Acta virol. **30** : 249—255, 1986

EFFECT OF MATERNAL VARICELLA-ZOSTER VIRUS INFECTION ON THE OUTCOME OF PREGNANCY AND THE ANALYSIS OF TRANSPLACENTAL VIRUS TRANSMISSION

J. TRLIFAJOVÁ, *R. BENDA, Č. BENEŠ

Institute of Hygiene and Epidemiology, 100 42 Prague
and *Military Institute of Hygiene, Epidemiology and Microbiology, Prague, Czechoslovakia

Received January 28, 1985; revised September 27, 1985

Summary. — In result of 20 pregnancies complicated by varicella 11 healthy and 6 defective offspring were born, 2 pregnancies were aborted by the physician and one pregnancy terminated by stillbirth of 3 siblings. Laboratory investigation of 16 pregnancies has shown that transplacental transfer of varicella-zoster (VZ) virus did not occur in 13 cases (81.2%). In 3 cases transplacental transmission of VZ virus was not inconsistent with the laboratory results, clinical course, and epidemiological analysis. Intrauterine VZ virus infection was suggested in one typical case of congenital varicella syndrome and in one healthy newborn according to the presence of high serum antibody levels at the time of maternal antibody decline. In the third case, VZ virus antigen was detected by indirect immunofluorescence in the foetal skin tissue after medical abortion.

Key words: varicella-zoster virus; transplacental transmission; intrauterine infection; teratogenic effect

Introduction

Twenty pregnancies complicated by varicella were followed in our laboratory of the Institute of Hygiene and Epidemiology, Prague, with the aim to analyse the possible teratogenic effect of VZ virus in human. Our laboratory data as compared with clinical observations in the course of these pregnancies are described in the present paper.

Subjects, Materials and Methods

Clinical characterization of pregnancies. Out of 20 women of the varicella group 10 had varicella during the first trimester, 7 in the second and 3 in the third trimester of their gravidity. Varicella in first trimester resulted in congenital malformation in 5 cases and one stillbirth of 3 siblings; the pregnancy was aborted twice and two times a healthy child was born. Out of 7 women who attracted varicella in the second trimester, 6 had healthy offspring, one newborn had congenital malformation. All 3 pregnancies with varicella in the 3rd trimester had a normal course and terminated by a regular delivery of healthy newborns.

Laboratory investigations were made in 16 cases. For technical failure 2 children with congenital disease were not examined, neither were the 3 stillborn siblings and one pregnancy which terminated by abortion. In 15 pregnancies serum antibodies were followed in the newborn. In one case foetal tissue was examined by indirect immunofluorescence.

Serological methods. Indirect haemagglutination (IHA) was made with fresh varicella-zoster (VZ) antigen (Trlifajová *et al.*, 1973); occasionally the complement-fixation reaction was performed. Titres < 1 : 8 were considered for negative. To confirm or exclude intrauterine infection, antibodies in the serum of the newborn must have been determined after the decrease of maternal antibody. According to our previous results maternal antibody levels to VZ virus as determined by IHA or by radioimmunoassay (Trlifajová *et al.*, 1982) in the offspring serum of healthy mothers considerably decreased during the first 10 months. Complete seronegativity has been found in 11—12-month-old babies. Because of the high antibody levels found in women experiencing VZ infection, maternal antibodies in babies of such women would reach negativity during the first trimester in the second year of age. At this age the positive titres of IHA antibodies to VZ virus could not be associated with maternal antibody persistence, but they should have been regarded for the sign of preceding offspring infection. Therefore, the antibody levels in this study were followed for a considerable long period after delivery.

VZ virus antigen detection in foetal tissues obtained at medical abortion was made by indirect immunofluorescence. Among the tissue fragments of the 13-week-old embryo only the limb could be recognized revealing no skin lesions. Kryostat sections were stained with human convalescent herpes zoster serum showing CF antibody titre to VZ virus of 128 and to herpes simplex virus (HSV) of 32. The human serum used as control contained no antibodies to VZ virus in dilution 1 : 8 but reacted with HSV at dilutions 1 : 16—32. Both sera were used in dilution 1 : 20, while the Sw-A-Hu IgG conjugate (Sevac, Prague) was diluted 1 : 40. The sections were contrastained with Evans-blue. The HSV-infected human embryonal lung cells (LEP) showed no positive immunofluorescence when stained with the sera in question diluted 1 : 20.

VZ virus isolation attempts were performed in LEP cells (Sevac, Prague) inoculated with 10% tissue suspensions prepared in serum-free MEM. The cells were observed for 3 weeks, but no subpassage was made.

Trypsinized fragments from foetal tissues obtained at abortion were cultured and propagated to establish diploid cell lines.

IgM class antibodies were determined by indirect immunofluorescence in a part of the sera examined after excluding the rheumatoid factor.

Histological examination of formalin-fixed and paraffin-embedded material obtained at abortion was made by conventional techniques.

Results

In 13 out 15 children the decrease of IHA antibodies to VZ virus to indetectable levels excluded the possibility of intrauterine infection with this virus (Table 1). The IgM class antibodies to VZ virus as tested in the umbilical cord blood in newborns No. 5 and No. 6 and in the child No. 13 by 3 months after birth (Table 1) were negative. In the child No. 10 traces of IgM class antibodies were found, but this results should have been regarded for negative because the later repeated serological investigation in this child was consecutively negative and excluded the possibility of intrauterine infection.

In 2 out 15 children the VZ virus antibody titres were positive at intervals when the maternal antibodies are certainly absent (case No. 4 and 15). One of these 2 children had typical congenital varicella syndrome, while the other did not show any symptoms. This indicated either intrauterine VZ infection related to maternal varicella or postnatal VZ infection of the newborn. The residence of the seropositive healthy child was examined by

Table 1. Serological follow-up of children from mothers who attracted varicella during pregnancy

No.	Onset of mother's varicella in pregnancy	Serological examination of the offspring[1] at delivery	IHA antibody titre	at the age of	IHA antibody titre	Transplacental transmission
Healthy children						
1	10 weeks			12 months	neg	none
2	12 weeks			12 months	neg	none
3	14 weeks			8.5 months	neg	none
4	18 weeks			22 months	256 (CF 8)	possible
				4 years	128	
				5 years	256	
5	19 weeks	umbilical cord	128	8 months	neg	none
6	22 weeks	umbilical cord	64	27 months	neg	none
7	23 weeks			27 months	neg	none
8	24 weeks			16 months	neg	none
				37 months	neg	
9	35 weeks			12 months	neg	none
10	38 weeks, delivey on 25th day of the disease	umbilical cord	neg 1 : 32 (CF)	3 years 52 months	neg neg	none
11	39 weeks, delivey on 2nd day of the disease	day 6 day 21	neg (CF) neg (CF)	38 months	neg	none
Children with congenital defects						
12	4 weeks			31 months	neg	none
13	8 weeks	3 months	16	6 months	neg	none
				16 months	neg	
14	9 weeks			30 months	neg	
15	10 weeks			21 months	256 (CF neg)	possible
				45 months	256	

* Serum dilution reciprocal

[1] Antibodies to VZ virus
CF — complement-fixation test; IHA — indirect haemagglutination test

Table 2. Congenital defects in the followed children

No.*	Onset of mother's varicella	Other infections	Intrauterine transmission of VZ virus	Clinical signs
12	4 weeks	angina penicillin oxymycoin	none	Hydrocephaly, central hypotonic syndrome, epilepsy, oligophrenia, psychomotoric retardation
13	8 weeks	parotitis	none	Left side lip and palatine cleft
14	9 weeks	none	none	Central hypotonia, epilepsy, oligophrenia, psychomotoric retardation
15	10 weeks	none	yes	Cicatricial skin scars, eye defects (Horner's syndrome), muscle defects, bone hypoplasia, dysphagia, psychomotoric retardation, absence of tendon reflexes (typical varicella embryopathy)
17	10 weeks	none	not tested	Dysphagia, repeated pneumonia, thigh skin vesicles (at 3 months). Several transfusions; death at age of 18 months
18	16 weeks	angina (5 months)	not tested	Sacrococcidal teratoma, death 2 days after birth

* See Table 1

the local pediatrist. From the birth till drawing the first serum sample (22 months) neither varicella nor shingles were registered in the small village, where the child was living and his parents were not avare of any contacts. This child was seropositive by the age of 5 years and has remained healthy although later on varicella contact could not have been excluded. No varicella contact with the stigmatized child (patient of Prof. Dietzsch, Dresden) has been registered since its birth until the first blood sample had been taken and the child did not attract varicella later on (Dietzsch *et al.*, 1978, 1980).

In one case embryonal tissues obtained at medical interruption in 13th week of pregnancy were investigated. Kryostat sections from the femoral area were stained by indirect immunofluorescence. VZ virus antigens were found in skin epithelial cells mainly in the basal layer of the stratified epithelium. The control section was negative. Sections from other tissue areas stained with immune serum did not show any fluorescence. No VZ virus was isolated from LEP cells inoculated with the 10% suspension of the embryonal fragments. Fibroblasts obtained from the trypsinized fragments were cultured like diploid cells for 3—11 passages. A cell line established from skin and muscle tissues propagated for 11 passages in culture was as sensitive to laboratory strain VZ virus infection as control (LEP) cells. No intranuclear inclusion bodies were found in the embryonal fragments at histological examination.

Summing up in result of 20 pregnancies complicated by varicella 11 healthy and 6 malformed children were born (Table 2). In addition, one pregnancy terminated by stillbirth of 3 siblings and another 2 by medical abortion. Laboratory findings from 16 pregnancies excluded transplacental transmission in 13 cases (81.2%), while in 3 cases (18.7%) the laboratory results, clinical symptoms and epidemiologic analysis were consistent with transplacental transmission. Intrauterine infection is supposed in one symptomless child, in one child with congenital varicella syndrome and in one pregnancy with medical interruption.

Discussion

The observed incidence of the teratogenic effect of VZ virus — namely 1 congenital defect with intrauterine VZ infection out 15 pregnancies complicated by varicella (7 out of these occurred during the first 4 months) — might be considered for fortuitous and influenced by accidental emergence of a rare varicella embryopathy.

Meyers (1974) investigated the incidence of transplacental transmission of VZ virus in children from mothers which experienced varicella during last 17 days of pregnancy as judged by the incidence of congenital varicella. Out of 46 children 11 showed congenital chicken pox (24%). If the illness of the mother occurred during the last 4 days before delivery, congenital varicella was observed in 17% of newborn. These clinical data roughly coincide with our laboratory results (18.7%). In contrast, Enders (1984) described decrease of VZ antibody levels detected by ELISA test in all 39 children, which mothers had varicella in pregnancy so that she denied

the possibility of transplacental infection. The clinical signs were not recorded in this study.

In 2 of 15 serologically investigated children persistence of high antibody levels to VZ virus beyond the age, when the presence of maternal antibodies can be certainly excluded led us to the assumption of intrauterine VZ virus infection. The possibility of postnatal acquisition of the VZ virus cannot be excluded in these cases. Inapparent VZ virus infection based on serological results, to our knowledge, has not been described till now. None of the children in question was in contact with VZ virus infection. Symptomless varicella without known contact seems improbable.

In the third case transplacental transmission was postulated due to the presence of VZ virus antigen in the skin epidermal cells from the lower extremity of the embryo removed at interruption. The possibility of cross--reaction with the herpes simplex virus antigen was minimalized as described in Materials and Methods. The possibility remains that the fluorescence was due to an antigen reaching with unknown antibodies present in the human serum. However, the women which underwent abortion due to varicella starting from the 2nd week of her pregnancy was not aware of any other exanthematic disease during that critical period. The failure to isolate the virus from cell lines established from the same embryo may be due to the localized character of skin varicella. This points to the usefulness of the above described complex approach in the diagnosis of human intrauterine infection.

The possible teratogenic effect of VZ virus was first suggested by Laforet and Lynch (1947) who described multiple congenital defects in association with maternal varicella in early pregnancy. Up to now 27 papers have been published in which 34 cases of congenital malformations are listed in newborn from mothers who attracted varicella during pregnancy. The real incidence of such teratogenic effect is not known and seems exceptionally rare. Our one case of serologically proven varicella embryopathy may be fortuitous, its frequency being fairly below of the proportion calculated from our observation group. It should also be taken into account that many symptomless seropositive babies are not registered perhaps because laboratory investigations are more often demanded in the case of an embryopathy.

Laboratory results confirmed intrauterine VZ virus infection only in one out four newborn with congenital defects. A nonspecific damaging effect of maternal varicella on the foetus cannot be excluded in the cases of these VZ seronegative congenital defects. The overall incidence of congenital malformations in our population ranges from 1—2%.

Acknowledgement. The authors thank Prof. MUDr. Bednář DrSc. from Dept. Pathology, Med. School, Charles University, Prague for histological examinations and MUDr. Fraňková CSc. from the Institute of Medical Microbiology and Immunology, Prague, for testing of IgM class antibodies.

References

Dietzsch, H. J., Rabenalt, P. and Trlifajová, J. (1978): Varizellen-embryopathie. *Dt. Gesundh.--Wes.* **33**, 410—413.

Dietzsch, H. J., Rabenalt, P., and Trlifajová, J. (1980): Varizellen-embryopathie. Klinische und serologisch Verlaufsbeobachtungen. *Kinderärztl. Prax* **3**, 139—145.
Enders, G. (1984): Varicella-zoster virus infection in pregnancy. *Prog. med. Virol.* **29**, 166—196.
Laforet, E. G., and Lynch, Ch. L. (1947): Multiple congenital defects following maternal varicella. *N. Engl. J. Med.* **236**, 534—537.
Meyers, J. D. (1974): Congenital varicella in term infants: risk reconsidered. *J. infect. Dis.* **129**, 215—217.
Trlifajová, J., Pokorný, J., Ryba, M., and Střížová, V. (1973): Indirect haemagglutination reaction with varicella-zoster virus antigen. *J. Hyg. Epidem. (Praha)* **17**, 26—36.
Trlifajová, J., Pokorný, J., Švandová, E., and Ryba, M. (1982): Study of persistence of maternal antibodies to varicella-zoster virus by indirect haemagglutination, with result control by radioimmunoassay. *J. Hyg. Epidem. (Praha)* **26**, 65—73.

Explanation of Figures (Plate XXIV):

Figs 1—2. Kryostat section from the embryonal skin obtained by abortion at 11 weeks after onset of mother s varicella. Positive immunofluorescence of VZ virus antigens in the basal epidermis (indirect staining).