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Relevance of Human Parechovirus Detection in Cerebrospinal Fluid Samples From Young Infants With Sepsis-Like Illness

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Background: The human parechoviruses (HPeVs) were recently recognized as important viral pathogens involved in various illnesses in young children. However, routine detection is not performed in most clinical laboratories. Therefore, in this study, we aim to assess the relevance of HPeV detection in cerebrospinal fluid (CSF) of infants, according to clinical presentation. *Methods:* A total of 120 CSF specimens collected during 2012 from infants aged less than 1 year and previously reported negative for Herpes simplex virus (HSV) and enterovirus were selected. HPeV detection was performed with a commercially available real-time RT-PCR and HPeV strains from positive samples were subsequently genotyped by sequencing. *Results:* HPeV RNA was detected in nine (7.5%) CSF samples. The median age of infected children was 41 days (range: 19–122 days). HPeV genotyping could be performed on five samples and three HPeV-3, one HPeV-1, and one HPeV-4 were identified. Hyperthermia associated with mottled skin was the predominant clinical presentation. Most clinical presentations of HPeV-infected infants were mild with a final diagnosis of sepsis-like illness. The median hospital stay was 3.5 days and five children received antibiotics. *Conclusion:* Routine detection of HPeV in CSF may allow differential diagnosis of enterovirus infection and improve etiologic identification of sepsis-like illness in children. J. Clin. Lab. Anal. 29:112–115, 2015.

Key words: human parechovirus; HPeV; cerebrospinal fluid; sepsis-like illness

INTRODUCTION

Attention to the clinical significance of human parechoviruses (HPeVs) has increased over the last decade. HPeV are nonenveloped, single-stranded, positive-sense RNA viruses that were previously classified within the *Enterovirus* genus of the *Picornaviridae* family. However, regarding some distinct genetic and biological properties, they were re-named and re-classified in the specific distinct genus *Parechovirus* that clusters now up to 16 HPeV genotypes (1). The clinical importance of HPeV infection was extensively explored worldwide (2–5). The majority of HPeV infections occur very early in life, mainly before the age of 1 year. HPeV infections are usually asymptomatic but may be associated to mild gastrointestinal and respiratory diseases or to encephalitis, meningitis, and sepsis-like syndrome in neonates (5–7).

Developments of sensitive and specific molecular tests for HPeV RNA have recently improved HPeV detection and typing and have allowed to better understand HPeV epidemiology. Several recent studies have highlighted the great interest of a more systematic testing for HPeV infection in infants (2–7). However, to date, few clinical laboratories have implemented HPeV molecular tests to their routine in addition to human enteroviruses (4, 8, 9). With the aim to set up the systematic detection of HPeV RNA in cerebrospinal fluid (CSF) collected from infants, we retrospectively investigated the presence of HPeV sequences in CSF specimens previously tested for routine diagnosis of viral infection.

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TABLE 1. Laboratory Findings From HPeV-Infected Children

	CSF				Blood			Viral data	
ID	WBC ($\times 10^3$ cells/μl)	Protein (g/l)	Glucose (mmol/l)	Interferon (UI/ml)	CRP (mg/l)	PCT (ng/ml)	WBC ($\times 10^3$ cells/μl)	HPeV amplification threshold (C_t)	HPeV genotype
7	nd	1.6	5.1	<2	2.7	0.17	2.9	32	HPeV-3
26	5	3.2	4	<2	6.8	1.25	7.7	35	HPeV-1
28	5	nd	nd	<2	21.5	0.23	6.6	37	HPeV-3
33	5	nd	nd	<2	72.6	3.66	2.7	35	HPeV-4
34	5	0.27	2.6	<2	101	0.29	12.7	38	nd
44	nd	0.57	2.9	4	14.3	0.14	6.4	37	nd
83	30	0.37	3	<2	121	1.47	7.0	33	Nontypable
88	nd	1.02	4	<2	2.9	0.15	6.6	36	HPeV-3
91	5	0.53	2.4	<2	99	1.42	nd	36	Nontypable

nd, not determined; WBC, white blood cells; C_t, cycle threshold.

MATERIAL AND METHODS

Patients and Samples

A total of 120 available CSF specimens collected during 2012 from infants aged less than 1 year were studied. These samples were previously tested and found negative for Herpes simplex virus (HSV) DNA and enterovirus RNA. Clinical and biological data were retrospectively recorded.

RNA was isolated from 200 μl of CSF spiked with the IC1 RNA internal control (bioMérieux—Argène, Verniolles, France) using the Nuclisens EasyMag extraction automated system (bioMérieux, Craponne, France). Extracts were stored at -80°C until use.

HPeV Detection by Real-Time PCR

HPeV detection was performed on 10 μL of RNA extracts using a commercially available real-time reverse-transcription PCR assay targeting the 5′ nontranslated region (Parechovirus r-gene, bioMérieux) on the LightCycler 480 system (Roche Diagnostics, Meylan, France) (4, 10).

HPeV Genotyping

HPeV genotyping was performed by VP1 sequencing (11). Sequences were blasted against GenBank for phylogenetic analysis.

RESULTS

HPeV RNA Detection and Genotypes

HPeV RNA was detected in nine (7.5%) CSF samples, with cycle threshold (C_t) values ranging from 31.9 to 38.7 cycles. Median age of these infants was 41 days (range: 19–122 days), with a male/female ratio of 2. Six infants with HPeV infections were admitted during summer months (June–August) whereas the three other HPeV cases occurred later in November. Seven patients had specimens available for genotyping. In two patients, the virus could not be typed. Three patients were infected with HPeV-3, one with HPeV-4, and one with HPeV-1.

Biological and Clinical Data

Laboratory results are displayed in Table 1. Infants with HPeV infection had a median white blood cells' count of 6.6×10^3 cells/mm^3 (range: 2.7–12.7 $\times 10^3$ cells/mm^3) with leukopenia ($<5 \times 10^3$ cells/mm^3) for only two patients. A mild inflammatory syndrome with a median C-reactive protein (CRP) level at 21.5 mg/l (range: 2.7–121 mg/l) was observed, whereas the procalcitonine (PCT) ranged from 0.14 to 3.66 ng/ml with a median at 0.29 ng/ml. None but one CSF displayed a pleiocytosis (30 cells/mm^3), the median CSF protein level was 0.53 g/l (range: 0.27–1.6 g/l, normal values: 0.15–0.45 g/l) and the median CSF glucose value was 2.9 mmol/l (range: 2.4–5.1 mmol/l, normal values: 2.5–4.5 mmol/l). Alpha-interferon levels in the CSF were <2 UI/ml except a discrete detectable level of 4 UI/ml in one sample.

Children were all hospitalized with a median hospital stay of 3.5 days (range: 2–8 days). At admission, all patients were febrile with a mean T_{max} of $39.2 \pm 0.8^\circ$C. The median duration of fever was 72 h (range: 48–96 h) and five infants received an antibiotics treatment. Hyperthermia was the predominant clinical presentation together with mottled skin. All children had neurological signs, seven of them had irritability, two of them had hypotonia, and one had tense fontanelle. Five patients presented a sepsis-like syndrome, with tachycardia and mottled skin. Other observed presentations were mild gastrointestinal disorders including diarrhea, vomiting, or food refusal

($n = 7$), respiratory signs ($n = 3$), and rash ($n = 1$, Table 2).

DISCUSSION

The HPeV detection rate of 7.5% in CSF collected from young infants observed in this study is similar with previous observations (2,4,5). In agreement with these reports, most infections occurred during summer months, however a second cluster of cases was detected in November suggesting that the period of HPeV circulation might be more extended than previously thought and that viral detection should be driven yearlong. Infants with HPeV detection in CSF were predominantly very young boys (median age of 41 days, sex ratio of 2), in line with similar observations of HPeV-3 infection affecting mainly male children under 3 months (4,12,13). This reinforces the interest for a focus on infants aged less than 3 months for HPeV testing.

In our retrospective study, a HPeV genotype could be identified in five of seven analyzed samples. As previously observed, HPeV-3 was the most prevalent genotype in CSF. HPeV-3 was recently shown to be a common cause of meningitis and neonatal sepsis in young infants and it is the most prevalent genotype detected in CSF samples (5,12–14). Other genotypes in CSF appear to be more sporadic and are mainly described in children older (median age 6.6 months) than those infected by HPeV-3 (median age <3 months) (1). The age of the two children infected with HPeV-4 or HPeV-1, although higher (84 and 98 days, respectively) than the median age was not significantly different from the other HPeV-infected children. HPeV-1, formerly echovirus 22, has been detected in CSF from infants with neurological diseases such as aseptic meningitis or encephalitis, or encephalomyelitis (15). Detection of HPeV-4 genotype in CSF of children with febrile syndrome has been also previously reported (4, 16). HPeV4 was, furthermore, recently described in neonatal sepsis with similar clinical manifestations as those observed in this study (17). However, the detection of these HPeV genotypes is less frequently reported than that of HPeV-3. Several studies have reported that HPeV-3-infected infants are younger and have more severe symptoms than those infected with other HPeV genotypes (7, 18). In this study, clinical presentations were mild for the three HPeV genotypes detected. Furthermore, the low HPeV viral loads in CSF observed in all patients are more likely to reflect a passive passage across the blood-brain barrier than an active replication within the CSF. This could be as well corroborated by results from most CSF showing no detection of alpha-interferon, absence of pleiocytosis, and a normal protein level. However, it must be specified that these markers are poor reliable indicators of CNS infection in children of less than 2 months (19).

TABLE 2. Hospital Course and Clinical Characteristics of HPeV-Infected Children

ID	Age (days)	Gender	Fever (h)	Temp. max. (°C)	Hospital stay (days)	Sepsis signs	Neurologic signs	Respiratory signs	Gastrointestinal signs	Other signs	Atb (yes/no)	HPeV genotype	Diagnosis
7	20	F	48	38.2	3		Irritability		Food refusal		No	HPeV-3	Sepsis-like illness/meningitis
26	98	M	72	39.2	3		Tense fontanelle	Rhinopharyngitis			Yes	HPeV-1	Sepsis-like illness/meningitis
28	47	F	96	39.5	3	Mottled skin	Irritability		Diarrhea		Yes	HPeV-3	Sepsis-like illness nephritis
33	84	M	96	40	4	Mottled skin	Irritability		Food refusal		Yes	HPeV-4	Sepsis-like illness
34	122	M	72	39.5	2		Irritability		Food refusal		No	nd	Febrile illness
44	35	M	96	38.9	8	Mottled skin	Irritability		Food refusal		Yes	nd	Sepsis-like illness
83	80	M	48	38.5	8		Irritability	Bronchiolitis respiratory distress			Yes	Nontypable	Bronchiolitis with sepsis-like illness
88	19	F	72	38.6	5	Mottled skin	Hypotonia		Emesis		No	HPeV-3	Sepsis-like illness
91	25	M	48	39.3	3	Mottled skin	Irritability, hypotonia	Respiratory distress	Diarrhea	Rash	No	Nontypable	Enteroviral infection with sepsis-like illness

Atb, antibiotics.

Dominant clinical presentation of the nine children at admission was high body temperature frequently associated to mottled skin. Mild neurological signs like irritability were also frequently recorded. Few children exhibited additional signs of a more severe form such as occurrence of rash or symptoms compatible with a meningitis (hypotonia, tense fontanelle). A significant inflammatory syndrome with high CRP (70–121 mg/l) and high PCT (1.25–3.66 ng/ml) was observed in five infants, whereas only one had a documented associated bacterial infection (nephritis). Thus, for all these children, the final retained diagnosis was a sepsis-like illness (not tolerated fever with peripheral vasoconstriction signs) and regarding current standard for care, hospitalization and administration of empiric antibiotics treatment were proposed. Furthermore, lumbar puncture was performed as part of workups to rule out sepsis and/or meningitis. Regarding the mild symptoms and presentations of HPeV-infected infant, our study suggests that routine detection of HPeV in available CSF is worth considering as differential diagnosis of enterovirus infection, even in noncomplicated sepsis-like illness of very young children. Furthermore, this diagnosis is now facilitated by the development of commercial solutions easy to set up in any clinical laboratory (4, 10). Length of hospital stay was not negligible for infants infected with HPeV and most of them received a likely useless antibiotics treatment. Therefore, detection of HPeV in such clinical situation of unknown etiology could reduce hospital stays, save antibiotics usage, and minimize overall care expenses.

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REFERENCES

1. Harvala H, Simmonds P. Human parechoviruses: Biology, epidemiology and clinical significance. J Clin Virol 2009;45(1): 1–9.
2. van der Sanden S, de Bruin E, Vennema H, Swanink C, Koopmans M, van der Avoort H. Prevalence of human parechovirus in the Netherlands in 2000 to 2007. J Clin Microbiol 2008;46(9):2884–2889.
3. Piralla A, Furione M, Rovida F, et al. Human parechovirus infections in patients admitted to hospital in Northern Italy, 2008–2010. J Med Virol 2012;84(4):686–690.
4. Schuffenecker I, Javouhey E, Gillet Y, et al. Human parechovirus infections, Lyon, France, 2008–10: Evidence for severe cases. J Clin Virol 2012;54(4):337–341.
5. Wolthers KC, Benschop KS, Schinkel J, et al. Human parechoviruses as an important viral cause of sepsislike illness and meningitis in young children. Clin Infect Dis 2008;47(3):358–363.
6. Sharp J, Harrison CJ, Puckett K, et al. Characteristics of young infants in whom human parechovirus, enterovirus or neither were detected in cerebrospinal fluid during sepsis evaluations. Pediatr Infect Dis J 2013;32(3):213–216.
7. Benschop KS, Schinkel J, Minnaar RP, et al. Human parechovirus infections in Dutch children and the association between serotype and disease severity. Clin Infect Dis 2006;42(2):204–210.
8. Renaud C, Kuypers J, Ficken E, Cent A, Corey L, Englund JA. Introduction of a novel parechovirus RT-PCR clinical test in a regional medical center. J Clin Virol 2011;51(1):50–53.
9. Escuret A, Mirand A, Dommergues MA, et al. Epidemiology of parechovirus infections of the central nervous system in a French pediatric unit. Arch Pediatr 2013;20(5):470–475.
10. Mirand A, Archimbaud C, Chambon M, et al. Diagnosis of human parechovirus infections of the central nervous system with a commercial real-time reverse transcription-polymerase chain reaction kit and direct genotyping of cerebrospinal fluid specimens. Diagn Microbiol Infect Dis 2012;74(1):78–80.
11. Nix WA, Maher K, Pallansch MA, Oberste MS. Parechovirus typing in clinical specimens by nested or semi-nested PCR coupled with sequencing. J Clin Virol 2010;48(3):202–207.
12. Selvarangan R, Nzabi M, Selvaraju SB, Ketter P, Carpenter C, Harrison CJ. Human parechovirus 3 causing sepsis-like illness in children from midwestern United States. Pediatr Infect Dis J 2011;30(3):238–242.
13. Walters B, Peñaranda S, Nix WA, et al. Detection of human parechovirus (HPeV)-3 in spinal fluid specimens from pediatric patients in the Chicago area. J Clin Virol 2011;52(3):187–191.
14. Boivin G, Abed Y, Boucher FD. Human parechovirus 3 and neonatal infections. Emerg Infect Dis 2005;11(1):103–105.
15. Legay V, Chomel JJ, Fernandez E, Lina B, Aymard M, Khalfan S. Encephalomyelitis due to human parechovirus type 1. J Clin Virol 2002;25(2):193–195.
16. Pajkrt D, Benschop KS, Westerhuis B, Molenkamp R, Spanjerberg L, Wolthers KC. Clinical characteristics of human parechoviruses 4–6 infections in young children. Pediatr Infect Dis J 2009;28(11):1008–1010.
17. Jääskeläinen AJ, Kolehmainen P, Kallio-Kokko H, et al. First two cases of neonatal parechovirus 4 infection with manifestation of suspected sepsis, Finland. J Clin Virol 2013;58: 328–330.
18. Harvala H, Robertson I, Chieochansin T, McWilliam Leitch EC, Templeton K, Simmonds P. Specific association of human parechovirus type 3 with sepsis and fever in young infants, as identified by direct typing of cerebrospinal fluid samples. J Infect Dis 2009;199(12):1753–1760.
19. Seiden JA, Zorc JJ, Hodinha RL, Shah SS. Lack of cerebrospinal fluid pleiocytosis in young infants with enterovirus infections of the central nervous system. Pediatr Emerg Care 2010;26(2):77–81.