

Molecular Epidemiology and Risk Factors of Herpes Simplex Virus Type 2 in Pregnant Women

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Abstract: *Background:* Herpes simplex virus type 2 (HSV-2) infection is the main cause of genital and neonatal herpes infections. It has considerable public health importance among women as the virus may lead to adverse outcomes in pregnancy and neonatal infection. This study determines the molecular epidemiology and risk factors of HSV-2 infection among pregnant women. *Methods:* In this cross-sectional study, all pregnant women admitted to three university hospitals for natural birth and Caesarean sections were enrolled. HSV detection and typing were carried out based on PCR and reverse dot blotting method, respectively. ANOVA and bivariate correlations were used to analyze the data. *Results:* In this study, the prevalence of genital herpes infection was 5.7%. A significant positive correlation was found between age group < 25 years and HSV-2 shedding ($P = 0.026$). Twelve participants (60%) with HSV-2 shedding were younger than 25. A significant correlation was found between the presence of genital lesion and HSV-2 ($P = 0.02$). Among participants with HSV-2 infection, the use of condom was low. Neonatal complications were not seen in newborns from mothers with HSV-2 shedding. *Conclusion:* PCR assay may help in promoting early diagnosis and more effective treatment for patients. Also, it shortens hospital stay and enhances patients' condition. HSV-2 transmission is rapid following the onset of sexual activity and likely to result in the significant prevalence of genital disease.

Keywords: Molecular epidemiology; Herpes simplex virus; Pregnant women; Risk factor

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1. Introduction

Herpes simplex virus type 2 (HSV-2) infection is the main cause of genital and neonatal herpes infections. It has considerable public health importance among women, worldwide ^[1]. It is estimated that 40% to 80% of the general population has been infected by HSV-2 ^[2,3]. The seroprevalence of HSV-2 varies in different

countries. In Scandinavia, the seroprevalence of HSV-2 among pregnant women is 33%, and in Canada, it is 17% [4]. Among the patients visiting a sexually transmitted disease clinic in the United Kingdom, 25% were found to be seropositive for HSV-2, seen in the general population in Asian countries. In a study in Iran, the prevalence of HSV-2 among pregnant women was found to be 8.25% [5]. The biggest concern about genital HSV-2 infections during pregnancy is the mother might transmit this virus to her fetus or neonate during delivery. It has been associated with severe consequences, such as premature delivery, low birth weight infants, fetal malformations, and significant morbidity to the newborn child [4]. Studies have shown that nearly 70% of newborns with neonatal herpes simplex virus infection were infected by their asymptomatic mothers. Therefore, early diagnosis and means of reducing the risk of infection in newborn are important [6]. Due to various clinical manifestations, suspected cases of herpes simplex virus infection should be confirmed by laboratory tests [7,8]. The aim of this study was to explore the molecular epidemiology of HSV-2 infection as the major cause of genital herpes among pregnant women.

2. Methods

2.1. Clinical samples

In this cross-sectional study from May 2017 to July 2018, all pregnant women (350), admitted to three university hospitals (Ghaem, Imam Reza, and OM Al-Banin) in Mashhad for natural birth and Caesarean sections, were studied. Informed consents were obtained from the participants. The demographic and clinical data of the mothers (including age, mode of delivery, fever, fatigue, sore, irritation, itching, vaginal lesions, and sexual transmitted diseases) and neonatal complications (hypothermia, skin lesions, and neurological symptoms) were collected by the physicians.

Sampling was done by trained nurses. All vaginal swabs were placed in 0.5 ml of 10% phosphate buffer solution. They were then sent to the virology laboratory within a maximum time of six hours and stored at -20°C until PCR analysis. The frequency of asymptomatic HSV-2 viral shedding was determined by PCR screening. The newborns were followed up for neonatal complications over two months.

2.2. DNA extraction and HSV detection and viral typing

DNA extraction from the clinical specimen was carried out using the High Pure Viral Nucleic Acid Extraction Kit (Roche, Germany) according to the manufacturer's instructions. The DNA samples were stored at -20 °C prior to diagnosing HSV-2 infection.

The detection of HSV was carried out by HSV 1/2 PCR Kit (AmpliSens, Russia). All PCR tests were performed in the same laboratory. The cycling parameters of the thermal cycler (ASTECH, Japan) were as follows: initial denaturation at 95°C for 5 minutes; then, 42 cycles at 95°C for 1 minute, 65°C for 1 minute, and 72°C for 1 minute, followed by a final extension at 72°C for 5 minutes. A 5 µl volume of each reaction mixture was subjected to electrophoresis. The sample was considered to be positive for herpes simplex virus type I and type II DNA if an amplicon of 430 bp was present in 1.5% agarose gel under ultraviolet illumination. The specific detection of HSV-2 was carried out using Herpes Simplex Virus Type 1 and 2 kits (GenID, GmbH, Germany) according to the manufacturer's instructions. A fragment of a highly conserved region of the HSV genome was amplified using a biotin labeled primer. Subsequently, the characterization and differentiation of HSV types were carried out by reverse hybridization assay. The results were analyzed using SPSS 11.5 (Illinois, USA). ANOVA and bivariate correlations were used to analyze the data. *P* value less than 0.05 was considered significant.

3. Results

In this cross-sectional study, the frequency of genital herpes infection was 5.7%. The age of the participants ranged from 14 to 45 years, with a median age of 27 years (SD ± 5.9). A significant correlation was found

between age group and HSV-2 shedding ($P = 0.026$). Decreasing HSV-2 shedding was observed with increasing age: 5.4% viral shedding in 25-30 years old and 1.6% in 31 years old and above, with a significant peak of 10.5% in those less than 25 years old. The majority of participants who tested positive for HSV-2 did not report symptoms commonly associated with HSV-2. The data showed that only 12 participants (60%) with HSV-2 shedding were younger than 25 and the rest aged 25 years or older (**Figure 1** and **Figure 2**).

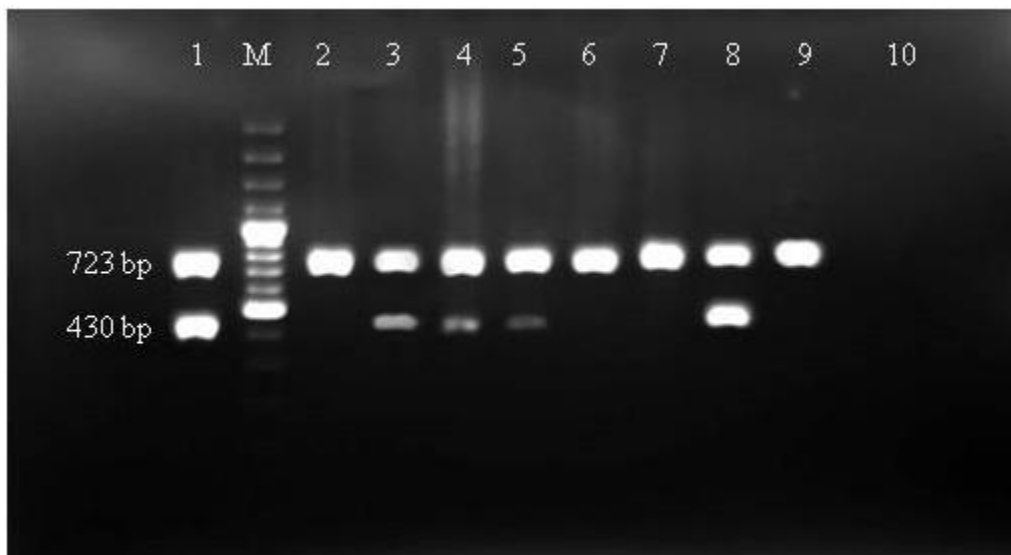


Figure 1. Agarose gel electrophoresis, showing the PCR amplified products for herpes simplex virus. Line 1: positive control; line M: 100 bp DNA ladder; line 3, 4, 5, and 6 are positive.

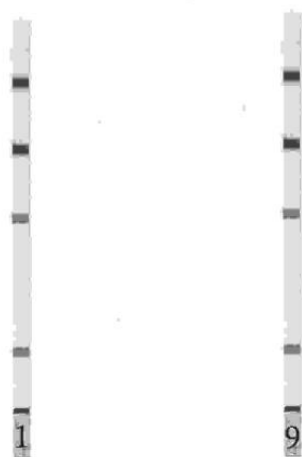


Figure 2. Herpes simplex virus typing by hybridization. From above, the first band is related to the conjugate control, the second band is related to the amplification control, the third band is related to HSV universal, and the final band is related to HSV-2.

In this study, 5% of the participants with HSV-2 shedding experienced vaginal pain, while vaginal itching and irritation were seen in 25% and 20%, respectively.

According to Pearson's chi-squared test, a significant correlation was found between the presence of genital lesion and HSV-2 ($P = 0.02$). Genital lesions were noted in 10% of women with HSV-2 shedding compared to 7% of non-shedding women. Vaginal pain ($P = 0.6$), vaginal irritation ($P = 0.26$), and vaginal itching ($P > 0.05$) were not associated with HSV-2 shedding.

Among the 20 participants with HSV-2 infection, the use of condom (as a risk factor) was low, with 85% of participants reporting not having used any condoms with their partners. Neonatal complications were not seen in newborns from mothers with HSV-2 shedding (**Table 1**).

Table 1. Descriptive and clinical characteristics of the pregnant women

Characteristic	Total number N (%)	HSV-2 positive N (%)	HSV-2 negative N (%)	Design-based Pearson's <i>P</i> value
Sample size	350	20 (5.7)	330 (94.3)	-
Age group				
< 25 (Group 1)	114 (32.5)	12 (10.5)	102 (89.5)	0.026
25-30 (Group 2)	111 (31.7)	6 (5.4)	105 (94.6)	
> 30 (Group 3)	125 (35.7)	2 (1.6)	123 (98.4)	
Vaginal manifestations				
Vaginal pain	10 (3.5)	1 (5)	9 (2.7)	0.6
Vaginal irritation	39 (11.1)	4 (20)	35 (10.6)	0.23
Vaginal itching	50 (14.2)	5 (25)	45 (13.6)	0.26
Vaginal lesion	4 (1.1)	2 (10)	2 (0.6)	0.02

4. Discussion

This study highlights asymptomatic HSV-2 shedding during delivery in 75% of participants. The majority of participants who tested positive for HSV-2 were younger than 25, and the use of condom, as a risk factor, was low in this study.

For women with genital lesions, viral culture or polymerase chain reaction (PCR) should be performed to detect and type the virus. Today, PCR analysis is preferred in view of the relative ease in specimen handling, its improved sensitivity, specificity, and turnaround time ^[9]. According to LeGoff and other researchers, when molecular testing is available, its use should be preferred over viral culture. Molecular testing helps to confirm viral shedding whether or not lesions are present ^[10].

Although the prevalence of herpes simplex infection is growing, there is little information about HSV prevalence in the Iranian population, indicating a necessity for more studies to be carried out ^[11]. This research was conducted in three university hospitals, providing useful insights for more definitive epidemiological studies about the extent of infection and the criterion for sample size estimates. Based on the results, 20 (5.7%) vaginal discharge specimens out of 350 pregnant women showed positive results for HSV-2 DNA. This is similar to studies in another part of Iran but lower than other countries. The levels of neutralizing antibodies against HSV-1 and HSV-2 in pregnant women during the period of delivery were reported to be 90.75% and 8.25%, respectively. The study also showed that the seroprevalence of HSV-2 increased with age ^[9].

Age is an important risk factor associated with the acquisition of genital HSV-2 infection. This study found a rapid rise in HSV-2 shedding coincident with the onset of sexual activity. This study also revealed that HSV-2 shedding decreased with increasing age: 5.4% viral shedding in those 25-30 years old and 1.6% in those 31 years old and above, with a significant peak of 10.5% in those less than 25 years old. Similar to a study conducted by Aliabadi and other researchers ^[6], in regard to age, twelve participants positive for HSV-2 were younger than 25, while the others were 25 or older. A reduction in HSV shedding is likely the result of the development of host immune response which leads to the clearance of virus ^[12].

Condoms appear to be 50% effective in reducing the risk of HSV transmission from men to women, and vice versa. However, pregnancy may increase the susceptibility to herpes simplex infection. It is

unknown whether the use of condom by a sexual partner with HSV-2 will have similar effects in pregnant women who are HSV-2 seronegative^[12]. In this study, since only a few married women (15%) use condoms consistently, the risk behavior of their husbands may be the primary risk factor. While neonatal herpes occurs in less than 1%, the risk of transmission increases to 25-50% among women infected during pregnancy^[13]. The disagreement between the low neonatal transmission rate and the high shedding rate among women with established HSV-2 infection suggests a role for transplacental antibody to abrogate the risk of infection^[14].

Neonatal herpes is known to be associated with serious outcomes. Therefore, in order to reduce the risk of neonatal herpes, it is recommended that women with asymptomatic genital HSV-2 infection are identified by examining HSV-2 shedding among pregnant women^[15]. In this study, there was no association between neonatal complication and HSV-2 shedding during pregnancy ($P = 0.99$). In a similar study^[16], it was reported that about three cases of neonatal herpes will occur per year, according to the estimate of 1 case per 3,200 live births in the USA^[17].

In this study, the majority of HSV-2 positive women did not report any symptoms associated with HSV-2. This is consistent with previous findings that HSV-2 is usually asymptomatic^[18].

Based on previous studies, during delivery, if skin lesions are present at the genital area, the risk of HSV-2 infection increases to 2-5% for the baby^[5]. In this study, an association was found between the presence of genital lesion and HSV-2 ($P = 0.02$). However, only 10% of HSV-2 positive individuals reported to have vaginal sores, highlighting the ability of HSV-2 to circulate undiagnosed in a population.

In conclusion, from the results, it can be appreciated that the occurrence of HSV-2 infection among pregnant women in this area is low. However, early diagnosis of such devastating illness using PCR is crucial in pregnant women for the health of their newborn. From this study, there is no evidence of any association between HSV-2 infection and fetal death.

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Disclosure statement

The authors declare no conflict of interest.

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