses PCR was positive in 37.1% (13) of neonates and 34.7% (8) of children. Pleocytosis of CSF was seen in 23% and 75% of EV positive neonates and children, respectively. Polymorphonuclear (PMN) dominance (PMN > 50%) of CSF was seen in 50% and 33% of EV positive neonates and children, respectively. There were three cases of bacterial meningitis in our group; EV PCR result was positive for one of these subjects. Concomitant bacterial infection (meningitis and sepsis) was seen in 9.5% (two cases) of EV positive CSFs in our study. Almost half of the available neonates (four of nine) with pure enteroviral meningitis (EVM) were discharged (in good condition) with final diagnosis of culture negative sepsis (CNS) and mean length of hospital stay (MLOS) of 4.3 days. One (12.5%) of the neonates with EVM, who had a very low birth weight (< 1500 mg), was expired, and two (25%) cases were discharged with brain damage and final diagnosis of severe asphyxia. The MLOS for children with pure EVM was 1.6 days (one to four days); they didn't have any sign of brain damage or mortality. Qualitative c-reactive protein (CRP) of serum was negative in 72.7% and 37.5% of EV positive neonates and children, respectively. The mean white blood cell count and PMN percentage in the peripheral blood was 11416/mm<sup>3</sup> and 60.8% for EV positive neonates, and 14500/mm<sup>3</sup> and 77.1% for EV positive children, respectively. Hyponatremia, due to possible syndrome of inappropriate antidiuretic hormone (SIADH), was seen in 30% of neonates and 57% of children with EVM.

**Conclusions:** Enteroviruses were a common cause (> 30%) of meningitis in our study group. Concomitant bacterial infection is not rare in neonates and children with EVM. Many of the neonates (50%) and almost all of the children with EVM did not require prolonged hospitalization. Both normal CSF and PMN dominance of CSF was common in neonates and children with EVM. Positive qualitative CRP of serum (up to two plus) was common especially in children with EVM. Non-symptomatic mild hyponatremia/SIADH was common in early life EVM.

**Keywords:** Enterovirus, Viral Meningitis, Aseptic Meningitis, Neonates, Infants, Children

## 1. Background

Meningitis is a common cause of admission to the neonatal intensive care unit (NICU) and pediatrics wards. Although aseptic/viral meningitis is much more common than bacterial, yet practically most meningitis cases especially in the neonatal period are treated as bacterial, even

when the child is not seriously sick, all the cultures are negative and CSF is compatible with viral infection. By doing enteroviruses (EV) polymerase chain reaction (PCR) for all cases of aseptic meningitis, the duration of admission can be shortened even in young infants, which saves money, reduces inappropriate antibiotic use and health care associated infections (1).

## 2. Objectives

This study aimed to investigate the role of EVs in neonatal and childhood meningitis in the Mashhad city of Iran, with a population of 4,000,000, making this city the second most populated city of the country.

## 3. Materials and Methods

This descriptive study was carried out during six months (March 21st, 2007 to September 22nd, 2007). During this time all the CSF samples from NICU and pediatric ward of Imam Reza educational hospital were prospectively collected and saved, after primary analysis (Gram staining, culture, and sugar and protein measurement). At the end of the six months, real time-polymerase chain reaction (RT-PCR) for EVs by a pan-enteroviral primer (Isogen Life Sciences, Maarssen, Netherlands), was done for all cerebral spinal fluid (CSF) samples. Clinical data was retrospectively extracted from the hospital files (deputy of research committee approval code 86013).

## 4. Results

Overall, we collected 58 CSF samples (35 neonates and 23 children) during six months. The mean age of the 23 children was 5.7 years (44 days to 17 years). Pleocytosis of CSF (neonates  $\geq 20$  white blood count (WBC)/mm<sup>3</sup>, children  $\geq 5$  WBC/mm<sup>3</sup>) was seen in 51.1% of cases (28% of neonates and 66.6% of children).

Enteroviruses PCR was positive in 37.1% (13 cases) and 34.7% (8 cases) of the neonates and children, respectively. There was a case with culture positive (15 years old) and another case with smear positive (two days old) results for bacterial meningitis; EV PCR was negative for both of them. In the EV positive group there was a 17-year-old boy with severe hypoglycorrhachia (CSF sugar: 6 mg/100 cc) and very high CSF protein (1285 mg/100 cc), which are highly against viral CNS infections and suggest simultaneous bacterial meningitis (Table 1). Table 2 compares the laboratory findings of neonates and children with EVM.

The outcomes of nine (of 13) EV positive neonates were available; four of them were discharged in good condition with final diagnosis of culture negative sepsis (CNS) and mean length of hospital stay (MLOS) of 4.3 days (mean). Two of these cases had a very low birth weight (< 1500 mg), neonates with CNS which one of them was expired. The two other cases were discharged with final diagnosis of severe asphyxia (with neurologic sequel). There was a case of simultaneous bacterial sepsis (Klebsiella) with EV positive CSF, without CSF pleocytosis.

**Table 1.** The Main Clinical Aspects of Fifty-Eight Neonates, Infants and Children Who Underwent Cerebral Spinal Fluid Analysis for Sepsis Workup or Clinical Suspicion of Meningitis

	Neonates	Children
CSFs without pleocytosis <sup>a</sup> , %	72	33.4
EV meningitis, No. (%)	13 (37.1)	8 (34.7)
EVM without pleocytosis, %	77	25
Bacterial meningitis	1	2
Concomitant EVM and BM	0	1
Concomitant EVM and bacterial sepsis	1	0
Death	1	0
Total number of CSFs	35	23

Abbreviations: BM, bacterial meningitis; CSF, cerebral spinal fluid; EVM, Enteroviral Meningitis.  
<sup>a</sup> < 20/mm<sup>3</sup> for neonates, < 5/mm<sup>3</sup> for children.

**Table 2.** Laboratory Findings of Neonates and Children with Enterovirus Meningitis

Laboratory Findings	Neonates	Infants and Children (44 days-17 y)
CSF pleocytosis <sup>a,b</sup>	23	75
WBC of CSF, (Mean), /mm <sup>3</sup>	13.5	172.5
PMN% of CSF (Mean) <sup>b</sup>	52	33.8
PMN > 50% in CSF <sup>b</sup>	50	33
Sugar of CSF (mean)	65.9	55.7
Protein of CSF (mean)	75.4	65.7
CRP of serum: negative <sup>b</sup>	72.7	37.5
CRP of serum: One plus <sup>b</sup>	18	37.5
CRP of serum: Two plus <sup>b</sup>	9	25
CRP of serum >+ <sup>b</sup>	0	0
ESR (Mean), mm/h	8.3 (1-21)	23 (2-42)
WBC of peripheral blood (Mean), /mm <sup>3</sup>	11416	14500
PMN% of peripheral blood (Mean), %	60.8	77.1
Hyponatremia, NA < 135 meq/L, %	30	57
Total numbers	13	8

Abbreviations: CSF, cerebral spinal fluid; CRP, c-reactive protein; PMN, polymorphonuclear; WBC, white blood count.  
<sup>a</sup> > 20/mm<sup>3</sup> for neonates, > 5/mm<sup>3</sup> for children.  
<sup>b</sup> Values are expressed as percentage.

The outcome of five (of six) children with EVM was available; all of them except one had CSF pleocytosis, three had non-complicated viral meningitis, one had a simple febrile disease with meningismus, and one had simultaneous EV and possible bacterial meningitis (due to CSF sugar

of 18). The Mean Length of Hospital Stay (MLOS) for children with pure EVM was 1.6 days (1-4 days).

## 5. Discussion

Viruses are by far the most common cause of childhood meningitis and their incidence is close to 20 times greater than bacterial meningitis, although because of mild and self-limited clinical signs and symptoms they are under-reported in most health statistics (2). The reported rate of bacterial meningitis (BM) to all cases of meningitis in children, which was 5.1% in our study, varies from 4.9% (in a cohort of 12,000 children) in Finland to 50% in the UK, and was 21.2% according to a report from the US (Baltimore) (2-4). The rate of BM was 6.9% and 4.5% in Mashhad and Shiraz, respectively, in (non-neonates) children who underwent LP with suspicion of meningitis, yet among children with confirmed meningitis (who had CSF pleocytosis) this rate was 16.3% and 12.3%. The rate of normal CSFs in children (not neonates) suspected of meningitis was 33.4% in this study, yet in Mashhad and Shiraz this was reported as 57.4% and 63.6%; the possible causes of this large mismatch can be neglect of reserving normal CSFs for further analysis or an unusual Enterovirus season in the current study (5, 6).

The cause of aseptic meningitis can be found in up to 50% of cases (7). Enteroviruses are responsible for up to 90% of etiologically-known aseptic meningitis in children (7). In a study of 1374 cases with meningitis/encephalitis (45% < 16 years) from New York, EVs were found in 15% of cases. Silva et al. found EVs in 37% of Brazilian children with aseptic meningitis (8). Hosseinasab et al. found the cause of aseptic meningitis (by PCR for seven viruses) in 46% of 65 Iranian children (two months to 15 years old). In the study of Hosseinasab et al. EVs were the cause of 43.3% of etiologically-known cases of aseptic meningitis (20% of all cases of aseptic meningitis) (9).

Concomitant bacterial infection has been reported in 3.1% of young infants with EV positive CSF (10); this rate was 9.5% in our study. Sferra et al. reported a rate of 2.8% for bacterial meningitis in EV positive CSFs (10 days to 22 years old) (11). In the study of Kobayashi et al., 10% of hospitalized children (< 15 years) with positive EV culture (CSF, rectum and throat) had bacterial co-infection (12). Hosseinasab et al. reported on a case of simultaneous haemophilus influenzae and EV meningitis among 13 children with EVM (9). In the study of Lee et al. on 233 cases of EVM, there was no case of concomitant meningitis with other organisms (13).

During the neonatal period, EVs are one of the most common causes of nonspecific febrile illness, accounting for approximately half of the hospital admissions for ruling out bacterial sepsis (14). Enteroviruses meningitis in

neonates is a common and benign disorder, unless when accompanied by multisystem involvement. Prematurity (as was in our cases) is the most important risk factor for severe illness and death from neonatal EV disorders (14, 15). In the study of Mistchenko et al. 77% of 142 infants (< 1 year old) with EV positive CSF were younger than three months old; however, only 7.8% of their 1242 children, who underwent CSF analysis with suspicion of EV central nervous infection, were neonates (16). In the study of Archimbaud et al. 18% of their 53 children with EVM were younger than 68 days (1). In the study of Tee et al. from Singapore the median age of 43 children (5 days - 12 years old) with EVM was two months (17). In our study 71.9% of all children with EVM were neonates.

The median length of hospital stay (MLOS) was two days in 90% of cases of aseptic meningitis (EV positive and negative) as reported by the study of Lee et al. In their study, only one of the 233 children (< 18 years) with EVM (0.4%) was admitted to the intensive care unit (ICU) and possible sequela was documented for two (0.85%) of them. There was no death due to EVM in the study of Lee et al. (13). In the study of Archimbaud et al., 95% of children with EVM were discharged within 24 hours, yet for the infants the MLOS was two days (1). In our study, for children with pure EVM, the MLOS was 1.6 days.

According to previous studies, the rate of CSFs with pleocytosis to all CSF samples (which is 51.1% in this study), varies between 3.8% (in 704 infants with first simple febrile seizure in US) to 43%, (in 471 febrile children (one month to 14 years old) from Spain) (18, 19). Lee et al. reported the absence of pleocytosis in EV positive CSFs (which in our cases was 77% in neonates and 25% in children) in 32.3% of neonates and 4.4% of children (older than 60 days) (13). This rate was 42% in infants (< 2 month) as reported by the study of Sawyer et al. (20). In the study of Archimbaud et al. 100% of infants and 84.4% of children with EVM had CSF pleocytosis (1). Seiden et al. suggested that CSF pleocytosis in young infants with EV positive CSFs increases with age from 59% (0 to 28 days old) to 90% (57 to 90 days) (10).

Polymorphonuclear (PMN) cell predominance of the first CSF sample was seen in half of the 151 children with EVM in the study of Shah et al. (21). Negrini et al. reported that 57% of children with aseptic meningitis have a PMN predominant CSF (22). In both of these studies the percentage of CSF PMN was not related to the duration of symptoms before LP. In the study of Archimbaud et al., median PMN percentage of CSF was 23% in infants and 56% in children with EV meningitis, while these values according to our results were 52% and 33.8%, respectively.

Hyponatremia (commonly due to SIADH) is a common event in infants and children with EVM, yet due to natural defect in urine concentration of young infants, it oc-

curs less often in neonates (23). In our study hyponatremia was seen in 30% of neonates and 57% of children with EVM, Chemtob et al. reported an incidence of 9% for SIADH in children with aseptic meningitis (24).

Serum c-reactive protein (CRP) of less than 20 mg/L (in the study of Sormunen et al.) indicated a negative predictive value of 99% for smear negative bacterial meningitis in children, yet the specificity of the test was not as high (93%), and high CRP of up to 40 mg/L was seen in 7% of their cases (25). In the study of Dommergues et al. the mean serum CRP in a group of 99 children with EVM was 25.5 mg/L (26). In our study qualitative CRP of serum was two plus in 9% and 25% of EV positive neonates and children, respectively, which indicates that positive CRP is more common in infants and children with EVM in comparison to neonates.

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