

THE FREQUENCY OF TRANSPLACENTAL VARICELLA-ZOSTER VIRUS TRANSMISSION AFTER PRIMARY MATERNAL INFECTION

UČESTALOST TRANSPLACENTARNE TRANSMISIJE VARICELLA-ZOSTER VIRUSA NAKON PRIMARNE INFEKCIJE TRUDNICA

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Abstract

Introduction: Varicella-Zoster Virus (VZV) is a very important cause of perinatal infections. The greatest risk for perinatal infection is a primary infection during pregnancy with possible transplacental transmission of the virus and the onset of congenital varicella syndrome or neonatal varicella.

Aim: The objective of this study was to evaluate the frequency of transplacental VZV transmission in pregnant women in our population.

Material and Methods: The study included 50 samples of amniotic fluid of pregnant women with suspected fetal varicella or VZV vertical transmission. The presence of the virus was determined by the PCR method with primers for DNA VZV gene 71. The procedure involved the extraction of DNA, PCR and gel electrophoresis for visualization of specific PCR products.

Results: The presence of VZV was demonstrated in 14% (7/50) of the amniotic fluid samples. Transmission of the virus was detected in all three trimesters of pregnancy. Statistical significance was not shown between the referral diagnosis, age of pregnant women and gestational age of the pregnancy and VZV positivity.

Conclusion: The results of this study showed a low prevalence of VZV vertical transmission during the eight-year period, the lowest in the second trimester, and no statistical significance in accordance with diagnosis, gestational age, and VZV positivity. Screening is significant for VZV during the generative period of women. Those who are seronegative for VZV during pregnancy are at high risk for vertical transmission and are indicative for immunoglobulin therapy. If intrauterine infection is confirmed, adequate antiviral therapy is required.

Keywords:

VZV infections,
fetal varicella,
vertical transmission

Sažetak

Uvod: Varičela zoster virus (engl. *Varicella-Zoster Virus*, VZV) je veoma značajan uzročnik perinatalnih infekcija. Najveći rizik za nastanak perinatalne infekcije je primarna infekcija u trudnoći, kada može doći do transplacentarne transmisije virusa i pojave kongenitalnog varičela sindroma ili neonatalne varičele sa mogućim fatalnim posledicama po fetus ili novorođenče.

Cilj: Cilj ove studije je ispitivanje učestalosti transplacentarne transmisije ovog virusa kod trudnica u našoj populaciji.

Materijal i metode: Istraživanje je obuhvatilo ispitivanje 50 uzoraka plodove vode trudnica sa sumnjom na fetalnu varičelu, odnosno vertikalnu transmisiju VZV sa majke na plod. Prisustvo virusa je dokazivano metodom polimerazne lančane reakcije (PCR) uz primenu prajmera za VZV DNK gen 71. Procedura je uključivala: DNK ekstrakciju, PCR i gel elektroforezu za vizuelizaciju specifičnih PCR proizvoda.

Rezultati: Prisustvo VZV je dokazano u 13% (7/50) uzoraka plodove vode. Transmisija virusa je dokazana u sva tri trimestra trudnoće. Nije pokazano postojanje statističke značajnosti između uputne dijagnoze, starosti trudnica i gestacijske starosti trudnoće i VZV pozitivnosti.

Zaključak: Rezultati ove studije ukazuju na nisku prevalencu VZV transmisije tokom perioda od 8 godina i bez značajnosti uputne dijagnoze, gestacijske starosti i VZV pozitivnosti. Skrining je značajan za VZV tokom generativnog perioda žena. One koje su seronegativne na VZV tokom trudnoće izložene su velikom riziku za vertikalni prenos i indikativni su za terapiju imunoglobulinom. Ako se potvrdi intrauterina infekcija, potrebna je odgovarajuća antivirusna terapija.

Ključne reči:

VZV infekcija,
fetalna varičela,
vertikalna transmisija

Introduction

Varicella-Zoster Virus (VZV) is a DNA virus which belongs to the family *Herpesviridae*, *Alphaherpesvirinae* subfamily, genus *Varicella Virus*. Like other herpes viruses, VZV establishes a persistent latent infection (1).

The virus is spread by respiratory route or by direct contact with vesicle lesions on the skin. After primary infection, which can manifest in the form of chickenpox (varicella) with the appearance of vesicular rash on the skin and mucous membranes, VZV establishes latency in sensory ganglia. Varicella most commonly occurs in childhood and presents as a mild disease. However, if the primary infection occurs in adulthood, varicella can be a serious disease with systemic manifestations. Reactivation of the virus manifests itself in the form of herpes zoster with the appearance of vesicular rash, which is identical to that of varicella, usually along one or more dermatomes—region of innervation of one ganglion (1). Varicella-Zoster Virus is a very significant cause of perinatal infection. During pregnancy, the virus may pass through the placenta as a result of the presence of the virus in the blood of the mother during primary infection. The largest number of *in utero* infection is asymptomatic, but varicella congenital syndrome may develop with the appearance of embryopathy with scarred skin, limb hypoplasia, microcephaly, cataracts, chorioretinitis and micro-ophthalmia. If a mother gets ill near term, neonatal varicella may occur due to a lack of protective antibodies, and the immaturity of the immune system of newborn. Neonatal varicella manifests in the form of vesicular and/or hemorrhagic lesions on the skin and mucous membranes, as well as in the form of systemic diseases (pneumonia, hepatitis,

encephalitis, coagulopathy) with a high mortality rate (2). Routine diagnosis involves the use of the serologic diagnosis, the detection of IgM antibodies in the blood. However, in cases of infection *in utero*, as well as neonatal infection, the most sensitive and specific method is the detection of virus DNA using molecular methods, most commonly polymerase chain reaction (PCR). Viral nucleic acid is evidenced in samples of the amniotic fluid, fetal blood, skin lesions swabs, cerebrospinal fluid, tissue, etc. (2,3). Intrauterine prevention of neonatal infections involves the use of VZV immunoglobulin having very high efficiency in the prevention of infection development (2). The aim of this study was to evaluate the frequency of transplacental transmission of the virus in pregnant women in the population where there was a suspicion of fetal varicella or vertical transmission of VZV from mother to fetus.

Material and methods

Patients and clinical samples

In the period from January 2008 to December 2016, at the Virology Laboratory of the Institute of Microbiology and Immunology, Faculty of Medicine University of Belgrade, samples of amniotic fluid were examined from 50 patients, aged 16 to 35 years (27.45 ± 4.97) where there was a suspicion of fetal varicella or vertical transmission of VZV from mother to fetus. Anamnestic data on the age of the patient, the gestational age of pregnancy weeks and referral diagnosis of suspected VZV transmission were taken from the documentation that came with the sent samples.

Detection of VZV

The presence of the virus in samples of amniotic fluid was confirmed with the use of PCR methods for the detection of viral DNA. The procedure consisted of 1) extraction of virus DNA, 2) the PCR method and 3) gel electrophoresis, for the visualization of obtained PCR products.

Extraction of virus DNA

Samples of amniotic fluid were first centrifuged for 10 minutes at 5000 rpm and resulting sediment was used for DNA extraction. The DNA extraction was done by using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA). Extraction was done according to the protocol of the manufacturers, with certain modifications. The resultant DNA extract was used for PCR reaction.

PCR for the detection of VZV DNA

Primers for the gene 71 of VZV DNA were used for the detection of VZV in the analyzed samples. Those primers are FW - 5' CGA GTC CTG AGC ACG ATC TA 3' and REV - 5' TTT GGA GACCTA GCA AGC TTC GTT C 3'(4). The total volume of PCR reaction mixture for each sample was 25 µl and was composed of 12.5 µl of Taq PCR Master Mix (QIAGEN, Valencia, CA, USA), 1 µl of FW primer, 1 µlREV primer, 5.5 µl of deionized water and 5 µl of the extracted DNA. The PCR program for gene 71 of VZV DNA was the following: (a) Initial denaturation 95 °C - 5 minutes; (b) the replication cycle which was repeated 40 times, and was carried out in three steps: denaturation of DNA - 30 seconds at 94 °C, the binding of the primers - 30 seconds at 58 °C and extension of the DNA chain - 1 minute at 72 °C; (c) an additional DNA extension chain 20 minutes at 72 °C, and (d) terminating the reaction at 4 °C.

Visualization of PCR products

Gel electrophoresis on a 2% agarose gel with added ethidium bromide (10 mg/ml) was used for visualization of the PCR product. Using standard DNA of 100 bp, the size of the resulting PCR product was determined, which for the reproduction of the VZV gene using the mentioned primers is 304 bp.

Statistical analysis

The statistics and data processing were done with the use of the Easy R program (SPSS For Windows XP - 10). Methods of descriptive statistics were used, in which the data were firstly processed in the Excel program, and afterwards, the measures of central tendency were calculated and presented in tables and graphics. Fisher's non-parametric test was used for the determination of statistical significance. Statistical significance considered the p value to be less than 0.05.

Results

Out of the total number of samples of the amniotic fluid, in 14% (7/50) the presence of VZV DNA was determined (**figure 1**).

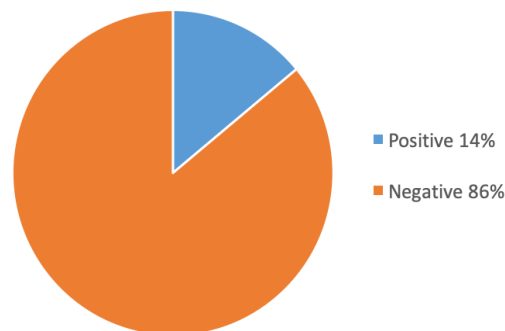


Figure 1 – Frequency of positive and negative VZV DNA PCR products

Medical history was available for 36 pregnant women. The majority of pregnant women (91%) was with the referral diagnosis of status post varicella, while pregnant women with the diagnosis Varicella (6%), and congenital anomalies (3%) showed a significantly lower frequency. Although all VZV positive samples were precisely the group with the diagnosis *status post varicella*, the existence of connections between the referral diagnosis and VZV DNA positivity was not shown ($p > 0.05$) (**table 1**).

Table 1. Presence of VZV DNA in accordance with the referral diagnosis

Referral Diagnosis	Number of VZV positive samples	Number of VZV negative samples	Total (%)
St. post Varicella	4	29	33 (91%)
Varicella	0	2	2 (6%)
Congenital anomalies	0	1	1 (3%)
Total	4 (11%)	32 (89%)	36 (100%)

Data on age were available for 38 pregnant women. The most of pregnant women were in the group of women of up to 30 years (71% vs. 29%). Although all VZV positive samples were in this group, this did not demonstrate the existence of statistical significance between VZV positivity and the age of pregnant women ($p > 0.05$) (**table 2**).

Table 2. Presence of VZV DNA in accordance with the age of pregnant women

Age of pregnant women	Number of VZV positive samples	Number of VZV negative samples	Total (%)
Less than or same as 30 years	4	23	27 (71%)
More than 30 years	0	11	11 (29%)
Total (%)	4 (10%)	34 (90%)	38 (100%)

Gestational age of pregnancy was available for 42 pregnant women tested. The majority of pregnant women were in the second trimester of pregnancy (78%). The VZV positive PCR findings amniotic fluid was detected in all trimesters of pregnancy, with the lowest frequency in the second trimester of pregnancy. However, this did not show the existence of statistically significant difference between the VZV positivity and gestational age of pregnancy (table 3).

Table 3. Presence of VZV DNA in accordance with the gestational age

Gestational age (weeks)	Number of VZV positive samples	Number of VZV negative samples	Total (%)
0-14	1 (20%)	4 (80%)	5 (12%)
15-27	3 (9%)	30 (91%)	33 (78%)
28-40	2 (50%)	2 (50%)	4 (10%)
Total (%)	6 (14%)	36 (86%)	42 (100%)

Discussion

Varicella-Zoster Virus is a very significant cause of perinatal infection. However, in the moderate climate zone, VZV infection occurs most commonly during childhood, so that about 90-95% of women in the reproductive period are VZV seropositive. This results in a very low seroprevalence of VZV infection in pregnancy (1-10 in 10,000 pregnancies) (2). Primary VZV infection during pregnancy can lead to infection of the mother, intrauterine fetal infection and neonatal infection of the newborn if the infection occurs near term. It is believed that pregnant women during the primary VZV infection are at higher risk for clinical image of varicella with complications, primarily pneumonia (5).

Intrauterine infection is caused by transplacental transmission of the virus, which is the result of the presence of the virus in the blood of the mother during primary infection. Frequency of vertical VZV transmission during primary infection mother goes between 12 and 30% (2, 6). During this research, the frequency of vertical transmission was 14%, which is in accordance with the aforementioned data. Numerous studies have examined the risk factors that are important for the vertical transmission during primary infection. It is not confirmed that the frequency of vertical transmission is affected neither by the age of the pregnant woman, VZV viremia in the mother's blood, nor by the clinical image of the mother having varicella (2). Most pregnant women in this study were examined after curing infection - mostly pregnant women younger than 30 years. The existence of significant difference between the age of pregnant women and VZV positivity was not shown. The gestational age of the fetus is of great importance for the development of congenital varicella syndrome. It is shown that the occurrence of this

syndrome correlates with the first or second trimester of pregnancy. Based on two prospective studies that have been done on 1423 pregnant women who were exposed to varicella, disease incidence was 0.55% in the first trimester, 1.4% the second trimester, and 0% in the third trimester (5, 7). After the first 20 weeks of pregnancy, the incidence of the disease is approximately 2% (3). It is believed that the greatest risk of developing varicella congenital syndrome is when a non-immunized pregnant woman is infected between 13th and the 20th week of pregnancy (7). So far, no cases of this syndrome have been reported after 28th week of pregnancy (8). However, previous studies have not been precisely defined whether the gestational age of pregnancy is associated with vertical VZV transmission. Within this research, vertical transmission has been demonstrated in all three trimesters of pregnancy, but the existence of statistically significant difference between age and pregnancy VZV positivity and transmission of the virus was not shown.

Conclusion

The results of this study indicate a low frequency of VZV transmission in the test period of eight years, with the lowest frequency in the second trimester of pregnancy. There was no statistical significance in accordance with gestational age, diagnosis and VZV positivity. Screening is significant for VZV during the generative period of women. Those who are seronegative for VZV during pregnancy are at high risk for vertical transmission and are indicative for immunoglobulin therapy. If intrauterine infection is confirmed, adequate antiviral therapy is required.

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