Inherited Chromosomally Integrated Human Herpesvirus 6: An Unexpected Finding in a Septic Neonate

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Abstract: Human herpesvirus 6 (HHV-6) can integrate its genome in human chromosomes and be germline transmitted (inherited chromosomally integrated HHV-6). We report a case of chromosomally integrated HHV-6 inherited from the mother unexpectedly diagnosed in a septic neonate. Since HHV-6 has recently been included in multiplex polymerase chain reaction assays for meningitis/encephalitis, diagnosing inherited chromosomally integrated HHV-6 status is essential to avoid misdiagnosis of active HHV-6 infection and unnecessary antiviral treatment.

Key Words: human herpesvirus 6, HHV-6, inherited herpesvirus, chromosomal integration, neonatal sepsis

(*Pediatr Infect Dis J* 2021;40:74–75)

Human herpesvirus 6 (HHV-6) is a ubiquitous β-herpesvirus that has been recently classified as 2 distinct viral species, HHV-6A and HHV-6B, sharing a sequence identity of about 90%.[1] Unique among human herpesviruses, HHV-6 genome can integrate into the host germline chromatin and be transmitted in a Mendelian manner [inherited chromosomally integrated HHV-6 (iciHHV-6)][1–3] but also latently infected cells in vitro.[4] Inherited ciHHV-6 is present in approximately 1% of the population worldwide, varying from 0.2% to 2.5% in different reports.[2,5] It has been reported that 86% of HHV-6 congenital infections is attributable to iciHHV-6, while transplacental transmission of the virus following reactivation or reinfection in the mother accounts only for 14% of them.[6] The clinical relevance of iciHHV-6 is little known, although some reports suggest an association between iciHHV-6 and different diseases.[5] Certainly, this condition is rarely recognized at birth.

Herein, we report a case of an incidental detection of iciHHV-6 in a neonate hospitalized in neonatal intensive care unit (NICU) and the downstream complex workup.

CASE PRESENTATION

The patient was a male neonate affected by severe left congenital diaphragmatic hernia treated in utero with fetal endoluminal tracheal occlusion procedure. He was delivered at 32 weeks of gestational age by cesarean section, using ex utero intrapartum treatment procedure because antenatal puncture of the balloon failed. His birth weight was 1800 g (55th percentile). After removal of the balloon and endotracheal intubation, he was transferred from the delivery room to our third level NICU.

Immediately after birth, he required high-frequency oscillatory ventilation, inotropic support and inhaled nitric oxide for pulmonary hypertension. A chest radiograph confirmed the diagnosis. On the day of life (DOL) 2, he underwent surgical repair of the defect.

Subsequently, his clinical condition slowly improved. On DOL 36, he was still on mechanical ventilation, but ventilator support had been reduced, drugs for pulmonary hypertension stopped and central venous catheter removed. Against this improvement, he developed high fever (temperature >38°C) together with tachycardia, with no other new clinical abnormality. A sepsis diagnostic workup, including a lumbar puncture, was performed, and empiric broad spectrum antibiotic therapy (amikacin plus vancomycin) was started. Blood count revealed leukocytosis (45,760 white blood cells/mm^3 with 74.4% neutrophils). C-reactive protein (CRP) was 14.06 mg/dL (normal value for age <1 mg/dL). Cerebrospinal fluid (CSF) biochemical characteristics were normal, with glucose level of 67 mg/dL (CSF/serum glucose ratio of 0.78), protein level of 83 mg/dL, 8 white blood cells/mm^3, and 0 red blood cells/mm^3. CSF specimen was tested using multiplex real-time polymerase chain reaction (PCR) assay to detect common pathogens, including herpes simplex virus 1, herpes simplex virus 2, varicella-zoster virus, enterovirus, parechovirus, cytomegalovirus, HHV-6, *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Escherichia coli*, *Listeria monocytogenes* and *Cryptococcus neoformans*. Multiplex PCR assay on CSF was negative for all pathogens except for HHV-6. Quantitative PCR (qPCR) testing for HHV-6 on the same specimen yielded 1202 copies/mL of HHV-6 DNA. Based on this result, intravenous ganciclovir (6 mg/kg per dose twice daily) was started, awaiting for confirmation of HHV-6 infection. The next day CRP raised to 18.72 mg/dL, and blood culture came back positive for *Enterobacter cloacae*. Vancomycin was stopped, and meropenem was added to amikacin, pending the result of CSF culture.

Considering unlikely that the patient could have a bacterial late-onset sepsis together with a HHV-6 infection, patient's blood was tested for HHV-6 by qPCR. PCR analysis yielded HHV-6 DNA both in plasma and serum, respectively, 239,555 and 187,672 copies/mL. With persisting doubts, expert virologists were consulted who suggested the patient might have iciHHV-6 rather than an acute viral infection. A whole blood sample and a hair follicle sample of the infant were collected on DOL 40 and analyzed. Whole blood qPCR yielded 46.05 million copies/mL of viral DNA, providing a high level of suspicion for iciHHV-6. DNA qPCR testing on the hair follicles was positive (144,630 copies/10^5 cells), definitively confirming the diagnosis of iciHHV-6. At the same time, PCR assay for HHV-6 DNA was retrospectively performed on patient's dried blood spots routinely collected on DOL 3 for screening of metabolic and genetic disorders (Guthrie-card). PCR assay was positive, corroborating a congenital rather than a postnatal infection. Ganciclovir was immediately stopped. In the next days, whole blood

Accepted for publication July 26, 2020.

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The authors have no funding or conflicts of interest to disclose.

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ISSN: 0891-3668/21/4001-0074
DOI: 10.1097/INF.0000000000002905

and hair samples were collected from both patient's parents. HHV-6 DNA was detected in the whole blood of the mother (147,600 million copies/mL) and in her hair follicles (192,456 copies/10^5 cells), while father's specimens were negative. The analyses performed did not distinguish between HHV-6A or HHV-6B.

Clinical conditions of the patient progressively improved, and CRP became negative on DOL 44. Meropenem was discontinued after 3 days because CSF yielded no bacteria, while amikacin was administered for a total of 10 days. The baby was discharged from the unit in good clinical conditions at 3 months and 18 days of chronologic age with the following diagnoses: congenital diaphragmatic hernia, pulmonary hypertension, late-onset sepsis caused by *Enterobacter cloacae*, and inherited chromosomally integrated HHV-6.

DISCUSSION

We report here a case of iciHHV-6 unexpectedly diagnosed in a neonate who underwent a diagnostic workup for suspected late-onset sepsis during his stay in NICU.

Inherited ciHHV-6 status is a condition little known to neonatologists and pediatricians, in contrast to primary HHV-6 infection that causes roseola infantum (exanthema subitum or sixth disease) in infants and children, typically between 6 months to 2 years of age. Both HHV-6A and HHV-6B can integrate their genomes into the telomers of host chromosome.[1–3,5]

Since subjects with iciHHV-6 have the viral DNA integrated in every nucleated cell, high viral DNA loads will persistently be found in polymorphonuclear cells and in whole blood. A HHV-6 DNAemia greater than 1×10^6 copies/mL of whole blood is strongly suggestive of iciHHV-6. Confirmation can be achieved if droplet digital PCR (ddPCR) assay performed on whole blood demonstrates 1 or more viral genomic copies per white blood cell. Body fluids containing small number of cells (ie, plasma, serum, CSF) will often be positive due to cell lysis, but HHV-6 DNAemia will be lower. Detection of HHV-6 DNA in hair follicles or nail cuticles definitively confirms a chromosomally integration of the virus. In our case, the presence of HHV-6 DNA in hair follicles of the neonate and his mother was diagnostic for iciHHV-6.[2,6]

Once iciHHV-6 is diagnosed, concerns about its clinical relevance and consequences may arise. Although many questions are still unanswered, recent studies suggest that the virus can reactivate and undergo lytic replication, especially in immunocompromised patients.[1–3,5] Reactivation of the integrated virus has also been documented in pregnant women with subsequent transplacental transmission of the virus.[6] Regardless, the causal relationship between iciHHV-6 and long-term morbidities, such as heart disease or central nervous system diseases, is still to be elucidated.

Meanwhile, we believe that knowledge of iciHHV-6 condition by neonatologists and pediatricians is desirable to not overreact to unexpected laboratory findings. As HHV-6 has recently been included in multiplex PCR assays for meningitis/encephalitis and about 0.8%–1% of newborns are iciHHV-6 carriers, incidental positivity of CSF for HHV-6 could occur quite frequently. Therefore, recognizing iciHHV-6 status would allow neonatologists and pediatricians to avoid misdiagnosis of active HHV-6 infection and consequently unnecessary antiviral treatment.

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