Identification of herpes simplex virus type 2 and risk factors associated with this infection in women Thi-Qar province

By Ahmed A. Nasir AL-Amery

WORD COUNT 3563
TIME SUBMITTED 13-JUN-2024 12:25PM
PAPER ID 109618980

Identification of herpes simplex virus type 2 and risk factors associated with this infection in women Thi-Qar province

Ahmed A. Nasir AL-Amery

Department of Physiology, College of Medicine, University of Thi-Qar,Iraq

ahmedsalihdr2008@yahoo.com

ABSTRACT

Background. For determining whether or not the herpes simplex virus type 2 (HSV2) is present in the female reproductive system, the research was carried out somewhere between November 2017 and March 2018.

Methods. In all, one hundred cervical samples were obtained from infected women. We used a Real-Time Polymerase Chain reaction to diagnose these samples. The individuals, whose ages ranged from 20 to 30 years, returned to private clinics located in the core of Nasiriya city in the Thi-Qar province of Iraq.

Results. Among those aged ≥30, the highest positive frequency for HSV-2 was 12, or 16.9%. The overall prevalence was 16 (24.6%). In the under-30 age bracket, 6.9% of cases were HSV-2 combinations. Out of all the women surveyed, 13.5% tested positive for HSV-2 in urban areas. When looking at viral infections and marital status, no significant differences were found. The highest HSV-2 level observed in this study was 4(33.3) in women who did not smoke. While 14 HSV-2 were detected at acidic pH, only two (18.2%) were detected at alkaline pH. Based on the number of births, the prevalence of HSV-2 varied between 8 (12.3%) and 2 (28.6%). In contrast, 10 cases (20.8% of the total) of vaginal secretions, 1 case (11.1%) of recurrent infection, 2 cases (5.7% of the total), and 1 case (33.3%) of post-coil and hysterectomy symptoms were found to be positive for herpes simplex virus.

Conclusion. Many individuals with genital herpes are unaware of their infection. This is because in most individuals, it either does not cause any symptoms or only causes very mild ones.

Keywords: herpes simplex virus type 2, virology, cervix, sexually transmitted diseases, cervicitis

Introduction

The term "cervicitis" describes an inflammatory condition affecting the cervix, the base of the uterus that protrudes into the vagina. Possible causes of inflammation include infection resulting from specific sexually transmitted diseases (STDs), cervical damage caused by the insertion of a foreign object into the vagina (such as birth control devices like the cervical cap or diaphragm), or the presence of cervical cancer [1].

Cervicitis can be acute or chronic, depending on the severity of the condition. Instantaneous manifestation of symptoms is one of the defining characteristics of acute cervicitis. Cervicitis

that is chronic typically lasts for a period of several months. Infection of the cervix with Herpes simplex type 2 has the potential to result in cervical cancer. Therefore, early detection of the infection can effectively prevent the progression of cancer in affected women. Cervical cancer is a malignancy that originates from the cervix. The cause of this phenomenon is the atypical proliferation of cells that possess the capacity to infiltrate or metastasize to different regions of the organism. Initially, the neoplastic lesions are limited to epithelium and do not cause any symptoms. Subsequent symptoms may encompass atypical vaginal bleeding, discomfort in the pelvic region, or pain experienced during sexual intercourse [1].

Herpes simplex viruses (HSV) are significant human pathogens that cause illnesses in various tissues and animal species. There are two distinct varieties of antigens, HSV-1 and HSV-2. HSV-1 is primarily spread through non-sexual means, while HSV-2 is typically transferred through sexual contact [2]. Herpes simplex virus 1 and 2 (HSV-1 and HSV-2), often referred to as human herpes virus 1 and 2 (HHV-1 and HHV-2), are two types of viruses belonging to the herpes virus family, Herpesviridae, which specifically target humans as their hosts [3] Both HSV-1 (which is responsible for the majority of cold sores) and HSV-2 (which is responsible for the majority of genital herpes) are widespread and easily transmitted. The transmission can occur when an individual who is infected is actively creating and shedding the virus [4].

Indications of herpes simplex virus infection encompass the presence of fluid-filled blisters on the skin or mucous membranes of the mouth, lips, nose, or genitals [3]. Lesions undergo the healing process by forming a scab that is typical of herpetic illness. Occasionally, viruses might result in minimal or unusual symptoms during periods of widespread occurrence. Nevertheless, they can also give rise to more problematic manifestations of herpes simplex. HSV-1 and -2, being viruses that affect the nervous system and invade nerve cells, can remain in the body by entering a dormant state and evading detection by the immune system within the cell bodies of neurons. It is possible for certain individuals to experience periodic episodes of viral reactivation or breakouts after the first or primary infection has occurred. During an outbreak, the virus that is contained within a nerve cell becomes active and is transported to the skin by the axon of the neuron. Once there, the virus reproduces and is discharged, which ultimately leads to the development of new sores. The virus that causes this illness is quite widespread, it is chronic, and it is commonly transmitted through sexual contact [5]. Like other herpesviruses, HSV possesses the capacity to persist in a dormant state within the host following an initial infection. During latency, the genetic material adopts an "inactive" condition as a circular episome inside

cells and remains there throughout the lifespan of the infected person, avoiding detection by the immune system. The reactivation from latency is frequently triggered by internal or external stimuli, resulting in a productive infection and the return of the disease [6].

Method

Primers and probes

The primer and probe designs utilized in this inquiry were generated using the NCBI Gene-Bank database and primer3 plus, an online program provided by the Pioneer business in Korea. The tables below present the details of these designs.

Table (1): Herpes Simplex type 2 (gpG) gene (GenBank: KU707769.1)

Primer	Sequence		Amplicon
HSV2 gpG primer	F	AACACATCCCCCTGTTCTGG	78bp
	R	TGTGGATGGTTGTGCTGATG	

The different methods used in this study, including viral DNA extraction, genomic DNA profile, and real-time polymerase chain reaction.

Samples Collection

One hundred women were dispatched to the private laboratory in Thi-Qar province that was managed by Noor-ALHussein. It was during the period of November 2017 to March 2018 that this research was held. These individuals' unique characteristics were documented using a questionnaire in the following ways: Factors such as number of births, residence, marital status, education, smoking, vaginal pH, and symptoms are taken into consideration. The samples were collected from the cervix of women who visited the Noor -ALHussein private laboratory/Thi-Qar province. The cervical swab putted in normal saline, then centrifuged at 3500 rpm to separated the cells. Until the Real-Time PCR technique was used to evaluate the obtained cells, they were kept at -20°C.

Viral DNA Extraction

Vaginal swab samples were used to extract viral DNA with the use of a gSYNCTM DNA Extraction Kit manufactured by Geneaid in the United States. The operation was carried out in a

manner that was consistent with the instructions that were supplied by the company, and the following is a summary of the procedure:

200 µL of supernatant obtained from vaginal swabs were transferred to a sterile 1.5 mL microcentrifuge tube. Next, a volume of 20 microliters of proteinase K was introduced into the mixture, which was then vigorously agitated using a vortex. Then, 200µl of GSB buffer was added to each tube and stirred well using vertexing to enhance the lysis efficiency. Subsequently, all tubes were subjected to incubation at a temperature of 60 degrees Celsius for a period of 10 minutes. An additional 200 microliters of ethanol were introduced into the mixture and well blended using a pipette. Subsequently, the mixture was centrifuged briefly to facilitate the adhesion of the drops to the lid. After transferring the lysate into a spin column that fit inside a 2-milliliter collecting tube, the tubes were sealed and spun at a speed of 8000 revolutions per minute for one minute. Following the disposal of the lysate, 500 microliters of washing buffer 1 (W1) were introduced into each spin column. The spin columns were thereafter rotated at a speed of 8000 revolutions per minute for a period of one minute. After disposing of washing buffer 1 into a container for waste, 500µl of Washing buffer 2 (W2) was added to each spin column. Next, the spin columns were subjected to centrifugation at a speed of 8000 revolutions per minute for a duration of 1 minute. Following the disposal of washing buffer 2 into a waste container, the tubes were subjected to centrifugation at a speed of 12000 revolutions per minute for a duration of 1 minute to completely remove ethanol. Afterwards, the spin column containing genomic DNA was moved to a sterile 1.5ml microcentrifuge tube. Next, 50µl of elution buffer was introduced and the tubes were left undisturbed for 5 minutes at room temperature until the buffer was completely absorbed into the glass filter of the spin Binding tube. Finally, all tubes underwent centrifugation at a speed of 8000 revolutions per minute for 1 minute to isolate DNA, which was then kept in a freezer at a temperature of -20 degrees Celsius.

Genomic DNA Profile

The extracted DNA was assessed using a Nanodrop spectrophotometer (THERMO, UK), which measures the purity of DNA by reading the absorbance at 260/280 nm. The following processes were followed:

1. When you open the Nanodrop software, choose the appropriate application (Nucleic acid, DNA).

2. The measuring pedestals were repeatedly cleaned with a dry Chem-wipe. Next, meticulously dispense 1 microliter of ddH2O onto the measurement pedestal and then hit the blank button.
3. Afterwards, the pedestals are cleaned, and a precise volume of one microliter of DNA sample is transferred using a pipette for the purpose of measurement.

Real-Time Polymerase chain reaction

The Real-Time PCR methodology was utilized to determine the presence of Human papillomavirus and Herpes. The amplification of the L1 gene and the gpG gene was accomplished using the simplex type 2 method. This approach was executed following the procedure outlined by [7], which consisted of the following steps:

Real-Time PCR master mix preparation:

A master mix for qPCR was made by utilizing the GoTaq®qPCR, and carried out in accordance with the instructions provided by the manufacturer, as shown in the following table:

Table (2): Real-Time PCR master mix

PCR Master mix	Volume
DNA template	5μL
Forward primer (10pmol)	1μL
Reverse primer (10pmol)	1μL
probe (20pmol)	1 μL
qPCR master mix	12.5 μL
PCR water	4.5 μL
Total volume	25μL

Subsequently, the PCR master mix components listed in Table (2) were placed in an Exispin vortex centrifuge and centrifuged at a speed of 3000 revolutions per minute for three minutes. Subsequently, all the components were placed inside a Real-time PCR Thermocycler manufactured by BioRad, located in the United States.

Real-Time PCR Thermocycler conditions:

The parameters of the Real-Time PCR thermocycler were modified based on the primer annealing temperature and the instructions provided by the qPCR TaqMan kit used with the Biorad Real-Time PCR thermocycler system, as indicated in table (3).

Table (3): qPCR thermocycler conditions

Step	Condition	Cycle
Pre-Denaturation	95 °C 5 min	1
Denaturation	95 °C 20 sec	45
Annealing/Extension	60 °C 30 sec	
Detection (Scan)		

Real-Time PCR Data analysis:

The qPCR data analysis involved determining the threshold cycle number (CT value) at which positive amplification occurred during the Real-Time PCR cycle.

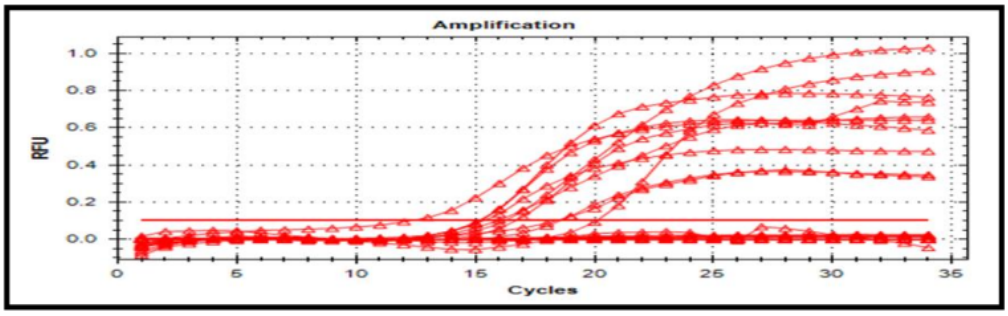

Figure (1): Real-Time amplification plots of Human herpesvirus 2 glycoprotein G (gpG) gene based on TaqMan probe. Where, the positive amplification samples which shown cross up the threshold cycle number.

Statistical Analysis

The data analysis was conducted using a computer equipped with the SPSS software version 11, which is designed for statistical analysis in the social sciences. The significant differences were evaluated using the chi-square test at a significance level of $p<0.01$ [8].

Results

The present study revealed that out of 100 cervical swab specimens from female participants from different regions of Thi-Qar province, aged 14 to 59 years were collected, the positive higher prevalence was 16 (24.6%) for HSV-2.

The levels of positive varied across different age groups, with the highest prevalence generally recorded in the age group of individuals over 30 years, with a rate of 12 (16.9%). In those under the age of 30, the occurrence of positive combination HSV-2 was 6.9%. The statistical analysis revealed that there was no significant correlation between viral infection in urban and rural areas ($P<0.05$). Specifically, the prevalence of positive HSV-2 in urban women was 13 (15.5) percent. There was no significant statistical difference observed between viral infections and marital status. This study shown that the prevalence of HSV-2 was 4 (33.3%) among non-smoking women, compared to 12 (13.6%) among smoking women. The prevalence of HSV-2 was 18.2% in alkaline pH, whereas it was 15.7% in acidic pH. The correlation between pH group and viral infection was statistically significant at a significant level of $P<0.05$. The prevalence of HSV-2 varied from 8 (12.3%) to 2 (28.6%) based on the number of births, namely less than 6 and non-birth, respectively. The positive frequencies of HSV were 20.8% for vaginal discharge, 11.1% for recurrent infection, 40.0% for menorrhagia, 5.7% for post coil, and 33.3% for hysterectomy symptom. The results indicated that there was no statistically significant correlation between symptoms and infection.

Table (4) Distribution of some risk factors associated with HSV-2 infection among women

Risk factors cases		Positive (%)	Negative (%)	Total (%)	Cal.X2
Age group	<30	4(13.8)	25(86.2)	29(10)	0.148
	>30	12(16.9)	59(83.1)	71(100)	
Residence	Urban	13(15.5)	71(84.5)	84(100)	0.107
	Rural	3(18.8)	13(81.3)	16(100)	
Marital state	Single	2(20.0)	8(80.0)	10(100)	0.132
	Married	14(15.6)	76(84.4)	90(100)	
Education	Education	11(15.3)	61(84.7)	72(100)	0.1
	Non	5(17.9)	23(82.1)	28(100)	

Smoking	Non smoking	4(33.3)	8(66.7)	12(100)	3.048
	Smoking	12(13.6)	76(86.4)	88(100)	
pH	Acidic	14(15.7)	75(84.3)	89(100)	0.044
	Alkaline	2(18.2)	9(18.8)	11(100)	
No. of birth	<6	8(12.3)	57(87.7)	65(100)	2.096
	>6	6(21.4)	22(78.6)	28(100)	
	Non. Birth	2(28.6)	5(71.4)	7(100)	
Symptoms	vaginal discharge	10(20.8)	38(79.2)	48(100)	6.56
	Recurrent infection	1(11.1)	8(88.9)	10(100)	
	Menorrhagia	2(40.0)	3(60.0)	5(100)	
	post coild	2(5.7)	33(94.3)	35(100)	
	Histrectomy	1(33.3)	2(66.7)	3(100)	

Discussion

The prevalence of HSV-2 positivity was highest in age groups over 30 years, while it was lowest in age groups under 30 years. However, the association between age and HSV-2 positivity was not statistically significant. These findings are consistent with previous reports Auvert et al. [9], Significant rises in HSV-2 seropositivity were noted as age increased, especially among women in the younger age group of 15-24 years.

The largest prevalence of HSV-2 was reported in urban areas, whereas the lowest prevalence was found in rural areas. This finding was consistent with the research conducted by Mohammed et al. [10] and Baloch et al. [11]. Urban and rural populations reside in distinct socioeconomic contexts, leading to variations in lifestyles and living standards. The prevalence of HSV-2 among rural residents was consistently high, comparable to urban residents, with no significant

disparities. This is likely since HSV-2 is prevalent among sexually active individuals, which aligns with the findings of [12].

HSV-2 infection was more prevalent in unmarried women as compared to married women. This finding in line with the research conducted by Kreider et al. [13] and Stevenson et al. [14], which demonstrates a decline in the marriage rate over the past 25 years. Currently, the marriage rate is at its lowest point ever recorded. Additionally, there has been a significant increase in cohabitation between unmarried partners, with a ten-fold increase between 1960 and 2000, and an 88% increase between 1990 and 2007. Postpartum chronic cervicitis is a frequently occurring condition. During pregnancy, elevated hormone levels can lead to increased blood flow to the cervix, which may result in cervical bleeding. Additionally, cervical bleeding can be caused by infections resulting from several reasons, such as allergies to spermicide or condom latex, the use of a cervical cap or diaphragm, or sensitivity to chemicals included in tampons. The correlation between educational attainment and the likelihood of testing positive for a viral infection, specifically HSV-2, revealed that non-educated women had the highest prevalence compared to educated women. This conclusion aligns with the research conducted by Fleming et al. [15]. Furthermore, the presence of HSV-2 antibodies was found to rise with age and decrease with lower levels of education. The presence of HSV-2 was significantly higher in non-smoking women compared to smoking women. This finding was consistent with the findings of Mzarico et al. [16]. The risk of negative health effects also escalates with a higher daily use of cigars, as corroborated by research conducted by Dickerson et al. [17], the study demonstrated that there was no significant interaction between smoking and genital herpes infection in relation to the risk of cervical abnormalities. HSV-2 was shown to be more prevalent among women with an alkaline pH compared to those with an acidic pH. When comparing the highest positive in Acidic pH, there was no significant association between asymptomatic cervical HSV-2 infection and the pH of the cervix or vaginal fluid. This finding is consistent with the results reported by Eggert-Kruse et al. [18].

Prevalence of HSV-2 Overall, there was a strong positive correlation between the presence of HSV-2 and the number of births in women. Specifically, women who had less than 6 births had a higher prevalence of HSV-2 compared to women who had more than 6 births. Additionally, women who had never given birth had the lowest prevalence of HSV-2 among all age groups. This study contradicts the findings of Munjoma et al. [19], who demonstrated that the incidence of HSV-2 is high after childbirth, suggesting that each time a woman gives birth, she experiences a

period of heightened risk for acquiring HSV-2. The main symptoms associated with positive HSV-2 infection were vaginal discharge, while the lowest occurrence was observed in those who had undergone hysterectomy. This finding contradicts the results reported by Reiter et al. [20].

Conclusion

Many individuals with genital herpes are unaware of their infection. This is because in most individuals, it either does not cause any symptoms or only causes very mild ones.

References

1. Tarney CM, Han J. Postcoital bleeding: a review on etiology, diagnosis, and management. Obstetrics and gynecology international. 2014;2014(1):192087.
2. Umene K, Kawana T. Molecular epidemiology of herpes simplex virus type 1 genital infection in association with clinical manifestations. Archives of virology. 2000 Mar;145:505-22.
3. Ryan KJ, Ray CG, Sherris JC. Sherris medical microbiology. (No Title). 2010 Jan 11.
4. Kimberlin DW. Management of HSV encephalitis in adults and neonates: diagnosis, prognosis and treatment. Herpes: the journal of the IHMF. 2007 Jun 1;14(1):11-6.
5. Gupta R, Warren T, Wald A. Genital herpes. The Lancet. 2007 Dec 22;370(9605):2127-37.
6. Nicoll MP, Proença JT, Efstathiou S. The molecular basis of herpes simplex virus latency. FEMS microbiology reviews. 2012 May 1;36(3):684-705.
7. Cubie HA, Seagar AL, McGoogan E, Whitehead J, Brass A, Arends MJ, Whitley MW. Rapid real time PCR to distinguish between high risk human papillomavirus types 16 and 18. Molecular Pathology. 2001 Feb;54(1):24.
8. Newman SC. Biostatistical methods in epidemiology. John Wiley & Sons; 2003 Apr 11.
9. Auvert B, Ballard R, Campbell C, Caraël M, Carton M, Fehler G, Gouws E, MacPhail C, Taljaard D, Van Dam J, Williams B. HIV infection among youth in a South African mining town is associated with herpes simplex virus-2 seropositivity and sexual behaviour. Aids. 2001 May 4;15(7):885-98.
10. Mohammed KA, Subramaniam DS, Geneus CJ, Henderson ER, Dean CA, Subramaniam DP, Burroughs TE. Rural-urban differences in human papillomavirus knowledge and awareness among US adults. Preventive medicine. 2018 Apr 1;109:39-43.
11. Baloch Z, Yuan T, Yindi S, Feng Y, Tai W, Liu Y, Liu L, Zhang A, Wang B, Wu X, Xia X. Prevalence of genital human papillomavirus among rural and urban populations in southern Yunnan province, China. Brazilian Journal of Medical and Biological Research. 2016 May 31;49:e5254.
12. Lin HaiJiang LH, He Na HN, Su MeiFang SM, Feng JiFu FJ, Chen Li CL, Gao MeiYang GM. Herpes simplex virus infections among rural residents in eastern China.

13. Kreider RM, Elliott DB. America's families and living arrangements: 2007. US Department of Commerce, Economics and Statistics Administration, US Census Bureau; 2009.
14. Stevenson B, Wolfers J. Marriage and divorce: Changes and their driving forces. Journal of Economic perspectives. 2007 May 1;21(2):27-52.
15. Fleming DT, McQuillan GM, Johnson RE, Nahmias AJ, Aral SO, Lee FK, St. Louis ME. Herpes simplex virus type 2 in the United States, 1976 to 1994. New England Journal of Medicine. 1997 Oct 16;337(16):1105-11.
16. Mzarico E, Gómez-Roig MD, Guirado L, Lorente N, Gonzalez-Bosquet E. Relationship between smoking, HPV infection, and risk of cervical cancer. Eur J Gynaecol Oncol. 2015 Jan 1;36(6):677-80.
17. Dickerson F, Adamos MB, Katsafanas E, Khushalani S, Origoni A, Savage CL, Schroeder J, Schweinfurth LA, Stallings C, Sweeney K, Yolken R. The association among smoking, HSV-1 exposure, and cognitive functioning in schizophrenia, bipolar disorder, and non-psychiatric controls. Schizophrenia Research. 2016 Oct 1;176(2-3):566-71.
18. Eggert-Kruse W, Mildenberger-Sandbrink B, Schnitzler P, Rohr G, Strowitzki T, Petzoldt D. Herpes simplex virus infection of the uterine cervix—relationship with a cervical factor?. Fertility and sterility. 2000 Feb 1;73(2):248-57.
19. Munjoma MW, Kurewa EN, Mapingure MP, Mashavave GV, Chirenje MZ, Rusakaniko S, Hussain A, Stray-Pedersen B. The prevalence, incidence and risk factors of herpes simplex virus type 2 infection among pregnant Zimbabwean women followed up nine months after childbirth. BMC women's health. 2010 Dec;10:1-8.
20. Reiter PL, Brewer NT, Gottlieb SL, McRee AL, Smith JS. Parents' health beliefs and HPV vaccination of their adolescent daughters. Social science & medicine. 2009 Aug 1;69(3):475-80.