Frequency of Parechovirus Serotype 1 Among Young Infants With Sepsis

Manoochehr Makvandi (✉ manoochehrmakvandi299@gmail.com)

Ahvaz Jundishapur University of Medical Sciences

Chiman Karami

Ardabil University of Medical Sciences

Rooya Pirmoradi

Ahvaz Jondishapour University of Medical Sciences

Ali Timori

Hamedan University of Medical Science

Ahmad Shamsizadeh

Ahvaz Jondishapour University of Medical Sciences

Research

Keywords: Parchovirus, Sepsis, PCR(Polymerase Chain Reaction)

Posted Date: July 13th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-39631/v1>

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Abstract

Background: Human parechovirus (HPeV) is recognized as a potentially severe viral infection such as gastrointestinal, respiratory and sepsis disease. In neonates and young infants, HPeV-1 is the most prevalent genotype and most commonly causes sepsis in young infants. The aim of study was to determine the prevalence of HPeV in hospitalized young infants with sepsis.

Methods: The sera of 100 samples were collected from young infants [46 (46%) females and 54 (54%) males < below 90 days] with clinical signs and symptoms of sepsis. The total RNA was extracted, cDNA was synthesized. The Nested PCR was carried out for detection of HPeV. The mean, chi square tests were used for distributions of HPeV genotypes among the gender, age group and season.

Results: 5/100 (5%) of patients including 2/46 (2%) females and 3/54 (3%) males showed positive for HPeV ($P=0.85$). The results of sequencing and phylogenetic tree revealed that the isolated HPeV were genotype 1.

Conclusion: Low prevalence of 5% HPeV were detected. HPeV was dominant in this region. The screening HPeV RNA in patients with sepsis may reduce the use of antibiotics and shorten the duration of hospitalization.

Introduction

Human parechoviruses (HPeVs) are non-enveloped, single stranded RNA, positive-sense and belong to the family Picornaviridae (1). The HPeV genome is 7350 nucleotides (nt), comprised of structural protein (VP0, VP3 and VP1), seven non-structural proteins (2A, 2B, 2C, and 3A-3D) and two Untranslated Region regions; 5' UTR and 3' UTR (2).

HPeVs have been classified in the genus *Parechovirus*, which is divided into two species: *Parechovirus A* and *Parechovirus B*. *Parechovirus A* is classified into 19 genotypes, HPeV-1 to -19, based on phylogenetic analysis of VP1 sequences, while *Parechovirus B* comprises Ljungan viruses 1 to 4 genotypes (3–4). The prevalence of HPeVs -A genotypic varies globally. HPeVs -A1 genotype is dominant in Europe and USA followed by PeV-A3 and PeV-A4, while PeV-A2, and A7-A19 genotypes are rarely reported (5). In Asia, similar to Europe and the USA, PeV-A1, A3, and A4 genotypes are the most prevalent and a higher diversity of genotypes has been reported in India and Pakistan (6, 7, 8). In the African continent, PeV-A1, A2, and A3 are the most prevalent but nearly all HPeVs genotypes have been detected, indicating a much wider circulation of genotypes in this continent (9). The prevalence of HPeVs have been reported in young children with aseptic meningitis, sepsis-like illness and gastroenteritis in Iran (10–12)

PeV-A1 has been detected in acute gastroenteritis, upper respiratory tract symptoms, fever, rash, paralysis and encephalitis in children age 6 months to 5 years (13–16).

HPeV 3, was recognized in children with respiratory, gastroenteritis and sepsis infections (5). HPeV-3 is the most important agent for asepsis, sepsis-like illness and CNS infection in infants > 3 months (17–20). HPeV-4 has been identified in patients with nervous system infection (21). HPeV-6 has been reported from a 1-year old fatally child with Reye syndrome (22) and isolated from a stool sample of a 2.5-month-old girl with fever, dehydration, bilateral otitis media, and anemia (23).

HPeVs can be Transmitted via the fecal-oral route and the respiratory tract (24, 25). At present There is no standard approach for diagnosis of viral sepsis but molecular approach such as PCR provides greater sensitivity and specificity than DFA and cell culture methods (26).

Serum samples are the most useful compared to CSF for detection of HPeV RNA from neonates and young infants with sepsis and encephalitis (27). Early recognition of HPeV infections is very important since it may reduce the use of antibiotics and shorten the duration of hospital admissions for neonates and young infants with sepsis (28).

This study was conducted to evaluate the frequency of HPeV genotype in hospitalized young infant with clinical sign and symptoms of sepsis like syndrome in Ahvaz city, Iran

Materials And Methods

In this prospective cross sectional study, 100 sera were collected from young infants [46 (46%) females and 54(54%) males < below 90 days] who had been admitted with clinical signs and symptoms of sepsis at the Abozar Children's Hospital, Ahvaz city, Iran during Sept 2016 until October 2017. The Exclusion criteria were congenital anomalies (including cerebral malformations) and positive for bacterial culture.

The patients ages were between 20–90 days with mean age of 54 ± 22 who were admitted to the intensive care unit with sepsis. The definition of sepsis was based on the following criteria ; Fever > 38 °C, Poor feeding (76%), Lethargy(47%), irritability(97%),Tachypnea(78%),Tachycardia(91%),rash(67%),vomiting(27%),diarrhea(41%), Poor Perfusion(54%), apnea (12%). The analyses of proteins, CBC and serological tests such as C-reactive were carried out for all the participants.

Statistical analysis

Data analysis was performed using the Statistical Package for the Social Sciences 22.0 (SPSS Inc., Chicago, IL, USA). Chi-Square test was used to calculate. $P < 0.05$ was accepted as significant.

Extraction of RNA and cDNA synthesis

The total RNA was extracted from each serum sample using high pure viral nucleic acid(Roche company, Germany) according to instructor manufacture. The cDNA was prepared using (Thermo scientific, USA) company, according to manufacture instructor.

Nested PCR

The Nested-PCR was carried using the following outer primers, forward: AN353(2126–2154) 5'-GACAATAGTTTTGAAATNACNATHCCNTA- 3', ,and reverse: AN358 (3116–3086) 5'-AACTATAATGCCATARTGYT TRTARAANCC- 3', and inner primers, forward: AN369 (2531–2559) 5'ACCAAGGTTGACAACATTTTYGGNMGNGC – 3', and inner primers, reverse AN357(2829–2798), 5'-GAATAAAATGGTACTGANARNG TCATYTGATC – 3', (21). First round PCR was performed with 25 µl volume, containing 5 µl of c DNA as template, 2.5 µl 10x reaction buffer, 0.75 µl MgCl₂ (50 mM), 0.5 µl (10 µM) forward/reverse primer, 1 µl dNTP (10 mM),0.2 Cinna Gen Taq DNA Polymerase (5 u/µl), and 13.55 µl double-distilled water. The reaction mixture was subjected to thermal cycler (TC-512, Techne, Staffordshire, UK) with the following program: 1 cycle with initial denaturation at 95 °C for 5 minutes, followed by 35 cycles, 94 °C for 30 s, 42 °C for 40 s, 72 °C for 60 s, and final extension at 72 °C for 3 minutes. 1 µl of PCR product of the first round of was used as the template for the second round. The amount of PCR components was the same as the first round. The thermal cycler was programmed as follows: initial denaturation at 95 °C for 5 minutes, 35 cycles consisting of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s, and final extension at 72 °C for 3 minutes. All reactions were performed in the presence of negative and positive controls. The PCR product of 293 bp shows the positive reaction (29).

Sequencing Of Isolated Hbv Dna

The results of two PCR products were sequenced using ABI 3730xl DNA sequencer (Applied Biosystems, USA). The partial sequences of parechovirus genome was sent to Gene Bank to obtain the accession number.

Phylogenetic tree

The phylogenetic tree was constructed by Neighbor joining method for partial VP1 region of two isolated HPeV and compared with different HPeV genotypes retrieved from GeneBanks. The Neighbor joining was built under Kimura two-parameter model, with reproducibility of 1,000 of bootstrap and scale bare = 0.005.

Results:

46/100(46%) of the patients were females and 54(54%) males < 90 days with a mean age of 67 ± 22 days. The sera of 5 (5%) samples [2/46 females and 3/54 males] were positive for HPeV RNA by Nested PCR. ($P = 0.85$). The patients profile present in the table 1. The incidence of HPeVe among the young neonates with sepsis were taken place in the 2(2%) in autumn and 3(3%) winter season. No HPeVe detection was found in the summer and spring seasons.

Table 2.Profile of neonates sepsis patients associated with HPeV.

Characteristic	HPeV positive	%	P value
<hr/>			
No. of patients (100)	5	5	
Sex			
Female	2/46	4.34	0.85
Males	3/54	5.55	
Age groups(days)			
1-19	-/19		0.48
20-29	2/41	4.78	
30-90	3/40	7.5	
Season			
Spring	--		0.87
Summer	--		
Autumn	2/32	6.25	
Winter	3/68	4.41	
Laboratory findings			
WBC(mm ³)	NA	9990-22000	
Absolute neutrophil count (mm ³)	NA	4766- 7233	
Platelet (×10 ³ /mm ³)	NA	355-7745	
CRP C-reactive Protein (mg/L)	NA 4.2±1.8		

Frequency of HPeV among males and females was not significant ($p=0.85$). Distribution of HPeV among different age groups was not significant ($p=0.48$), Frequency of HPeV in winter and autumn seasons wasnot significant (P.87).

Results of Sequencing

The sequences of two isolated HPeVs were deposited in GenBank with accession numbers, MN845069 and MN845070. Both isolated HPeVs showed 97% nucleotide identity with HPeV genotype1 (MK792785.1) isolated from Tehran, Iran.

Phylogenetic Tree

The analysis of phylogenetic tree revealed that both the isolated HPeV (MN845069 and MN845070) are cluster with HPeVs serotype 1 isolated from different regions of the world (Figure A).

Discussion

Sepsis due to HPeV may result in notable morbidity in neonates and young children. Although, most of HPeV infections are self-limited, but long-term neurological deficits such as learning disability, paralysis, epilepsy and encephalitis have been reported in patients with HPeV infections (16–22, 30). It has been reported the prevalence of HPeV3 is much more associated with sepsis disease than HPeV1 (17–20).

In the present survey, the prevalence of HPeV1 was 5/100 (5%) [2(2%) Females and 3(3%) males] in young infants with sepsis ($P = 0.85$). No HPeV3 was detected in the patients. The studies have shown HPeV1 was dominant in young children with aseptic meningitis and sepsis-like illness and gastroenteritis in Iran (10–12). The results of sequencing of two VP1 region of isolated HPeV1 (MN845069 and MN845070) showed 97% nucleotide identity with HPeV genotype 1 isolated from Tehran, Iran (MK792785.1). The results of phylogenetic tree of two isolated HPeV1 (MN845069 and MN845070) were cluster with HPeV1 isolated from different regions of the world. In this study, the incidence of HPeV1 was found in 2(2%) cases in autumn and 3(3%) cases winter season. No HPeV1 detection was detected in summer and spring seasons.

Rahimi et al, (2013) have detected HPeV 1 infections throughout the years with no significant difference in infection rate between seasons. However, the most HPeV 1 detections were found during summer and the lowest HPeV detection rate was in spring in Tehran, Iran (10).

In a multinational epidemiological study have been described the etiology of one-third children with severe sepsis, were viral infections (31). The most frequent sites of infection were the respiratory tract (40%) with rhinovirus, Respiratory syncytial virus, and adenovirus most commonly isolated in children with sepsis (31). In Australia and New Zealand the etiology of 50% of patients with sepsis were RSV, HVR, CMV, EBV, HSV, VZV

and influenza (32). The previous study have reported that 15% of neonates with sepsis, were bacterial etiology (33).

In the present study the role of other viruses such as human rhinovirus, human Respiratory syncytial virus, human Enterovirus (EV), HAAd, CMV, EBV, HSV, VZV and influenza have not been investigated in patients with sepsis, but it requires comprehensive investigation.

To date, no antiviral drug has been shown to be effective against HPeV, and no vaccines are currently available to protect against infection. The IVIGs have been used for the treatment of severe disease. An infant with severe, dilated cardiomyopathy caused by HPeV-1 was fully recovered after treatment with Intravenous immunoglobulin) IVIG (34).

Although low detection of 5% HPeV were found among young infant but the screening of HPeV RNA should be implemented in young infants with sepsis. The implementation of molecular detection of HPeV is very pivotal as it reduce the use of antibiotics and shorten the duration of hospitalization. the role of other viruses such as HRV, RSV, and HAdV, HCMV, EBV, HSV, VZV and influenza have not been investigated in patients with sepsis, but it requires comprehensive investigation.

Conclusion

The main goal of the current study was to determine **Frequency of Parechovirus serotype 1 among young infants with sepsis**. This study has identified low prevalence of 5% HPeV were detected. HPeV was dominant in this region. The screening HPeV RNA in patients with sepsis may reduce the use of antibiotics and shorten the duration of hospitalization. Further studies need to be carried out in order to validate these finding.

Abbreviations

Human parechovirus HPeV, Untranslated Region UTR, Polymerase Chain Reaction PCR, Direct Fluorescent Antibody DFA, human respiratory syncytial virus RSV, human Enterovirus EV, human adenovirus HAdV, HVR, human cytomegalovirus CMV, Epstein-Barr virus EBV, herpes simplex virus HSV, varicella zoster virus VZV, Intravenous immunoglobulin IVIG.

Declarations

Ethic consent

The project was approved by ethic committee of Ahvaz Jundishapur university of Medical Sciences, Ahvaz Iran. The informed consent was obtained from all the parents-neonates who were participated in this study.

Acknowledgment

This project with the registration number OG-95108 was recorded by Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Authors' contributions

Authors' contributions M M, conceptualized and designed the study, reviewed and revised the manuscript and approved the final manuscript as submitted. CH K, carried out the data collection, analyses, reviewed and revised the manuscript and approved the final manuscript as submitted. CH K, carried out the data

collection and analyses. R P, carried out the data collection and analyses. A SH carried out the data collection and analyses. A T , All authors read and approved the final manuscript

Consent for publication

"Not applicable"

Availability of data and materials

"Not applicable"

Authors' information

1 Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

2 Virology Department, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran

3 Pediatric Infectious Division, Abuzar pediatrics hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

*Corresponding author: Manoochehr Makvandi, Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran IR Iran. Tel: +98-9166181683, Fax: +98-6113738313, E-mail: manoochehrmakvandi299@gmail.com

Competing interests

No Competing interests

Funding

This project was financially supported by Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

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Figures

Fig 1

Figure 1

A phylogenetic tree was constructed with Neighbor joining method for partial VP1 region of two isolated HPeV from two patients with sepsis in Ahvaz city. The two isolated HPeV RNA with accession numbers (MN845069, MN845070) were compared with the different HPeVs genotypes retrieved from GenBank. The results of phylogenetic revealed that the Iranian isolates with black circle color are cluster with HPeV genotype 1 isolated from different regions of the world. The accuracy was assessed by 1000 bootstrap replicates. Scale bar=0.005