

Frequency of herpes simplex virus in Syria based on type-specific serological assay

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ABSTRACT

Objective: This study aimed to determine the seroprevalence of herpes simplex virus types 1 and 2 antibodies in Syrian populations. Serum samples collected between 1995 - 1998 from a total of 1293 healthy persons, neonates, pregnant women, labial herpes patients, kidney transplantation patients, atherosclerosis patients, cervical cancer patients, patients with suspected herpetic keratitis, viral encephalitis patients, patients with sexually transmitted diseases, AIDS patients, female prostitutes and bar girls.

Methods: Serum samples were studied by enzyme immunosorbent assay using herpes simplex virus type-specific antigen, glycoprotein (gG).

Results: In the healthy group, herpes simplex virus type 1 infections correlated mostly with age and was prevalent among subjects >30 years (approximately 95% of persons

in our country have herpes simplex virus type 1 antibodies by 30 years of age), while they varied moderately among other groups. Herpes simplex virus type 2 prevalence varied greatly among the groups defined by sexual activity and was associated with risk behavior for prostitution. Herpes simplex virus type 2 seroprevalence was highest among prostitutes (34%) and bar girls (20%); lowest in healthy persons (0.15%); and intermediate in sexually transmitted disease patients (9.5%), cervical cancer patients (8%) and AIDS patients (4%).

Conclusion: It can be concluded that infection of herpes simplex virus type 2 is very rare in persons in our country.

Keywords: Herpes simplex virus, prevalence, population, seroepidemiological study, infection, antibodies.

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Infection with herpes simplex virus (HSV) is extremely common and widespread occurring in a worldwide distribution. Although HSV is among the most common infectious agents in humans, the epidemiological characteristics of HSV infections in Syria have remained unknown so far. Herpes simplex virus infections are caused by the 2 antigenic types, HSV-1 and HSV-2, with HSV-1 being most often thought to be transmitted non-sexually and HSV-2 sexually.¹ They show a wide variety of clinical manifestations, from recognizable symptomatic diseases to sub-clinical, asymptomatic infections. Herpes simplex virus causes

mucocutaneous and nonmucocutaneous infections. Mucocutaneous HSV infections primarily involve oral and genital sites, and less commonly other cutaneous areas. Herpes simplex virus infections in neonatal, immunocompromised, and pregnant individuals are occasionally fulminant, but these types of infection are not common. Seroepidemiologic studies in the United States for example, showed that more than 90% of some US populations had antibodies to HSV by the 4th decade of life.² Other studies showed that the prevalence of HSV-2 antibodies vary considerably with age.² The prevalence of HSV-2 antibodies have been shown to

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range from 3% in nuns to 80% in prostitutes, with up to 25% of higher and 60% of lower socioeconomic groups being seropositive.² Age and race were demographic factors associated with the presence of HSV-2 antibody.² Herpes simplex virus type 2 antibody increased from less than 1% in the under-15 age group to 20% in the group 30 to 44 years old.² High rates were observed in the black population (41%) while 31% in the white population.² Of the subjects with HSV-specific antibodies only 20-30% reported a positive history of clinical symptoms, while more than 70% of HSV infections may be unapparent and therefore require serological determination.³ However, the serological assessment of type-specific immune status of HSV infections has been difficult.^{4,5} Many serological assays, including neutralization tests, passive hemagglutination tests, radioimmunoassay and enzyme-linked immunosorbent assay have been developed to determine the HSV antibody type (types 1 or 2 or both). However, none of these serological methods specifically discriminated between the antibodies to the two types, particularly in individuals infected with both viruses, due to the extensive antigenic cross-reactivity.⁶ Recently, the antibody response to HSV glycoprotein G (gG) has been shown to be entirely type-specific primarily due to the presence of a 526 aminoacid insertion in gG of HSV-2 (gG2) relative to that of HSV-1 (gG1).⁶ The use of type-specific proteins, gG1 and gG2 as antigens in immunological assays now allows differentiation of prior infection with HSV-1 and HSV-2 or both virus types.⁷⁻¹¹ This is the first report from Syria on the type-specific seroepidemiology of HSV-1 and HSV-2 based on gG antigens. It is of interest to compare the prevalence of HSV-1 and HSV-2 in Syrian populations with that in different parts of the world reported previously.

Methods. *Sera.* A total of 1293 serum samples were collected from different cities in Syria, especially from Damascus City and Swida, between 1995-1998. Samples were collected from 654 healthy persons, divided according to their age into 5 subgroups (Table 1) - 100 neonates (their age between 1-45 days); 55 pregnant women (mean age 26 years) who visited the pregnant care clinic; 36

labial herpes patients (mean age 20 years); 32 kidney transplantation patients (mean age 27.25 years); 60 atherosclerosis patients (mean age, 49.76 years) who were admitted to the Cardiac Catheterization Center in Al-Assad University Hospital; 51 cervical cancer patients (mean age, 49 years) who were admitted in the oncology clinic in the Obstetric University Hospital and Nuclear Medicine Center in Moassat Hospital; 14 patients (mean age, 32.6 years) with suspected herpetic keratitis who visited ophthalmologic clinics in Damascus City; 51 viral encephalitis patients (their age between 1-25 years) who were admitted in Al-Assad University, Children, and Moassat Hospitals; 21 sexually transmitted disease clinic attendants in the dermatology hospital and other clinics in Damascus City; 25 AIDS patients (mean age, 38.68 years) from the AIDS treatment center in the Ministry of Health, 101 female prostitutes who were divided into subgroups: 47 from cheap hotels in Damascus City (mean age, 23.57 years) and 54 (mean age, 25.6 years) from Doma prison that were accused of dealing with prostitution; 125 bar girls who worked at night clubs in Damascus City and were also divided into 2 subgroups: 50 arab bar girls (mean age, 27.3 years) and 75 foreign bar girls (mean age, 23.17 years). Cerebrospinal fluid (CSF) samples beside serum samples for viral encephalitis patients group were also collected.

Micro-enzyme immunosorbent assay was used for detecting HSV type-specific antibodies: HSV-1 and HSV-2.^{8,12} The used kits were for RADIM company which used glycoprotein G2 (gG2) as specific antigen in the kit which detects HSV-2 antibodies. Briefly, tested sera dilute (1/300) while CSF dilute (1/10) and then 100 ul of each diluted sera added into the corresponding wells and the wells incubated for 60 minutes at 37°C. Specific antibodies bind to the antigen in the wells. Unbound material is washed away 3 times with 350 ul of diluted washing solution and anti-human IgG or IgM conjugated with horseradish peroxidase added, according to the type of assay, and incubated 30 minutes at 37°C. Conjugate binds to the human antibodies bound to the antigen. Unbound material is again washed away. On addition 100ul of the substrate (tetramethylbenzidine) into the wells a color will develop only in those wells in which enzyme is present, indicating the presence of human anti-HSV antibody. Wells are then incubated for 10 minutes at 37°C. Then, the enzyme reaction is stopped by the addition of 100 ul of sulphuric acid and the absorbance is then measured at 450nm. Positive, negative and cut-off controls were included in each assay. The presence or absence of anti-HSV antibodies is defined by comparing the sample absorbance with the absorbance of the cut-off control samples with an optical density lower than the cut-off control are to be considered non-reactive for anti-HSV antibodies. Samples with an optical density higher than the cut-off control are to be considered

Table 1 - Distribution of healthy persons group according to age and sex.

Age (year)	1 - 5	6-10	11-20	21-30	>30	Sum
Male	55	59	57	48	86	305
Female	46	58	134	68	43	349
TOTAL	101	117	191	116	129	654

Table 2 - Prevalence of herpes simplex virus 1 and 2 antibodies in the Syrian populations between 1995 - 1998.

Study Group	Total number of cases	Percent of antibodies to herpes simplex virus type		
		HSV1 (IgG)	HSV2 (IgG)	HSV (IgM)
1. Healthy Persons				
Males: 1 - 5 years	55	60	0	0
6 - 10 years	59	74.5	0	1.69
11 - 20 years	57	77.1	0	1.76
21 - 30 years	48	85.4	0	2
> 30 years	86	94.1	1.2	2.3
Females: 1 - 5 years	46	50	0	2.1
6 - 10 years	58	81.1	0	0
11 - 20 years	134	83.6	0	4.4
21 - 30 years	68	88.2	0	7.3
> 30 years	43	95.3	0	9.3
2. Neonates	101	100	0	0
3. Pregnant women	55	100	0	5.45
4. Viral encephalitis patients				
In serum	51	58.8	0	9.8
In CSF	51	21.5	0	3.9
5. Aseptic meningitis patients				
In serum	21	85.7	0	0
In CSF	21	0	0	0
6. Labial herpes patients	36	100	0	2.7
7. Artherosclerosis patients	60	100	0	0
8. Kidney transplantation patients	32	96.87	0	21.81
9. Patients with suspected herpatic keratitis	14	85.7	0	0
10. Sexually transmitted disease patients	21	90.48	9.52	14.2
11. Cervical cancer patients	51	100	8	13.7
12. HIV positive patients	25	96	4	0
13. Female prostitutes				
Doma prison prostitutes	54	100	13	7.4
Cheap hotel prostitutes	47	100	34	0
14. Bargirls				
Arab bargirls	50	98	22	14
Foreign bargirls	75	92	18.6	9.3

reactive for anti-HSV antibodies. Samples with absorbance values ranging within $\pm 10\%$ of the cut-off control are to be considered a borderline result.

Statistical analysis. The Fisher's exact probability test was carried out for comparison of antibody prevalence in each population group.

Results. Prevalence of HVS-1 and HSV-2 antibodies. The prevalence of HSV type-specific antibody is shown in Table 2.

For the HSV-1 antibody, in the healthy persons group, the highest prevalence of HSV-1 antibody was observed in >30 years subgroup (94% in males and

95% in females), and the lowest in the 1-5 years subgroup (60% males and 50% in females). The HSV-1 seroprevalence tends to increase with age (Table 3). The HSV-1 antibody prevalence was associated with age (P value=0.000) and none with

Table 3 - Relationship between age and HSV1 (IgG) percentage.

Age (year)	1 - 5	6 - 10	11 - 20	21 - 30	> 30
Percentage	55%	77.75%	80.39%	86.8%	94.7%

Table 4 - Association of prevalence of herpes simplex virus antibodies and risk factors, with control for age.

	HSV1 (IgG) antibody P value	HSV2 (IgG) antibody P value
Healthy persons group versus age	< 0.001	NS*
Healthy persons group versus sex	0.72 (NS)	NS
Pregnant group versus healthy group	NS	NS
Atherosclerosis group versus healthy group	NS	NS
Kidney transplantation group versus healthy group	0.193 (NS)	NS
Cervical carcinoma group versus healthy group	NS	0.15 (NS)
AIDS patients versus healthy persons	NS	0.735 (NS)
Doma prison prostitutes versus healthy girls	NS	0.0003
Cheap hotels prostitutes versus healthy girls	NS	0.0000
Arab bargirls versus healthy girls	NS	< 0.001
Foreign bargirls versus healthy girls	NS	< 0.001
* Not significant		

sex ($P=0.72$). In other groups, the prevalence of HSV-1 antibody varied from 58% to 100% according to the studied group. For instance, it was 59% in serum of viral encephalitis group and 100% in cervical carcinoma. It has been found that 11 cases of cerebrospinal fluid (CSF) were positive to HSV-1 (IgG) antibodies and 2 cases to HSV (IgM) antibodies. The prevalence of HSV-1 IgG, HSV IgM, in viral encephalitis group, was 21.5% and 4%. HSV-2 seroprevalence correlated markedly with sexual activity. The highest prevalence was found in female prostitutes, 34% in cheap hotel prostitutes and 13% in Doma prison prostitutes, and in bar girls (22% in Arab bar girls and 19% in foreign ones); intermediate in STD patients (9.5%); lowest in the healthy persons group (1%) and other groups (0%). Association of HSV antibody prevalence and risk factors. After adjustment of age, the association between several risk factors and HSV infections was explored (Table 4). Herpes simplex virus type 1 antibody prevalence increased with age ($P < 0.001$) in the healthy group. Herpes simplex virus type 2 seroprevalence was associated with prostitution. If we compare the HSV-2 (IgG) antibodies between prostitutes groups, bar girls groups and healthy

control group, we find that P-value in Doma prison prostitutes group was 0.0003, cheap hotel prostitutes was 0.0000, Arab bar girls was < 0.001 , and foreign bar girls was < 0.001 , patients with STD was 0.017. We do not find significant correlation between HSV-1 (IgG) antibody prevalence and sex. There was no significant correlation between HSV antibody prevalence and other agents such as atherosclerosis and kidney transplantation.

Discussion. This is the first study on HSV-1 and HSV-2 epidemiology in Syria based on type-specific serological tests. The use of type-specific antibody assays for HSV-1 and HSV-2 seroprevalence among general populations in several high-risk communities has been performed. By comparing the results of this study with the results of other studies performed in other countries, we found the following. 1. Healthy group. If we compared the prevalence of HSV-1 (IgG) with that in Hashido's study in Japan,⁷ HSV-1 (IgG) prevalence rate was 47% in the 20-29 year-old subgroup; 61.5% in the 30-39 year-old subgroup; 86% in the 40-49 year-old subgroup; 91% in the 50-59 year-old subgroup; and 100% in the 60 year-old subgroup. If we compared our results with Hossain's study in Saudi Arabia,¹³ HSV-1 (IgG) prevalence rate was 60% in the 1-15 year-old group, and 90% in the adult group. In comparison with Meguenni's study in Algeria, the prevalence of HSV-1 antibody was 81% in persons in the 15 year-old group.¹⁴ We conclude that the age group at which HSV-1 antibody prevalence exceeds over 50% was 30-39 year-old group in Hasido's study, 1-15 year-old in Hossain's study, and 1-5 in our study. In healthy persons, we find that the prevalence of HSV-1 antibodies in Saudi Arabia, Algeria and our country were somewhat similar but it was lower in Japan. This is probably due to better sanitary conditions and fewer family members in Japan, while it has not reached to the desired level in our countries. Thus, we see that HSV-1 prevalence differs according to the socio-economical levels, such as crowdedness and sanitary conditions.

Herpes simplex virus type 2 antibody prevalence, in our study, was 0% in all age groups, except in males of age > 30 years. In other studies the prevalence was 30%. In women aging 25-59 years in Oberle's study in Costa Rica,¹⁵ 20% in the group 30 to 44 years old in Johnson in the United States,¹⁶ 11% in the 40-49 year-old group and 14% in the age group 60 years in Hashido's study in Japan.⁷ This difference is probably due to the differences in sexual practices among these cultures. In our Moslem culture, it is still limited to legal marriage, while it is not in other cultures. We conclude that the prevalence of HSV-2 antibodies differs from country to country. 2. Pregnant women group. Herpes simplex virus

type 1 IgG antibody prevalence was 100% in our study. It was somewhat similar to that in other countries. According to Nahmias and colleagues,¹⁷ HSV-1 antibody prevalence rates among pregnant women who visited prenatal clinics around the mid 1980s was >90% in blacks in Atlanta, whites in Spain and Italy, and in Orientals in Taipei. The rate was 70-80% in Rey Kjvik, Lyon, and Sydney; about 60% in Sweden; 90% in Turkey;¹⁸ 50% in Tokyo and 73% in Kagoshima in Japan.⁷ Herpes simplex virus type 2 antibody prevalence was 0% in our study, 33% in Sweden,¹⁹ 14.5% in Australia,²⁰ 14% in Singapore,²¹ 40% in blacks, 10% in whites in England,²² 7% in Tokyo and 17% in Kagoshima in Japan.⁷ It has been found that the HSV-2 prevalence in our study was lower than in other studies. 3. Neonates group. The HSV-2 antibody prevalence was also 0%. This is a normal result because the prevalence of HSV antibody was 0% in the pregnant women group in our country. We conclude that the infection with HSV-2 in neonates is very rare in Syria. We did not see any cases in the Children Hospital during the period 1995-1998 of our study. 4. Viral encephalitis group. We have found 12 CSF samples were positive for HSV antibodies (10 were positive for HSV-1 (IgG), 1 for HSV-1 (IgG) and HSV (IgM), and 1 for HSV(IgM)). We conclude that 23.5% of viral encephalitis cases were caused by HSV-1. 5. Labial herpes patients group. We have not found any significant differences of HSV-1 (IgG) antibody prevalence between this group and the healthy persons group. Another interesting finding is that the titer of HSV-1 (IgG) antibodies did not increase in recurrent labial herpes infection. This result was concluded from the follow-up of 6 patients with recurrent labial herpes persons for 3 weeks after recurrence. The titer of HSV-1 (IgG) antibodies in samples which were taken immediately after the recurrence after 2 and 3 weeks was similar in all patients. 6. Cervical carcinoma group. Herpes simplex virus type 2 antibody prevalence was 8% in our study, 71% in India,²³ 73% in Malawi,²⁴ 26% in Denmark and 76% in Greenland.²⁵ Here, also, we see that the HSV-2 (IgG) antibody prevalence was the lowest among those in other countries. By comparing the HSV-2 antibody prevalence in this group with that in the healthy women control group (0%), we have not found any relationship between HSV-2 and cervical cancer (P=0.15). 7. The STD patients group. Herpes simplex virus type 2 antibody prevalence was 9.5% in our study, 43% in Tanzania,²⁶ 23% in Japan,⁷ 40% in Australia in 1993²⁷ and 65% in Australia in 1994.²⁰ It has also been noticed that the prevalence of HSV-2 antibody in this group of our study was the lowest among those in other studies. 8. Female prostitutes group. Herpes simplex virus type 2 antibody prevalence was 34% in our study, 85.5% in Singapore,²⁸ 80% in Japan,⁷ and 55.5% in Mexico.²⁹ We have found that the

prevalence in this group was also the lowest among those in other countries.

We can conclude from this study that the prevalence of HSV-1 and HSV-2 antibodies varied from country to country, depending on sanitary and socio-economic conditions. Herpes simplex virus type 2 epidemiology in populations in our country seems to be very rare, except in prostitutes and bar girls. These groups are the greatest source of HSV-2 infections in Syria.

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