Serological evaluation of herpes simplex virus type 1 and type 2 infections in pregnancy

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Objective: Serological evaluation of herpes simplex virus infections during pregnancy.
Methods: 2991 serum samples were obtained during 1st, 2nd, and 3rd trimester from 997 pregnant women. Baculovirus expressed glycoproteins gG1 (HSV-1) and gG2 (HSV-2) were used as antigens in ELISA for HSV-1 and HSV-2 IgG and IgA antibodies.
Results: The prevalence of HSV-1 gGl antibodies was 70% and that of HSV-2 gG2 antibodies 16%. Among susceptible women we found five (0.6%) cases with serological evidence of primary HSV-2 infection during pregnancy. Evidence of active HSV-1 infection was found in nine (0.9%) cases. Decline of HSV-2 gG2 IgG antibody levels during pregnancy was pronounced compared with HSV-1 gG1 IgG antibody levels (p<0.01); also the proportion of seroreversions was considerably higher among HSV-2 seropositives (25%) than among HSV-1 seropositives (3%) (p<0.001).
Conclusions: HSV-2 gG2 IgG antibodies were readily distinguished from HSV-l gG1 IgG antibodies by the glycoprotein gG ELISAs. Serological assays for gG2 antibodies should guard against the decline of specific antibodies during pregnancy.
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Keywords: glycoprotein gG; herpes simplex virus; pregnancy; neonatal herpes

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Introduction

Genital herpes simplex virus (HSV) infection in pregnant women is associated with a risk of viral transmission to the infant at delivery, but identification of the risk pregnancies remains enigmatic.[1] While reactivation of maternal HSV infection and primary infection during pregnancy are common, the risk of associated neonatal HSV infection is 10 times higher for offspring of women with primary infection near labour and with no time to develop an adequate immune response.[2–4] Primary HSV type 2 (HSV-2) infections, particularly in the last trimester of pregnancy, are associated with high morbidity and mortality of the infant.[5 6] Clinical examination is likely to miss many cases of genital herpes[7] and antepartum cultures may not accurately predict viral shedding at the time of delivery.[8] Serological evaluation of HSV-2 infections during pregnancy might provide a way to identify risk deliveries.

Standard serological methods do not distinguish HSV-2 antibodies from HSV-1 antibodies.[9] However, determination of type specific antibodies to HSV-2 glycoprotein G2 (gG2) has made it possible to establish a specific diagnosis of past or current HSV-2 infections.[10–13] Using gene technology large quantities of gG2 and gGl can be produced[14 15] and applied for screening of HSV-2 antibodies. We have used the gG2 and gGl antigens in an ELISA test to study the prevalence of HSV-2 and HSV-l antibodies in pregnant women and to screen for HSV-2 and HSV-l infections during pregnancy.

Methods

Sequential serum samples of pregnant women, collected in each trimester from 16 733 pregnant women in the Helsinki area from January 1988 to May 1989, were available from a population based screening trial for congenital infections with a participation rate of 90.2%.[16] For the present study three serum samples, one from each trimester of 997 randomly selected women (altogether 2991 sera) were retrieved from −25°C. The mean age of the women was 30 years and their age distribution is shown in table 1.

Production of the HSV-1 and HSV-2 specific gG1 and gG2 by recombinant baculoviruses AcDSMgG-1 and AcDSMgG-2 has been described elsewhere.[14 17] The recombinants carry 871 bp and 3779 bp fragments of HSV-1 gG1 and HSV-2 gG2 genes, respectively, and express in Sf9 cells 37 to 42 kDa (gG1) and 118 kDa (gG-2) proteins to cell culture supernatants. The supernatants containing gG1 and gG2, a generous gift from Dr Philip E Pellet (CDC, Atlanta, GA, USA), were centrifuged (500 g ×10 minutes), and stored in aliquots at −70°C.

IgG and IgA antibodies to HSV-1 gG1 and HSV-2 gG2 were determined by ELISA as follows. The antigens were diluted in phosphate buffered saline (PBS) (pH 7.2) at a final concentration of 5 μg/ml and used for coating

Table 1 *Age related prevalence of HSV-2 and HSV-l specific IgG antibodies in pregnant women during the first trimester*

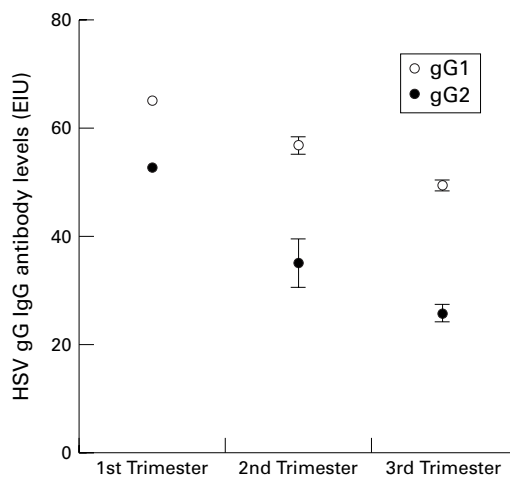| Age group	No of subjects	No (%) seropositive	
		HSV-2 gG2	HSV-1 gG1
–20	36	5 (13.9)	26 (72.2)
21–25	181	29 (16.0)	132 (72.9)
26–30	344	60 (17.4)	323 (67.4)
31–35	297	46 (15.5)	211 (71.0)
36–40	121	13 (10.7)	82 (67.8)
41–	18	4 (22.2)	15 (83.3)
Total	997	157 (15.7)	689 (70.0)

Figure 1 Changes over time of HSV-1 gG1 and HSV-2 gG2 IgG antibody levels in initially seropositive pregnant women at the second and the third trimester. Mean antibody levels at each of the three trimesters plus or minus 2 SEM decline at the second and third trimester are shown for both gG1 and gG2 IgG class antibodies.

of microtitre plates (Macrosorb F-plates, NUNC a/s, Copenhagen, Denmark) overnight at 4°C. The next morning the plates were washed with a washing buffer (PBS containing 0.5% Tween 20) and saturated for 1 hour at 37°C with 10% fetal calf serum (FCS) in PBS. Prediluted serum samples were added into duplicate wells (final dilution 1/100) and incubated for 1 hour at 37°C. After the incubation the serum dilutions were recollected for further use. After washing, alkaline phosphatase conjugated antihuman IgG (Dakopatts a/s, Glostrup, Denmark) diluted 1:500 was added; the plates were incubated for 1 hour at 37°C and washed. Paranitrophenyl phosphate substrate (Sigma, St Louis, MO, USA) was added to the wells for 30 minutes after which the optical densities (ODs) were photometrically read at 405 nm. For the detection of IgA antibodies, the same plates were washed three times both with diethanolamine buffer (Orion Diagnostica, Espoo, Finland) and PBS, after which the preserved serum samples were added to the same individual plate wells (final dilution 1/80) and incubated at 37°C for 1 hour. The plates were washed, and IgA peroxidase conjugate (Dakopatts) diluted 1:500 was added for 1 hour incubation at 37°C. After washing the substrate solution, o-phenylenediamine (Sigma), was added for 15 minutes. After stopping the reaction with 1 M H_2SO_4 the ODs were read at 492 nm. Mean ODs of the positive reference sera were 0.683, 0.420, 0.447, 0.417 for HSV-1 and HSV-2 IgG and IgA antibodies, respectively. Intra-assay variation between duplicates of the same samples was low, with mean coefficient variation of 4.9%, 5.2%, 8.1%, and 8.4% for HSV-1 and HSV-2 IgG and IgA antibodies, respectively.

ELISA of HSV IgG antibodies using HSV-1 infected cell lysate antigen was as described.[18]

To avoid interassay variation antibody reactivity was expressed as background corrected percentage—that is, enzyme immuno units (EIU). The EIU is obtained by subtracting the OD of a known negative reference serum from the OD of a given serum sample, dividing it by the OD of a known reference positive serum minus OD of the negative reference serum and multiplying the result by 100.[19] Cut off levels for HSV-1 and HSV-2 seropositivity were calculated using sera from 45 one year old children as a reference material. All were negative for HSV-2 IgG antibodies, and the cut off level for the presence of HSV-2 IgG antibodies was calculated as a mean plus three standard deviations (SD). For HSV-1 IgG antibodies outliers (HSV-1 seropositive children) were excluded by iterating calculation of the cut off level (mean +3 SD) until no outliers were left.[20] Thereafter the cut off level for the presence of HSV-1 IgG antibodies was calculated from the remaining sera as a mean +3 SD. As a result, the cut off level for HSV-2 was 28 EIU and for HSV-1 21 EIU. A seroconversion was defined as a rise equal to or higher than 20 EIUs,[19 21 22] within the three consecutive samples the overall rise had to be equal to or higher than the cut off level.

Statistical significance of changes in the antibody levels between different trimesters, and differences in the frequencies of seroconversions and seroreversions were tested with paired t test and χ^2 test using the BMDP statistical software (BMDP Inc, Cork, Ireland).

Results
In the 997 first trimester serum samples the prevalence of IgG antibodies to HSV-2 was 16%, and to HSV-1 70% (table 1). Of the 997 mothers, 280 (28%) were seronegative for both HSV-1 and HSV-2, 560 (56%) had HSV-1 antibodies only, and 19 (2%) had HSV-2 antibodies only. The prevalence of HSV-2 IgG antibodies was 14% in the youngest age group, 17% in the age group 26–30 years, and 22% in the age group over 40 years. Altogether, there was little variation in the prevalence of HSV-1 antibodies between the different age groups.

In the first trimester the range of HSV-2 IgG antibody levels was from 0 to 388 EIU (median among the HSV-2 seropositives was 42 EIU) and that of HSV-1 IgG antibody levels from zero to 248 EIU (median among HSV-1 seropositives was 54 EIU). All the second and third trimester serum samples were also analysed. Among the seropositives a general decline of IgG antibody levels was observed during the pregnancy both for HSV-2 and HSV-1 (fig 1). As a result of this decline some seropositive cases reverted to seronegative. The reversion to seronegative occurred in 24 cases (3%) with HSV-1 antibodies and in 40 cases (25%) with HSV-2 antibodies. For HSV-2 both the absolute decrease of antibody levels and proportion of seroreversions were more pronounced than for HSV-1 (p<0.01 and p<0.001, respectively).

Of the 840 individuals who were seronegative for HSV-2 in the first trimester five (0.6%) seroconverted during pregnancy (fig 2A). In four of the five individuals the HSV-2 antibody response occurred between second and third trimester, and in one individual between first and second trimester. Three women with HSV-2 seroconversion were initially negative for antibodies to HSV-1 gG1 and the cross reactive HSV-1 infected cell lysate antigen. The
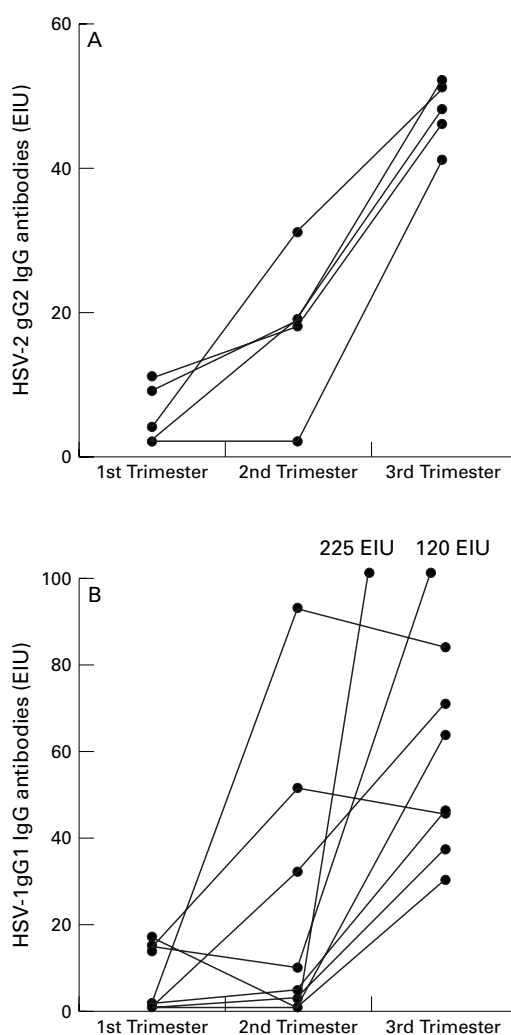
Figure 2   (A) HSV-2 gG2 IgG antibody responses in five women with primary HSV-2 infection during pregnancy. (B) HSV-1 gG1 IgG antibody responses in nine women with a significant increase in the specific antibody levels during pregnancy.

HSV-1 gG1 antibody levels did not change with HSV-2 seroconversion. IgA antibodies to HSV-2 gG2 were present in all five seroconverters after IgG seroconversion. In three cases IgA antibodies were detectable already in the previous sample, and one case showed an increase in HSV-2 gG2 IgA antibody level before the IgG seroconversion. In the remaining two individuals the IgA antibody responses were concomitant with the HSV-2 IgG seroconversion.

Of the 299 women who were negative for HSV-1 gG1 antibodies in the first trimester, nine (3%) seroconverted (fig 2B). However, only one of the nine cases was initially seronegative for HSV as measured by an HSV-1 infected cell lysate ELISA. In three of these individuals a moderate albeit insignificant increase of the HSV-2 gG2 IgG antibody levels was observed concomitantly with the HSV-1 gG1 seroconversion. IgA antibodies to HSV-1 gG1 and HSV-2 gG2 were observed in three individuals already in the first trimester. In three other individuals the IgA antibody responses were comparable with the IgG responses.

Discussion

The prevalence of HSV-2 (16%) antibodies in Finnish women, as measured by gG2 ELISA, is comparable with that in many other countries.[23] Most HSV-2 seropositive women had acquired HSV-2 infection by the age of 25 years. This is in line with what has been reported for similar American and Swedish populations of pregnant women.[24–26] The prevalence of HSV-2 antibodies increased by 2.1% within the first 5 years and by 1.4% within the next 5 years. Thus, the crude incidence of HSV-2 infections in Finnish women between ages 21 and 30 years was 0.3–0.4%. In non-pregnant populations primary HSV-2 infections have been suggested to occur at a constant rate throughout the years of sexual activity[13 27 28]; in our study this could not be evaluated owing to decline of antibody levels over time.

The observed frequency of HSV-2 seroconversions during pregnancy in this study was 0.6%, which is line with other studies.[4 25] Of the pregnant women who were initially seronegative for HSV-2, 28% had no HSV-1 gG1 antibodies either. Both HSV-1 and HSV-2 antibodies have been reported to protect from acquisition of heterologous infection among women.[4 29] In our study the frequency of HSV-2 seroconversions was 0.4% among pregnant women with serological evidence of earlier HSV-1 infection, compared with 1.1% among those without HSV-1 antibodies. Duration of pregnancy may also predispose to primary HSV infection.[4] This was suggested for HSV-2 by the excess of seroconversions (four against one) in late pregnancy, but not for HSV-1 (four and five seroconversions in early and late pregnancy, respectively).

The HSV-2 gene for gG2 contains a long unique DNA sequence with no counterparts in the HSV-1 genome.[30] It seems that human antibody responses to the unique epitopes of the gG2 are dominant[9 12 14 15] but variable.[31] The former explains the lack of cross reactivity of HSV-2 gG2 IgG antibodies with the gG1 antigen, while the latter is associated with the transience of HSV-2 gG2 IgG antibody response. On the other hand, three of the nine cases with HSV-1 seroconvertants also showed increasing HSV-2 gG2 antibody levels. Cross reactive low avidity IgG or IgA antibody response[32 33] to the gG1 protein following HSV-1 infection may be transiently detected by the gG2 antigen.

Although gG1 and gG2 western blot assays are the gold standard for the determination of type specific HSV antibodies,[9 13] the sensitivity of these assays is not superior to that of ELISA techniques based on similar antigens.[34 35] Using baculovirus gG1 and gG2 dot blot assays for comparable serial samples, reversion from HSV-1 or HSV-2 seropositive to seronegative has been described in 5%–12% of individuals (Pellet P, personal communication). We found a highly significant difference between the reversion rates of HSV-1 gG1 and HSV-2 gG2 antibodies. Although the general decline of antibody levels during pregnancy[16] might play a role, the nature and impact of the specific

decline of HSV-2 gG2 IgG antibody levels warrant further investigation.

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