CONGENITAL ECHO VIRUS INFECTION—MORPHOLOGICAL AND VIROLOGICAL STUDY OF FETAL AND PLACENTAL TISSUE*

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SUMMARY

A prospective study of 78 pregnant women was undertaken to detect maternal enterovirus infection. Maternal faecal specimens and blood samples, placental and fetal tissue were taken for viral study, electron microscopy, histochemistry, and morphological examination.

We present the post-mortem findings in three fetuses whose maternal infection was detected before delivery by isolation of ECHO virus type 33 and type 27 from faecal specimens and/or placental and fetal tissues. The morphological aspects were similar in all cases and included an acute infection of the placenta and hypoxic/hypotensive injury to fetal organs. In one case, viral particles were detected by electron microscopy of the fetal liver.

This series of cases of intrauterine ECHO virus infection confirms the potential gravity of such infection during pregnancy and the need to prevent enteroviral disease.

KEY WORDS—ECHO virus, congenital infection, morphological and virological study, placental inflammation.

INTRODUCTION

Although in the late 1950s the effects of ECHO virus on the human fetus (spontaneous abortion and congenital defects) were already considered the most important consequence of infection with this group of viruses, there have been few recorded cases of direct maternal to fetal infection. Moreover, little is known about risk factors, sources of infection, modes of transmission, and other epidemiologic features of perinatal ECHO virus infection. There is serological and virological evidence that ECHO virus can cross the placenta to reach the fetus. Modlin reviewed the literature and concluded that the mechanism of direct transmission of ECHO virus from mother to fetus was likely. Some infants become infected in utero before delivery and others are infected by exposure to maternal sources of the virus in the peripartum period.

This paper is based on the morphological study of placental and fetal tissues obtained at post-mortem examination from which ECHO virus was isolated. This material was studied by light, electron microscopy, immunofluorescence, and immunohistochemical techniques.

METHODS

In a prospective study of enterovirus infections in pregnancy, 78 pregnant women were investigated: three pregnancies produced two abortions and one full-term stillbirth. These three cases were investigated further.

Viral studies

Placentae, fetal organs, and stool samples were processed to obtain tissue extracts and faecal

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suspension. Each extract was inoculated into cell cultures (VERO, LLC-MK2, HEP2). The positive extracts, suspected of containing enteroviruses because of their cytopathogenic effects, were titrated and the viruses identified in the LLC-MK2 lineage, using Melnick’s standard sera against enteroviruses.

SeroLOGY

Maternal blood samples collected before and after abortion were tested against the respective isolated virus, by the neutralization test in cell culture LLC-MK2.

Indirect immunofluorescence and immunoperoxidase techniques (IF and IP)

These were performed on paraffin-embedded tissue samples. The sections were deparaffinized with xylene for 20 min, washed in buffer, and processed for IF and IP tests following standard techniques, using virus specific and purified rabbit antiserum [anti-rabbit fluorescein (Behring) or peroxidase (Sigma) labelled sera].

Electron microscopy

Fresh tissue samples were fixed immediately after delivery in 2.5 per cent glutaraldehyde (Merck) for 2 h in pH 7.2 cacodylate-sucrose buffer, post-fixed with 1 per cent osmium tetroxide (Sigma), dehydrated in a series of alcohols and embedded in polyethylene resin (Resina). The sections were stained with uranyl acetate and lead citrate (Merck) and observed in a Philips EM 301 electron microscope.

Morphological study

The autopsy material and placenta were stained with haematoxylin and eosin. Selected tissues were stained with phosphotungstic acid–haematoxylin, periodic acid Schiff (PAS), Gomori’s one-step trichrome, Gomori’s method for reticulin, and Weigert’s method for elastic fibres.

CASE REPORTS

Case 1

A 25-year-old graveda 3, para 2, was delivered of a female fetus at 17 weeks. Her pregnancy had been uncomplicated. As part of the prospective study, ECHO virus type 33 was isolated from maternal faecal specimens in the week of the abortion. Serological examination of paired maternal sera (fourth and fifth month, 1/8–1/640) showed acute infection by ECHO virus type 33.

At autopsy, the non-macerated fetus weighed 128 g, with a length of 19 cm and a foot length of 2.5 cm. No external or internal malformations were found; visceral congestion and focal haemorrhagic areas were seen in the liver and adrenal parenchyma. Virus isolation was attempted from the placenta only; the same type of virus was isolated.

Case 2

A 33-year-old graveda 7, para 2, was delivered of a male fetus at 21 weeks. ECHO virus type 33 had been isolated from maternal faecal specimens 10 days before the abortion and the serological examination of paired sera (fourth and fifth month, 0–1/640) confirmed the infection by this type of virus. Five hours before delivery, the patient had presented with a high temperature, chills, bloody vaginal discharge, uterine contractions, and low back pain.

At autopsy, the fresh stillborn fetus weighed 150 g, with a length of 20 cm and a foot length of 2.2 cm. No external or internal malformations were observed. In the hepatic and adrenal parenchyma, visceral congestion and haemorrhagic foci were seen. Virus isolation was achieved from placenta, brain, liver, kidneys, and adrenal glands.

Case 3

A 33-year-old multipara was delivered of a female infant at 38 weeks’ gestation by Caesarean section for suspected abruptio placenta. Virus isolation was not attempted on maternal faecal specimens and maternal serological tests were not performed.

At autopsy, the fresh stillborn infant weighed 3260 g with a length of 49 cm and a foot length of 7.5 cm. No external or internal malformations were detected. A large retroplacental haemorrhage was found as well as visceral congestion and petechial haemorrhages. ECHO virus type 27 was isolated from the placenta, heart, kidney, brain, and liver.

RESULTS

Pathological findings

The histological findings were similar in both fetuses and infant and are shown in Table I. The
liver and adrenal glands were abnormal in all cases. The liver was enlarged in all cases, presenting extensive areas of red discoloration; in the infant (case 3), there was mild lymphocytic infiltration of the portal spaces with periportal fibrosis. In the liver and adrenal glands of case 2, fibrin thrombi were seen. The adrenal glands showed focal areas of cortical necrosis or haemorrhagic necrosis; similarly, focal areas of haemorrhagic necrosis were always present in the peripheral muscle. In all placentae, a septic type of inflammation was observed with acute villitis and intervillitis (Fig. 1); in case 3, in which the placenta was at term, besides the areas of acute inflammation, proliferative-necrotic and reparative types of villitis were seen (Fig. 2).

Virological findings

In cases 1 and 2, ECHO virus type 33 was isolated from maternal faecal specimens just before abortion and from the placentae and fetal organs. ECHO virus type 27 was isolated from the organs in case 3. The neutralization test in cell culture LLC-MK2 against the viruses showed positive maternal serological conversion in cases 1 and 2. The presence of viral antigens from ECHO viruses 33 and 27 in tissues was confirmed by immunofluorescence and immunoperoxidase. Morphological alterations in the placentae and fetal organs, possibly related to viral replication, were demonstrated by electron microscopy; viral particles were observed in the liver parenchyma in case 2 (Fig. 3).

DISCUSSION

Although the spectrum of disease produced by ECHO virus is not fully defined, the importance of enteroviral disease in the human fetus and the newborn infant is growing. Modlin¹ considers that the pathogenic effects of the ECHO viruses may be a more important cause of fetal and neonatal mortality than previously suspected. The high prevalence of this group of viruses and their ability to produce infection of minor severity with viraemia explain their potential for causing fetal damage. If illness in the mother is associated with viraemia, maternal disease carries a greater risk of transplacental transmission of virus to the fetus and subsequent illness in the perinatal period.

In this study, there were three cases of intrauterine ECHO viral disease in non-mucosal sites, due to ECHO virus 33 or 27. Pregnancy ended in the second trimester as late fetal abortions in two cases and at full term in one. The maternal infection in all three cases was subclinical even though the virus was isolated from maternal faecal specimens and there was maternal seroconversion; the fetal infection was established by isolation of the virus from

Table I—Summary of post-mortem findings in three cases of intrauterine ECHO virus infection

<table>
<thead>
<tr>
<th>Placenta</th>
<th>Liver</th>
<th>Adrenal glands</th>
<th>Muscle</th>
<th>Lungs</th>
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<tr>
<td>Case 1</td>
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<tr>
<td>Acute placentitis</td>
<td>Focal cytolysis</td>
<td>Focal cytolysis</td>
<td>Interstitial haemorrhage</td>
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<tr>
<td>IV Thrombosis</td>
<td>Haemorrhagic necrosis</td>
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<td>IV Leucostasis</td>
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<td>Acute villitis</td>
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<td>Case 2</td>
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<tr>
<td>Acute placentitis</td>
<td>Focal cytolysis</td>
<td>Focal haemorrhagic necrosis</td>
<td>Interstitial haemorrhage</td>
<td>NL</td>
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<tr>
<td>IV Thrombosis</td>
<td>Haemorrhagic necrosis</td>
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<td>IV Leucostasis</td>
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<tr>
<td>Fibrin thrombi</td>
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<td>Case 3</td>
<td></td>
<td>Portal fibroplasia</td>
<td>Focal cytolysis</td>
<td>NL</td>
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<td>Acute placentitis</td>
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<td>Haemorrhagic necrosis</td>
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<td>IV Thrombosis</td>
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<td>Pneumonitis</td>
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<td>IV Leucostasis</td>
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<td>Acute, proliferative-necrotic and reparative villitis</td>
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</table>

IV = Intervillous; NL = no lesions.
viscera and placentae. The fetuses and infant probably died during labour, as blood was observed inside the bronchi and autolysis was not present, even on microscopy.

In the cases of this series, the pathological findings were similar to the previously reported fatal perinatal infection with ECHO virus. The most striking morphological features were focal hepato-adrenal haemorrhage and necrosis. There was evidence of disseminated intravascular coagulation with focal haemorrhage in many organs associated with sinusoidal fibrin thrombi. As Kurnetz et al., Berry and Nagington, and Speer and Yawn have all stated, the isolation of ECHO virus from many viscera reflects viraemia; it is, however, difficult to separate cytopathic effect from damage secondary to disseminated intravascular coagulation.

In our review of the literature, we found little information on the placental morphology in intrauterine enteroviral infection. In our cases, gross examination disclosed areas of hypotransparency of the membranes of the chorionic plate and the presence of blood clots adherent to the basal plate. On microscopy, a septic type of inflammation of villous tissue was found. In the light of our present understanding of the pathogenesis of congenital ECHO virus infection, it seems that this type of virus causes a more acute reaction in villous tissue than that described in intrauterine rubella. Allied to the necrotic villous and decidual tissues there were areas of detachment of the placenta, which, in two cases, were the cause of vaginal haemorrhage. In one case, there was suspicion of abruptio placenta, which prompted a Caesarean section. Although there are some associated factors with abruptio placenta, the mechanism which causes the placental separation is unknown; in this respect, it is possible that an acute reaction of the placental tissue to ECHO virus could cause focal detachment of the organ and bloody vaginal discharge. Modlin found that 5 of 22 mothers, who gave birth to infants who developed fatal perinatal ECHO virus infection, had had
a similar illness late in pregnancy and three had had emergency Caesarean sections performed.

In our study, isolation of ECHO viruses from placentae could be interpreted as faecal contamination, but finding the same viruses in fetal tissues suggests that infection probably occurred by the transplacental route. Although in one case serological evidence of infection was not obtained, isolation of virus from several infant tissues leaves little doubt about the infection. As Butterfield et al. stated, the presence of lesions in several organs emphasizes the versatile organotropism of ECHO viruses.

These three cases of intrauterine ECHO virus infection confirm both the potential gravity of this infection during pregnancy and thus the need to prevent enteroviral disease in pregnant women.

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REFERENCES