

Serological Evaluation of HSV-1 and HSV-2 Infection In Pregnancy

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Abstract: We performed a serological evaluation of herpes simplex virus (HSV) infections at the time of labor. In order to determine the seroprevalence of asymptomatic HSV infections in pregnant women, blood samples were collected for antibody measurement from 130 randomly selected pregnant women. Presence of HSV IgG and IgM antibodies in the serum samples was tested by ELISA. The prevalence of HSV-1 gG1 antibodies was 90% (117/130), that of HSV-2 gG2 antibodies was 63.1% (82/130) and that of HSV-2 IgM antibodies was 13.8% (18/130). Eight (44.4%) of the pregnant women with HSV-2 IgM were at the same time found to have HSV-2 IgG antibodies, and 10 (55.6%) women were determined to have HSV-2 IgM antibodies. Furthermore, 6 (4.6%) out of the 130 pregnant women were seronegative for HSV.

In conclusion, the results of our study show that the prevalence of HSV-2 infection (63.1%) is relatively high among asymptomatic pregnant women in Adana, Turkey. Therefore, to take precautions for reducing the risk of neonatal herpes, the investigation of HSV-2 type specific antibodies in pregnant women should be offered by using HSV-2 gG2 type specific serological tests that allow the identification of women with asymptomatic or subclinical genital HSV-2 infection and women susceptible to primary genital HSV-2 infection.

Key Words: Genital HSV infection, HSV-2, ELISA, seroprevalence, pregnancy.

Introduction

The incidence of herpes simplex virus (HSV) infection has been increasing steadily in recent decades, and concerns about perinatal HSV infection are growing among women of reproductive age because of the risk of transmission of the virus to their babies during pregnancy, with potentially devastating consequences to the fetus (1-3).

Genital HSV infection in pregnant women is of particular importance because of the risk to the fetus and newborn. Data from several prospective trials have shown that the major risk to the fetus results from primary maternal genital HSV infection. Over 95% of infected babies are born to women who are unaware that they have genital herpes. The most severe forms of neonatal disease occur when infection of the mother occurs during the last stage of pregnancy, at a time when maternal antibodies develop much more slowly for

passive transfer of the protective antibodies to the fetus (1,3-5).

The most severely affected population is neonates, who acquire HSV infection after exposure to the virus during birth (5). This is a relatively rare infection, occurring in about 1 in 3,000 births in the northwestern United States and with a somewhat lower frequency in other areas. However, these infections result in permanent neurological damage for many infants despite appropriate antiviral therapy. The increasing acquisition rate of genital herpes among women of childbearing age suggests that more neonates may be exposed to the virus than in past decades (3,5).

Previous studies have suggested that genital HSV infection acquired during pregnancy is associated with preterm labor, intrauterine growth retardation, and spontaneous abortion (1,5).

The best method for detecting HSV infections in the general population is measuring type-specific serum antibodies (6).

In this study, we used serological methods to investigate the seroprevalence of HSV infection among pregnant women.

Materials and Methods

This study was carried out in Adana, Turkey, between September 1999 and May 2001. In order to determine the seroprevalence of asymptomatic HSV infections in pregnant women, blood samples for antibody study were collected from 130 randomly selected pregnant women. The existence of HSV IgG and IgM antibodies in the serum samples was tested by ELISA. Blood samples were centrifuged at 1500 rpm for 5 min to obtain serum samples.

ELISA procedure

Determination of HSV-2 IgG Antibodies: The reactions were carried out on polystyrene plates (Costar, USA). One hundred milliliters of antigen diluted in sodium bicarbonate-carbonated 0.05M buffer pH 9.6 was added to each well. After incubation for 18 h at 4 °C, the excess antigen was removed by 3 washes with PBS Tween 80 at 0.05%. The blocking step was carried out using 100 µl of PBS bovine serum albumin 5%, pH 7.6, over 1 h at 37 °C. After washing, the plate was incubated at 37 °C for 60 min, with 100 µl of each serum sample diluted in PBS Tween (1:100). Unbound antibodies were removed by washing the plates as described above. One hundred microliters of monoclonal anti-human IgG Conjugate (Sigma), diluted in PBS-Tween, was added to each well and the plates were incubated for 60 min and washed. The anti-human IgG conjugate (Sigma) was used at a dilution of 1:10,000. The plate was washed after incubation for 1 h at 37 °C, and 100 µl of orthophenylene-diamine (OPD) substrate solution was added. The reaction was interrupted using sulfuric acid 1N (50 µl per well). Absorbances were read using a microplate reader (Sigma) with a 450 nm filter.

The determination of HSV-2 IgM, HSV-1 IgG and HSV-1 IgM antibodies was carried out as for the determination of HSV-2 IgG antibodies. The anti-human IgM conjugate (Sigma, USA) was used at a dilution of 1:10,000 and the results were evaluated as above.

The average absorbance (OD) value of the cutoff serum run in duplicate was calculated. Then the cutoff index (COI) of each serum sample was determined by dividing the OD obtained for that serum sample by the average OD of the cutoff serum. A COI below or equal to 1 was considered negative, a COI above 1.1 was considered positive, and a COI between 1 and 1.1 was considered borderline.

Results

In this study the pregnant women were aged between 17 and 44 (26.7 ± 5.6). Considering the age distribution, 21 of the pregnant women were 17-20 years old, 37 were 21-25 years old, 40 were 26-30 years old and 32 were above 30 years old.

All pregnant women without an apparent history of genital herpes enrolled in the study. It was found that the prevalence of HSV-2 IgG was 63.1% (82/130), and the prevalence of HSV-1 IgG was 90% (117/130). In addition, 13.8% (18/130) of the pregnant women had HSV-2 IgM antibodies. Eight (44.4%) of the pregnant women with HSV-2 IgM were at the same time found to have HSV-2 IgG antibodies, and 10 (55.6%) women were determined to have HSV-2 IgM antibodies. Furthermore, 6 (4.6%) out of the 130 pregnant women were seronegative for HSV.

Evaluation of the HSV-2 IgG antibodies according to age group showed that 9 (42.8%) out of the 21 pregnant women in the 17-20 age group, 20 (54.1%) out of the 37 women in the 21-25 age group, 28 (70%) of the 40 pregnant women in the 26-30 age group and 25 (78.1%) of the 32 pregnant women above 30 years old had HSV-2 IgG (Figure 1).

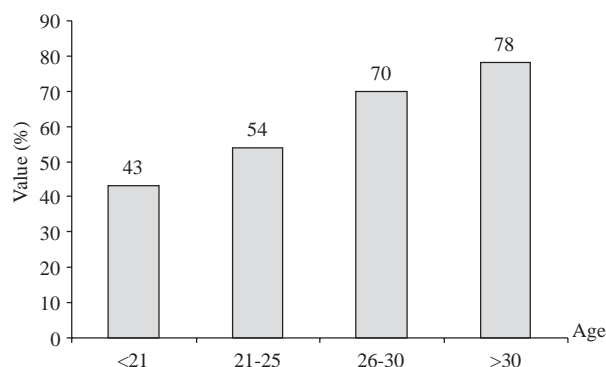


Figure 1. The distribution of HSV-2 IgG antibodies according to age.

Thirty-four percent (39/117) of the 117 pregnant women with a history of oral herpes had antibodies to HSV-1 IgG only; 3.4% (4/117) of these women had antibodies to HSV-2 only, and 59.8% (70/117) had antibodies to both HSV-1 and HSV-2. Furthermore, 3.4% (4/117) of the pregnant women were seronegative for HSV (Figure 2).

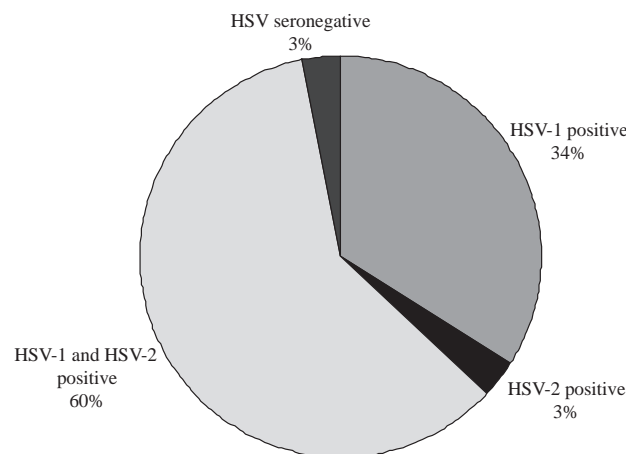


Figure 2. The antibody distribution of HSV-1 and HSV-2 IgG in a total of 117 pregnant women with an oral herpes history.

Three (23.1%) of 13 pregnant women without an oral herpes history had only HSV-1 IgG antibodies, and 7 (53.8%) of these pregnant women had both HSV-1 IgG and HSV-2 IgG antibodies. One (8%) of them had only HSV-2 IgG antibody. In addition, 2 (15%) of these 13 pregnant women were seronegative for HSV (Figure 3).

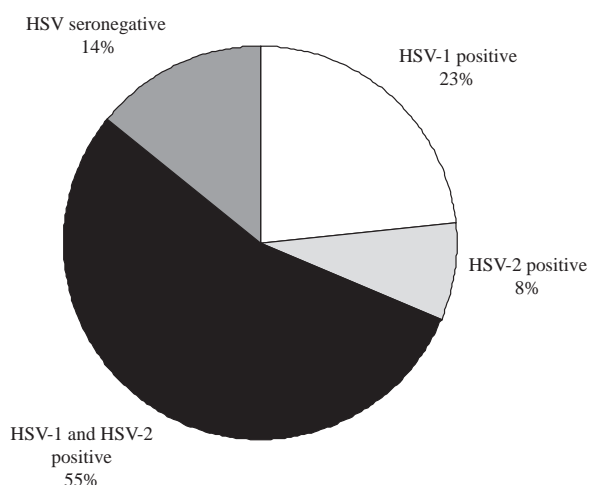


Figure 3. The antibody distribution of HSV-1 and HSV-2 IgG in a total of 13 pregnant women without an oral herpes history.

Examination of the 18 HSV-2 IgM positive pregnant women by age group showed that 2 (9.5%) out of the 21 of the 17-20 age group, 11 (29.7%) out of 37 in the 21-25 age group, 3 (7.5%) out of 40 in the 26-30 age group and 2 (6.3%) out of 32 in the above 30 age group had the HSV-2 IgM antibody (Figure 4).

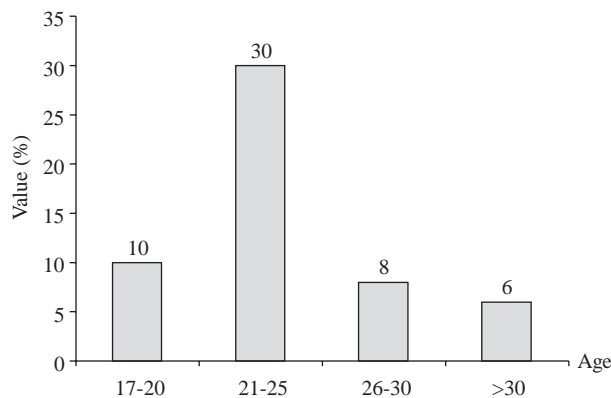


Figure 4. The distribution of HSV-2 IgM in a total of 18 pregnant women according to age group.

Discussion

HSV infection occurs worldwide but its epidemiology varies between different countries and between groups of individuals. The seroprevalence of HSV-1 and HSV-2 antibodies is an accurate method of determining the epidemiology of this infection (6,7). Even though genital herpes infection in pregnant women is common and rarely serious, the risk of vertical transmission to the infant when the mother develops a primary infection during the third trimester is high and this risk increases the closer to the time of delivery (7-12).

The identification of pregnant women with genital herpes infection is necessary for the protection of babies from the risk of neonatal herpes. Type-specific HSV serologic screening has been recommended to identify women at risk of acquiring genital HSV-1 or HSV-2 infections close to term, a setting in which there is high risk (30-50%) of neonatal herpes (6-13,14).

In this study, we investigated the incidence of HSV infections in pregnant women at delivery. While the prevalence of HSV-2 IgG was 63.1% (82/130) in the pregnant women, the prevalence of HSV-1 IgG was 90% (117/130).

Among the 117 pregnant women with an oral herpes history 34% and 3% were found to have antibodies against HSV-1 and HSV-2, while 60% had antibodies against both HSV-1 and HSV-2, and 3% were seronegative (Figure 2).

Seven (39%) of the 18 pregnant women with HSV-2 IgM positivity had an oral herpes history while 11 (61%) of these women had no oral herpes history. This seems to be in line with the view that the presence of HSV-1 antibodies results in a reduction in the development of HSV-2 infections, no matter how small (10-14).

HSV-2 seroprevalence increased significantly with age ($p < 0.01$). Examination of the distribution of the prevalence of the HSV-2 IgG by age revealed an increase from 43% in the 17-20 age group to over 78% in the above 30 age group (Figure 1).

Studies on the prevalence of HSV-2 infection among pregnant women are limited in Turkey. Arseven et al. (15) investigated the distributions of anti-HSV-1 and HSV-2 IgG and IgM antibodies in sera obtained from 296 pregnant women. While 24 (8.11%) of 287 HSV-1 IgG antibody positive patients were IgM positive, anti-HSV-1 IgM alone was not detected in any patients. In 16 (5.41%) of 125 (42.23%) patients who were positive for the HSV-2 IgG antibody HSV-2 IgM antibodies were detected as well. The presence of HSV-2 IgM was found in only 4 (1.3%) of the pregnant women (15).

Cengiz et al. (16), in their study on 73 mothers with various obstetric problems like abortion, stillbirth, premature birth and intrauterine developmental retardation, reported HSV-1 positivity in 71 (97.3%), HSV-2 IgG positivity in 65 (89%) and HSV-2 IgM positivity in 6 (8%) (16).

With regard to studies from other countries on the seroprevalence of HSV-2 in asymptomatic pregnant women, Frenkel et al., in the USA in their study on 1355 pregnant women without histories of genital herpes, detected HSV-2 seropositivity in 439 (32.4%) cases (17).

Enders et al. (18), reported an 8.9% HSV-2 infection ratio among 1999 pregnant women examined in Germany. They found that 20.7% of the pregnant women were seronegative for both HSV-1 and HSV-2 IgG antibodies, hence the conclusion was that an increased risk of the development of both types of primary HSV infection existed (18).

Eskild et al. (19), in their study on 961 pregnant women, reported HSV-2 antibodies in 256 (26.6%) cases, and an increase in prevalence with age (17% in the 20-24 age group and 34% in the above 30 age group (19)).

Avraja et al. (20) reported a prevalence of 16% for the HSV-2 IgG antibody and 70% for the HSV-1 gG1 antibody among pregnant women in Finland and also found active HSV-2 and HSV-1 infection during the course of pregnancy in 5 (0.6%) and 9 (0.9%) of the women, respectively (20).

Hashido et al. (21) in their study on pregnant women in Japan reported the seroprevalence of HSV-2 as 7%. Due to the downward trend in the incidence of HSV-1 infections during childhood in Japan, primary genital HSV-2 infections have been estimated to have become more common (21).

Dong et al. (22) reported the prevalence of HSV-2 IgG as 69.1% in 233 pregnant women and that of HSV-2 IgM as 3%. In this group HSV-1 IgG and HSV-1 IgM antibodies were respectively 66.7% and 2.5% (22).

Carvalho et al. (23) reported a 22.6% HSV-2 seropositivity in Brazil among 102 pregnant women studied, with only 1 of them having a history of genital herpes. These data demonstrate that regardless of the presence or absence of a history of genital herpes viral spread could be very common (23).

In our study, the 63.1% HSV-2 antibody prevalence found in the asymptomatic pregnant women approaches the seroprevalence of HSV-2 reported by Arseven et al. (15). That reported by Dong et al., however, is similar. In contrast to this, the prevalence of HSV-2 infection among pregnant women varies from 7% to 32%. Demographic criteria and socio-economic factors are possible determinants of differences in the seropositivity ratios of HSV-2. The prevalence of HSV-2 infection increases with advanced age, more sexual partners, early age of first sexual intercourse, history of sexually transmitted disease and lower socio-economic status (24,25).

In a study by Eskild et al. (19) from Norway, an increase in the prevalence of HSV-2 with advancing age has been reported. In our study group, the observation that the HSV-2 IgM antibody response was highest in the 21-25 age group among the pregnant women with a ratio of 30% demonstrates clearly that this age group is especially at risk of developing primary or reactive genital HSV-2 infection.

In conclusion, these results confirm a high prevalence of HSV infection in Adana. To improve recognition of genital herpes near term, obstetricians should counsel pregnant women about the high prevalence and mild and

diverse symptoms of genital HSV-2 infection. In addition, it is recommended that pregnant women who are susceptible to primary genital HSV-2 infections should be identified by HSV-2 IgG 2 type specific serological tests.

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