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Investigation of herpes simplex virus type 2 seroprevalence in all age groups

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Abstract

Aim: Herpes simplex virus type 2 (HSV-2) is one of the most common causes of sexually transmitted genital infections and remains latent in neuronal cells for the lifetime of humans. This retrospective study aimed to determine the seroprevalence of HSV-2 and evaluate its relationship with age and gender.

Materials and Methods: In this study, 976 serum samples sent to Virology Laboratory between October 2016 and December 2018 were evaluated. HSV-2 IgM and IgG antibodies were tested with VirClia EIA/CLIA device by using a commercial kit.

Results: The median age of 976 patients included in the study was 31 (0—88). Forty-three point three percent (423) of the patients were male. HSV-2 IgM was found in 0.3% (3/976) of the patients and HSV-2 IgG was found in 3.7% (36/976). HSV-2 IgG was detected in 2.8% of male patients (12/423) and 4.3% (24/553) of female patients ($p = 0.120$). The highest HSV-2 IgG seropositivity rate was between the ages of 40 and 59 in both genders (8.3% in males and 8.3% in females) ($p = 0.010$ and $p = 0.250$ respectively).

Conclusion: The HSV-2 IgG seropositivity rates we detected in this study were consistent with other study data in our country and in the world. Studies on HSV-2 IgG seroprevalence and determination of the age groups at risk will be guides for public health studies aimed for protection against HSV-2 infection.

Introduction

Herpes simplex virus type 2 (HSV-2) is classified in Simplexvirus genus belonging to the subfamily Alfaherpesvirinae of family Herpesviridae [1]. HSV-2 remains one of the most common causes of sexually transmitted genital infections in both developed and developing countries. It causes primary or recurrent infections and remains latent in lifelong sensory neurons. Genital herpes is associated with morbidity and even mortality [2, 3]. Although genital herpes infections are generally asymptomatic, clinically symptomatic ones are characterized by recurrent, painful, vesicular and ulcerative lesions [4, 5]. HSV-2 can cause rare but serious diseases such as encephalitis and newborn infections [6]. In these highly contagious viruses, transmission occurs through unprotected oral or genital sexual intercourse with an infected person [7-10].

Improvements in type-specific HSV serological tests have ensured the increase in the information about HSV-2 epidemiology. Seroepidemiological findings from previous studies revealed that most of the people infected with this virus do not have any symptoms associated with herpes [5, 11, 12]. Most of the studies involve high risk groups such as patients with a sexually transmitted disease, female sex workers, HIV positive individuals and pregnant women presenting to the hospital before delivery.

HSV-2 is epidemic in the whole world. In 2016, more than 490 million people worldwide had HSV-2 infection, equivalent to a global prevalence of 13.2% in 15-49 year olds [13]. Previous studies reveal that HSV-2 prevalence is considerably higher in African and Latin American countries than in some European countries and the United States [14].

The prevalence of HSV-2 infection varies by region, but has tended to increase in the last decades. Social and sexual attitudes, ethnic origin, education level, socioeconomic conditions, age, gender, number of sexual partners, early age of sexual intercourse, are important risk factors for this infection and affecting seropositivity rates. It is reported that HSV-2 infection is more common in women all over the world [5, 11-13, 15]. Age strongly correlates with the rate of HSV-2 seropositivity. Antibodies are rarely de-

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tected before puberty. In most populations, the prevalence of HSV-2 infection increases with age [5, 16].

Commercial test kits developed to detect the antibodies against glycoprotein gG-2 special to HSV-2 have made a considerable improvement in the serological detection of HSV-2 infection. When these tests are compared with Western blot, their sensitivity is reported to be between 93% and 100% and the specificity is between 95% and 100% [3].

In our country, published studies on HSV-2 seroprevalence are quite limited. In this study, it was aimed to determine the seroprevalence of HSV-2 in patients admitted to our hospital and to obtain regional epidemiological data by investigating the age and gender distribution.

Materials and Methods

This retrospective, cross-sectional study includes the period from October 1, 2016 to December 31, 2018. All patients with suspected HSV-2 infection in different departments of the Gulhane Training and Research Hospital and whose HSV-2 enzyme immunoassay (EIA) test was studied were included in the study. The sample number was calculated between 65 and 358 based on the seroprevalence values (4.4%-63.1%) determined in previous studies in our country. Patients were selected using a random sampling method. Subjects with recurrent patient results, insufficient samples and no test results were excluded from the study. Demographic data such as date of application, diagnosis codes, age and gender of the patients were obtained from the hospital database. In order to better understand the distribution of HSV-2 seroprevalence according to age, patients were divided into five age groups: $<$ 18, 18-39, 40-59, 60-79, $\geq$80. This study was performed with the approval of the Non-Interventional Clinical Research Ethical Committee of the University of Health Sciences Gulhane Training and Research Hospital (Reference Number: 2019/19/41).

HSV-2 IgM and IgG antibodies were analyzed with VirClia EIA/CLIA (chemiluminescent immunoassay) device (Vircell, Granada, Spain) by using Herpes Simplex 2 VirClia® IgM and IgG Monotest commercial kit (Vircell, Granada, Spain) in accordance with the recommendations of the manufacturer.

The test kit investigated HSV-2 IgM and IgG antibodies with indirect CLIA principle. The current kit uses gG-2 protein obtained from HSV-2 through purifying as the solid-phase antigen. While the test results are valid in conditions that RLU (relative light unit) of the calibrator is between 2 and 7 and RLU of negative control is $<$ 2, otherwise, the test is repeated. The antibody index value was calculated for example by dividing its RLU by the calibrator's RLU. The index value was evaluated as negative at $<$ 0.9, intermediate value at 0.9—1.1, and positive at $>$ 1.1. Results of the blood samples sent for HSV-2 IgM and IgG analysis were retrospectively evaluated.

Statistical analysis

Based on the data collected in the study, SPSS 25 software (SPSS Inc, Chicago, IL, USA) was used for statistical evaluation. Continuous data were given as mean and standard deviation while categorical data were given as counts and percent. Compliance of the variables with the normal distribution was assessed by visual methods (histogram and probability graphs) and Kolmogorov-Smirnov test. The dependent variables of the study were HSV-2 seropositivity (HSV-2 IgM and IgG) and the independent variables were gender and age group. The relationship between dependent variables and independent variables was examined by chi-square test for categorical variables. The Mann-Whitney-U test, a non-parametric test, was used to determine whether there was a statistical difference between age (median) and variables (sex, HSV-2 IgG positive and negative groups). The results were evaluated at 95% confidence interval. The results with P value under 0.05 were accepted statistically significant.

Results

The ages of the patients were between 0-88 (median age 31), 423 of them were male (43.3%) and 553 were female (56.7%). HSV-2 IgM antibodies were detected in 3 out of 976 patients (0.3%) (95% Cl: 0.1-0.9) and HSV-2 IgG antibodies were in 36 patients (3.7%) (95% Cl:2.6-5.1). HSV-2 IgM antibodies were at limit values in one male patient and HSV-2 IgG antibodies in five patients, one female and four male patients.

HSV-2 IgM seropositivity was 0.2% (1/423) (95% Cl: 0.0-1.3) and 0.4% (2/553)(95% Cl: 0.0-1.3) in male and female patients respectively (p = 0.760). HSV-2 IgG seropositivity rate was 2.8% (12/423) (95% Cl: 1.5-4.9) in male patients and 4.3% (24/553) (95% Cl:2.8-6.4) in female patients (p = 0.120).

Median ages of HSV-2 IgG negative and positive patients were 31 (range = 0-88) and 40 (range = 0-73) respectively (p = 0.020). While the highest rate of HSV-2 IgG seropositivity was 8.3% (18/217) in the 40-59 age group, IgG was not detected in patients aged 80 and over (p = 0.008). When the seropositivity rates by gender were evaluated, the highest HSV-2 IgG was detected in the 40-59 age group for both genders (8.3% in male patients; 95%Cl: 3.9-15.2 and 8.3% in female patients; 95%Cl: 3.8-15.1) (p = 0.010 and p = 0.250, respectively) (Table 1).

Three patients, one male and two female patients, with HSV-2 IgM seropositivity were in the age group of $<$18 (p = 0.060). Demographic information about 36 HSV-2 IgG positive patients were given in Table 2.

There was pregnant state in 29.2% of 24 HSV-2 IgG positive female patients.

Discussion

It is estimated that there are more than 500 million people infected with HSV-2, which is a sexually transmitted pathogen, in the World and 23 million new infections are added every year [17]. Investigation of the epidemiology of HSV-2 infections is important for the severity of symptomatic clinical findings and infection-induced psychosocial diseases [18].

HSV-2 infection is endemic in many regions of the World. It is estimated that HSV-2 prevalence worldwide is 11.3%. When the regional seroprevalence rates are evaluated, it is

Table 1. HSV-2 IgG and IgM seropositivity rates according to the age groups[a]

Antibody	Age groups	No. tested	Sero-positive		Sero-negative		Equivocal	
			F	M	F	M	F	M
IgM	<18	163	2(2.5)	1(1.2)	78 (97.59)	82 (98.8)	-	-
	18-39	494	-	-	314 (100)	179 (99.4)	0 (0.)	1(0.6)
	40-59	217	-	-	109 (100)	109 (100)	-	-
	60-79	92	-	-	44 (100)	48 (100)	-	-
	≥80	10	-	-	6 (100)	4 (100)	-	-
	Total	976	1(0.2)	2(0.4)	421(99.5)	551 (99.6)	0 (0.0)	1(0.2)
IgG	<18	163	2 (2.5)	0 (0.0)	78 (100)	83 (97.5)	-	-
	18-39	494	12 (3.8)	3 (1.7)	301 (95.9)	176 (97.8)	1 (0.6)	1 (0.3)
	40-59	217	9 (8.3)	9 (8.3)	100 (91.7)	97 (89.8)	0 (1.9)	2 (0.0)
	60-79	92	1 (2.3)	0 (0.0)	43 (97.7)	47 (97.9)	0 (0.0)	1 (2.1)
	≥80	10	-	-	6 (100)	4 (100)	-	-
	Total	976	24 (4.3)	12 (2.8)	528 (56.5)	407 (43.5)	1(20)	4 (80)

F, female; M, male; a, count (%)

Table 2. Demographic characteristics of HSV-2 IgG seropositive patients (n= 36)

	Median age		
	HSV-2 positive case (n=36)	40 (range 0-73)	
	Male (n=12)	43 (range 36-59)	p = 0.020
	Female (n=24)	35 (range 0-73)	
	Diagnosis		
	Male	Anogenital wart	8.3% (1/12)
		Herpes virus infection	8.3 % (1/12)
		Other*	83.3% (10/12)
	Female	Pregnant state	29.2% (7/24)
		Herpes virus infection	4.2% (1/24)
		Other *	66.7% (16/24)

*Anemia, dermatitis, crohn's disease, arthralgia, diabetes mellitus, kidney-transplant, acute vaginitis, Guillain-Barre, idiopathic thrombocytopenic purpura, splenomegaly, epilepsy.

reported that Africa has the highest prevalence rate with 32%, followed by America with 14%. Although Southeast Asia (8%) and West Pacific (8%) have a low prevalence, it has a considerable effect on the total global infection because they have a high population density [19].

In HSV-2 prevalence studies on different patient populations in different regions of Turkey, seropositivity rates range between 4.4% and 63.1% (Table 3) [20-27]. In this study, HSV-2 IgG seropositivity rate was 3.7% (36/976) in general population. Our seroprevalence rates are under the results of both HSV-2 seroprevalence studies around the world and studies in our country. This may be because similar studies were performed in different populations with different demographic characteristics or because of the selection criteria of patient groups included in the study. Therefore, we consider it more proper to evaluate our results according to the studies performed on the general population. In a comprehensive study involving global and regional studies between 2003 and 2012, HSV-2 seroprevalence rates were emphasized to be generally high in women. Moreover, it was reported that HSV-2 infection was more common in women (14.8%) than in men (8.0%) in all continents [19]. It is not known for certain whether women are more susceptible to HSV-2 [28]. In this study, while HSV-2 seropositivity was higher in female patients (4.3%) than in male patients (2.8%), the difference was not statistically significant (p = 0.120).

Although there are studies reporting that HSV-2 seropositivity rate correlates positively with increasing age, there are also some studies reporting that HSV-2 seropositivity rate decreases with increasing age [28, 29]. In a worldwide meta-analysis study evaluating the seropositivity rates in the population aged 15-49, they found that the rate of seropositivity in the 15-19 age group (4.9%) was higher than in the 45-49 age group (1.4%). These researchers reported that HSV-2 seropositivity rates decreased globally with increasing age [19]. In contrast, a study conducted in Germany reported that HSV-2 seroprevalence increased from approximately 3% in children aged 10-15, to 7% in children aged 16-18, and to 14% in adults [30]. In Colombia, a strong relationship between HSV-2 seropositivity and increasing age was found and while seropositivity was 9.8% in the age group of ≤24 it was 25.6% in women in the age group between 45 and 54 [13]. In another study in Taiwan, Shen et al. [31] found that HSV-2 seropositivity was 7.7% in 1072 patients, 1.6% under the age of 30, 10.1% between the age range of 30 and 39, and 31.2% above the age of 60. In the same study, female gender and

Table 3. Results of HSV-2 IgG seroprevalence studies in different population groups in Turkey

City	Population	Date	Age group	Number	HSV-2 IgG(%)	Reference
Erzurum	Pregnant women	1992	unspecified	296	42.23	Arseven et al.(24)
Ankara	Pregnant women	2004	18-40	96	62.5	Cengiz et al.(25)
Adana	Pregnant women	2004	17-44	130	63.1	Duran et al.(27)
Istanbul	Sexually active adult	2006	17-56	725	4.8	Dolar et al.(26)
	Hotel staff		17-50	264	8.3	
	Sex workers		18-50	483	60	
	Patients with genital warts		18-54	110	17.3	
	Pregnant women		19-43	300	5	
	Blood donors		21-47	200	5.5	
Trabzon	General population	2008	20-49	1983	7.6	Topbaş et al.(22)
Konya	Pregnant women	2008	18-44	249	4.4	Özdemir et al.(20)
Izmir	Pregnant women	2009	18-40	158	8.2	Özdemir et al.(21)
Ankara	Female population	2009	≥15	1115	53.5	Maral et al.(23)
This study	General population	2019	0-88	976	3.7	

rural settlement were determined as risk factors for HSV-2 seropositivity. In this study, it was found that seropositivity rate rose significantly with increasing age and while seropositivity rate was 1.2% in the age group of <18, it rose to 8.3% in the age group between 40 and 59, which is consistent with the literature.

There are also epidemiological studies on HSV-2 IgG positivity in the pregnant. In our study, out of 24 pregnant patients, seven had HSV-2 IgG positivity (29.2%). When we looked at the studies in this field, the rate was found in a wide range between 20% and 99.4%, which makes us consider that seropositivity is demographically affected by many risk factors such as age, age of first sexual experience, number of sexual partners and education status. Although the number of pregnant patients included in our study was small, the rate of 29.2% was evaluated as compatible with the data of other studies conducted with pregnant women [25, 32-34]. Epidemiological studies aimed at investigation of HSV-2 seropositivity rate provide the determination of asymptomatic populations and detection of reservoirs that have not been diagnosed yet. Epidemiological studies, especially on the risk groups, contribute to the control of HSV-2 infection and improvement of protection methods.

Limitations

Limitations in our study; the change in HSV-2 prevalence according to the years could not be revealed as the duration when the samples were included in the study consisted of only 2 years. In some studies published on this subject, over the years, while HSV-1 seropositivity decreased, it could not be determined whether HSV-2 seropositivity changed [35]. Additionally, it was revealed that other demographic information such as education level or profession would also contribute to the epidemiology of the disease as well as the relationship between the age and gender and HSV-2 seropositivity [36]. Since this study was a retrospective laboratory study, the education level or professional knowledge of the patients could not be questioned.

Conclusion

HSV-2 IgG positivity was 3.7% in the all age groups and our data were consistent with other studies in our country and in the world. The highest rate of seropositivity was in the 40-59 age group, and this rate was higher in women. In conclusion, HSV-2 infection is a disease in which the number of infected people may reach serious levels with silent spread. Therefore, it is highly important to detect the risk groups by means of HSV-2 seroprevalence studies. Detection of high-risk groups will be a guide for struggle strategies on protection against HSV infection and future health care studies.

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