Analysis of Herpes Simplex 1 and 2 IgG and IgM Antibodies in Pregnant Women and their Neonates.

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Abstract

Objectives: (1) Assessment of prevalence of herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2) antibodies in pregnant mothers visiting the antenatal clinic and delivery room in King Fahd Hospital of the University (KFHU) in Al-Khobar, Saudi Arabia and (2) assessment of prevalence of HSV-1 and HSV-2 antibodies in cord blood in the delivery room in KFHU.

Material and methods: Laboratory methods used included the type-specific enzyme linked immunosorbent assay to assess HSV-1 and HSV-2 IgM and IgG antibodies in the sera.

Results: generated from the samples of pregnant mothers (N=459) showed that 90.5% have detectable levels of HSV-1 IgG antibodies, 6.5% have detectable level of HSV-2 IgG antibodies, 4.3% have detectable levels of HSV-1 IgM antibodies and 0.5% have detectable level of HSV-2 IgM antibodies. As for the cord blood samples (N=459), the IgG antibody reactivity was exactly the same as the corresponding mothers for both HSV-1 and HSV-2. However, only three cord blood samples have detectable levels of HSV-1 IgM antibodies, and none have detectable antibodies for HSV-2 IgM antibodies.

Conclusions: Because both HSV-1 and HSV-2 can infect pregnant women and their neonates, assessment of HSV infection in pregnant women and neonates will help in proper management of HSV infection and will also be useful for epidemiological purposes.

Keywords: Herpes simplex virus 1 and 2, antibody screening, pregnant women.
INTRODUCTION

Herpes simplex virus (HSV) is classified in the alpha virinae subfamily within the family Herpesviridae. Two closely-related viruses are designated HSV types 1 and 2. HSV-1 is the usual cause of orolabial infection (gingivostomatitis or herpes labialis), whereas HSV-2 is the major cause of genital infection. However, either virus can infect either location (1-4).

Several modalities are available for the diagnosis of HSV infections (5-7). The benchmark method is viral culture, but it is not widely available in Saudi Arabia. Serology can establish current and past infection with HSV. It has also been used in research studies of the epidemiology of HSV and is very useful in unusual clinical situations (8-15).

The antibody response to HSV glycoprotein G (gG) is highly specific, and gG-based assays can accurately determine whether individuals have past infection with HSV-1 and/or HSV-2 (12-14).

Because genital HSV-2 infection is much more likely to recur than genital HSV-1 infection, the presence of antibody to HSV-2 and a compatible clinical history would be strong presumptive evidence that the disease was recurrent genital herpes (17-19).

Testing of pregnant women for HSV antibodies is usually done with a type-specific assay for HSV antibodies (20). Studies of neonatal HSV infections have generally shown that most infected infants are born to women who have no clinical history of recurrent genital herpes but who are HSV-2 antibody positive at term (21). Early identification of these women by serologic testing might be used as part of a strategy to prevent some perinatal transmission of HSV. It is generally agreed that identification of both unrecognized HSV-2-positive pregnant women and pregnant women who are HSV antibody negative but in danger of becoming infected is essential (22).

When a primary HSV-2 infection is contracted during pregnancy, the fetus is at high risk of acquiring HSV-2 infection at delivery. In the study population of almost 200 infants, an estimated 5% of neonatal HSV infections were intrauterine (23-25).

In one study specific antibody to type 2 herpes simplex virus was detected in 439 of 1355 pregnant women without a clinical history of genital herpes (32%)(18). 280 Asymptomatic shedding of virus in late pregnancy and at delivery was detected in 5 of 1160 cultures (0.43%). During the pregnancy 43 of the women who had antibody
to type 2 virus recognized their first symptomatic genital infection. Detection of HSV IgM antibody in cord blood obtained by cordocentesis and in the blood of the neonate during the first week of life is also diagnostic of in-utero HSV infection (26). Worldwide the number of individuals seropositive for HSV-1 and HSV-2 increases with age (27-28). Various studies have been published that evaluate the prevalence of HSV-1 and HSV-2 antibody in Saudi population (29-35).

Hossain A (36) investigated the seroepidemiology of infection due to HSV-1 in 224 Saudi children and 452 adults (healthy male blood donors and pregnant women) indirect immunofluorescence. The overall prevalence of antibodies was 60 per cent for HSV-1 in children whereas about 90 per cent of the adults showed the presence of antibodies to HSV-1. However, the author did not assess antibodies to HSV-2 and no comparison was made between HSV-1 and HSV-2 in this population. Hossain A et al (37) found genital HSV infections in asymptomatic pregnant women and in male patients to vary between 61.1% and 16.6% depending on the technique. However, the authors did not assess antibodies to HSV-1 or HSV-2 and no comparison was made between HSV-1 and HSV-2 in this population. Ghazi et al (38) assessed the seroprevalence of TORCH agents in 926 pregnant Saudi women and detected HSV-1 IgG antibodies in 90.9% of samples and detected HSV-2 IgG antibodies in 27.1% of samples. The authors did not assess IgM antibodies to either HSV-1 or 2 and they did not analyze the cord blood of the neonates born to the pregnant women in the study. To our knowledge, there is no published data about the seroprevalence of HSV-1 and HSV-2 antibody in Saudi pregnant women AND their neonates. Because both HSV-1 and HSV-2 can infect pregnant women and neonates, assessment of HSV infection in both will help in proper management of HSV infection and will also be useful for epidemiological purposes.

The current paper presents a hospital-based assessment of prevalence of HSV-1 and HSV-2 antibodies in pregnant mothers and their neonates who delivered in King Fahd Hospital of the University (KFHU) in Al-Khobar.

**MATERIALS AND METHODS:**

(a) Type of study:

A hospital-based cross-sectional study
(b) Study area, population and period of study:
All pregnant mothers who delivered in the third trimester at KFHU were included in the study. In addition, all neonates delivered at KFHU were included in this study. The period of the study was one year.

(c) Techniques Used for Data Collection:
Blood samples were collected from the subjects by venepuncture and serum samples were analyzed for HSV-1 and HSV-2 IgG and IgM antibodies using type-specific ELISA (Gull, USA). Briefly, patient serum is diluted with specimen diluent. The diluted serum is incubated with purified HSV antigens bound to ELISA plate wells. If antibodies to HSV are present they bind to the antigen and do not rinse off. Subsequently when enzyme labeled anti-human IgG/IgM is added to the reaction site, it binds to the immobilized antibodies. After washing and the addition of a chromogenic substrate and stopping reagent, specimens containing antibodies to HSV produce a color endpoint reaction which can be read with a standard ELISA reader. Results were interpreted according to the manufacturer’s recommendations.

(d) Statistical Analysis:
Data were entered to a d-Base file. Bivariate analysis between the dependent and the independent variables were done through Chi-square, t-test and paired t-test as appropriate. Analysis of variance ANOVA was used to test difference in means for qualitative variables. Level of significance were set to be < 0.05 throughout the study.

RESULTS:
HSV-1 and HSV-2 IgG Antibodies in pregnant mothers:
Samples of pregnant mothers (N=459) were analyzed for IgG antibody levels against HSV-1 and HSV2 using ELISA.
As shown in Figure 1, 90.5% of samples have detectable levels of HSV-1 IgG antibodies whereas 6.5% of the samples have detectable level of HSV-2 IgG antibodies.

HSV-1 and HSV-2 IgM Antibodies in pregnant mothers:
Samples of pregnant mothers (N=459) were analyzed for IgM antibody levels against HSV-1 and HSV2 using ELISA.
As shown in Figure 2, 4.3% of samples have detectable levels of HSV-1 IgM antibodies and 0.5% have detectable level of HSV-2 IgM antibodies.

HSV-1 and HSV-2 IgG and IgM Antibodies in cord blood:
Samples of cord blood (N=459) were analyzed for IgG antibody levels against HSV-1 and HSV-2 using ELISA. The IgG antibody reactivity was exactly the same as the corresponding mothers for both HSV-1 and HSV-2. However, only three cord blood samples had detectable levels of HSV-1 IgM antibodies, and none had detectable antibodies for HSV-2 IgM antibodies (Figure 3).

**DISCUSSION:**

When a primary HSV infection is contracted during pregnancy, the fetus is at high risk of acquiring HSV infection either intrauterine or at delivery. Therefore, screening of pregnant mothers for HSV-1 and HSV-2 is an important part of the antenatal check.

The seroprevalence of HSV-1 antibody in pregnant women in France was 68%. This is considerably lower than the values obtained in our study (90.5% for HSV-1). This could well be due to the fact that all our samples were from adults, whereas the French study was conducted in a general population of all ages. HSV-1 seropositivity is known to increase with age. The seroprevalence of HSV-2 in the French study in the same population was 17.3%\(^\text{28}\) whereas it was 6.5% in our study. Since HSV-2 is mainly sexually transmitted, the difference in the HSV-2 seropositivity between the two studies could be explained by the differences in the sexual habits between the two communities. The Saudi community is a conservative community.

In Zaire, the seroprevalence of HSV-1 and HSV-2 antibodies in pregnant women was 85% and 32% respectively. The difference in the HSV-2 positivity between our studied population and the Zaire study could well be due to the assay used. The ELISA used in our report is highly specific to HSV-2.

The seropositivity for HSV-1 IgG antibodies in our study (90.5%) confirms the findings of previous investigators (33, 38).

The estimated seropositivity of HSV-2 IgG antibodies in our study (6.5%) is significantly less than the HSV-2 IgG antibodies reported by Ghazi et al (27.1%). This could well be due to regional differences within Saudi Arabia.

Using cell culture, Hossain et al estimated the positivity of HSV isolation in pregnant women to be 50%. The difference in positivity between our data and data generated using the cell culture may well be due to the difference in sensitivity between
serological diagnosis of HSV and detection of HSV by cell culture. Serological assays are far more sensitive than cell culture techniques. Our data showed that the seropositivity of HSV IgG antibodies in pregnant women and their babies are identical. This is due to the placental transfer of IgG. Only three cord blood samples have detectable HSV-1 IgM antibodies. This is considerably less than the IgM positivity in their corresponding mothers because IgM does not cross the placental barrier. These three neonates may have HSV-1 infection in utero. Follow up of these babies is currently underway.

Detectable IgM antibodies in HSV infection usually reflect current or recent infection. In fact, most IgM-positive pregnant mother gave symptoms and signs suggestive of HSV infections although there were some IgM-positive asymptomatic mothers. The IgM-negative symptomatic mothers may well be suffering from reactivation of a latent HSV infection. IgM is usually negative in HSV reactivation.

**CONCLUSION:**

HSV-1 and HSV-2 can infect pregnant women and their neonates, assessment of HSV infection among them will help in the proper management of HSV infection, besides being useful for epidemiological purposes.

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