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Frequent Evaluation of Herpes Simplex Virus Type 2 in Women with Genital Herpes by Realtime PCR

Behnaz Montazeri [1], Haniyeh Bashi Zadeh Fakhar [2,3], Babak Shaghaghi [3], Forouzan Rostami [4]*

1 Islamic Azad University, Chalus Branch, Department of Medical Science, Chalous, Iran.

2 Department of Human Geneticse, Science and Research Branch, Branch, Islamic Azad University, Tehran, Iran.

3 Department of Laboratory Science, Chalous Branch, Islamic Azad University, Chalous, Iran.

4 Department of Nursing, Faculty of Nursing and Midwifery, Islamic Azad University, Chalous Branch, Chalous,Iran.

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ABSTRACT

Background: Herpes simplex type 2 is a common infection worldwide. This disease is common in both developed and developing countries. Early detection of infection is very important to reduce the risk of infection. Real-time reliable PCR is a very sensitive and specific method that can be used as the best marker in determining the therapeutic effect by identifying a viral genome in an individual. The prevalence of herpes simplex virus type 2 in women with genital herpes was evaluated by Real time PCR method.

Methods: From January 1999 to March 2010, 45 samples of vaginal swabs and cervix of women with genital herpes were examined for HSV virus DNA detection using Real Time PCR.

Results: The mean age of the patients was 35.9 + 5.9. The percentage of positive cases of herpes simplex virus type 2 in the studied women was 22.2% and the history of infection with hpv was 33.3% vs. 12.5%. = 0.094 which was significant.

Conclusion: Clinical specimens of vaginal swabs from genital herpes caused by herpes simplex virus 2 can be quantitatively analyzed instead of nucleic acid extraction and amplification by PCR.

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*Corresponding Authors: Haniyeh Bashi Zadeh Fakhar, Department of Human Genetics, Science and Research Branch, Branch, Islamic Azad University, Tehran, Iran.

Tel: +98-21-22749213, *E-mail:* haniyehfakhar@yahoo.com

Introduction

Cervical cancer is the second most common cancer among women, affecting approximately 1.6% of all women in their lifetime. Herpes simplex type 2 is a common infection worldwide. It is common in developed countries as well as in developing countries (2). Herpes simplex virus is a member of the herpes viridae family, a subfamily of the genus Herpesviruses and a genus of simplex. Herpes simplex virus's genum contains a 152.261-kilo base double-stranded spiral chain (3). There are two types of herpes simplex viruses which are similar in some traits such as DNA similarity and antigenic markers, tissue orientation and disease symptoms (4). Clinically, herpes viruses cause a wide range of diseases. skin and mucous infections are among common HSV infections that can cause lesions on the face, mouth, lips, genital area or other parts of the body (5). HSV is the leading cause of herpes infection in the mouth and lips, which can usually be transmitted through normal contact (6). Genital herpes ranks third among sexually transmitted diseases in terms of prevalence after gonococcal and chlamydial infection (7, 8). According to the World Health Organization, an estimated 492 million people aged between 15 to 49 live with HSV-2 worldwide, and it is reported that this disease has the most burden in Africa (9). Various factors contribute to HIV infection, including gender, race, marital status, age and polygamy. HCV-related infection can be symptomatic or asymptomatic. Primary infection in people without anti-HCV antibodies occurs after the first exposure to HCV (5). HSV infection type 2 involves genital area and it is sexually transmitted. The biggest concern regarding genital herpes relates to pregnancy period in which the mother may pass on the infection to the fetus during childbirth (10). Therefore, early detection of the infection is important so that the risk of infection can be reduced. Real Time PCR is a valuable diagnostic method to diagnose HSV genome. This reliable method is very sensitive and specific that can be used as the best marker in determining the therapeutic effect by determining the viral load in an individual (11,12). Therefore, this study was carried out to evaluate the frequency of herpes simplex virus type 2 in women with genital herpes by RT-PCR method.

Materials and Methods

Patients and Samples

45 women with genital herpes are studied in this research. These women referred to multiple gynecological offices in Tehran since January 1999 to March 2010 for vagina examination. Primarily, a gynecologist discussed with all patients before sampling.

Each patient was examined with one swab, then it was placed in 2.5 viral transport medium (VTM) which contained minimal essential medium (MEM), 25 mM Hepes (BioWhittaker,Europe) along with 10% bovine serum albumin(BSA), 7.5% sodium bicarbonate, penicillin (500 U/ml),streptomycin (1 mg/ml) and amphotericin B (5mg/ml). Samples were centrifuged for 30 seconds and divided into five 500ml-aliquots for PCR application in the Laboratory.

DNA Extraction

A DNA blood it was used for home-brew molecular assay according to the manufacturer's instructions that required 20 µl of sample. A rapid DNA extraction protocol was used for the real-time PCR assay. 100 µl of sample was added to 300 µl of a suspension with 20% (wt/vol) Chelex 100 resin (Bio-Rad Laboratories, Richmond, Calif.) in 10 mM Tris-HCl (pH 8.0)–0.1 mM EDTA–0.1% sodium azide in a 1.5-ml tube. The sample was centrifuged for 10 seconds, then it was incubated at 100°C for 10 min and centrifuged for another 10 second and then allowed to cool to room temperature. When the resin was completely

settled, 5 µl of the supernatant was directly applied for amplification.

Quantitative and qualitative evaluation of extracted DNA

Before performing any PCR, it is necessary that the concentration and quality of the extracted DNA were examined for corrosion and the size of the purified DNA fragments after DNA purification. It is required to examine the concentration and quality of the extracted DNA before performing PCR for corrosion and the size of the purified DNA fragments after purification of DNA.

DNA was extracted using a pair of PCO3 primers quantitatively (1.6 <OD <1.9) and qualitatively: 5'-ACACAACTGTGTTCACTAGC-3 'and PCO4 (3'-CAACTTCATCCACGTTCACC-5 ') in which a fragment of the human B-globulin gene was amplified, assayed simultaneously with multiplex PCR.

Primer design

Oligonucleotides were deduced from the published sequence of the DNA polymerase gene-coding region from HSV and then used for the real-time PCR assay (38-38).This set of primers was selected within a highly conserved region of the DNA polymerase gene from the herpes virus group. It is possible to amplify a 92-bp fragment from each of the HSV-2 DNA polymerase genes in clinical samples with this set of primers (39-40).The 5′ end of TaqMan probe (TIB MOLBIOL, Berlin, Germany) was labeled with 6FAM and the 3′ end with TAMRA. The sequences and characteristics of primer and sequences are shown in Table 1.

Real-time PCR on the LC instrument

The real-time PCR was performed on the specially designed LC instrument (Roche Diagnostics, Mannheim, Germany). Evaluation of the different assay formats has been described in detail elsewhere (41). the Specially designed LC instrument (Roche Diagnostics, Mannheim, Germany) was used to perform real-time PCR. TaqMan probe along with the hot start technique were used to test all samples by the LC-DNA Master Hybridization Probes assay (Roche Diagnostics) in the present study (table 1).adding TaqStart antibody to the 10* DNA Master solution to be incubated for 5 min at room temperature.In the following, primers, TaqMan, probe and water was added to the solution. Each capillary was filled with 15 ul of master mix 5 ul of DNA template. Capiilaries were sealed and centrifuged using a microcentrifuge and then were put into LC rotor. 55 PRC cycles were performed after denaturation for two minutes at 95 degrees.

Statistical analyses

The logistic regression model and data analysis suing SPSS 16 was simultaneously used to evaluate the significant effective factors.

Results

In this study, 45 women with genital herpes were selected. The mean age of patients was 35.8. 5.9, the smallest sample was 28 years old and the largest was 49 years old, the majority in the age group under 30 to 39 years. The mean marriage duration of the study sample was 8.02 33 5.33, the majority of women had a marriage duration of 5 years or less (44.4%). The majority of women (84.4%) had only one sexual partner. 20% of the samples had a history of positive smear for malignancy, 46.7% had a history of HPV, 40% had a history of genital ulcers and 57.8% had a history of genital warts. The mean duration of genital ulcers and warts in the studied women were 2.28 ± 1.78 and 2.85± 1.95 respectively. The percentage of positive cases of herpes simplex virus type 2 in the studied women is 22.2% with a 95% confidence interval of generalization to the study

Table 1. Oligonucleotides used for real-time PCR assay.

	HSV 2 Gene	Length (nucleotides)	G+C content (%)	Melting temp (°C)
Primer Forward	5′-CATCACCGACCCGGAGAGGGAC -3′	22	68.2	66.9
Primer Reverse	5′-GGGCCAGGCGCTTGTTGGTGTA -3′	22	63.6	69.0
TaqMan probe	5′-6FAM-CCGCCGAACTGAGCAGACACCCGCGC-TAMRA-3′	26	73.1	79.8

Table 2. Frequency distribution of HSV- 2.

		Num	%	95.0% Lower CL for Column N %	95.0% Upper CL for Column N %
HSV- 2	Negative	35	77.8%	64.2%	88.0%
	Positive	10	22.2%	12.0%	35.8%
	Total	45	100.0%	.	.

Table 3. Comparison of the percentage of positive cases of Hsv-2 virus according to the study variables.

		HSV- 2										P*
		Negative				Positive				Total		
		Num	%	95.0% CL		Num	%	95.0% CL		Num	%	
				Lower CL	Upper CL			Lower CL	Upper CL			
history of positive smear for malignancy	No	29	80.6%	65.6%	90.9%	7	19.4%	9.1%	34.4%	36	100.0%	0.313
	Yes	6	66.7%	34.8%	89.6%	3	33.3%	10.4%	65.2%	9	100.0%	
	Total	35	77.8%	64.2%	88.0%	10	22.2%	12.0%	35.8%	45	100.0%	
history of HPV infection	No	21	87.5%	70.3%	96.4%	3	12.5%	3.6%	29.7%	24	100.0%	0.094
	Yes	14	66.7%	45.4%	83.7%	7	33.3%	16.3%	54.6%	21	100.0%	
	Total	35	77.8%	64.2%	88.0%	10	22.2%	12.0%	35.8%	45	100.0%	
history of genital ulcers	No	23	85.2%	68.5%	94.8%	4	14.8%	5.2%	31.5%	27	100.0%	0.137
	Yes	12	66.7%	43.7%	84.7%	6	33.3%	15.3%	56.3%	18	100.0%	
	Total	35	77.8%	64.2%	88.0%	10	22.2%	12.0%	35.8%	45	100.0%	

Ulcers duration (mean ± SD)		.97±1.69				1.50±1.96				1.09±1.74		0.354
history of genital warts	No	15	78.9%	57.4%	92.4%	4	21.1%	7.6%	42.6%	19	100.0%	0.584
	Yes	20	76.9%	58.5%	89.7%	6	23.1%	10.3%	41.5%	26	100.0%	
	Total	35	77.8%	64.2%	88.0%	10	22.2%	12.0%	35.8%	45	100.0%	
Warts duration (mean ± SD)		1.97±1.89				2.50±2.12				2.09±1.93		0.436
number of sexual partners	1.00	29	76.3%	61.2%	87.6%	9	23.7%	12.4%	38.8%	38	100.0%	0.506
	2.00	6	85.7%	49.9%	98.4%	1	14.3%	1.6%	50.1%	7	100.0%	
	Total	35	77.8%	64.2%	88.0%	10	22.2%	12.0%	35.8%	45	100.0%	

Table 4. Comparison of the percentage of positive cases of Hsv-2 virus according to the study variables.

		B	S.E.	Sig.	Odds Ratio	95% C.I .for OR	
						Lower	Upper
Unadjusted Model	Duration of marriage	-.291	.202	.150	.747	.503	1.111
	Age	.146	.166	.381	1.157	.835	1.601
	history of positive smear for malignancy	.389	1.044	.709	1.476	.191	11.414
	history of HPV infection	1.505	.930	.105	4.506	.728	27.882
	history of genital ulcers	.836	1.160	.471	2.307	.237	22.407
	Ulcers duration	.005	.288	.986	1.005	.572	1.766
	history of genital warts	.429	1.069	.688	1.536	.189	12.484
	Warts duration	.167	.277	.547	1.182	.686	2.035
	number of sexual partners	-.675	1.332	.612	.509	.037	6.922
	Constant	-6.082	4.918	.216	.002		
Adjusted Model	history of HPV infection	1.253	.772	.104	3.500	.772	15.878
	Constant	-1.946	.617	.002	.143		

population equal to 12% to 35.8% (Table 2). According to Fisher's Exact Test, the percentage of positive cases of herpes simplex virus type by age group (P = 0.500) and duration of marriage (P = 0.329), history of positive smear for malignancy (33.3% vs. 19.4%) (P = 0.313), history of genital ulcers (33.3% vs. 14.8%) (P = 0.137), history of genital warts (P = 0.584) and number of sexual partners (P = 0.506)) None of them were statistically significant differences. But the history of HPV infection (33.3% vs. 12.5%) (P = 0.094) was significant (Table 3). Based on the Back Ward LR logistic regression model, in the multiple analysis of the studied variables, in the Adjusted model, only the history of HPV conflict variable remained (Table 4).

Discussion

Cervical cancer is the third most common cancer in the world and the fourth leading cause of death in women. Every year 530,000 cases are diagnosed and 265,653 deaths occur annually due to cervical cancer (13). Cervical cancer is mainly effective on countries with low or moderate levels of human development and the incidence varies from

country to country (14). Several independent epidemiological studies have linked HSV 2 infections to an increased incidence of cervical cancer (15). Herpes simplex virus type 2-related Genital infections have been reported in many parts of the world, and the prevalence of HSV 2 among women varies from region to region. 17% in the United States, 80% in sub-Saharan Africa and the risk of HSV 2 infection is higher in women than men generally (17.16). Outbreaks in European countries is reported to be 13% in Germany, 16% in Finland, 18% in and 20% in the United Kingdom (18). McQuillan et al. (2018) showed the prevalence of Hermes simplex Type 2 in the United States is 11.9% (19). A study was performed on 380 women referred to cevicare clinics in hospitals in Ghana reported that the prevalence of HSV 2 is 78.4 percent (20). Another study was conducted in Sudan on pregnant women reported the prevalence of HSV 2 (34.6) (21). Also the prevalence rate is reported in studies in Uganda 58% (22), in Zimbabwe 68% (23), in Zambia 55% (24), in Gambia 28% (25). It seems that the infection rate is high in Western societies due to non-compliance with ethical principles, and it is much low in developing Asian countries due to socio-cultural issues in the community than in other countries which is reported to be 10 to 30% (26). In the present study, positive cases of herpes simplex virus were reported to be (22.2%) by RT.PCR. Another study at Chennai Medical College in 2020 conducted on 60 patients with genital herpes by the RT.PCR method showed 42 cases of positive HSV 2 (27). In 2021, a study conducted on 60 patients in Iraq showed that 90% of patients were infected with HSV 2 (28). In 2020, another study conducted on 895 people in Spain, of which 126 individuals had genital herpes reported 58 cases of HSV infection (52.7%) (29). The present study did not find a significant relationship between the percentage of positive cases of herpes simplex virus type 2 in terms of age group and duration of marriage and the number of sexual partners. In a study conducted in Urmia, 6.5% of 170 samples were reported to be herpes simplex virus positive. Based on the findings of this study, no significant differences were found between the positive groups based on any of the variables including age and the number of sexual partners (30). In another study conducted on 380 Sudanese pregnant women in October 2014 to 2015, no significant differences were found between age and HSV 2 infection (21), which is consistent with our study. HPV is the main proven cause of the pathogenesis of cervical cancer. The HPV virus alone is not enough to cause cervical cancer because there is a long latent period between infection and clinical manifestations. There are some common factors that contribute to cervical cancer. A recent epidemiological study showed that a biological interaction between HSV 2, HPV 16 and HPV 18 occurs during the development of cervical cancer (31). In this study, a history of involvement with HPV has a greater impact on prevalence of HSV based on the logistic regression model. The differences seen in this study compared to other studies may be due to differences in regional race and small sample size. In a 2002 study by Xu et al., people with a history of genital herpes had a prevalence of HSV 16.6% higher than other people (32). In another study in 2017, 8184 samples were taken from Russians. Based on logistic regression analysis, a significant relationship was found between HPV and the risk of HSV 2 (33). In another study on 105 samples whose age was between 20-49 years in Nigeria, a significant relationship was found between HSV 2and HPV (34). In another study on 151 women, most of whom were from Canada and Africa, a significant relationship was found between HSV and HPV. (35) Another study on 50 women-related tissue samples in Baghdad in 2018 found a significant association between HSV 2 and HPV (36), which is consistent with our studies.

Conclusion

Herpes simplex virus type 2 (HSV-2) is prevalent globally, and is a significant concern for women's health due to its association with genital herpes and potential transmission during childbirth. Real-time PCR is a crucial diagnostic tool for early detection and effective management of HSV-2, highlighting the importance of routine screening and prompt treatment to mitigate infection risks. This study confirms that 22.2% of women with genital herpes tested positive for HSV-2, with a notable correlation to HPV infection history. It is clear that more research is needed. Further studies should also be performed on a larger community of patient with HSV-2 infection in this geographical area.

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Ethics approval and consent to participate

Not needed.

Conflict of interest

Authors declare no conflict of interest.

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