Asymptomatic herpes simplex virus type 2 (HSV-2) infection among pregnant women in Turkey

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Background & objectives: A large proportion of individuals with serologic evidence of infection with herpes simplex virus type 2 (HSV-2) are asymptomatic. HSV-2 is the main cause of genital herpes infections. The acquisition of genital herpes during pregnancy has been associated with spontaneous abortion, premature labour and congenital and neonatal herpes. The present study was undertaken to determine asymptomatic genital HSV-2 shedding and seroprevalence of HSV-2 infection among asymptomatic pregnant women at the time of delivery in Adana, Turkey.

Methods: Asymptomatic 130 pregnant women without a history of genital herpes were enrolled in the study. HSV-2 shedding was determined by viral culture of the swabs collected from cervix and vulva and HSV-2 antigen was detected by direct immunofluorescence assay (IFA), HSV-2 IgG and IgM antibodies were detected by HSV-2 type specific IgG and IgM enzyme-linked immunosorbent assay (ELISA).

Results: HSV-2 IgG and IgM antibodies were found in 82 (63.1%) and 18 (11.3%) of 130 pregnant women. HSV-2 type-specific antigen was detected in 22 (16.9%) pregnant women by IFA test, 17 (13.1%) of whom had HSV-2 IgM antibodies. HSV-2 was isolated only in 3 women.

Interpretation & conclusion: The seroprevalence of HSV-2 (63.1%) and genital HSV-2 infection (16.9%) was high among asymptomatic pregnant women in Adana, Turkey. Therefore, to reduce the risk of neonatal herpes, HSV-2 type-specific antibodies should be detected in pregnant women using serological tests that allow to identify women with asymptomatic or subclinical genital HSV-2 infection and those susceptible to primary genital HSV-2 infection.

Key words: Cell culture - ELISA - genital herpes - herpes simplex virus type 2 - IFA - pregnant women - seroprevalence

Herpes simplex virus type 2 (HSV-2) is the major cause of genital herpes; 78-97 per cent of HSV-2 infections are asymptomatic1,3. Unrecognized virus shedding from the mother’s genital tract may have occurred at the time of delivery in as many as 70 per cent of cases of neonatal herpes3,5.

The worldwide prevalence of HSV-2 seropositivity is alarmingly high6, especially among women of reproductive age group. Antibodies to HSV-2 have been detected in approximately 20 per cent of pregnant women; however, only 5 per cent reported a history of symptomatic infection. Primary genital HSV infections during pregnancy occur at rates similar to those in non pregnant women and often these infections are asymptomatic1,6,7. The seroconversion rate is about 2-3 per cent in pregnant women. Genital herpes usually spread through patients with asymptomatic or subclinical infections8. Unfortunately, 70 per cent of the newborns with neonatal herpes infection were infected by their asymptomatic mothers during birth. Although antiviral therapy exists, HSV infection is still the major cause of...
the morbidity and mortality in the newborns. Due to high occurrence of HSV-2 infection among adults, physicians should be aware of the risk of a primary HSV infection in pregnant women and its consequences to the foetus. Information on the occurrence of asymptomatic HSV-2 infection among pregnant women in our region is scanty. This study is undertaken to determine the occurrence of genital HSV-2 infection among asymptomatic pregnant women and HSV-2 shedding during the birth of the child.

**Material & Methods**

Genital swabs samples were collected from 130 pregnant women with no apparent history of genital herpes, attending Obstetrics & Gynaecology Department of Balcali Hospital of Cukurova University, Adana, Turkey, during September 1999 to May 2001. These women voluntarily gave consent to participate in this study. At the time of swab collection, blood samples were also obtained from these pregnant women. During the medical consultation, women were counseled for 5-10 min about genital herpes. At the end of the consultation, history were taken.

A dacron or cotton swab was vigorously passed over the cervix and vulva and was then placed in 2 ml of Hanks’ transport media (Sigma, USA). Swab samples were used for the isolation of HSV-2 and determination of HSV-2 antigen. Existence of HSV-2 IgG and IgM antibodies in the serum samples were tested using ELISA method.

Swab samples were centrifuged in transport media at +4°C (2000 g) for 30 min. The supernatant was used for viral isolation and subnatant (precipitate) for direct IFA (Immuno Fluorescence Assay) method. An aliquot of each sample was placed onto HEp-2 cells for viral isolation using standard tissue culture techniques. Cultures were investigated daily with the inverted microscope (Nikon, USA) to see cytopathological effect (CPE) for a week. The samples having CPE were centrifuged at 500 g for 10 min, then placed onto glass slides and fixed with cold acetone for 10 min and were stored for IFA study at -20°C.

**ELISA procedure:** HSV-2 antigen (Amico Laboratories, USA) diluted in sodium bicarbonate-carbonate buffer (0.005 M, pH 9.6) was added in each well on polystyrene ELISA plates (Costar, USA). For IgG measurement, monoclonal anti-human IgG conjugate (Sigma, USA) was used. Colour was developed using ortho-phenylenediamine (OPD) as substrate. Absorbance was read using a microplate reader (Sigma, USA) with a filter of 450 nm.

The determination of HSV-2 IgM antibodies was carried out with anti-human IgM conjugate (Sigma, USA) in a similar manner.

Positive and negative control serum samples were used in each experiment. The cut-off was determined by dividing the optical density (OD) of positive and negative controls. The average absorbance (OD) value of the cut-off serum run in duplicate was calculated. The cut off index (COI) of each serum sample was determined by dividing the OD obtained for that serum sample by the average OD of the cut-off serum. A COI below or equal to 1 was considered negative, above 1.1 was considered positive, and a COI between 1 and 1.1 was considered borderline.

**Statistical analysis:** Statistical analysis was done using the \( \chi^2 \) test or Fisher’s exact test for association and Student’s ‘t’ test when appropriate; the odds ratios and 95 per cent confidence intervals (95%CI) were calculated. Statistical significance was assigned to \( P < 0.05 \).

**Results & Discussion**

The most devastating consequence of genital herpes is neonatal herpes. It is known that the majority of newborns acquire this infection by contact with infected genital secretions during delivery from asymptomatic mother who acquired the first episode of genital herpes near the time of labour. Since the majority of cases of first episode genital herpes during pregnancy are unrecognised, the prevention of neonatal transmission will depend upon the identification of the HSV-2.

In this study the pregnant women were in the age group of 17-44 yr (26.7 ± 5.6 yr). Of the 130 women, 21 were 17-20 (18.8 ± 1.0) year old, 37 were in the age group of 21-25 (23.1 ± 1.4) years, 40 in 26-30

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(28.3 ± 1.4) years, and 32 were above 30 (35.5 ± 3.6) years old.

All pregnant women had no history of genital herpes at the time of enrolment. HSV-2 IgG was found in 82 of 130 women (63.1%) (95% CI: 61.2-64.7) and HSV-2 IgM in 18 (3.8%) (95% CI: 11.5-15.1). Eight of the 18 women with HSV-2 IgM had HSV-2 IgG antibodies.

Of the 130 swab samples, 22 (16.9%, 95% CI: 15.6-17.3) were found to have the HSV-2 type-specific antigen by IFA test. HSV-2 was isolated in 3 women by the culture method. These 3 women were also positive for HSV-2 IgM antibodies and HSV-2 type-specific antigen. Two of these women were positive for HSV-2 IgG antibody. The number of HSV-2 isolation by the cell culture method was lower than the HSV-2 antigen positivity. This was probably due the inactivation of the virus by local antibodies, low titre of the virus in the samples or inactivation of the virus during transportation of the samples13,14. The sensitivities and specificities of IFA, ELISA and culture were found to be 84.67, 81.8 and 96.4, 96.6 and 16.7, 100 per cent respectively.

Evaluation of the HSV-2 IgG antibodies according to age group showed that 11 (52.4%) of the 21 women in the 17-20 yr age group, 20 (54.1%) of the 37 women in the 21-25 yr age group, 28 (70%) of the 40 women in the 26-30 yr age group, and 25 (78.1%) of the 32 pregnant women above 30 yr had HSV-2 IgG. HSV-2 seroprevalence increased significantly with age (P<0.001). In a study by Eskild et al15, the prevalence of HSV-2 IgG was reported to increase with advancing ages.

Of the 21 women in the 17-20 yr age group, 2 (9.5%) were positive for HSV-2 IgM, 11 (29.7%) of 37 in the 21-25 yr age group, 3 (7.5%) of 40 in the 26-30 yr age group, and 2 (6.25%) of 32 in the above 30 yr age group had the HSV-2 IgM antibody. HSV-2 type-specific antigen was detected in 4 (19.1%) of 21 women in the 17-20 yr age group, 9 (24.3%) of 37 in the 21-25 yr age group, 4 (10%) of the 40 in the 26-30 yr age group, and 5 (15.6%) of 32 pregnant women in the above 30 yr age group.

The frequency of HSV-2 IgM antibody was highest in the 21-25 yr old women. These findings demonstrate that this age group is especially at risk for primary or reactive genital HSV-2 infection. In addition, the presence of HSV-2 antigen was also found to be highest in this age group.

Studies on the prevalence of HSV-2 infection among pregnant women are limited in Turkey. Arseven et al16, investigated the distribution of anti-HSV-1 and HSV-2 IgG and IgM antibodies in serum samples obtained from 296 pregnant women. In 16 (5.41%) of 125 women who were positive for the HSV-2 IgG antibody, HSV-2 IgM antibodies were also detected. Cengiz et al17, in their study on 73 mothers with various obstetric problems like abortion, stillbirth, premature birth and intrauterine developmental retardation, reported HSV-2 IgG positivity in 65 (89.1%) and HSV-2 IgM positivity in 6 (8.2%).

Frenkel et al18, in their study on 1355 pregnant women without history of genital herpes, detected HSV-2 seropositivity in 439 (32.4%) cases. Eskild et al15 reported HSV-2 antibodies in 256 of 961 (26.6%) pregnant women, and an increase in prevalence with age (17% in the 20-24 yr age group and 34% in the above 30 age group). Dong et al19, reported the prevalence of HSV-2 IgG as 69.1 per cent in 233 pregnant women and that of HSV-2 IgM as 3 per cent. In this group HSV-1 IgG and HSV-1 IgM antibodies were 66.7 and 2.5 per cent respectively.

In our study, presence of HSV-2 antibody in 63.1 per cent asymptomatic pregnant women in similar to that reported by others16,19. In contrast, the prevalence of HSV-2 infection among pregnant women varies from 7 to 32 per cent in other studies15,18. Demographic criteria and socio-economic factors are possible determinants of differences in the seropositivity rates of HSV-2. The occurrence 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 status9,10,20.

HSV shedding was reported to vary from 0.09 to 4 per cent in asymptomatic pregnant women by cell culture2-4. In a study in USA, in which samples for culture from HSV-2 seropositive pregnant women in their third trimester were taken everyday for HSV-2 determination...
and polymerase chain reaction (PCR) studies, Boggess et al., found the spread of asymptomatic virus to be 2.3 per cent by culture and 13.8 per cent by PCR. Cone et al., found asymptomatic HSV spread in 100 pregnant women to be 9 per cent by PCR but failed to isolate the virus by cell culture. It is reported that PCR is more sensitive than culture method for diagnosis of genital herpes.

In conclusion, the results of our study show that the occurrence of HSV-2 infections among pregnant women in our area (Adana, Turkey) is high. Though HSV-2 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. Neonatal herpes is known to be associated with serious consequences. For this reason to take precautions for decreasing the risk of neonatal herpes it is recommended that women with asymptomatic or subclinical genital HSV-2 infection and those susceptible to primary genital HSV-2 infections be identified by examining the HSV-2 antibody status of pregnant women.

References


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