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ORIGINAL ARTICLE

Neonatal acute liver failure due to enteroviruses: a 14 years single NICU experience

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ABSTRACT

Background: Neonatal acute liver failure (ALF) is a severe condition with a mortality rate up to 70%. Human enterovirus (HEV) infections are associated with serious diseases in newborns, including myocarditis, meningoencephalitis and, more rarely, ALF with a fulminant course.

Methods: Cases of neonatal-onset ALF were identified using the institutional clinical database. The history and clinical data of infants with HEV infection were collected by medical record revision. Viral testing by nested real- time PCR (nRT-PCR) was performed by the Bambino Gesù Children's Hospital Clinical Laboratory and by National Institute of Public Health in Rome.

Results: Among ten infants referred to our Institution with neonatal-onset ALF in the 2004–2018 period, we identified five cases due to HEV. In three of these, the mother reported an episode of mild fever and diarrhea during the last trimester of gestation, suggesting fetal-maternal transmission. All were late preterm infants (32–36 weeks). Two infants died as a result of ALF; the other three survived with full normalization of liver function. In four, the causing agents were coxsackie B serotypes 3 ($n = 1$), 4 ($n = 1$) and 5 ($n = 2$), in the fifth case we identified echovirus serotype 11.

Conclusions: Human enterovirus (HEV) are a rare but relevant cause of ALF in neonates. HEV testing should be systematically performed in cases of neonatal ALF for diagnostic and management purposes.

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Introduction

Acute liver failure (ALF) in the neonatal period is a rare but potentially fatal condition, characterized by massive liver cytolysis, coagulopathy, and encephalopathy, with a mortality rate up to 70%. In case of neonatal ALF, prematurity and early onset represent poor outcome predictors [1]. ALF typically manifests during the first 4 weeks of life with nonspecific symptoms such as fever, restlessness, poor feeding, and transient respiratory distress, mimicking late-onset bacterial sepsis. Within one to two days, jaundice and coagulopathy appear, followed by metabolic failure and encephalopathy. If not reversed, the disease progresses towards multiorgan failure, profuse hemorrhages, coma, and death. Liver transplantation might improve the outcome [1].

Human enterovirus (HEV) infections in neonates present with a spectrum of manifestations [2]. Among these fulminant hepatitis and myocarditis are the most severe, characterized by a high mortality rate. The aim of the present study is to describe the diagnostic markers, clinical course and prognosis of HEV fulminant hepatitis in a series of neonates hospitalized in a single level IV neonatal intensive care unit over a 14-year period.

Materials and methods

Through the institutional clinical database, we identified all subjects hospitalized for ALF in the Neonatal Intensive Care Unit at the Bambino Gesù Children's Hospital in Rome, Italy, from January 2004 to December 2017. Infants with a concomitant diagnosis of HEV infection were selected. Data on medical history, clinical course, laboratory results, and pathology records were collected by individual medical record revision. Standard pathology analysis was performed by the hospital clinical pathology laboratory. Standard human enterovirus species testing was performed by nested real-time polymerase chain reaction (nRT-PCR) at

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Table 1. Neonates' laboratory characteristics.

	case 1	case 2	case 3	case 4	case 5
Total bilirubin (mg/dl)	22.49	16.29	18.7	5.45	6.54
AST (n.v. 5–40 UI/L)	1.046	1076	13.187	147	150
ALT (n.v. 5–40 UI/L)	1.167	487	3.510	347	187
gammaGT (n.v. 4–45 UI/L)	115	201	91	54	57
Ammonium (n.v. 0–75 μg/dl)	170	159	200	45	40
INR (n.v. 0.5–1)	1.9	9.78	17.48	0.5	< 0.5
Fibrinogen (n.v. 200–500 mg/dl)	80	64	50	148	150
D-Dimer (n.v. <0.50 μg/ml)	>20	>20	7.45	<0.5	<0.5
Platelets (n.v. 150–450 × 103 mc/L)	5000	20.000	30.000	240.000	180.000
Ferritin (n.v. 17–390 ng/ml)	8.640	719	1000	300	270

the institution's clinical laboratory and at the National Institute of Public Health in Rome. Specific group and serotype testing was performed by specific capside protein VP1-encoding sequence determination and comparison with GenBank reference sequences [http://blast.ncbi.nlm.nih.gov/Blast.cgi], using the Enterovirus Genotyping Tool version 0.1 software [http://www.rivm.nl/mpf/enterovirus/typingtool] as published [3,4], and by serotype-specific antibody testing at the department of infectious, parasitic and immune-mediated diseases, National Institute of Public Health in Rome, Italy. We reviewed the available scientific literature on this topic in the medicine literature database.

Results

We identified 10 cases of ALF occurring in the neonatal period, five caused by HEV, one by cytomegalovirus, one by hemophagocytic lymphohistiocytosis (HLH) and three with no identified cause. All neonates underwent comprehensive diagnostic procedures including liver function tests, metabolic workup, ferritin and transferrin measurements, Coomb's test, bone marrow aspirate, and extensive serology and/or molecular testing for viruses (human herpes simplex, herpes virus 6, Varicella zoster, Epstein-Barr virus, cytomegalovirus, hepatitis A, B and C, parvovirus, adenovirus and enteroviruses). Laboratory data are listed in Table 1. We describe here the five cases related to HEV.

Case 1

A 35 weeks preterm infant presented at 6 days of life with lethargy, poor feeding, jaundice, and hepatosplenomegaly. The mother reported an episode of fever and mild diarrhea 2 months before delivery. The baby showed marked hepatic cytolysis, cholestatic jaundice, hyperammonemia and diffuse intravascular coagulopathy. Since ferritin level was elevated and iron saturation was 99%, a salivary gland biopsy was performed, which did not show traces of hemosiderin, ruling out neonatal hemochromatosis (NH). Bone marrow aspirate and liver biopsy did not show hemophagocytosis. Blood, cerebrospinal fluid, and bone marrow aspirate were positive for HEV. The causative agent was subsequently identified as Coxsackie B3. The virus could not be detected in maternal stool and vaginal samples by nRT-PCR at that time, suggesting that transmission occurred earlier during the third trimester of pregnancy rather than peri/postnatally, since enterovirus shedding in stools persists up to 7–11 weeks after an acute infection [5]. The infant died at 27 days from multiorgan dysfunction. Post mortem findings included acute myocarditis, congestive heart failure, and cerebral edema. Liver microscopy revealed extensive necrosis (Figure 1, Panel A), confirming the diagnosis of fulminant HEV hepatitis.

Case 2

A late preterm male infant who was born at 36 weeks by cesarean section showed marked direct hyperbilirubinemia, hepatic cytolysis, acute liver failure, and coagulopathy on day one. The mother reported an episode of fever and diarrhea 6 weeks before delivery. Coxsackie B4 virus was identified in blood, cerebrospinal fluid, and bone marrow. Maternal stool, blood, and vaginal samples were negative. Liver biopsy showed marked iron deposits in hepatocytes and macrophages, absent in other tissues (Figure 1, Panel B). Considering the massive liver necrosis, the neonate was listed for emergency liver transplantation, but before a donor was available, his condition improved and he recovered with supportive treatment. Liver function tests at 7 months were normal and liver ultrasonography showed only minor structural anomalies.

Case 3

This infant was born at 32 weeks of gestation by cesarean section. The mother reported a febrile episode 1 month prior to delivery. The baby developed marked hepatic cytolysis, direct hyperbilirubinemia, acute liver failure, intravascular coagulopathy, metabolic acidosis, and hypertrophic cardiomyopathy in the

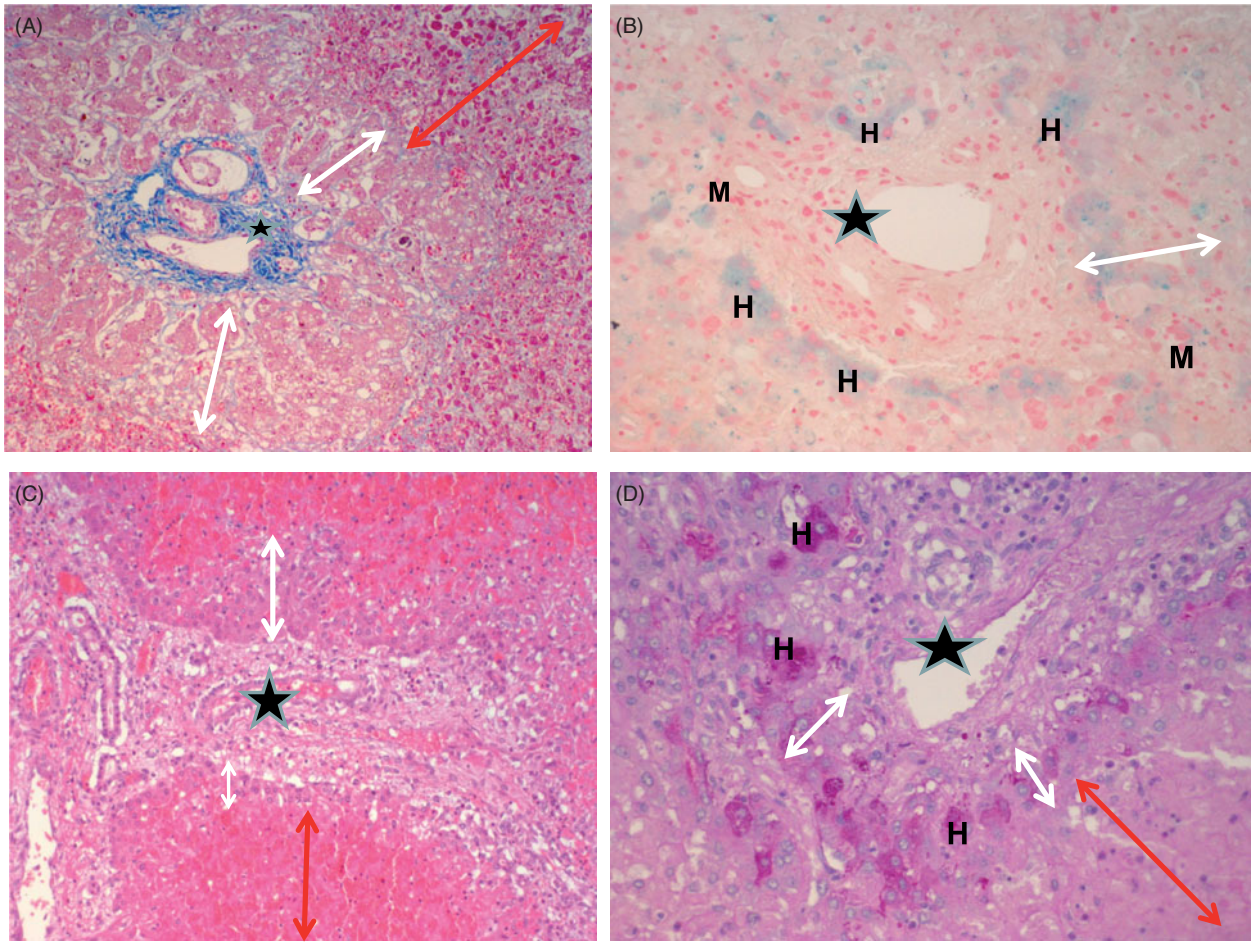

Figure 1. **(A)** Patient 1: *Post mortem* liver biopsy. The liver shows normal portal spaces (black star), sinusoidal congestion, plump hepatocytes in periportal zone 1 (close to portal vein ramification and terminal branches of afferent vessels) (white arrow) and extensive necrosis in zones 2 (the triangular area centred around the parenchymal lobule) and 3 (the centrolobular zone around the centrolobular vein) (red arrow) (Masson trichrome, 10X). **(B)** Patient 2: percutaneous liver biopsy. Vital hepatocytes (H) in periportal zone (white arrow). Small iron deposits are present both in macrophages (M) and in periportal hepatocytes (H). (Prussian blue, 20X). **(C)** Patient 3: *Post mortem* liver biopsy. Extensive hemorrhagic hepatic necrosis: a rim of hypotrophic hepatocytes is visible in central zone 1 (white arrow) and extensive hemorrhagic necrosis in peripheral zone 2, (red arrow) (HE, 20X). **(D)** Patient 3: *Post mortem* liver biopsy. PAS staining highlights scattered vital hepatocytes (H, deep violet) in the periportal space (white arrow) among extensive parenchymal necrosis (red arrow); note that necrotic hepatocytes are not labeled with PAS (PAS, 20X).

first days of life. Liver biopsy showed massive centrolobular necrosis with periportal sparing. Echovirus 11 (HEV-B, serotype E11) was identified in blood. Maternal blood and stool were negative for HEV. The infant died at 12 days of life despite multiple transfusions, hemodynamic support, and peritoneal dialysis. The autopsy confirmed fulminant hepatitis with massive hepatocellular necrosis (Figure 1, Panel C–D), myocardial hypertrophy, congestive heart failure, and cerebral edema.

Cases 4 and 5

Two dizygotic twin siblings (one male, one female) born uneventfully at 35 weeks were hospitalized at 1 month of age for hepatosplenomegaly, hepatic cytolysis, and direct hyperbilirubinemia. Although a mild coagulopathy was found on admission, liver function tests normalized and hepatosplenomegaly resolved within 1 month with supportive therapy. Coxsackie B5 was identified in neonatal blood. The mother had no stool shedding demonstrated by PCR, but serology tests showed a 1/125 anticoxsackie B5 neutralizing antibodies titer, suggesting again maternal-fetal transmission during late third trimester of pregnancy, given anticoxsackie antibodies become undetectable 8–16 weeks after acute infection [6].

Discussion

Neonatal ALF is a life-threatening condition with high mortality rate [7,8]. Frequent causes of neonatal ALF include: (1) infections (HAV, HBV, HCV, Herpes simplex,

HHV6, CMV, EBV, Enterovirus, Adenovirus, Parvovirus B19, Dengue fever); (2) metabolic diseases (galactosemia, tyrosinemia, hemochromatosis, Wilson disease, Reye syndrome, fatty acid oxidation disorders, mitochondrial diseases); (3) hematologic diseases (familial HLH); (4) neoplastic diseases (neuroblastoma, congenital leukemia); (5) vascular diseases (veno-occlusive disease, Budd–Chiari syndrome, perinatal asphyxia); (6) autoimmune diseases (giant cell hepatitis, liver cytosol-1 antibody-mediated (LC1) hepatitis, liver/kidney microsome Type 1 antibody-mediated (LKM) hepatitis); (7) idiopathic diseases ("le foie vide", hepatic nonregeneration syndrome), among others [1]. Fulminant hepatitis is highly unusual outside the neonatal period, the most common presentation being within the first week after birth. This could be explained by poor macrophage function, Th-1 cell-mediated immunity, and by the insufficient production of interferon in immature tissue cells [9], all together contributing to explain the prevalence of preterm infants in our series. In 2013 Liu pointed out the role of specific host cell surface receptors, namely Coxackie-Adenovirus Receptors (CARs), in initiating the infectious process. CARs are expressed more in the liver than in any other tissue around birth, and decrease with increasing age. The lack of CARs expression in the placenta may help to prevent vertical transmission of the virus to the fetus [10].

Enteroviruses are small single stranded RNA viruses, belonging to the broad picornavirus family. They original classification system consists of four classes based on antigen expression: polioviruses 1–3, coxsackieviruses A1–22 and 24, coxsackieviruses B1 to B6, and echoviruses 1–7, 9, 11–27, 29–33 and 68–71. The current, genome-based classification of EVs includes four human species (HEV A–D), plus the related human rhinovirus species A–C. The majority of severe infections are caused by HEV-B [11]. HEV (including echoviruses and coxsackie viruses), although typically affecting the airway, heart, and central nervous system, can potentially lead to serious liver disease in neonates, although only a few reports show a causal relationship with ALF [2]. Each year in the USA symptomatic HEV infections affect 10–15 million subjects, 10% developing in the neonatal period, which suggests a particular susceptibility in this age group [12]. Myocarditis and/or pneumonia are commonly associated in HEV sepsis. Only 21% of infected newborns have symptoms, and just a minor subset develops severe illness. The lack of systematic diagnosis makes it difficult to estimate the real incidence and morbidity of HEV infection in this age group, but mortality can be as high as 80% when complicated by myocarditis [13]. Whereas the source of infection is most likely maternal, mechanisms of infection remain controversial. In humans, transplacental echovirus infection has been occasionally reported [14]. About 65% of women who give birth to infants with HEV infection report a viral illness episode during the last trimester, similarly to our observation. The neonatal outcomes of intrauterine coxsackie infection during the first and second trimesters are less known and include early spontaneous abortions and fetal myocarditis [2]. Evidence for ascending or contact HEV infection around birth is scant in the literature. We did not isolate HEV genome from maternal feces. We did not investigate vaginal secretions for HEV since all neonates were outborn, but several prospective human studies failed to detect HEV in the female genital tract, suggesting that ascending infections are, if anything, uncommon [15]. Placental exams were also not available being the neonates outborn. Another transmission mechanism is postnatal contact infection, as horizontal clusters have been reported in nurseries [16]. EV transmission by means of breastfeeding has also been described [17]. In this series, transplacental infection is highly likely given the presumptive timeframe of the mother's infection.

Diagnosis of HEV infection is best achieved by viral RNA PCR amplification on serum, pharyngeal secretions, stools, cerebrospinal fluid, urine, or other materials and tissues. Abundant HEV copies are present in the acute phase and viral shedding from the respiratory tract and feces continues for more than 5 weeks [18]. Serology is not a practical early diagnosis approach, given the many serotypes with no common group antigens. However, it may contribute to confirming or dating an infection once the infecting serotype has been identified by PCR, or to suggest the transmission route as in cases 4 and 5.

Liver histology is important for the differential diagnosis and for management and goals of care since, for example, liver transplantation is contra-indicated in HLH but indicated in emergency in other instances such as NH. In case of HEV-related hepatitis, the dominant features are hepatocellular damage and inflammation, prevalent in perivenular areas. At the subcellular level, hemosiderin granules are seen mainly in macrophages and in sinusoids, whereas they colocate with hepatocyte lysosomes in NH. Myocardial calcifications, ventricular aneurysms, and hepatic calcifications may also occur [19].

Therapeutic options for HEV hepatitis are scarce beside supportive therapy. Large doses of IGIV have been used in affected neonates showing favorable outcome with early administration within 3 days from

illness onset [20]. Liver transplantation has been successfully used to bypass the acute phase of the disease and allow recovery [21,22]. Pleconaril, an oral antiviral agent active on picornaviruses, has shown some potential. Abzug et al. have reported beneficial effects of pleconaril in infants with severe HEV infections, although larger trials are need in order to establish safety profile, proper regimens and clinical efficacy in reducing mortality and morbidity [23–25]. For now, the management of neonatal HEV disease remains primarily supportive. Acute mortality is elevated, but full recovery is possible. In our series, mortality related to HEV hepatitis was 40% (2/5), consistent with the rates reported in the literature [7,8].

In conclusion, HEV are relevant causative agents of ALF in neonates, and early diagnostic ALF workup protocols should include HEV, given its acute severity but its potential for recovery with proper supportive treatment. Patients with a suspected or confirmed diagnosis of ALF should be emergently transferred to highly specialized pediatric institutions, where fast and broad diagnostic testing can be performed, and where intensive care facilities, multidisciplinary and subspecialty consultants, and transplantation capacities are available in order to maximize chances of survival in case of a fulminant course.

Disclosure statement

No potential conflict of interest was reported by the authors.

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