Original Article

PCR Detection of Herpes Simplex Virus in Human Placenta and Aborted Materials in Patients with Spontaneous Abortion

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Abstract

Objective: Herpes simplex virus (HSV) infection during pregnancy may lead to fetal loss. The aim of this study was to explore the infection rate of HSV type 1 and 2 infection associated with spontaneous abortion in a pilot case-control study in two cities of Iran in 2010.

Patients and Methods: Placenta and curettage samples from 35 healthy full term neonates and 35 cases of spontaneous abortion with related questionnaires were collected. Sample DNA was extracted using Qiagen extraction kit, and then PCR was applied for detection of both types of viruses. In parallel, results were checked using SinaClon detection kit.

Results: In this study 2.8% (1/35) of samples taken from aborted materials were found to be HSV positive (p=0.497). Differences were detected between case and control groups regarding vaginal infection (p =0.001) and having previous spontaneous abortion (p =0.018).

Conclusion: Our findings revealed that HSV can be an abortion-related factor in pregnancy.

Key words: Herpes Simplex Virus, PCR, Placenta, Aborted material, Spontaneous Abortion

Introduction

The incidence of fetal death seems to demonstrate a stable or even upward trend during the last decades despite major improvements in perinatal care (1). It is estimated that 10-20% of recognized pregnancies will end in miscarriage. The etiology of early pregnancy loss is multifactorial (2).

Herpes simplex virus (HSV) is a member of *Alphaherpesvirinae,* a subfamily of the *Herpesviridae* family, and is among the most ubiquitous viruses in the adult population. A characteristic of this family is life-time latency after primary infection and reactivation of the latent virus can reoccur in infected individuals at any time (3). In spite of the fact that a large proportion of women at childbearing age are seropositive to this virus, primary or secondary infections may occur during pregnancy (4). The danger of intrauterine HSV transmission is highest during the first 20 weeks of gestation because it can lead to abortion, stillbirth and congenital anomalies (5).

Pregnancy induces a transient immunosuppression which increases the vulnerability of pregnant women to viral infections (6), and may lead to fetal death. So, the higher incidence of HSV infection (72%) in HIV-positive women that had spontaneous abortion (SA) is reasonable (7); However, consequences of primary HSV-1 or HSV-2 infections on pregnancy outcome are thought to be more severe than of secondary infections (4,8,9), but some studies showed the relation between intrauterine latent HSV infection and SA (10); In cases of SA that HSV is a causative agent, there is a risk for later SAs in spite of the fact that HSV infection is not primary and this may occur even in the absence of clinical signs and symptoms (11).

Placenta is an important barrier between maternal and fetal circulations. So placenta is likely to be altered by transplacental transmission of viruses (12). Deciduitis and villitis have been described in relation to HSV infections (13). Moreover, early infection of extravillous trophoblast cells may prevent normal placental attachment to the uterine wall, predisposing women to miscarriage (4).

Because there is no epidemiologic study about the incidence of HSV in cases of SA in Iran so far, this pilot study was carried out to investigate the detection of HSV-1 and HSV-2 placental infection associated with SA using PCR test during 2009-2010.

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Patients and Methods

Specimens

In abortion group, 35 cases of women with spontaneous abortions at a gestational age of 4–20 weeks were examined. Placenta and products of conception in some cases were obtained, however, therapeutic abortions were excluded from the study. The control group included 35 placentas of full term neonates at 33–40 weeks gestation. All fresh frozen samples were collected from Arash Hospital in Tehran and Dezyani Hospital in Gorgan between January 2009 and November 2010 after signing a written informed consent, evaluated and proved by Avicenna Ethical Committee. The following data were obtained in two groups: gestational age, maternal age, maternal job and education status, gravidity, parity, presence of influenza-like symptoms, vaginal or urine infection, smoking, consumption of alcohol and medications during pregnancy and history of infertility, abortion, stillbirth and STD. The samples were preserved at -70°C after collection.

DNA extraction

Tissues were briefly washed in sterile 1x PBS in order to remove blood. Spin-column based QIAamp Mini Kit (Qiagen, Germany) protocol extraction was used as indicated by the manufacturer. DNA was extracted from 0.3 to 0.5 g of placental tissue. Extractions were aliquoted and stored at -20°C until testing.

PCR

Each amplification run contained a negative and a positive control. HSV DNA as positive control was obtained from the extraction of culture-grown HSV from Tarbiat Modares University, Iran. HSV DNA was extracted using QIAamp®DNA Mini Kit (Qiagen, Germany) as described by the manufacturer. Extractions were stored at –20°C for further study.

PCR was used in order to detect both types of HSV. The primers were based on the sequence of the DNA polymerase gene as described (14). HSV PCR was carried out on CMV, HHV-6 and HHV-7 DNA positive controls to ensure specificity of PCR for HSV detection.

The final PCR mixture for a 25µl reaction contained 1x PCR buffer (Roche, Germany), 2mM $MgCl_2$ (Roche, Germany), 0.6 mM dNTP (Roche, Germany), 0.2 µM of each primer , 1 U/µl Taq DNA polymerase (Roche, Germany) and 5µl of extracted template. After an initial 5 min denaturation at 94°, 35 cycles of 95°C for 30 sec, 69°C for 45 sec, 72°C for 1 min was carried out, followed by a 5 min extension at 72°C using a thermal cycler (Eppendorf, Germany). The 292bp amplified product was electrophoresed on 2% agarose gel. In parallel, results were checked using SinaClon detection kit (Herpes Simplex virus I & II Detection Kit Cat No: PR8240C).

Statistical Analysis

Analysis was performed using SPSS 11.5 software. The descriptive analysis results were shown as means ± SD. For comparison between study groups, Chi square, Fisher exact and independent t tests were used. P-values of <0.05 were considered statistically significant.

Results

Assessment of monoplex PCR

An electrophotogram of the monoplex PCR product showed amplified fragment of the expected size (292 bp) when using the primer sets specific to HSV-1, 2 (Figure I). Results from SinaClon detection kit and monoplex PCR was the same. The PCR which used with HSV primers was not able to detect CMV, HHV-6 and HHV-7 in samples implies specificity of PCR for HSV detection. Thus, we confirmed the amplification of the target virus DNA.

Detection of Viral DNA

Among participants with SA, HSV infection rate was 2.8% (1 out of 35) while no HSV-1/2 DNA was found among controls. The differences for HSV infection between study groups were not significant (Fisher exact test p values: 0.497). Overall HSV was detected in 1.4% of whole samples.

Characteristics of the Study Population

Seventy persons in two equal groups were investigated in this study. The mean maternal age of control group was 24.8±8.1 years and 26.1± 6 in abortion group (p= 0.442). Maternal age ranged between 18-41 years and 17-40 years in controls and SA cases respectively. In addition, the mean gestational age of full term neonates was 38.2±1.5 weeks and in aborted fetuses were 11.5± 6 weeks. Thirty four percent of women in abortion group were primigravida (G1), 40% were secundigravida (G2) and 25.7% were multigravida. In control group these rates were 44.1%, 41.2%and 14.7% respectively. Also 42.9% and all of women in abortion and control groups, respectively, had children. No women in the two groups had smoking or alcohol consumption during their pregnancy. Vaginal infection was more prevalent in mothers with abortion (48.5%) as compared with mothers with full term neonates (2.8%); OR=32.1(4-261)(Fisher exact test p =0.001). Also 34.3% of women in abortion group reported previous SA while this rate was 8.5% in the other group; OR=5.6(1.4-22) (Fisher exact test p =0.018). In one HSV positive case, a 19 years old primigravidae mother had vaginal infection and showed influenza-like symptoms during her pregnancy. She lost her fetus at gestational age of 9 weeks.

Discussion

According to our knowledge, this is the first report of HSV infection rate in cases of first trimester pregnancy loss using PCR in Iran. In this study 2.8% (1/35) of

samples taken from aborted materials were found to be HSV positive. Although HSV infection in this study was not found commonly and detection of the virus did not differ significantly between spontaneous abortion cases and controls, this infection rate should be considered. Most participants (88%) were from rural regions with low socioeconomic status. So they have been at higher risk of HSV infection before their pregnancy (2). It may explain the reason of the lower infection rate of HSV in our study population regarding other studies. Since HSV-1 has emerged as a principle causative agent of genital herpes (15) and also same antiviral drugs are used to treat both types of HSV, in this study both types of the virus were examined using the same primers.

Some previous studies showed no relation or low relation between HSV infection and SA. Chow et al (12) examined 105 pregnant women using multiplex PCR to explore prevalence of HSV and other viruses during pregnancy in three cohorts. There were 2 cases of miscarriage in the cohorts. They did not detect any HSV infection however they could find CMV infection in this number of cases. No detection of HSV infection in their study may be due to low number of abortion cases. Moreover and similar to our study, they did not detect HSV infection in their control group.

Sifakis et al (16) examined 102 cases of women with spontaneous abortion by PCR and serological assays and found HSV genome in 3 cases (2.9%), even in cases without any seropositivity or clinical symptoms for a primary infection. They concluded that the detection of HSV in 3 out of a total number of 102 cases does not support HSV infection as a major abortion-related factor. The HSV infection rate in Sifakis study is similar to our work with difference in number of cases.

Eskild et al (17) examined 281 women with a fetal death after the 16th weeks of gestation and 961 controls of live-born child to assess the association of fetal death with herpes simplex virus type-2. They found no evidence of an association between HSV-2 infection during pregnancy and fetal death using serological assays. This finding might be explained partly by the method, type of the virus and gestational age of aborted fetuses. As concluded in the study of Sifakis et al, serological assays were not very useful for elucidation of the role of HSV in inducing SA.

In other studies, however, higher relation was found. Robb et al (18) examined 380 cases (200 cases: SA /180 cases: endometrial curettage) for detection of HSV antigen in nonpregnant and pregnant endometria, placentas, umbilical cords, and neonatal tissues. They found placental HSV positivity was significantly correlated with SA (39% positive) versus therapeutic abortions (14% positive). Syridou et al (1) using nested PCR found 6% (1/18) of specimens in SA group were HSV positive. This detection rate was not significantly different from those of term newborns (5%). Similar to our work, they did not detect HSV infection in their control group. The infection rate of HSV in their study is higher than ours. In the study of Kapranos et al (19) using nested PCR, HSV was detected in 41 out of the 95 cases (43.2%) of early pregnancy loss and the HSV-positive cases were observed between 6th and 12th gestational week (mostly in 9th, 8th and 6th week). The author concluded HSV may play a significant role in first trimester pregnancy loss. In our study the HSV-positive case was in 9th week that is similar to their study but the rate of HSV infection in SA is about 15 times lower than their study. Since the study of Sifakis et al (16) and Kapranos et al (19) both were carried out in Greece but in different cities and the incidence of HSV infection was considerably different (43.2% versus 2.9%); similarly, other studies in different cities of Iran should be carried out to find the incidence of HSV infection in cases of SA in Iran with different socioeconomic status.

Conclusion

HSV can be a abortion-related factor in the studied population. Further large case-control studies are needed to elucidate the actual risk of HSV in cases of SA in Iran.

Acknowledgement

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Figures:

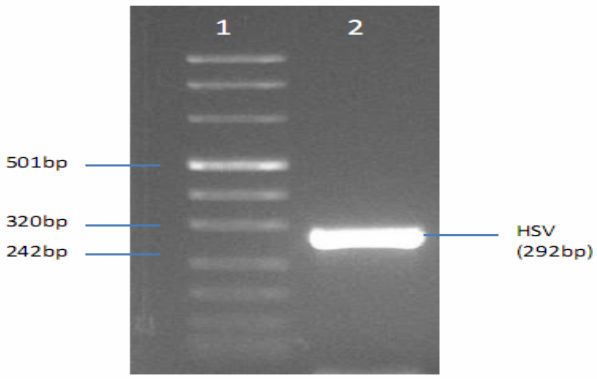

Figure I: Electrophotogram of PCR product.
Lane 1: molecular size marker (VIII)
Lane 2: HSV positive sample (292bp)

Legends

HSV: Herpes Simplex Virus
SA: Spontaneous Abortion
PCR: Polymerase chain reaction

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