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Prevalence of human papillomavirus and herpes simplex virus in amniotic fluid from pregnant women of Eastern Poland*

Częstość występowania wirusa brodawczaka ludzkiego i wirusa opryszczki ludzkiej w płynie owodniowym ciężarnych we Wschodniej Polsce

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Summary

Background: The exact route of human papillomavirus (HPV) and herpes simplex virus (HSV) transmission from a pregnant woman to her fetus has not been clearly established thus far. The data of many studies raise the possibility of intrauterine infection. In order to clarify our knowledge about virus vertical transmission in pregnant women, viral prevalence in amniotic fluid cannot. To the best of our knowledge, this is the first study on HPV DNA and HSV DNA detection in amniotic fluid in Poland.

Material and methods: The study covered 138 samples of amniotic fluid from patients undergoing invasive prenatal diagnostic procedures (for medical indications) during the second trimester of gestation. The aim of the study was to assess the prevalence of HPV and HSV in the amniotic fluid samples obtained from asymptomatic women with intact amniotic membranes. To identify viral DNA of HPV and HSV in collected material, polymerase chain reaction (PCR) was performed.

Results: We did not find HPV or HSV DNA in any of the examined specimens of amniotic fluid.

Conclusion: Our investigation did not confirm the prenatal transmission of HPV and HSV to the amniotic fluid.

Key words: **amniotic fluid • herpes simplex virus • human papillomavirus • prenatal transmission.**

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Background

Human papillomavirus (HPV) infection is a well-recognized risk factor for anogenital warts and cancers among adults [8,12]. Low-risk HPV types, e.g. HPV-6 and HPV-11, are most common in neonates with laryngeal papillomas – recurrent respiratory papillomatosis [8,19]. The infection spreads mainly via sexual contacts, although other routes are also possible. It is still unknown how the fetus becomes HPV positive. *In utero* transmission has been confirmed, but the trials that have assessed the prevalence of HPV infection differ considerably [3,8]. The frequency of vertical transmission from mother to child is low and ranges from 1 to 20% [20-23,25], although many investigators suggest that this mode of transmission is common [12,15,23].

Herpes simplex virus (HSV) is the term used to describe the common, cold sore-causing virus. Two types of HSV may be distinguished, and they are thought to affect different parts of the body. HSV-1 infection usually involves the mucous membranes of the mouth, the lips and facial skin, while HSV-2 affects mainly the genital area [9]. HSV-1 and -2 are transmitted by contact with an infectious area of the skin during reactivation of the virus. However, sometimes HSV can be transmitted during latency, though this is less likely to occur [5,7]. Primary infection with HSV-1 usually occurs in early childhood and, after initial replication at the site of inoculation (usually the lips), the virus is transported along nerve cells and becomes latent in the peripheral nervous system [10]. HSV-2 is primarily a sexually transmitted infection, but rates of HSV-1 genital infections are increasing [5]. In latent HSV-1 and -2 infections, the viral genome is still present but only one set of transcripts is formed with no viral proteins detectable. The virus may be reactivated from latency, at which time replication and synthesis of viral proteins occur, resulting in the production of complete virions and an ensuing acute infection [14]. The risk of vertical transmission from the mother to the fetus or to the newborn during pregnancy is a serious health problem; however, this is uncommon and probably depends on the gestational age and the form of the infection (primary infection, non-prime first episode or recurrent infection). Approximately 85% of perinatal transmissions are observed during the delivery. In the third trimester of pregnancy, women with genital herpes may infect their newborns during the vaginal delivery depending on the infection form (50% in primary infection, 33% in the non-prime first episode and 0-3% in recurrent infection) [4].

The aim of this study was to assess the prevalence of HPV and HSV in the amniotic fluid samples obtained from asymptomatic women with intact amniotic membranes in the second trimester of gestation.

Methods

All study participants (n=138) attended the Department of Obstetrics and Pathology of Pregnancy, Medical University of Lublin (Eastern Poland) between June 2005 and August 2010, and underwent invasive prenatal diagnostic procedures for medical indications. All the procedures were approved by the Local Ethics Committee of the Medical University of Lublin. The subjects provided written, informed consent prior to any procedure.

The study covered 2 mL samples of amniotic fluid. The samples were stored at -70°C until further analysis. To identify viral DNA, collected material was transported to the Institute of Experimental Biology, Department of Molecular Virology at Adam Mickiewicz University in Poznan. Genomic DNA was isolated from amniotic fluid and healthy cells by proteinase digestion and phenol extraction or by using the QIAamp DNA Mini Kit (QIAGEN) in accordance with the manufacturer's instructions. The specimens were homogenized with the addition of l mL buffer containing: 0.01 M Tris-HCI (pH 7.5), 0.01 M EDTA, and 0.6% SDS. The homogenate was incubated for 30 minutes at room temperature. Following this, K proteinase was added at a final concentration of 50 mg/mL and incubated for 24 hours at 37°C. After the incubation had finished, half the volume of phenol, chloroform and isoamyl alcohol (in a ratio of 25:24:1) mixture was added to the solution; it was shaken for 15 minutes at room temperature and centrifuged for 15 minutes at 3000 rpm. Half the volume of phenol, chloroform, isoamyl alcohol mixture was again added to the obtained water phase, shaken vigorously and then centrifuged. This was repeated until complete purification of DNA, manifested as a lack of interphase, was achieved. Then, half the volume of isopropyl alcohol and 0.1 of 3M acetate (pH 7.0) were added to the obtained water phase.

The DNA samples that were attained in this manner were then rinsed in 80% ethanol and dissolved in distilled water after drying. The samples with dissolved DNA were stored at -20°C. Quantitative determination of the DNA obtained was carried out by the spectrophotometric method using an automatic spectrophotometer (Pharmacia Co). In order to determine the amount of DNA in a given sample, l mL of the sample was dissolved in 69 mL of re-distilled water and, after calibration of the spectrophotometer, placed in its measuring chamber. After automatic processing of the data measured, the result was read in mg/mL.

HPV identification

Genomic DNA was isolated from study tissues using the QIAamp DNA Midi Kit (QIAGEN) according to the manufacturer's protocol. HPV infection was identified in PCR amplification of the HPV gene sequence, using primers MY09 and MY11 complementary to the genome sequence of at

least 33 types of HPV viruses. The reaction mixture contained 15 ng/µl of DNA and the following reagents: 0.5 U Taq DNA polymerase (Fermentas), PCR Buffer and magnesium chloride in the final concentration of 1.5 mM (Fermentas), primers in the final concentrations of 0.25 mM each, and dNTPs (Promega) in the final concentration of 0.2 mM. PCR products were run in 1.2% agarose gel electrophoresis with ethidium bromide and visualized in UV light. The product's length was assessed according to the Mass Ruler marker (Fermentas). PCR products were randomly eluted from agarose gel using QIAquick Gel Extraction Kit (QIAGEN) and sequenced in order to confirm expected product presence. The sequencing results were computationally analyzed using the BLAST database.

HSV identification

In order to identify viral DNA in the DNA isolated from amniotic fluid, PCR was performed using HSV1 (5'-CATCACCGACCCGGAGAGGGAC-3') and HSV2 (5'-GGGCCAGGCGCTTGTTGGTGA-3') starters (product size – 92bp) with sequences complementary to HSV.

Results and discussion

The amniotic fluid was collected from 136 singleton pregnancies and two twin pregnancies. The results of laboratory tests performed before admission were normal for the second trimester of pregnancy. Patients' ages ranged from 16 to 47 years (mean 32.9 years). Average age of gestation was 18 weeks, with a range from 15 to 26 weeks. In every case patients had intact membranes. Neither HPV nor HSV infection was found on PCR in the samples of amniotic fluid. The method that was applied is acknowledged as sufficiently sensitive to detect DNA of HPV or HSV. There were no known obstacles to prevent the detection of this infection.

To the best of our knowledge, this is the first study on HPV DNA and HSV DNA detection in amniotic fluid in Poland. Our research detected neither HPV nor HSV infection in the examined samples from asymptomatic women with intact amniotic membranes in the second trimester of gestation.

Several studies agree with the findings of our study concerning either HPV or HSV infections. Ruffin et al. [17] did not detect HPV in any of 146 paired samples from 142 women. Worda et al. [26] examined 153 pregnant women without clinical signs of HPV infection and did not detect HPV DNA in the amniotic fluid or cord blood; however, 8 out of 153 placental specimens were HPV positive. Absence of contact with cervical or vaginal secretions may protect the fetus from viral infection in some cases when the pregnant woman is HPV positive [13]. We cannot rule out the possibility that our patients have been infected, since also women with no clinical signs of HPV infection may be positive for viruses [26]. Moreover, viral load is an important factor responsible for the rate of HPV transmission [6]. Some authors have suggested the possibility of *in utero* HPV transmission. HPV DNA was detected in amniotic fluid by Xu et al. [27] in 23.1% of specimens, but in 13 cases the samples were collected during cesarean section. Wang et al. [24] reported presence of HPV types 16 and 18 in 6 out of 39 specimens (15.4%).

Concerning HSV prevalence in amniotic fluid, the result of our survey is also consistent with the results of other studies. In the study by Baschat et al. [2], amniotic fluid obtained in the second trimester by amniocentesis was analyzed, and it was found that in all 686 samples, no HSV was identified by PCR. Similarly, when DNA was extracted from the amniotic fluid from 77 pregnant women in the study by Rota et al. [16], no HSV expression was shown by RT-PCR. No HSV occurrence has been observed in amniotic fluid of pregnant women without symptoms of the infection. However, in the available literature, frequent detection of HSV in the vaginal swab and blood also correlates with no HSV detection in the amniotic fluid. Recently, the case report by Orsini et al. [11] described a 36-year-old pregnant woman with genital infection of HSV. Nested polymerase chain reaction (PCR), positive on vaginal swab, gave a negative result for the maternal serum and the amniotic fluid (at the 21st week). Similarly, repeated clinical examinations during the delivery were negative for HSV in laboratory investigations. Likewise, in the study by Alanen et al. [1] 21 pregnant women were studied to determine the distribution of HSV. Ten had symptomatic genital herpes, including one case with primary cervical HSV infection, and 11 patients had asymptomatic genital herpes. Samples from vesicles, the cervix, and amniotic fluid were analyzed with PCR. All amniotic fluid samples were negative and only 4 cervical samples were positive by PCR.

Conclusion

In conclusion, our investigation did not confirm the prenatal transmission of HPV and HSV to the amniotic fluid.

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