

ASSESSMENT OF SEROLOGICAL DATA OF HERPES SIMPLEX VIRUS TYPE 1 AND 2 INFECTIONS IN SAMSUN, TURKEY, 2012-2016

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Abstract. Herpes simplex virus type 1 and 2 (HSV-1 and HSV-2) are worldwide the most common human pathogens with a lifelong latency. HSV-1 more commonly causes orofacial infection while HSV-2 typically genital infection in both males and females of all age groups. The aim of this study was to determine the prevalence of IgG and IgM against HSV-1 and HSV-2 among various age groups in Samsun, Turkey between 2012 and 2016. Overall the prevalence of IgG and IgM for HSV-1 was 89% and 4%, respectively, and that against HSV-2 92% and 6%, respectively. The prevalence of IgG against HSV-1 and -2 was 69-94% for all age groups, and that of IgM for HSV-1 and -2 4-8% for ages >1 to 55 years, with no IgM detected for ages >55 years. Interactions with other sexually transmitted diseases and an increasing prevalence of HSVs have made these infections a major public health concern worldwide in the past few decades. Hence, early diagnosis and initiation of therapy are important for the management of these infections.

Keywords: HSV-1, HSV-2, IgG, IgM, seropositivity

INTRODUCTION

Herpes simplex virus (HSV) is a ubiquitous human pathogen in the family Herpesviridae within the subfamily Alphaherpesvirinae under the *Simplexvirus* genus. Two well-known types, which are closely related but differ in epidemiology are herpes simplex virus type-1 (HSV-1)

and herpes simplex virus type-2 (HSV-2) (Sauerbrei *et al*, 2011; Desai and Kulkarni, 2015; Shen *et al*, 2015). HSV-1 is transmitted person-to-person by physical, non-sexual contact during childhood and adolescence, while HSV-2 is almost always been transmitted through sexual contact and mainly affects adults and adolescents (Smith and Robinson, 2002; Bradley *et al*, 2014; Looker *et al*, 2015a). Both viruses lead to several distinct medical disorders for people of all ages.

HSV-1 more commonly affects face, mouth, eyes, and hands, with many symptoms, such as blisters and cold sores,

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gingivitis, stomatitis, keratitis and conjunctivitis (Igde *et al*, 2007; Igde *et al*, 2011; Vilibic-Cavlek *et al*, 2011). However, studies in recent years have indicated increasing evidence of emerging genital HSV-1 infections in adolescents and adults (Bradley *et al*, 2014; Shen *et al*, 2015). Furthermore, most HSV-1 infections are subclinical and many people carrying this type of virus are not aware they are infected.

HSV-2 more commonly causes genital lesions with painful genital ulcers and blistering sores (Wald and Asley-Morrow, 2002; Dolar *et al*, 2006; Tayyebi and Sharifi, 2010; Sauerbrei *et al*, 2011; Vilibic-Cavlek *et al*, 2011). Similarly to HSV-1, no clinical symptoms can be recognized in many people infected with HSV-2. They can shed the virus and there is a potential risk of viral transmission to their sexual partners (Sauerbrei *et al*, 2011).

Although rare, complications as a result of HSV-1 and -2 infections in infants can have several serious sequelae, leading to a common infection with neurological symptoms and mortality (neonate herpes) (Vilibic-Cavlek *et al*, 2011; Bradley *et al*, 2014). In addition, pregnant women with unrecognized HSV-2 infection pose a major risk for their neonates, with approximately 70-85% of neonate herpesvirus infection reported to have been caused by HSV-2 (Sauerbrei *et al*, 2011; Vilibic-Cavlek *et al*, 2011).

Both types of viruses can establish a lifelong latency following primary infection and are never quite removed from the infected individual (Shivaswamy *et al*, 2005; Clemens and Farhat, 2010; Sauerbrei *et al*, 2011, Shen *et al*, 2015). HSV infections, in particular with HSV-2, represent a significant public health concern due to their interactions with human immunodeficiency virus (HIV) and human

papillomavirus (HPV) (Kramer *et al*, 2008; Desai *et al*, 2015; Shen *et al*, 2015). There also are facilitative effects of HSV infections for the acquisition and transmission of HIV (Kramer *et al*, 2008; Igde *et al*, 2011; Desai *et al*, 2015). Furthermore, HSV-2 can accelerate disease progression of invasive cervical carcinoma in women infected with HPV (Clemens and Farhat, 2010).

In Turkey, studies of the serological status of HSV-1 and 2 are limited and the vast majority of these studies are based on detection of neutralizing antibodies (Duran, 2004; Dolar *et al*, 2006; Igde *et al*, 2007; Igde *et al*, 2011). Hence we assessed and updated outcome of HSV-1 and -2 prevalence among different age groups in Samsun Province, Turkey between 2012 and 2016.

MATERIALS AND METHODS

Study population

The study was conducted with the approval of the Scientific Research Assessing Committee, Samsun Training and Research Hospital (SRAC), Turkey, approval no.BADK-2016/09. Assessment of type-specific serologic outcome of HSV-1 and -2 infections in Samsun, the most populated city on the Black Sea coast of Turkey, was conducted between 2012 and 2016. Serum samples were obtained from patients attending Samsun Training and Research Hospital. Ages of the patients ranged from < 1 to 83 years, and were categorized into three groups, namely, children <1-17 years old, adults 18-55 years old and elderly >55 years old.

Serological assays

Sera were obtained from 133 females and 99 males for testing of anti-HSV-1 IgG, from 153 females and 121 males for anti-HSV-1 IgM, from 99 females and 77 males

for anti-HSV-2 IgG, and from 108 females and 87 males for anti-HSV-2 IgM. Commercially available IgG enzyme-linked immunosorbent assay (IgG ELISA) kits (Alegria, Orgentec, Germany) were used to detect neutralizing antibodies against glycoprotein (G) of HSV-1 and -2 (gG1 and gG2, respectively) in serum samples according to the manufacturer's instructions. Sensitivity and specificity of these assays was reported (by the manufacturer) as 99.1% and 98.5%, respectively for HSV-1

IgG, and 95.2% and 99.2%, respectively for HSV-2 IgG. Commercially available IgM ELISA kits (Alegria) were used to detect antibodies against HSV-1 and -2 in serum samples according to the manufacturer's instructions. Sensitivity and specificity was 83% and 100%, respectively for both HSV-1 and -2 IgM.

Statistical analysis

Determination of differences between age, gender and serological parameters of HSV-1 and HSV-2 was conducted using chi-square test.

RESULTS

Age distribution

For evaluation of anti-HSV-1 IgG, sera used according age group was 7% (16/232) in children, 79% (183/232) in adults and 14% (33/232) in the elderlies. For anti-HSV-1 IgM evaluation, the distribution

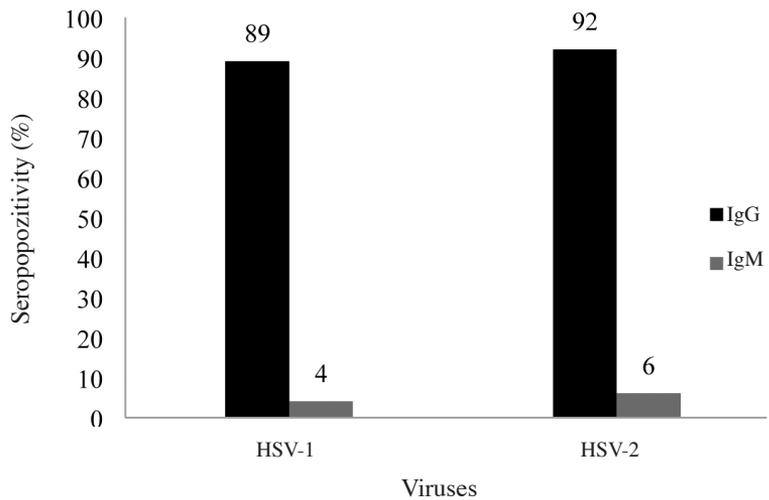


Fig 1—Overall prevalence of IgG and IgM against HSV-1 and HSV-2 in patients attending Samsun Training and Research Hospital, Samsun Province, Turkey between 2012 and 2016. ELISA kits (Alegria, Orgentec, Germany) were used to detect anti-HSV-1 and -2 IgG and IgM.

of sera was 5% (14/274) in children, 81% (220/274) in adults and 15% (40/274) in the elderlies. As for HSV-2 evaluation, the distribution of sera used for detecting IgG and IgM was 6% (11/176) and 5% (11/195), respectively in children, 81% (143/176) and 80% (156/195), respectively in adults, and 12% (22/176) and 14% (27/195), respectively in the elderlies.

Seroprevalences of HSV-1 and HSV-2

The overall seropositivity of HSV-1 and HSV-2 in all patients was as 89% (206/232) and 92% (162/176), respectively (Fig 1). No co-infection of HSV-1 and HSV-2 was detected.

Frequencies of anti-HSV-1 and anti-HSV-2 IgG were higher in females (57% and 55%, respectively) than in males (43% and 45%, respectively) (Fig 2). Furthermore, anti- HSV-1 IgG prevalence in females was higher than that of anti-HSV-2 IgG, while anti-HSV-2 IgG prevalence in

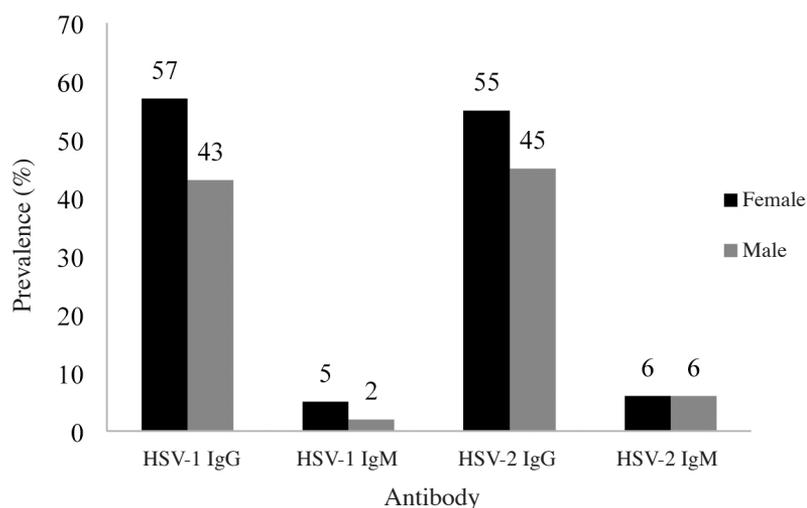


Fig 2–Anti-HSV-1 and –HSV-2 IgG and IgM according to gender of patients attending Samsun Training and Research Hospital, Samsun Province, Turkey between 2012 and 2016. ELISA kits (Alegria,Orgentec,Germany) were used to detect anti-HSV-1 and -2 IgG and IgM.

male was higher than that of anti-HSV-1 IgG. The overall prevalence of anti-HSV-1 and -2 IgM was 4% and 6%, respectively (Fig 1). The anti-HSV-1 IgM prevalence in females (5%) was higher than that in males (2%), while that of anti-HSV-2 IgM prevalence in females (6%) was the same as that in males (6%). As shown in Table 1, there was statistically significant difference between age groups for IgG and IgM prevalence of HSV-1 and HSV-2, ($p < 0.001$ and $p < 0.05$). HSV1 and HSV 2 IgG prevalence was statistically significantly higher in 18-55 and > 55 age groups than in $< 1-17$ age group ($\chi^2 = 19.86$, $p = 0.000049$ for HSV-1 IgG and $\chi^2 = 21.42$, $p = 0.000022$ for HSV-2 IgG). HSV-1 and -2 IgM prevalence was found statistically significant for $< 1-17$ age group as compared to other age groups ($\chi^2 = 6.983$, $p = 0.0305$ for HSV-1 IgM and $\chi^2 = 7.792$, $p = 0.0203$ for HSV-2 IgM). Adult patients had 91% and 94% seropositivity for HSV-1 and HSV-2, respectively, but no

difference in children (69% and 73%, respectively) or the elderly (88% and 91%, respectively). On the other hand, there are no statistical significant differences between children and adults for prevalence of IgM against HSV-1 (7% and 4%, respectively). No anti-HSV-1 and -2 IgM were detected in the elderly.

DISCUSSION

HSV-1 and -2 are defined as neurotropic viruses that lead to recurrent infections

throughout life due to neuronal infection resulting in latency (Smith *et al*, 2006). HSV-1 and -2 infections occur worldwide with a wide age spectrum in humans. Clinical manifestations caused by both types of viruses can change between asymptomatic and symptomatic (Smith *et al*, 2006; Clemens and Farhat, 2010).

In general, seroprevalence among adults is $\geq 60\%$ and 11.3% for HSV-1 and -2, respectively (Clemens and Farhat, 2010; Looker *et al*, 2015a,b). The highest HSV-1 (87%) and HSV-2 (31.9%) seroprevalence are in Africa, while the lowest HSV-1 and HSV-2 seroprevalence (40-50%) is in the Americas and Middle-Eastern countries. HSV-1 and HSV-2 seroprevalences are higher in females (68% and 14.8%, respectively) than in males (66% and 8%, respectively), ranging between 52% to 84% for HSV-1 and 4% to 24% for HSV-2 in Europe (Looker *et al*, 2015a,b).

Both types of HSVs in Turkey have

Table 1
Seropositivities of anti-HSV-1 and -HSV-2 IgG and IgM among patients admitted to Samsun Training and Research Hospital, Samsun Province, Turkey between 2012 and 2016.

Age group (years)	HSV-1		HSV-2	
	IgG%	IgM%	IgG%	IgM%
<1-17	69 ^b	7 ^{a**}	73	8 ^{a**}
18-55	91 ^{a*}	4 ^{ab}	94 ^{a*}	6 ^{ab}
>55	88 ^{a*}	0 ^b	91 ^{a*}	0 ^b

Columns with different letters indicate statistically significant difference.

^{a*} $p < 0.001$, ^{a**} $p < 0.05$.

similar prevalence compared to other countries across the world, with seroprevalence of HSV-1 and HSV-2, respectively of 85.3% and 4.8% in sexually active adults, 96% and 5.5% in blood donors, 98% and 5% in pregnant women, 93.6% and 17.3% in patients with genital warts, 97.3% and 8.3% in hotel staff, and 99% and 60% in sex workers (Dolar *et al*, 2006). Igde *et al* (2011) reported that the overall HSV-1 seroprevalence in randomly selected patients was 59.7% in Turkey and, as in the case with other published studies, HSV-1 seroprevalence is higher in females than males.

Commercially produced serological assays provide an opportunity to confirm a clinical diagnosis of viral infections without using time-consuming and expensive virus identification and isolations methods (Venkateshwaran *et al*, 2011). For laboratory diagnosis of HSV-1 and HSV-2, type-specific ELISA kits have proven to be highly specific and sensitive and thus are widely used.

In Turkey, there is limited research on HSV-1 and -2 prevalence that incorporate data on presence of serum IgG and IgM (Cengiz *et al*, 1993a,b). According to the data obtained from the current research, the overall neutralizing antibody distri-

bution of HSV-1 and -2 were 89% and 92%, respectively. These rates are close to the serological data reported in previous studies that had been conducted in HSV-suspected patients in Turkey (Cengiz *et al*, 1993a,b; Dolar *et al*, 2006). Age-related seroprevalence of both viruses was higher in females than in males, similar to other reports (Clemens and Farhat, 2010; Looker *et al*, 2015a,b). The lower seroprevalence of both viruses among children compared to the other the age groups (adult and the elderly) could be seen as normal for the Turkish population owing to the conservative social structure, with thus, the prevalence of HSV can be correlated to increasing sexual activity because people in the age group <1-17 years are considered to be sexually less active compared to the same age group in Europe and the Americas. Other studies showed the highest seroprevalence for both viruses in the age group 18-55 years, whose members are considered to be more sexually active than other age groups (Smith and Robinson, 2002; Bradley *et al*, 2014).

The presence of serum IgM in patients and neonates is an important indicator for the diagnosis of recent and intrauterine infection, respectively, which was similar in both groups (~5%). In this survey IgM

prevalence of HSV-2 was slightly higher in males than in females and vice versa for HSV-1. In particular, anti-HSV-2 IgM positivity in males was significant (6%) and poses a serious risk of transmitting HSV-2 to their sexual partners. Higher prevalence of anti-HSV-1 and -2 IgM (7% and 8%, respectively) in the age group <1-17 years than adults and the elderly reflect acute and recent infection of both viruses and that the transmission of HSV-1 was more permissive than HSV-2 because of the former virus non-sexual route of transmission. On the other hand, it could be thought that HSV-2 transmission was more difficult for this age group due to the conservative family structure of Turkish society and transmission route. We interpret these prevalence data in this age group as resulting from perinatal transmission (mother-to-child) occurring during the intrapartum period (Straface *et al*, 2012). The absence of anti-HSV-1 and -2 IgM positives among the elderly of both sex suggests that there were no active HSV-1 and -2 infections in this age group.

In conclusion our survey data are consistent with other studies demonstrating HSV-1 and HSV-2 epidemiology is rapidly changing depending on age, sex, socioeconomic status, race and cultural structure. There are four important points that should be considered regarding HSV infection: (i) HSV-1 is mainly responsible for oral and labial infections and HSV-2 is a cause for genital infections; however, genital infection involves not only HSV-2 but also HSV-1, in particular in industrialized countries. (ii) The vast majority of HSV infection are asymptomatic, and therefore, many people most likely are unaware of their infection status and thereby contribute to a large percentage of HSV-1 and -2 infections. (iii) HSV infections can lead to serious complications in adults,

such as blindness, aseptic meningitis and encephalitis; and having HSV-2 infection can increase the risk of contracting HIV. HSV-1 and -2 infections can lead to fatal neonatal infections. In particular, there is a correlation between an increasing number of genital HSV infection and incidence of neonatal herpes. (iv) It is important to continue to monitor the prevalence of HSV-1 and -2 due to their rapidly changing epidemiology landscape. Hence, we recommend that further studies be conducted to investigate the role of HSV-1 in incidences of genital herpes, the interaction between HSV and STDs and the development of effective strategies for the prevention of HSV infections.

CONFLICTS OF INTERESTS

All authors declare no conflicts of interest and that they are solely responsible for the content and writing of the manuscript.

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