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Prevalence of Herpes Simplex Virus in Pregnant Women in Ismailia City

Shimaa A S Hasan[a,*], Maha Anani[b], Nashwa Harb[a], Amal H A Gomaa [c], Hanaa H A Gomaa [a]

aDepartment of Botany and Microbiology, Faculty of Science, Suez Canal University, Ismailia, Egypt
bDepartment of Clinical Pathology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt
cDepartment of Dermatology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt

Abstract

Introduction: Herpes simplex virus infections are usually asymptomatic, however infection during pregnancy is life-threatening for pregnant women and their newborns. Herpes simplex virus infection is one of the most common viral infections worldwide. Both herpes simplex virus type 1 and type 2 are neurotropic viruses that can be transmitted congenitally. HSV-2 infection is the primary cause of genital herpes and is the most common cause of genital ulcer disease worldwide.

Objectives: We aimed to assess both the seroprevalence of herpes simplex virus type 1 and 2 among pregnant women in Ismailia City as well as the impact of pregnancy duration on their prevalence.

Methods: A descriptive cross-sectional study was conducted on 94 serum samples of pregnant women in Ismailia City. The presence of herpes simplex virus type 1 and 2 antibodies (IgG and IgM) and viremia were evaluated by commercial indirect ELISA and real-time polymerase chain reaction (RT-PCR) techniques.

Results: Our findings revealed a very high frequency of herpes simplex virus HSV-1 and 2 infections among the studied pregnant women with percentages of 74.5% and 98.9%, respectively. Upon the interpretation of the HSV serological profiles, the past latent infection with HSV-1 and 2 were the most prevalent types of infection representing 74.5% and 92.5%, respectively, followed by HSV-2 recurrent infection which was more prevalent (6.4%) than HSV-1 recurrent infection (0%), and no primary infection was found. HSV-2 co-infection was detected in all the HSV-1 positive cases (n= 70, 74.5%). Moreover, by using The Chi-square ($\chi 2$) test to test the significance of qualitative variables, there was no correlation between pregnancy duration and HSV-1 and 2 seroprevalences. In addition, the real-time PCR confirmed the positivity of the HSV-2 IgM subjects (n=6), while ruling out the equivocal sample as HSV-2 IgM negative.

Conclusion: Pregnancy screening for herpes simplex viruses is recommended to control the high viral seropositivity found among pregnant women in Ismailia City. Furthermore, the real-time PCR technique confirm improves the diagnostic value of serological tests.

1. Introduction

Pregnancy is a unique stage of life that most women go through. One of the most common characteristics of pregnant women is an immune system deficit. As a result, harmful microbes like viruses can overwhelm the host's defensive system. Human herpes simplex viruses (HSVs) are a well-known microbial pathogen that causes a variety of diseases [1]. They are double-stranded DNA viruses that are, enveloped, and belongs to the Herpesviridae family and subfamily alpha herpesvirinae [2]. They are transmitted through mucosal membranes and nonintact skin and migrates to nerve tissues, where it remains in latent status. HSV-1 is frequently acquired orally in infancy. It can also be transmitted sexually. Whereas, a person can only get HSV-2 through genital contact [3].

* Corresponding author.
Email address: sh_abas89@yahoo.com (Shimaa A S Hasan)

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Both viruses have the potential to be transferred vertically during childbirth [4]. However, the risk is significant when a mother becomes infected with the virus for the first time during late pregnancy [5] .

Up to 80% of HSV infections are asymptomatic [2] and a large percentage of HSV infections have no or mild manifestations with no detection; hence, pregnant women are the most vulnerable patients to HSV infections. A small number of patients get clinical symptoms such as vaginal lesions and herpetic ulcers [1, 4, 6].

A first primary infection occurs when a susceptible person (without preexisting HSV-1 and HSV-2 antibodies) is exposed to HSV. A first nonprimary episode happens when a person who has preexisting HSV antibodies (type 1 or 2) has a first episode with the opposing HSV type. Recurrent infection develops when a person has already antibodies against the same HSV type [7]. All three kinds of HSV infections are prevalent in pregnant women, which may lead to infection transmission to fetuses and newborns causing eye or skin lesions, meningoencephalitis, fetal abnormalities, and even death [8, 9].

Thus, the present study aimed to assess the seroprevalence of HSV-1 and 2 in pregnant women in Ismailia City from January 2019 to January 2020 and in addition, to assess the impact of virus on pregnancy duration. To our knowledge, this is the first report from Ismailia City and gives clues regarding the seroprevalence of the HSVs in Egyptian pregnant women.

2. Materials and methods

2.1. Study Population and data collection

This is a cross-sectional descriptive study conducted in Ismailia city which is located in northeastern Egypt, near the midpoint of the Suez Canal. Ninety-four apparantly healthy pregnant women were randomly chosen from those attending the health care centers in Ismailia city, the sample collection continued from January 2019 to January 2020. After having received written and verbal permission from the participants, they were asked about the following data: name, age and residence, data about the pregnancy semester, and associated complications.

2.2. Collection and storage of samples

Each participant's blood sample (5.0 ml) was taken in a sterile plane tube and centrifuged at 3000 rpm for 5 minutes. Then, the serum obtained from each study subject was divided into three different Eppendorf tubes. One was used for serological tests, the second was used for the real-time PCR test, and the third one was a backup sample. Sera were stored at less than -20°C until being used.

2.3. The serological assessment

Sera from all the participants were subjected to serological studies by indirect ELISA for HSV-1 IgG detection (MyBioSource HSV-1 IgG ELISA kit, Cat no. MBS494292, MyBioSource, Inc., San Diego, California, USA); HSV-1 IgM (MyBioSource HSV-1 IgM ELISA kit, Cat no. MBS495633, MyBioSource, Inc., San Diego, California, USA) and HSV-2 IgG/IgM (gG2 purified) (VIRCELL kits for HSV-2 IgG/IgM, Cat.No.G/M1013, VIRCELL, S.L. Pza, Spain). The technique was done according to the manufacturer′s instructions. The absorbance of each well was read at 450nm and determined by ELISA system using ELISA Reader (Hyperion, USA). The concentrations were determined using standard curves. Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph.

2.4. The molecular assessment

For confirmation of HSV-1/2 infection, all serum samples positive for IgM were tested to detect the presence of HSV-1/2 DNA using qualitative real-time PCR (RT-PCR) assay.

2.5. DNA Extraction and Qualitative real-time PCR:

Since no HSV-1 IgM was detected in any sera, only DNA from all positive HSV-2 IgM serum samples were manually extracted and purified using DNA-sorb-AM nucleic acid extraction kit, Cat no. K1-12-100-CE, supplied by AmpliSens®, Russia.

The HSV-2 DNA was detected by using qualitative real-time polymerase chain reaction (RT-PCR) using AmpliSens® HSV-typing-FRT PCR kit, Cat. No. R-V38-F(RG,iQ)-CE, Supplied by Inter Lab Service Ltd, Russia. The DNA target amplification specific region for HSV-2 was gpB gene by using applied biosystem Step On, USA real-time PCR.

In the real-time PCR, the amplified product was detected with the use of fluorescent dyes. These dyes were linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allowed the detection of accumulating product without re-opening the reaction tubes after the PCR run (Table 1 and 2).

2.6. Statistical analysis

Data analyses were performed using SPSS version 23. Quantities data were expressed as means ±SD and qualitative data were expressed as numbers and percentages. The Chi-square ($\chi 2$) test was used to test the significance of qualitative variables. $P < 0.05$ was considered to denote statistical significance.

3. Results

3.1. Study population

The study population comprised 94 pregnant women; their ages ranged from 16 years up to 42 years (mean ± SD =25.9 ± 5.73 years). Regarding the pregnancyduration at the time of the study, the studied population (n=94) was classified into three groups as follows: only 14.9% were during the 1^{st} trimester; 52.1% were during the 2^{nd} trimester and 33% were during 3^{rd} trimester (**Table 3**).

3.2. Serological profiles of HSV-1 and 2

Regarding the serological markers for both HSV-1 and 2, the most prevalent immunoglobulins were HSV-1 IgG and HSV-2 IgG which were found to be positive in 74.5% and 98.9% of the studied population, respectively. Whereas the HSV-2 IgM was detected in only 6.4% of the studied cases who had also HSV-2 IgG, and there was no HSV-1 IgM seropositivity detected at all (**Table 4**).

3.3. Detection of HSV-2 DNA in all positive HSV-2 IgM serum

The Qualitative RT-PCR technique confirmed the HSV-2 infection of the six IgM seropositive subjects, while the unknown (equivocal) sample was negative for HSV-2 DNA, and thus it was confirmed to be a negative HSV-2 IgM case (Table 5).

3.4. Interpretation of the serological profile of HSV-1 and 2

The interpretation of the performed serological tests of HSV-1 and 2 revealed that most of the studied subjects had serological evidence of past latent infection with HSV-1 and 2 that representing 74.5% and 92.5%, respectively. HSV-2 recurrent infection was more prevalent (6.4%) in the studied subjects than recurrent HSV-1 which was not detected at all. Otherwise, no primary infections of HSV1 and 2 were detected. Indeed, the results showed a very high prevalence of HSV-1 and 2 infections among the studied pregnant women with percentages of 74.5% and 98.9%, respectively (**Table 6**).

3.5. Relation between pregnancy duration and the HSV-1 and 2 infections among the studied population

Our results showed that regarding the HSV-1 and 2 serological profiles and infection types, there was no significant difference between the studied three groups of pregnant women (p-value > 0.05) (**Table 7 , 8**). Thus, there was no relationship between the pregnancy duration and the seroprevalences of HSV-1 and 2.

3.6. HSV-1 and 2 co-infections among the studied pregnant women

Interestingly, our results showed that all the HSV-1 positive cases (n= 70, 74.5%) had also HSV-2 co-infection. They were classified as follows: 6 (6.4%) had recurrent HSV-2 infection and HSV-1 latent infection, and 64 had evidence of both HSV-1 and 2 past coinfections.

Table 1: Primers and probes used for Taqman detection of the gBregion [a]

Primer or probe	Sequence	Label	Tm (°C)	μM
HSV2-F	TGCAGTTTACGTATAACCACATACAGC		59.5	0.9
HSV2-R	AGCTTGCGGGCCTCGTT		60.5	0.9
HSV2-probe	CGCCCCAGCATGTCGTTCACGT	5′FAM or JOE, 3′ TAMRA	70.0	0.2

[a]FAM, 6-carboxyfluorescein; JOE, 6-carboxy-4′,5′-dichloro-2′, 7′-dimethoxyfluorescein, TAMRA, 6-carboxytetramethylrhodamine.Nucleotides that differ from HSV-1 are underlined.

Table 2: Real-timePCR amplification program for amplifying HSV-2 gpB gene

step	Temperature °	Time	cycles
1	95	15 min	1
	95	5 s	
2	60	20 s	5
	72	15 s	
	95	5 s	
3	60	20 s fluorescent signal detection	40
	72	15s	

Table 3: Basic Characteristics of the study population

Characteristics	(n=94)
Age\ year	
Mean ± SD	25.9 ± 5.73
Range	16 – 42
Age of pregnancy\ months	
Mean ± SD	5.5 ± 2.13
Range	1 – 9
Trimester	
First no. (%)	14 (14.9%)
Second no. (%)	49 (52.1%)
Third no. (%)	31 (33%)
Healthy status	
Apparantly healthy	94 (100%)
complains	0 (0%)

4. Discussions

The prevalence of HSV-1 IgM observed in this study was 0%. Similar to the previous study in other different countries such as Saudi Arabia [10], Brazil [11] and Turkey [12]. On the contrary the high level of HSV-1 IgM, seropositivity was reported also by other countries researchers such as in Serbia 6.25% [13], Saudi Arabia two separate studies (5.9%, 4.3%) respectively [14, 15], Nigeria 2.8% [16] and India 0.55% [17].

The present study found that the prevalence of HSV-1 IgG was 74.5%. This is considerably close to the values obtained in other studies such as in India 69.44% [17], Serbia68.75% [13] and Switzerland 79.4% **[18].**

This is also considerably lower than the values obtained in other studies such as in Turkey98% [19]**,** Saudi Arabia four separate studies(90.9% , 90.5% , 84.1% , 94.7%) respectively [10, 14, 15, 20], Ghana 99.2% [21], Yemen 99.4% [22], Germany 82% [23] and Brazil 82% [11]. This is also considerably higher than values obtained in other studies such as in united states two separated studies(59.3%, 63%) respectively [24, 25]
.

In the current study it was observed that the prevalence of anti-HSV-2 IgM was 6.4%.Different studies had presented different results; in Saudi Arabia 0.5% [15], Serbia 6,25% [13], Iraq three separate studies (6.2%, 2.2%, 0.6%) respectively [26–28], Brazil 1.2% **[11], I**ndia 2.1% [29] and Turkey 11.3% **[12]. I**n contrast, some studies showed that all the studied cases had no recent HSV- 2 infection with negative IgM titre such as in Saudi Ara-

Table 4: Serological results ofHSV-1 and 2 among the studied groups

		Seropositivity in the studied group. (n=94)			Titer (U/ml)	
			N	%		
HSV-1	IgM	Negative	94	100%	Mean ± SD	0.39 ± 0.197
		Positive	0	0.0%	Range	0.1 – 0.9
	IgG	Negative	24	25.5%	Mean ± SD	13.26 ± 6.1
		Positive	70	74.5%	Range	3 – 40
HSV-2	IgM	Negative	87	92.5%	Mean ± SD	1.41 ± 2.93
		Positive	6	6.4%	Range	0.1 – 0.9
		Equivocal (unknown)	1	1.1%		
	IgG	Negative	1	1.1%	Mean ± SD	49.3 ± 31.7
		Positive	93	98.9%	Range	8 – 200

Table 5: PCR for cases with recent herpes virus type IIinfection and unknown sample among the studied population

Variable	Cases (n=7)
Positive (%)	6 (85.7%)
Negative (%)	1 (14.3%)

Table 6: Interpretation of the serological results ofHSV-1 and 2

	Interpretation status	N	%
HSV-1	No infection	24	25.5%
	Primary infection	0	0%
	Latent infection	70	74.5%
	Recurrent HSV-1 episode	0	0%
	Total HSV-1 prevalence	70	74.5%
HSV-2	No infection	1	1.1%
	Primary infection	0	0%
	Latent infection	87	92.5%
	Recurrent HSV-2 episode	6	6.4%
	Total HSV-2 prevalence	93	98.9%

bia [10, 14], Turkey [30], Sudane [31] and India [17]. The present study also contrasts with other studies such as in Iraq28.9% [32] and Turkey28.6% [33].

Moreover, some studies found that the state of pregnancy may predispose to the reactivation of latent HSV-2 infection which might result in fetal infection with spontaneous abortion [34, 35].

In this study, seroprevalence of anti HSV-2 IgG antibodies among pregnant women was 98.9%. This is considerably close to the values obtained in other studies such as in Nigeria 99.4% [16], Zaria 82.2% [36] and Turkey 73.8% **[33].**

Regarding the low level of HSV-2 IgG, seropositivity was reported also by other countries researchers such as in India five separate studies found that the seropositivity of anti HSV-2 IgG among pregnant women was (64.9% , 16.66% , 8.7% , 7.5% , 6.7%) respectively [6, 17, 29, 37, 38], Nigeria two separate studies (58.9%, 33.3%) respectively [39, 40], Zimbabwe two separate studies (51.1%, 49.1%) respectively [41, 42], Sudan 32.1% [31], Ethiopia 32.1% [43], Turkey two separate studies (63.1%, 4.4%) respectively [12, 30], Saudi Arabia five separate studies (27.1%, 7.6%, 6.8% , 6.5%, 0.5%) respectively [10, 14, 15, 20, 44], Serbia 12.5% **[13], I**raq 9% [45], 2.2% [27, 28], Yemen two separate studies (5.1% , 6%) respectively [22, 46] and Brazil 9.5% [11] **.**

The higher prevalence in our study might be due to various factors. First, the prevalence of HSV might be high among general population especially in men. Financial constraints might be the second factor as it was difficult to cover screening cost in our country and simultaneously creating awareness. Educational status might be the other factor. Lack of health information might play important role to increase the prevalence.

In this study, real-time PCR confirmed the positivity of the HSV-2 IgM cases (n=6), while it cleared that the equivocal sample was HSV-2 IgM negative.

Table 7: The relation between thepregnancy duration and the serological profile of HSV-1 and 2

			pregnancy duration			P-value
			1st trimester (14)	2nd trimester (49)	3rd trimester (31)	
HSV-1	IgG	Positive (n=70)	9 (64.3%)	38 (77.6%)	23 (74.2%)	0.664 (NS)
		Negative (n=24)	5 (35.7%)	11 (22.4%)	8 (25.8%)	
	IgM	Positive (n=0)	0	0	0	—
		Negative (n=94)	14 (100%)	49 (100%)	31 (100%)	
HSV-2	IgG	Positive (n=93)	13 (92.9%)	49 (100%)	31 (100%)	0.06 (NS)
		Negative (n=1)	1 (7.1%)	0 (0.0%)	0 (0.0%)	
	IgM	Positive (n=6)	0 (0.0%)	3 (6.12%)	3 (9.7%)	0.5 (NS)
		Negative (n=87)	14 (100%)	45 (91.84%)	28 (90.3%)	
		Equivocal (n=1)	0	1 (2.04%)	0	

Table 8: The relation between thepregnancy duration and HSV-1 and 2 infection types

	Interpretation status	Pregnancy duration						P-value
		1^{st} trimester (n=14)		2^{nd} trimester (n=49)		3^{rd} trimester (n=31)		
HSV-1	No infection	5	35.7%	11	22.4%	8	25.8%	0.5 (NS)
	Primary infection	0	0%	0	0%	0	0%	
	Latent infection	9	64.3%	38	77.6%	23	74.2%	
	Recurrent HSV-1 episode	0	0%	0	0%	0	0%	
	Total HSV-1 prevalence	9	64.3%	38	77.6%	23	74.2%	
HSV-2	No infection	1	7.1%	0	0	0	0	0.1 (NS)
	Primary infection	0	0%	0	0	0	0	
	Latent infection	13	92.9%	46	93.9%	28	90.3%	
	Recurrent HSV-2 episode	0	0%	3	6.1%	3	9.7%	
	Total HSV-2 prevalence	13	92.9%	49	100%	31	100%	

This proved the importance of the DNA assays in cases of doubtful serological findings, due to their sensitivity and specificity**.**

Our study showed high HSV PCR positivity compared with the previously published studies such as in Iraq 1.2% [47], Brazil; 0% [11], 1.49% [48].

In contrast, another study performed by [49] in Brazil reported about 28% and 12.6% positivity results of HSV-1 DNA and HSV-2 DNA respectively.

[50] reported that about (16% and 12.3%) of pregnant women in Brazil had positive result to HSV-1 DNA and HSV-2 DNA respectively.

In this study we found that all IgM positive cases with recent HSV-2 infection had old HSV-1 and HSV-2 infection too with positive IgG test result and about 75.3% of cases with old HSV-2 infection gave positive results for old HSV-1 infection. Also one case showed to be negative for old infection of both types.

In contrast to this, [12] found that about 44.4%

of cases with recent herpes type 2 infection had old infection too with positive IgG test results. Another study by [28] reported that only one case with recent herpes type 2 infection had also old infection too with positive IgG test result.

Also, other studies showing that none of the cases that had old infection with herpes type 2 had also recent infection with negative IgM test results such as [12. 14, 30, 31, 10, 17].

The present study found that none of the demographic factors age and duration of pregnancy has significant influence on the HSV positivity rate, these results are in agreement with [27, 46] and are inconsistent with certain studies such as [11, 37, 38].

Our results clearly suggested that the prevalence of HSV were high and can contribute to various congenital infections and other complications. Periodic screening for antenatal mothers thus becomes necessary to avoid HSV complications, which results in pregnancy-related and neonatal morbidity.

5. Conclusion

The current study results showed that there was a high seroprevalence of both HSV-1 and HSV-2 among pregnant women in Ismailia city. Thus, pregnancy screening for HSVs is recommended to manage the complications caused by these viruses. HSVs can be a significant problem in pregnancy since they can infect newborns vertically and produce difficulties that can affect mother health and even cause maternal mortality. Furthermore, we discovered that detecting DNA in maternal serum using PCR improves the diagnostic value of serological testing.

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