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Human parechovius and enterovirus in neonates: Distinct infections with overlapping features☆

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ABSTRACT

Introduction: Human parechoviruses (HPeVs) have recently been added to the family Picornaviridae, where Enteroviruses (HEV) belong. The specific characteristics of HPeV infection in the neonate are not clear, and their involvement in neonatal infection is believed to be largely underestimated. HPeV type 3 has been recently linked to sepsis-like illness and neurological involvement in the newborn.
Objective: To assess the involvement of HPeV in central nervous system (CNS) infections throughout the neonatal period in term newborns, describe their clinical, analytical and cerebrospinal fluid (CSF) characteristics, and compare them to HEV infections.
Methods: Term newborns admitted for neurological symptoms or a suspected infection, aged 0–30 days were prospectively recruited (September 2012–August 2014). Bacterial cultures were performed in all patients. Viral tests were performed in CSF including: RT-PCR for cytomegalovirus, Herpes simplex type 1 and 2, Epstein–Barr virus, HEV and HPeV. HEV and HPeV positive samples were genotyped.
Results: Fifty-seven newborns were diagnosed of sepsis-like illness and/or CNS alteration. HEV (8.7%) and HPeV-3 (3.5%) were the two most common viral agents involved during the study period. The most frequent symptom at admission was fever. Irritability was present in 1/2 of HPeV and 1/5 of HEV cases. There were no other neurological symptoms. Blood and CSF analysis were unremarkable in HPeV infections. All cases resolved favorably.
Conclusions: HPeV infection was clinically very similar to that of HEV, while it featured normal blood and CSF analysis.
Practice implications: HPeV should be considered by clinicians in the differential diagnosis of neonatal infection, particularly when blood and CSF analysis are unremarkable.

1. Introduction

Human parechoviruses (HPeV) and Enteroviruses (HEV) are RNA viruses within the family of *Picornaviridae*. HPeV genotypes 1, 3 and 6 are considered to be widely prevalent worldwide. Although there is clear evidence of the clinical relevance of these infections in young children, knowledge about the specific characteristics of HPeVs as neonatal pathogens is scarce. The clinical symptoms of neonatal HPeV infection have been described as superimposable to that of HEV infection. Interestingly, HPeV genotype 3 in particular has been associated with sepsis-like illness and central nervous system (CNS) involvement in the newborn, which can have a complicated course and cause distinctive cerebral white matter damage [1,2].

The involvement of HPeVs in neonatal infection is believed to be largely underestimated. HEV specific polymerase chain (PCR) reaction techniques do not detect HPeVs, which makes HPeV specific assays essential for diagnosis [1,3,4].

The objective of this prospective study was to assess the involvement of HPeV in central nervous system (CNS) infections in term neonates, describe their clinical, analytical and cerebrospinal fluid (CSF) characteristics, and compare them to HEV infections.

2. Methods

For 24 months, from September 2012 to August 2014, term neonates aged 0–30 days of postnatal life admitted to the Neonatal Unit at Tertiary Burgos University Hospital for a sepsis-like illness or neurological symptoms were prospectively recruited.

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Newborns were considered for study if they met at least one of the following criteria (1) fever (rectal temperature above 38 °C), (2) clinical and/or analytical signs compatible with infection, and (3) neurological symptoms. Newborns with exclusive gastrointestinal or respiratory tract infections at presentation, as well as cases of nosocomial sepsis, were excluded. Late onset infections were defined as episodes appearing in newborns aged >72 h.

Antenatal and perinatal data and details of the clinical course were recorded. In order to accurately recruit our target population of infected patients, newborns later diagnosed of a condition other than infection were not included in the results. These conditions are shown in Fig. 1.

Bacterial cultures were performed before the beginning of antibiotic treatment. Viral tests were performed in CSF including: PCR for cytomegalovirus, herpes simplex 1 and 2, Epstein-Barr virus, and HEV. In pathogen-negative cases, HPeV specific reverse transcription-polymerase chain reaction (RT-PCR) PCR in CSF was performed. HEV and HPeV positive samples were typed at the Spanish National Centre of Microbiology, by VP1 amplification and sequencing [5].

The study protocol was approved by the Hospital's Ethics Committee.

3. Results

Out of the 94 recruited candidates [Fig. 1], 57 neonates were diagnosed of sepsis-like illness or suspected neurological infection. There was a male predominance (male:female ratio of 2.2:1). The median age at presentation was 1.1 days (IQR 0–11) and 63% (36/57) of the neonates presented at age ≤72 h.

There were eight episodes of viral infection: seven of the 57 newborns were positive for HEV (5/7) or HPeV (2/7); and there was a Herpes simplex virus type 1 infection (1/8).

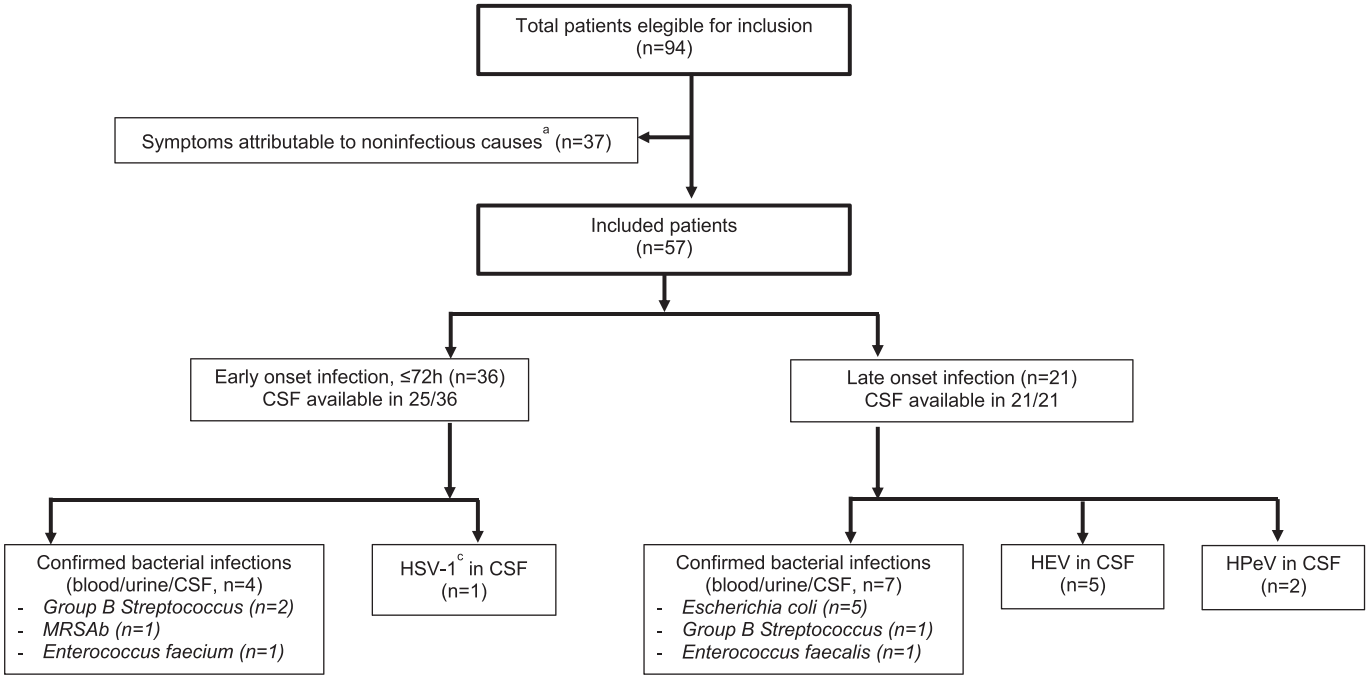
There were no cases of early onset HPeV or HEV infection. In the newborns with late onset infections, HEV and HPeV were diagnosed in 5/21 and 2/21 respectively. The median age at admission of the HEV or HPeV positive cases was 12 days (IQR 6–23). HEV genotyping was performed in four cases, in which Echovirus-11, Echovirus-16 and Echovirus-18 were identified. HPeV-3 was genotyped in both HPeV infections.

There was a seasonal distribution of cases in our series, with all HEV and HPeV infections occurring in spring–summer months (March to August). HPeV cases were detected in May–June 2013, whereas HEV was detected in both 2013–14.

Epidemiological, clinical and analytical characteristics collected during the hospitalization of the newborns positive for HPeV or HEV are shown in Table 1. All the newborns were born at term, had a normal immediate perinatal period, and were being breastfed. The most frequent symptom at presentation was fever (100%). Intense irritability appeared in one of the two HPeV cases, while mild irritability appeared in 1/5 of HEV infections. Total duration of fever was less than 48 h in all cases. All HPeV or HEV positive newborns remained hemodynamically stable and did not require intensive care support. Apart from the cases with irritability, they did not show any other neurological symptoms. A case of HPeV-3 infection developed apneic spells which required non-invasive oxygen support for the first 3 days of hospitalization. The median duration of hospitalization in the HEV or HPeV positive patients was 7 days (IQR 4–8) and neurological examination was normal at discharge in all cases.

4. Discussion

Our work provides comprehensive data on the clinical course, blood and CSF analysis of a subgroup of newborns diagnosed of HPeV and HEV



[a] Symptoms attributable to noninfectious causes (n=37):
- Perinatal asphyxia (n=11), perinatal stroke (n=2), intraventricular hemorraghe (n=1), epileptic encephalopathy (n=1), apparently life-threatening event (n=1).
- Fever only at birth in cases of maternal fever (n=3). Dehydration (n=4), hypoglicemia (n=1).
- Transient respiratory distress: neumothorax (n=4), pulmonary maladaptation (n=7), meconium aspiration (n=2).

[b] MRSA: Meticilin-resistant *Staphylococcus aureus*.

[c] HSV-1: Type 1 Herpes simplex virus.

Fig. 1. Patient inclusion and exclusion algorithm.

Table 1
Characteristics of the neonates with HPeV or HEV infection.

Patient number	1	2	3	4	5	6	7
Sex (male/female)	M	F	M	M	M	F	M
Gestational age (weeks)	39	39	41	38	38	40	40
Birth weight (g)	3090	3470	3780	3115	3410	3660	3630
Apgar score (1st/5th min)	9/9	8/9	9/10	9/10	9/10	9/10	9/10
Perinatal period	Uneventful	Fractured clavicle, mild jaundice	Uneventful	Uneventful	Uneventful	Uneventful	Uneventful
Date of admission (month/year)	03/2013	05/2013	06/2013	08/2013	04/2014	05/2014	08/2014
Age of presentation (days)	24	19	6	5	23	11	12
Signs and symptoms	Fever	Fever, vomits, diarrhea, apneas	Fever	Fever	Fever and altered perfusion	Fever	Fever
Neurological symptoms	No	Marked irritability	No	No	No	Irritability	No
Max temperature (°C)/duration of fever (days)	39/1.5	38.3/1.5	38.9/1	39/2	38.4/1.5	39.2/0.75	38/0.5
Min/Max blood WBC count, cells $\times 10^9$/L	5.4/5.7	6.8/7.1	4.9/7.1	5.8/15.5	5.8/5.8	10.2/10.2	11.0/10.3
Max C-reactive protein (mg/L)	10	1	1	40	26	34	1
CSF	9	3	15	2	1	15	643
Cell count, cells/mm^3	57	37	64	81	33	65	73
Protein mg/dl	69	47	47	52	55	44	48
Glucose mg/dl							
HEV/HPeV serotype	E-11	HpEV 3	HpEV 3	E-16	E-16	E-18	Enterovirus not typed
Symptoms of infection in close relatives	Sibling respiratory infection	No	Sibling respiratory infection	Fever in caregiver, siblings fever and skin rash.	Gastrointestinal infection in cohabitants.	No	No

infection in a population of term newborns with sepsis-like illness and/or CNS involvement.

The overall prevalence of HPeV infection in our series was 3.5%, which is slightly lower than what other groups have found [6]. Still, HPeV was the second most common viral infection involved in neonatal sepsis-like illness. HPeV-3 was the only HPeV genotype found in our sample.

Interestingly, there were no cases of severe disease, and the clinical course was favorable with normal neurological examination at discharge in all cases. This is in accordance with pediatric series that show that severe central nervous system infection by HPeV is rare [4]. Irritability and skin rash have been linked to HPeV-3 infections [1,7]. In our series, irritability was intense in one of the two HPeV-3 cases, and there were no cases of skin rash.

Certain studies suggest that CNS infection by HPeV shows significantly less pleocytosis and a higher protein count in CSF than HEV infection; with a lower blood absolute lymphocyte count [9]. We did not find such analytical differences in our series and CSF analysis was unremarkable in both viruses, except for a case of HEV meningitis with important pleocytosis. Similar to what others have found [7,8], CRP levels in blood remained normal in both HPeV cases while they were elevated (cutoff of 10 mg/L) in 4/5 of the cases of HEV infection.

This study included both early and late onset episodes of infection. The median age at presentation of HEV or HPeV infection was 12 days with the earliest infections occurring in newborns as young as 5 (HPeV-3) and 6 (HEV) days. Although we did not find any case of HEV or HPeV early onset sepsis, and do not suspect a vertical transmission in our cases, we did not perform viral tests to the mothers so this possibility could not completely be ruled out. There are reports suggesting that HEV and HPeV infections can be acquired perinatally [8,10].

This is one of the first prospective studies designed to analyze the importance and features of HPeV in sepsis or CNS infection exclusively in the newborn. In this particular period of life the signs of sepsis are nonspecific and this is especially remarkable with viral infections. Most of the data known to date was extracted from large retrospective studies with a variety of clinical inclusion criteria, and different HPeV testing procedures. We believe that the strict patient inclusion protocol which targeted term neonates, and the HPeV detection assay used in this study, are important strong points. On the other hand, although this is a population study and we believe that all the infants who met the inclusion criteria for the two-year study period were included, the small sample size does not allow to draw definitive clinical and epidemiological conclusions about HPeVs from our data. It is possible that our study might have underestimated the prevalence of HPeV infection: performing viral diagnosis tests in both CSF and plasma could have increased the detection rates in patients with a low CSF viral load [3,12]; in addition, testing HPeV in newborns who were positive for other pathogens could have detected cases of concurrent infection.

In conclusion, we recommend that clinicians should include HPeVs in the differential diagnosis of neonatal sepsis-like illness or CNS infection, particularly in cases presenting with fever, irritability and normal blood CRP and CSF analysis. Further prospective studies are needed to clarify the epidemiological and clinical spectrum of neonatal HPeV infection. It has been shown that rapid diagnosis of HEV infection by PCR can reduce hospital stay and duration of antibiotic use [4]. Experts advocate for the implementation of a readily available HPeV PCR assay [11], which could improve and optimize the management of these patients, while at the same time minimizing unnecessary procedures and reducing the risk of viral transmission within the Neonatal Unit.

Conflict of interest statement

The authors report no conflict of interest.

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