Lower-Limb Hypoplasia Due to Intrauterine Infection with Herpes Simplex Virus Type 2: Possible Confusion with Intrauterine Varicella-Zoster Syndrome

A.-B. Johansson,1 A. Rassart,1 D. Blum,1 D. Van Beers,2 and Corinne Liesnard3

1Neonatal Intensive Care Unit, Hôpital Universitaire des Enfants Reine Fabiola, 2Laboratory of Virology, Hôpital Erasme, and 3Division of Virology, Hôpital St.-Pierre, Université Libre de Bruxelles, Brussels, Belgium

A neonate with lower-limb hypoplasia, cutaneous scars, bilateral chorioretinitis, and multiple brain abnormalities is presented. Intrauterine herpes simplex virus type 2 (HSV-2) infection was established on the basis of serological testing of the mother and viral cultures of the child's cutaneous lesions, obtained soon after birth. This is, to the best of our knowledge, the first case of a patient with in-utero-acquired HSV-2 infection presenting with a limb hypoplasia. It illustrates that, in addition to congenital varicella-zoster syndrome, HSV-2 infection should also be considered in patients presenting with limb hypoplasia.

In the newborn, herpes simplex virus (HSV) infection is a severe disease associated with significant morbidity and mortality. The estimated incidence is 1 case per 2000–5000 deliveries per year [1]. Herpes simplex virus type 2 (HSV-2) is responsible for 70% of all cases of HSV infection in neonates [2]. HSV infection of the newborn can be acquired in utero, intrapartum, or postnatally. In 85%–90% of cases, HSV is acquired at the time of delivery, most frequently through direct contact of the newborn with infected maternal genital secretions. Prolonged rupture of membranes could increase the risk of virus transmission to the fetus, probably as a consequence of ascending infection from the cervix. Early postnatal acquisition of the virus has been described. Transmission from a mother presenting with HSV lesions of the breast has been reported, as well as transmission of the virus to infants of seronegative mothers from fathers or relatives excreting the virus. Finally, in 5% of the cases, HSV infection is acquired in utero. The diagnostic criteria of intrauterine HSV infection consist of identification of infected infants in the first 48 h of life, virologic confirmation of infection, and exclusion of other pathologic conditions (such as other congenital infections). The classic triad of findings associated with this syndrome includes skin vesicles or scarring, lesions of the eye, and neurologic damage (frequently including microcephaly or hydranencephaly) [3]. We report a case of HSV-2 infection acquired in utero and characterized by fetal growth impairment, scarred skin, chorioretinitis, CNS involvement, and lower-limb hypoplasia (a condition generally seen in cases of varicella-zoster embryopathy and, to the best of our knowledge, not described before in association with HSV infection acquired in utero).

Case report. A 1450-g white female infant was born after 34 weeks gestation in a local primary care center. The mother, a 25-year-old woman (gravida III, para III), underwent a cesarean section because of abruptio placentae and breech presentation.

At 8 weeks, the pregnancy was complicated by a 2-week-long maternal flulike illness consisting of fever, nausea, and intractable vomiting. Laboratory evaluation revealed abnormal liver function. The results of serological testing for hepatitis A, B, and C, HIV, cytomegalovirus, toxoplasmosis, and rubella antibodies were unremarkable. The patient had no previous history of genital or oral herpes lesions. In childhood, the mother and her brother had chickenpox at the same time.

At 24 weeks’ gestation, fetal growth impairment was discovered by ultrasound examination, and it was confirmed at 30 weeks’ gestation. The morphologic findings were considered normal. Triple test results (i.e., results of second trimester serum screening for Down syndrome) were normal, and no further investigation was performed.

Physical examination at birth revealed that the infant was noted to have scaphocephaly, scoliotic posture, and left lower-limb hypoplasia with cutaneous inguinal and popliteal synechiae. Examination of the skin revealed scarred skin lesions in crops all over the body, involving the chest, back, abdomen, and limbs. The oral mucosa and nails were normal. Weight and length at birth were below the third percentile of Gairdner growth curves, and head circumference was at the 25th percentile. A complete clinical evaluation disclosed bilateral chorioretinitis and multiple brain abnormalities, which were shown
by MRI and consisted of ex vacuo ventricular dilatation, porencephaly, and cerebellar lobe hypoplasia (figure 1). The findings of an MRI of the lower part of the spinal cord were normal. Radiography of the left lower-limb (figure 2) showed osteopenia, delayed ossification, and a recent tibial fracture. There were no intrathoracic, intraabdominal, or cerebral calcifications. Blood chemistry values were as follows: aspartate aminotransferase, 34 U/L (normal range, 10–35 U/L); alanine aminotransferase, 7 U/L (normal range, 10–35 U/L); alkaline phosphatase, 363 U/L (normal range, 100–350 U/L); γ-glutamyl-transpeptidase, 376 U/L (normal range, 5–36 U/L); total bilirubin, 7.1 mg/dL (normal range, 1–3 mg/dL). The WBC count was 4560 cells/mm³, with 34% polymorphonuclear neutrophils, 56% lymphocytes, 6% monocytes, and 4% eosinophils. The hematocrit was 45%, the platelet count was 225 platelets/mm³, and the caryotype was normal (46XX).

The observed malformations suggested an extrinsic insult during the first or second trimester of gestation. The clinical presentation and the findings of the neonatal investigation (particularly the presence of limb hypoplasia) led us to first suspect a varicella-zoster embryopathy. The placenta showed fibrinoid deposits in the chorionic villi and in the perivillous spaces. After a 3-day course of phototherapy for hyperbilirubinemia, crops of bullous vesicular lesions developed rapidly within the scarred lesions over the entire body (figure 3). HSV-2 grew <24 h after fluid samples obtained from the lesions were inoculated on MRC5 cells in shell vials; its identity was confirmed with use of an anti-HSV-2 monoclonal antibody (HSV1/2 typing DFA kit; Chemicon International). The virus was also isolated from skin samples obtained by biopsy. Microscopic examination of a skin lesion showed intraepithelial vesicles with numerous epithelial ballooned cells and an inflammatory infiltrate rich in polymorphonuclear cells, typical of herpesvirus infections (figure 4). Electronic microscopy demonstrated typical herpeslike particles within the keratinocyte nucleus (figures 5 and 6).

The child experienced apneas as a result of severe brain damage and died on the ninth day of life. Autopsy was not performed. Using PCR analysis, HSV-2 was detected in a CSF sample obtained postmortem. A percutaneous liver biopsy sample did not show any sign of HSV hepatitis, although HSV-2 grew from a culture of the liver sample. No maternal serum samples were available that were obtained before gestation, but...
maternal serum samples obtained after the febrile episode were retrospectively investigated for the presence of HSV antibodies (human HSV IgG or IgM) and varicella-zoster virus (VZV) antibodies (human VZV IgM or IgG). HSV-2 IgM antibodies were present at the 11th week of gestation but were no longer detectable at the 24th week of gestation. Maternal HSV-2 IgG titers increased 3.8-fold between the 11th and 24th week of gestation. Results of tests performed on samples of maternal serum for VZV IgM antibodies were negative, and VZV IgG titers were stable (table 1). HSV-2 and VZV IgG were detected in serum samples obtained from the child at birth, but HSV-2 and VZV IgM were not.

**Discussion.** Intrauterine HSV infection is a rare disorder and accounts for 5% of HSV infections in newborns. Among HSV infections acquired in utero, 90% of those with an identified type are due to HSV-2 [4]. Risk factors associated with intrauterine transmission of the virus have not been identified. Both primary and recurrent maternal infection can result in congenital infection [5]. The “flulike syndrome” the mother experienced at 8 weeks’ gestation probably repre-
presented a primary HSV-2 infection, given that systemic symptoms, mild hepatitis, and HSV IgM antibodies were present. However, this diagnosis was made retrospectively, illustrating the fact that maternal infection is frequently unrecognized [6]. Fifty-nine percent of live newborns infected in utero are born prematurely, 85% are born with low birth weight, and 36% are born with short stature [4]. Signs and symptoms of intrauterine infection are manifest within the first 48 h of life, whereas in intrapartum-acquired infections, skin and brain manifestations of the disease appear at 1 week and 2 weeks of age, respectively (or even later) [1]. Cutaneous involvement is present in 94% of cases of intrauterine HSV infection, eye lesions (principally chorioretinitis) are present in 59%, and brain anomalies (especially microcephaly and hydranencephaly) are present in 79% [7]. The characteristic clinical triad—associating skin, eye, and brain involvement—is present in 39% of cases. Abdominal, thoracic, and cerebral calcifications may be present. At birth, scarred skin lesions are observed and vesicular lesions appear (usually within 48 h), reactivated by the stress of delivery and frequently localized in areas of the skin that are subjected to pressure [1, 4].

In our case, phototherapy could have played a role in the...
reactivation of the skin vesicles; alternatively, cutaneous involvement could have been atypical or delayed. Fagnant and Monif [8], using stringent criteria for intrauterine HSV infection, selected 15 cases from the literature. Requirements for inclusion comprised evidence of some effect on embryogenesis, virologically and/or histologically demonstrated herpetic infection within 24 h of membrane rupture, and evidence of viral placentalitis. Among these 15 cases, only 10 were associated with the presentation of a cutaneous involvement, including 1 atypical rash. In 3 of these 10 cases, typical vesicles became apparent on day 4 or 5 after birth. The heterogeneity of the cutaneous manifestations leads to differential diagnosis, including bacterial and other viral infections, with pathogens such as VZV, enterovirus, cytomegalovirus, as well as other disorders, including intrauterine aplasia cutis, epidermolysis bullosa, and incontinentia pigmenti [9].

Our neonate presented with a limb hypoplasia, a condition never described, to the best of our knowledge, in association with a case of intrauterine HSV infection. This anomaly is part of the fetal varicella-zoster syndrome, consecutive to a maternal primary VZV infection during the first part of pregnancy. This syndrome has common features with intrauterine HSV infection. Infants with this syndrome are generally small for gestational age and present cicatricial skin lesions with dermatomal distribution, neurologic abnormalities (such as cortical atrophy, microcephaly, or myoclonic seizures), ophthalmologic anomalies (such as chorioretinitis, microphthalmia, or optic atrophy), or skeletal sequelae (such as limb hypoplasia or equinovarus). Limb hypoplasia is probably due to in utero cicatricial cutaneous VZV lesions causing synchiae and/or neurologic VZV involvement, with denervation of the limb resulting in diminution of muscle mass and bone growth. Higa et al. [10] suggested that VZV-related bone malformations, such as limb hypoplasia and skin lesions that show a dermatomal distribution, are not related to the initial fetal viremia but to the in utero reactivation of the virus. After a latent period in the ganglia of the posterior roots of the spinal cord, the virus reactivates and replicates along the nerve roots, resulting in cutaneous, nervous, and musculoskeletal lesions. One can suspect that, in our case, a similar mechanism was responsible for the limb hypoplasia.

As with all other herpesviruses, HSV established latency in the organism after primary infection. Animal models and clinical observations suggest that mucosal inoculation of the virus results in infection of sensory nerve endings and transport of the virus to dorsal root ganglia. When reactivation occurs, the virus is transported down axons to mucocutaneous sites where it replicates, causing cutaneous lesions [1]. In this event, also, damage to the dorsal root ganglia could lead to denervation of a limb and result in hypoplasia. By contrast, other features of this case, such as disseminated skin scars and the involvement of CNS, are probably related to initial fetal viremia.

The diagnosis of intrauterine HSV infection rests on the isolation of the virus, either from samples obtained from a fresh vesicle as the virus load decreases in late lesions or, if cutaneous lesions exist, from skin samples obtained by biopsy [3]. When skin lesions are present, the results of culture are positive in 93% of cases [4]. In the absence of skin lesions, the virus can be recovered from conjunctiva, pharynx, or CSF samples [3]. Absence of specific IgM in this neonate can be explained by the fact that the fetal infection occurred too early in the pregnancy for the fetus to synthesize IgM; the fetal synthesis begins at ~6 months of gestation [11]. Moreover, the limb hypoplasia we observed could indicate that the virus reactivates in utero, suggesting that lack of appropriate immune response to the virus in a severely infected fetus or immune tolerance toward the virus are other possible explanations for the absence of IgM. PCR detection of HSV DNA is a useful, rapid technique with good sensitivity and specificity, particularly for the detection of the virus in CSF samples (culture of which often yields no pathogen) [12]. Early antiviral treatment is recommended in cases of neonatal infection because it decreases risk of mortality and reduces the frequency of neurologic sequelae [3]. However, there is little experience with cases in which HSV infection has been acquired in utero. Antiviral treatment would probably have no effect on established irreversible CNS lesions, such as those found in cases of microcephaly and hydranencephaly, yet some beneficial effects could be expected on reversible lesions as a result of preventing ongoing viral replication.

In summary, we described a neonate with intrauterine HSV-2 infection who presented with limb hypoplasia, mimicking intrauterine varicella-zoster syndrome. This differential diagnosis should be kept in mind in the presence of limb hypoplasia.
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