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Herpes simplex virus type-2 antibodies in pregnant women: the impact of the stage of pregnancy

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SUMMARY

In this study the impact of pregnancy duration on the measured level of HSV-2 antibodies was assessed. The study population comprised 35940 pregnant women in Norway, in 1992–4, followed during pregnancy. A random sample of 960 women was selected. A mean of 2·6 serum samples from each woman were analysed for HSV-2 specific IgG antibodies at different times in pregnancy. Crude and adjusted odds ratios were estimated in logistic regression models taking all observations per women into account. Twenty-seven percent of the pregnant women had antibodies against HSV-2 in the first trimester. The adjusted odds ratio of being HSV-2 antibody positive decreased during the pregnancy and was 0·5 (0·2–0·9, 95% confidence interval) in the 40th as compared to the 10th week of pregnancy. About 50% of initially HSV-2 positive women did not have detecable antibodies by the end of the pregnancy. This may be explained by haemodilution during pregnancy. Our findings have diagnostic implications and should encourage further studies.

INTRODUCTION

Studies of the prevalence of herpes simplex virus type-2 (HSV-2) in different populations in industrialized countries have shown various results from less than 10% to more than 40% [1–18]. The prevalence varies according to ethnicity within one area [1, 12] and between geographical areas [7, 11, 13, 14, 16, 18]. An increasing prevalence during the past decades has been suggested [8, 19, 20]. Very few population based prevalence studies have yet been performed [21]. On the individual level, the presence of HSV-2 antibodies has been associated with age [8, 9, 14, 17] and number of sexual partners [3, 7, 10, 15].

Less than 50% of HSV-2 infected subjects have clinical signs of infection [2, 3, 12]. In both symptomatic and asymptomatic HSV-2-infected subjects, viral shedding occurs [3, 6, 22–26]. It is assumed that most HSV-2 infected subjects have at least one episode of viral shedding per year [4]. These asymptomatic infectious episodes may be responsible for the silent spread of HSV-2.

In addition to clinical symptoms in infected adults, HSV-2 infection may cause fatal infection in the newborn child. Studies suggest that only women with incident HSV-2 infection during the last part of pregnancy may transmit the infection to the child, regardless of clinical symptoms in the mother [27]. The overall risk of serious HSV-2 infection in newborns is, however, low [27, 28].

The possible clinical use of HSV-2 typespecific serology is creating debate [22, 29]. Despite the controversy, such testing may be increasingly used for identification of pregnant women at risk of transmitting HSV-2 to the neonate. Reliable serological HSV-2 diagnosis in pregnant women depends on identification and understanding of the factors that

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influence the level of HSV-2 antibodies. Yet, there is little knowledge on the natural variation in antibody levels in general, and in pregnant women in particular.

The aim of this study was to assess the impact of the stage of pregnancy on the presence HSV-2 antibodies in a random sample of 960 pregnant women followed with repeated HSV-2 antibody tests.

METHODS

Source population

The source population comprised 35940 pregnant women in Norway. These mothers had participated in a prospective study of *Toxoplasma gondii* infection in pregnancy performed by the National Institute of Public Health from June 1992 and until the last birth in the study, May 1994 [30]. The toxoplasmosis study included almost 100% of the pregnant women in 11 out of 19 counties in Norway, approximately 60% of all pregnant women in Norway during the study inclusion period [31].

A random study sample

The presence of HSV-2 antibodies was studied in a random sample of 970 women, drawn from the source population after linkage between the Toxoplasmosis Study Registry and the Medical Birth Registry of Norway [32]. The Medical Birth Registry has been in operation since 1967 and comprises information on all births in Norway after the 16th week of gestation. For 10 women in the study sample, sufficient serum for antibody analysis was not available at recruitment. Thus, 960 women could be included in the analysis.

The mean age was 28·7 years (median 27·5 years, range 16–47 years). A total of 406 women (42%) had no previous births reported to the Medical Birth Registry (birth after >16 weeks of pregnancy). Additionally 359 women (37%) had one previous birth, 138 (14%) had two and 57 (6%) had three or more. 205 women (21%) were from Oslo, the capital and the largest city in Norway, with a population of 500000.

Follow-up during pregnancy

The women were included in the study at the first antenatal visit at 10th–12th pregnancy week (mean 10·2, standard deviation (S.D.) 3·2 weeks). Additional serum samples were requested in the 22nd week (mean 23·8, S.D. 3·3 weeks) and the 38th week (mean 37·8, S.D. 1·2 weeks). For 60 women with *Toxoplasma gondii* IgG in the first serum sample, follow-up serum samples were not requested. For an additional 46 women, only one serum sample was available for HSV-2 antibody analysis due to incomplete follow-up, pre-term birth, insufficient serum amount or recruitment late in pregnancy. Thus, 854 of the 960 women (89%) had more than one serum sample. 159 women had two serum samples and 646 had three. A few women had four or more serum samples. A total of 2515 serum samples from 960 women (mean 2·6) were analysed for HSV-2 antibodies. For 47 serum samples (2%) the pregnancy week of serum collection was missing.

Testing for HSV-2 antibodies

The serum samples had been stored at the National Institute of Public Health at -20 °C since 1992–3 and were analysed for HSV-2 antibodies during the autumn of 1996. The means of serum collection, transportation and storage remained the same throughout the study period.

For determination of HSV-2 specific IgG antibodies, a protocol described by Ades [1] was followed. In brief, microtitre plates were coated with purified HSV gG-2 antigen [33] in an optimal dilution and used in a conventional enzyme immunoassay (EIA) system. Sera were tested at 1 in 200 dilution on antigen coated plates. Bound IgG antibodies were detected by adding horseradish peroxidase-conjugated; affi Pure goat anti-human IgG (H & L) (Jackson Immuno Research Laboratories Inc., West Baltimore Pike, PA). For determination of cut-off, three control sera with HSV-1 antibodies, but negative for HSV-2 antibodies were used. Each control serum was tested in duplicate. The cut-off was calculated as the mean absorbance for negative controls plus three standard deviations. The absorbance of the tested serum samples is given as percent of cut-off. Absorbance, measured as optical density, equal to or more than 100% of cut-off was defined as positive. The sensitivity of this HSV-2 antibody test has been estimated to above 97% and the specificity to above 94% [34–35]. All sera were sampled, stored and tested under identical conditions.

Definitions

HSV-2 antibodies were defined as present if the optical density was $\geqslant 100\%$. Incident HSV-2 in-

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fection/seroconversion was defined as more than 100% increase in optical density, from below cut-off in the first serum sample to above cut-off in the last available sample from the pregnancy period.

Loss of antibodies was defined as having detectable antibodies in the first serum sample (optical density $\geqslant 100\%$ and no detectable antibodies (optical density $< 100\%$) in the last available serum sample from the pregnancy period.

Explanatory variables

The impact of the following variables on the presence of HSV-2 antibodies were studied:

Main explanatory variable

Pregnancy duration. Coded: < 10, 10–19, 20–29, 30–39, > 39 weeks, according to pregnancy week of serum collection.

Confounding variables

Parity. Coded: 0, 1, 2, 3, > 3 number of previous births.
Age. Coded: < 20, 20–24, 25–29, 30–34, 35–39, > 39 years at study entry
Living in Oslo. Coded: yes/no.

The information on age was derived from the Toxoplasmosis Study Registry and obtained by questionnaires to physicians or midwifes in charge of the antenatal care. The information on place of living and parity was obtained from the Medical Birth Registry.

Statistical analysis

A logistic regression model was specified for the relationship between presence of HSV-2 antibodies and the explanatory variables. The 2515 measurements of presence of HSV-2 antibodies (optical density $\geqslant 100\%$, yes/no) were the units of analysis. However, it is important to note that the measurements were nested within 960 women. It is reasonable to assume that measurements across women are independent whereas repeated measurements within a woman are dependent. Hence, standard statistical analysis based on independent observations is problematic. Such an analysis would entail an exaggeration of the effective sample size and consequently underestimated standard errors and confidence intervals. It follows that a statistical method accommodating dependent observations is called for. Thus, the logistic regression models were estimated by means of generalized estimating equations (GEE), using an unstructured working correlation matrix [36]. This approach provides consistent estimators of the regression parameters and robust estimators for the corresponding standard errors under dependence. The software used was OSWALD [37] which runs under SPLUS [38]. Both crude and adjusted odds-ratios for presence of HSV-2 antibodies according to the explanatory variables were estimated.

Ethics

This study was approved by the Norwegian Data Inspectorate, the Regional Medical Ethical Committee and the National Board of Health.

RESULTS

Twenty-seven percent (256/961) of the women had HSV-2 antibodies in the first serum sample collected during pregnancy. In Oslo, the proportion was 32% (66/205) ($P = 0{\cdot}04$, χ^2 test).

Table 1 shows the percent of all sera that were anti-HSV-2 positive and estimated crude and adjusted odds ratios for presence of HSV-2 antibodies according to the stage of pregnancy, parity, age and place of living.

Stage of pregnancy

Among the HSV-2 antibody-positive women in early pregnancy, only 55% (127/231) were also positive in the last available sample from the pregnancy period. When studying all available sera according to stage of pregnancy, 24% (109/449) of the samples from before the 10th pregnancy week, 29% (145/508) from the 10–19th week and 14% (11/77) from after the 39th week were anti-HSV-2 positive. The adjusted odds ratio of being HSV-2 antibody positive was 0·5 (0·3–0·9, 95% confidence interval (95% CI)) in the 39th pregnancy week or later as compared to before the 10th pregnancy week (Table 1, Fig. 1).

There was a strong association between having a low HSV-2 antibody titre in the first sample taken in pregnancy and subsequent loss of antibodies. Among the women with optical density level 100–149% in the

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Table 1. *Crude and adjusted odds ratios of having antibodies against herpes simplex virus type-2 in a random sample of 960 pregnant women in Norway (1992–4) estimated by generalized estimating equations (GEE). A total number of 2515 (mean 2·6 per woman) serum samples from the pregnancy period are included in the analysis*

	No. of women	Number of sera	Percent with anti-HSV-2	Crude OR (95% CI)	Adjusted OR (95% CI)
Stage of pregnancy (weeks)					
< 10	449	449	24·3	1·0	1·0
10–19	508	508	28·5	1·3 (0·9–1·5)	1·2 (0·9–1·4)
20–29	768	768	23·7	0·9 (0·7–1·1)	0·9 (0·7–1·1)
30–39	666	666	20·0	0·8 (0·6–0·9)	0·7 (0·6–0·9)
> 39	77	77	14·3	0·5 (0·3–0·9)	0·5 (0·3–0·9)
Missing data	47	47	25·5		
Number of previous births					
0	406	1061	23·7	1·0	1·0
1	359	941	22·5	1·0 (0·7–1·3)	0·8 (0·6–1·1)
2	138	370	23·5	1·0 (0·7–1·4)	0·7 (0·5–1·1)
3	40	78	25·5	1·1 (0·6–2·1)	0·7 (0·5–1·1)
> 3	17	45	37·8	1·8 (0·8–3·6)	1·1 (0·5–2·5)
Age					
< 20	40	106	17·9	1·0	1·0
20–24	230	592	16·4	0·9 (0·5–1·8)	0·9 (0·5–1·9)
25–29	335	872	22·2	1·3 (0·7–2·5)	1·4 (0·7–2·7)
30–34	240	650	28·8	1·8 (1·0–3·5)	2·1 (1·1–4·2)
35–39	104	268	31·7	2·1 (1·0–4·2)	2·5 (1·1–5·3)
40+	11	27	37·0	2·7 (0·8–9·6)	3·0 (0·8–11·0)
Living in Oslo					
No	756	1983	22·4	1·0	1·0
Yes	205	532	27·6	1·4 (1·0–1·8)	1·2 (0·8–1·6)

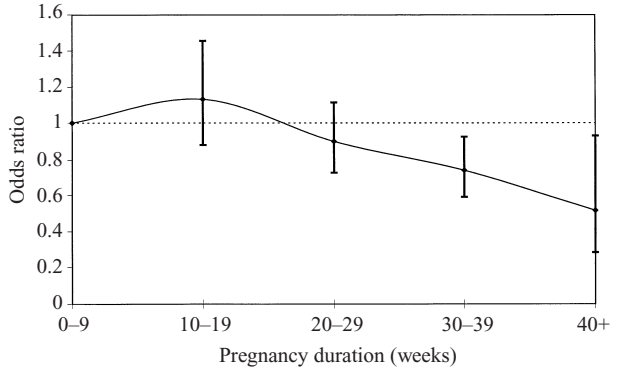

Fig. 1. The odds ratios of being diagnosed as HSV-2 antibody positive in a random sample of 960 Norwegian pregnant women (with a total of 2515 HSV-2 antibody tests) according to stage of pregnancy, adjusted for age, parity and living in Oslo (yes/no). < 10th pregnancy week is used as the reference category.

first serum sample, 72% (83 out of 115) had seroreverted in the last serum sample from the pregnancy period (optical density < 100%). Among the subgroups of women with initial optical density 150–199%, 200–299% or ⩾ 300% of cutoff, 57% (16/28), 15% (2/13) and 4% (2/75) seroreverted respectively (χ^2 test, $P < 0{\cdot}001$). The overall correlation between optical density in the first and the last serum from the pregnancy period was 0·82.

Despite an overall decreasing odds ratio for presence of HSV-2 antibodies according to pregnancy duration, women with incident HSV-2 infection during pregnancy were identified. Among the 623 initially HSV-2 antibody-negative women, with more than one sample from the pregnancy period, 16 (2·6%) seroconverted. The mean observation time, from the first to the last sample in this group was 26 weeks (S.D. 5·6), giving a 4% cumulative incidence during 40 pregnancy weeks.

Parity

The risk of being HSV-2 antibody positive decreased according to parity (Table 1). The adjusted odds ratio

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was 0·7 (0·5–1·1, 95% CI) for women with three previous pregnancies as compared to primipara women. Women with four or more previous pregnancies deviated from the trend. This group, however, was small.

Age

Nineteen percent of the women 20–25 years old and 36% of the women 35 years old or more were HSV-2 antibody positive in the first serum sample collected in pregnancy ($P = 0{\cdot}002$, χ^2 test of independence). When controlling for the other independent variables and taking all HSV-2 observations into account, the impact of age on the odds ratio of HSV-2 seropositivity increased almost linearly (Table 1). Women less than 20 years deviated from the trend.

DISCUSSION

In this study of a random sample of 960 pregnant women in Norway, 27% of the women were HSV-2 antibody positive in the first serum sample collected in pregnancy. The presence of HSV-2 antibodies was influenced by duration of pregnancy, age and location. The estimated HSV-2 antibody prevalence in our study was high compared to earlier studies of pregnant women from industrialized countries [11]. However, recent studies of women in childbearing age give similar HSV-2 antibody prevalence estimates [6, 7, 19, 20]. Most HSV-2 antibody tests used worldwide are based on the HSV gG2 antigen which is also used in our study [1, 34, 35].

Stage of pregnancy

In our study, probability of being HSV-2 antibody positive decreased by about 50% during pregnancy. Lower antibody levels against specific infectious agents in pregnant as compared with non-pregnant women have been described [39, 40]. Also, reduction in other antibodies during pregnancy has been reported [41–43]. The degree of reduction has, however, not previously been estimated in a large population based sample of pregnant women.

The level of antibodies against most infectious agents decreases as a function of time since infection. Because of the chronic nature of the HSV-2 infection, it is unlikely that such large decrease in antibody concentration during the pregnancy period, as observed in our study, can be explained entirely by a natural decrease as a function of time. HSV-2 antibody titres have been reported to be stable over time [44].

Selection bias in follow-up in our study could be an explanation of the estimated decrease in HSV-2 antibodies during pregnancy. However, only 11% were not followed with additional serum and the main reason for not being followed, was presence of antibodies against *Toxoplasma gondii* in the first serum sample collected. There is little reason to believe that toxoplasma antibody status at study entry should be associated with changes in HSV-2 antibody status during follow-up. No significant association between presence of antibodies against *Toxoplasma gondii* and HSV-2 in the first serum sample was found (data not shown).

In our study there was an observed increase in anti-HSV-2 prevalence between serum samples drawn in $<$ 10th and 10th–19th pregnancy week, from 24·3 to 28·5%. This difference is not statistically significant, but is, however, interesting and could be explained by a selection of women with low HSV-2 risk to early pregnancy care.

The most likely explanation of the decreasing risk of being HSV-2 antibody positive during advancing pregnancy, is dilution of immunoglobulins caused by the increasing blood volume during pregnancy [41]. However, active transportation of IgG across the placenta is described [43] and may also contribute to the maternal reduction in antibodies.

Our observations indicate that misclassification of women in late pregnancy with regard to HSV-2 antibody status, and perhaps also antibody status against other infectious agents, may occur. Serological diagnosis in clinical practice today do not control for haemodilution during pregnancy.

According to the definitions of serological changes used in our study, 2·6% (16/623) of the HSV-2 antibody negative women seroconverted. This may be an underestimate, because of lack of control for haemodilution, as discussed above. The HSV-2 incidence during pregnancy in our study is high, higher than expected when calculating the yearly average incidence on the basis of HSV-2 prevalence and age at sexual debut [45]. However, high rates of seroconversion have also been reported from other studies of pregnant women [27]. Whether the risk of HSV-2 seroconversion in pregnant women differs from a non-pregnant population may therefore be a relevant question. Standardized criteria for diagnosis of HSV-

2 seroconversion during pregnancy are lacking and should be developed.

Parity

Although not statistically significant, presence of HSV-2 antibodies showed a negative association with the number of previous pregnancies, except in the group of women with four or more previous pregnancies. This latter group was, however, small and the confidence limits around the estimate wide. The association between HSV-2 and parity may be explained by selection of low HSV-2 risk women to repeated pregnancies.

If parity truly is associated with decreased risk of being HSV-2 positive, it may be explained by sustained low antibody levels from previous pregnancies. The low HSV-2 antibody level, which is demonstrated in the last part of pregnancy may partly be maintained after the delivery.

We are not aware of any other reported studies of antibody level according to parity. Further studies of antibody levels and other immunological parameters according to parity may be of importance. Pregnancy complications, such as preeclampsia, are most common in primipara [46] and the risk may be influenced by changing immunological competence according to parity.

Age

The presence of HSV-2 specific antibodies increased with age, except for the youngest age group. The relatively high HSV-2 antibody prevalence among the youngest women in our study may reflect a selection of high HSV-2 risk women with teenage pregnancies. This group was, however, small and the confidence limits around the estimates wide. The association with age has been reported in other studies [8, 9, 14, 17] and may be explained by increasing cumulative risk of HSV-2 infection according to time since sexual debut. The relatively high HSV-2 seroprevalence in our study may therefore be explained by earlier sexual debut or higher number of sexual partners in Norway, as compared to other countries. In population based surveys on sexual behaviour in Norway in 1987 and 1992, the median age at sexual debut in women 18–35 years of age was estimated to 16·5 years [45]. This is lower than in most other European countries [47]. Also the number of reported sexual partners was higher [47]. The median number of lifetime partners among married/cohabiting women 18–35 years were four for Norway as a whole, and six for women living in Oslo. The proportion with more than ten lifetime partners was also higher for Oslo than for the rest of the country (40% versus 26%) [48].

The decrease in HSV-2 antibodies during pregnancy is likely to be explained by normal changes in pregnancy. Such changes in antibody levels during the pregnancy period have diagnostic implications and could affect clinical management. Assessing HSV-2 status may not be valid in the last part of pregnancy.

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