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CLINICAL REPORT

# Decreasing Prevalence of Herpes Simplex Virus-2 Antibodies in Selected Groups of Women in Sweden

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**High estimates of herpes simplex virus-2 (HSV-2) seroprevalence have been reported for women who were pregnant in Sweden in the 1980s, probably reflecting sexual risk-taking during the 1960s and 1970s. The aim of the present study was to evaluate the HSV-2 seroprevalence in pregnant women and in the female attendees at a clinic for sexually transmitted infections in Sweden at the beginning of the 21st century and to compare the results with those of earlier Swedish studies. Sera were collected during the period 2000 to 2002 from 299 pregnant women at an antenatal clinic and from 290 female attendees at a clinic for sexually transmitted infections in Gothenburg. To enable comparison with earlier seroprevalence data the same test method was used; *Helix pomatia* antigen in an enzyme-linked immunoassay. The overall HSV-2 prevalence was 10.4% for the pregnant women and 25.2% for the female attendees at the clinic for sexually transmitted infections. The seroprevalence of HSV-2 in pregnant women appears to have decreased in Sweden during the past decade, which may reflect changes in sexual behaviour. *Key words: herpes simplex virus; seroprevalence; pregnancy; sexually transmitted diseases*.**

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Since serological tests discriminating between herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) became available in the 1990s (1), it has been possible to follow changes in the epidemiology of HSV infections. Most cases of recurring genital herpes are caused by HSV-2, and the presence of HSV-2 antibodies is mainly considered to reflect genital herpes infection. Genital herpes is a sexually transmitted infection (STI) and changes in the seroprevalence of HSV-2 antibodies have been proposed to mirror changes in sexual behaviour over time (2–4). In contrast to the bacterial STIs, chlamydia and gonorrhoea, genital herpes is a chronic infection. While the prevalence of bacterial infections may reflect ongoing sexual risk-taking in a society, HSV-2 antibodies reflect temporal phenomena such as changes in sexual behaviour. A high seroprevalence of HSV-2 has been reported in Swedish women who were pregnant during the 1970s and 1980s, and is presumed to reflect sexual risk-taking during the 1960s and 1970s (5–7).

The aim of this study was to estimate the age-related HSV-2 seroprevalence for Swedish women at the beginning of the 21st century, both in an antenatal clinic and an STI clinic, and to compare the findings with previous studies from Sweden.

## PATIENTS AND METHODS

### *Antenatal clinic*

The study included consecutive attendees at an antenatal clinic during 2002. The socio-economic status of the inhabitants in the catchment area was estimated to be representative of the general population of Gothenburg. The majority of the women were in their first trimester. Blood was drawn from all 661 women for rubella and HIV testing, as is routine in Sweden, and a random sample of 299 (45%, age range 17–45, mean 30.5 years) sera was tested anonymously for HSV-2 antibodies.

### *Clinic for sexually transmitted infections*

During the period January 2000 to May 2001 (apart from the summer months June to August) consecutive patients attending the STI Clinic at Sahlgrenska University Hospital were offered a blood test for HSV-2 antibodies besides the routine testing for *Chlamydia trachomatis*, HIV and syphilis. Clinical data and an analysis of drop-outs have been published earlier (8). Of the 483 women offered testing 290 (60%) accepted and were included in the present study. The mean age of those included was 27.7 years (range 16–57 years).

### *Laboratory tests*

*Herpes simplex virus-2*. Sera were initially tested with an in-house enzyme-linked immunoassay (ELISA) test, based on *Helix pomatia* lectin-purified glycoprotein G (gG) 2 antigen (9). A high titrating HSV-1 positive serum was used as control and the cut-off was set to the mean value of this control plus 0.2 absorbance units. For verification, all positive sera were further analysed with a commercial kit approved by the US Food and Drug Administration (FDA); Herpes Select HSV-2 ELISA (Focus Diagnostics), which is based on recombinantly produced gG2 (1). Results of the *Helix pomatia* based gG2-test are presented here to increase the precision in the comparisons with earlier studies.

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*Statistical analyses*

Point estimates of proportions are given with 95% confidence intervals (CI).

The study protocol was approved by the ethics committee at the Medical Faculty at Gothenburg University.

## RESULTS

### *Herpes simplex virus-2 antibodies*

Of the 299 pregnant women 31 (10.4% (CI 7.4–14.3)) were positive in the type-specific in-house HSV-2 ELISA. These sera were further tested in the HSV-2 Focus test and 27/299 (9.0% (CI 6.3–12.8)) were verified as positive, with the remaining four testing negative. Of the 290 sera from the STI clinic attendees 73 (25.2% (CI 20.5–30.5)) were positive in the in-house test and 66 of these were verified positive by the HSV-2 Focus test, giving a seroprevalence of 22.8% (CI 18.3–27.9).

The age-related prevalences of HSV-2 antibodies are shown in Table I.

## DISCUSSION

In this study we estimated the prevalence of HSV-2 antibodies in two different populations of women. The prevalence among pregnant women was 10% which is in accordance with contemporary reports from Norway and Finland (10, 11). In the 1980s, high prevalences of HSV-2 antibodies (> 30%) were reported for pregnant women from Stockholm, the largest city, and Malmö, the third largest city in Sweden. There are no previous studies of HSV-2 seroprevalence in pregnant women from Gothenburg, Sweden's second largest city. Table II presents the results of the earlier HSV-2 seroprevalence studies of pregnant women in Malmö and Stockholm along with our results from Gothenburg. The prevalences reported during the 1980s are considerably higher than those reported from other countries around the same time, for example Germany, Finland and Norway, where the HSV-2 seroprevalences among pregnant women were 8.9% in 1996 to 1997 (12), 16% in 1988 to 1989 (13) and 27% in 1992 to 1994 (14), respectively.

Data on time trends in HSV-2 seroprevalence are limited. A repeated cross-sectional study in USA showed a statistically significant decrease in the overall prevalence of HSV-2 antibodies from 21% (CI 19.1.1–23.1) in 1988 to 1994 to 17% (CI 15.8–18.3) in 1999 to 2004 (15). Similarly, a study from Israel showed a decline in the prevalence of HSV-2 between 1984 and 2002 (16). However, a cross-sectional study of Swedish populations found no significant change in HSV-2 prevalence between 1990–1991 and 1996–1997 (17). According to the data presented in Table II, the HSV-2 seroprevalence increased in Sweden during the 1970s and 1980s. Most impressive was the increase in seroprevalence in pregnant women in Stockholm, with a near doubling of the seroprevalence from 1969 to 1989 (6). The highest prevalence, up to 40%, was reported among women aged over 30 years of age in the 1980s and has been considered to reflect unprotected sex during the sexually liberal 1960s and 1970s, coinciding with the increased spread of gonorrhoea and chlamydia also reported at that time.

In the present study a decrease in the HSV-2 prevalence in pregnant women in Gothenburg was obvious in all age groups compared with previous reports from Sweden. The overall HSV-2 prevalence of the 1990 to 1993 Malmö cohort was 20.7% (CI 18.5–23.1) compared with 9.4% (CI 6.6–13.2) in the present study (after age-adjusting our population to the age distribution of the 1990 to 1993 Malmö cohort).

The HSV-2 prevalence among our STI clinic attendees was as high as 25%. A previous study of sera collected from the same clinic in the late 1980s showed an HSV-2 seroprevalence of 26.3% (CI 22.6–30.5) among female attendees (5). Although the catchment area for the STI clinic has not changed since the time of this earlier study, the 1990s saw the introduction in Sweden of so-called "youth clinics", which has led to fewer young women attending the STI clinics. The majority of the women included in the previous study from our clinic were in the 21–25 year age group, and thus were younger than

Table I. *Prevalence of herpes simplex virus-2 seropositives in 299 pregnant women in an antenatal clinic and 290 women attending a clinic for sexually transmitted infections*

| | Pregnant women | | STI female attendees | |
|---|---|---|---|---|
| Age-group (years) | *n* | % positive | *n* | % positive |
| < 25 | 52 | 5.8 | 136 | 13.2 |
| 26–30 | 95 | 8.4 | 71 | 22.5 |
| 31–35 | 102 | 7.8 | 37 | 32.4 |
| >35 | 50 | 24.0 | 46 | 58.7 |
| Total | 299 | 10.4 | 290 | 25.2 |

Table II. *The herpes simplex virus-2 seroprevalence (%) in different populations of pregnant women in Sweden from 1970 to 2002*

| | Age group (years) | Years of publication | | | |
|---|---|---|---|---|---|
| | | *1970–1973* | *1979* | *1987–1989* | *1990–1993* |
| Person et al., Malmö (7) | <25 | 23 | 16 | 18 | 11 |
| | 26–30 | 18 | 25 | 31 | 15 |
| | 31–35 | 25 | 24 | 36 | 31 |
| | >35 | 14 | 19 | 33 | 37 |
| | | *1969* | *1983* | *1989* | |
| Forsgren et al., Stockholm (6) | <25 | 17 | 23 | 32 | |
| | 26–30 | 17 | 32 | 30 | |
| | 31–35 | 21 | 40 | 39 | |
| | >35 | 23 | 39 | 31 | |
| | | | | | *2002* |
| Berntsson et al., Gothenburg (present study) | <25 | | | | 6 |
| | 26–30 | | | | 8 |
| | 31–35 | | | | 8 |
| | >35 | | | | 24 |

those in the current study. HSV-2 prevalence increases with age and adjusting for age would result in a lower prevalence than the estimated 25.2%; this indicates that there was also a decline in HSV-2 prevalence among STI clinic attendees.

One explanation for this decline in the HSV-2 seroprevalence, which was also seen in the 1990 to 1993 Malmö cohort, could be changes in the sexual behaviour in the population. The 1980s saw widespread campaigns and information about how to avoid being infected with HIV, and interview data have confirmed that this resulted in a change in sexual behaviour towards safer sex and a lower number of sexual partners (18). Other explanations for the lower incidence of HSV-2 could be the increasing use of antiviral therapy, awareness of atypical presentation and silent transmission of the infection (8). The incidence of genital herpes caused by HSV-1 has increased (19). Since infection with concurrent genital HSV-1 and HSV-2 seems uncommon, genital HSV-1 infection may protect against being infected with HSV-2 (20).

There are many factors to consider when making comparisons between HSV seroprevalence studies; type of population, age and sex distribution, test methods and selection bias as in our STI group. We are aware of the limitations in the comparisons presented here, especially concerning definition of the populations. The higher HSV-2 occurrence in pregnant women in Stockholm compared with Malmö and Gothenburg may reflect different socioeconomic backgrounds and ethnic variation in the populations. Similar geographical differences have been reported from the Netherlands (21). Furthermore, sera were collected postpartum in the Malmö study, but during the first trimester in the Stockholm study and the present study. Sero-reversion of HSV-2 antibodies in the second and third trimester has been reported (15), which might have contributed to the lower occurrence in Malmö.

Concerning test method, the same antigen, *Helix pomatia* lectin purified gG2 antigen, was used in an ELISA test in all the Swedish studies referred to. This "in-house" test may be too sensitive and so to avoid false positives it is now recommended that positive results are confirmed with a Western blot or a US FDA-approved ELISA test, such as Focus. In order to make the best possible comparisons with previous studies we used the results from the *Helix pomatia* test. Of the 104 sera that tested positive in the "in-house" test with *Helix pomatia* as antigen, 93 (89%) were verified by the Focus test. This indicates that the HSV-2 seroprevalence figures given in earlier studies using *Helix pomatia* antigen without further confirmation may have been somewhat overestimated.

In conclusion, our study, together with earlier Swedish studies, shows a decreasing HSV-2 seroprevalence in pregnant women in Sweden. Since the end of the 1990s the spread of chlamydia has increased in Sweden, especially among young individuals, which could reflect increased sexual risk-taking (22). A follow-up of the HSV-2-seroprevalence in selected population groups in coming years will reveal the extent to which there is a concomitant rise in genital HSV-2 infections.

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