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 gG1 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-1 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
HSV-1 and HSV-2 infections in pregnancy

infected cell lysate antigen was as described. 18

8.1%, and 8.4% for HSV-1 and HSV-2 IgG

Y
cient variation of 4.9%, 5.2%

mean coe

plifies of the same samples was low, with

for HSV-1 and HSV-2 IgG and IgA antibodies,

were read at 492 nm. Mean ODs of the positive

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

multiplied by the result by 100. 19 Cut off level

for HSV-1 and HSV-2 seropositivity were

were calculated using sera from 45 year old chil-

dren 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 devia-

tions (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 EIU, 19 21 22

within the three consecutive samples the over-

all rise had to be equal to or higher than the cut

off level.

Statistical significance of changes in the anti-

body levels between different trimesters, and
differences in the frequencies of seroconver-

sions and seroreversions were tested with

paired t test and χ2 test using the BMDP statisti-

cal 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 anti-

bodies 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 sero-

positives 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 abso-

lute 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

quarter, 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.
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. 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. 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, 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. 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. 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. 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. It seems that human antibody responses to the unique epitopes of the gG2 are dominant but variable. 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 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, the sensitivity of these assays is not superior to that of ELISA techniques based on similar antigens. 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 might play a role, the nature and impact of the specific
HSV-1 and HSV-2 infections in pregnancy