HERPES

Prevalence of infection with herpes simplex virus types 1 and 2 in Australia: a nationwide population based survey

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Background: Studies demonstrating previous herpes simplex virus (HSV) type 2 infection as a risk factor for HIV transmission, and the development of a HSV vaccine candidate, have emphasised the need for worldwide population based studies of HSV seroprevalence. The only nationwide seroprevalence studies have been conducted in the United States.

Methods: An Australia-wide, population based study of HSV-1 and HSV-2 seroprevalence was conducted, using serum and sociodemographic data collected between 1999–2000, for a representative study of risk factors for diabetes in over 11 000 adults. A stratified random sample of 4000 was tested for HSV-2 and 1000 for HSV-1, with sampling and weighting for various demographic factors.

Results: Seroprevalence of HSV-2 in Australian adults was 12%. Prevalence in women (16%) was twice that in men (8%). Rural populations had a lower prevalence (9%) than metropolitan (13%), and Indigenous had a higher prevalence (18%) than the non-Indigenous populations (12%). The seroprevalence of HSV-1 was 76% with significant differences by age group, sex and Indigenous status.

Conclusion: These are the first nationwide data to compare with US studies. HSV-2 infection is less common in Australia than the United States, and this will allow planning for combating HIV transmission in high prevalence populations in northern Australia. In addition, the high HSV-1 seroprevalence will be important for future deployment of genital herpes vaccines.

I nfections caused by herpes simplex viruses (HSV) types 1 and 2 are common throughout the world, with considerable variation from country to country and within population groups.[1] Studies in selected populations, considered to be at high risk for the acquisition of sexually transmitted infections (STIs), have shown that HSV-2 occurs in 13–75% of patients attending STI clinics,[2–6] 24–87% of men who have sex with men,[2 6–9] and 74–98% of female sex workers.[10 11] In populations considered to be at lower risk for the acquisition of STIs, the prevalence of HSV-2 is generally lower. In women attending antenatal clinics, reported prevalence ranges from 5–55%,[4 5 12–17] in women attending family planning clinics 11–38%,[12 18] and in blood donors 2–25%.[2 5 19] Demographic risk factors and behaviours associated with HSV-2 antibodies include increasing age, female gender, black race, previous STIs, young age at first intercourse, lower educational level, and lower social class.[2 3 6 8 18–22] In the few studies of HSV-1 seroprevalence that have been conducted, HSV-1 infection occurs in 30–100% of adults aged 20 or above.[5 9 13–17 19 20] Many of these seroprevalence studies have been small or conducted in highly selected or convenience samples and some have used suboptimal serological assays.

The only representative, nationwide studies, using HSV validated type specific serological assays to establish the prevalence of HSV in the general population, have been conducted in the United States. These studies used serum derived from the National Health and Nutrition Survey (NHANES), a multistage, stratified, cluster sample of the non-institutionalised civilian population aged between 6 months and 74 years. In the first study (1976 and 1980), a random subsample was tested for antibodies to HSV-2 and 16.4% of the population aged 15–74 were HSV-2 positive.[21] A second survey (1988 to 1994) showed that the seroprevalence of HSV-2 in individuals aged 12 years or older was 21.9% and compared with the earlier study, the age adjusted seroprevalence of HSV-2 rose by 30%. Independent predictors of HSV-2 seropositivity were female gender, black race or

Mexican-American ethnic background, older age, less education, poverty, cocaine use, and a greater lifetime number of sexual partners.[22] There have been a number of other population based studies. However, these have been in regions or individual cities and cannot be considered to be national.

With the potential availability of a vaccine to reduce HSV-2 infection and HIV transmission,[23] accurate determination of nationwide HSV prevalence and its demographic variability is essential.

METHODS

The study used serum and sociodemographic data already collected for AusDiab, a nationwide, representative study designed to investigate diabetes and related risk factors in the Australian population (age ≥25 years), derived from subjects residing in 42 randomly selected urban and non-urban areas, based on 1996 census collection districts in each state and territory.[24] The study was conducted between 1999 and 2000 and included over 11 000 participants (0.05% of the Australian population). The survey involved a short household interview, followed by a biomedical examination at a local survey centre. Householders not available at the initial interview were approached again, on up to four occasions. Household questionnaires were completed in 67% of the households (n = 11 479) that could be contacted and contained at least one eligible person. A total of 20 347 eligible individuals were interviewed in these 11 479 households. The final survey sample (those attending the biomedical examination) included 11 247 adults (5049 men and 6198 women), representing 55% of those completing the household interview; 44.9% were male, with the mean age being 51.5 years. This compares with 49.0% male in the 1998

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Table 1 Demographic variation in HSV-2 seroprevalence in Australia

HSV-2	No	Rate (%) and 95% CI	Weighted†	Univariate RR	Multivariate RR*
Age (years)		$\chi^2(5) = 33.6\ p<0.001$			$\chi^2(5) = 34.0\ p<0.001$
25–34	496	10.7 (8.0 to 13.4)	10.2 (12.8 to 7.5)	1.00	1.00
35–44	935	16.4 (14.0 to 18.7)	15.5 (13.1 to 7.8)	1.53 (1.00 to 2.35)	1.59 (1.20 to 2.12)
45–54	1020	15.8 (13.6 to 18.0)	14.4 (12.3 to 6.6)	1.48 (0.96 to 2.26)	1.53 (1.16 to 2.03)
55–64	678	11.9 (9.5 to 14.4)	10.8 (13.1 to 8.4)	1.12 (0.69 to 1.80)	1.18 (0.86 to 1.61)
65–74	571	9.3 (6.9 to 11.7)	8.3 (10.5 to 6.0)	0.87 (0.51 to 1.47)	0.94 (0.66 to 1.32)
⩾75	300	7.7 (4.7 to 10.7)	8.7 (11.8 to 5.5)	0.72 (0.37 to 1.41)	0.79 (0.51 to 1.24)
Sex		$\chi^2(1) = 47.4\ p<0.001$			$\chi^2(1) = 49.5\ p<0.001$
Male	1739	8.9 (7.6 to 10.3)	8.4 (9.7 to 7.1)	1.00	1.00
Female	2261	16.3 (14.8 to 17.8)	15.6 (14.1 to 7.1)	1.83 (1.45 to 2.32)	1.81 (1.52 to 2.14)
Geography		$\chi^2(2) = 24.1\ p<0.001$			$\chi^2(2) = 26.4\ p<0.001$
Capital city	2162	14.4 (12.9 to 15.9)	13.7 (12.2 to 5.1)	1.00	1.00
Other metropolitan	804	15.3 (12.8 to 17.8)	13.3 (10.9 to 5.6)	1.06 (0.80 to 1.42)	1.05 (0.87 to 1.27)
Rural and remote	1034	8.7 (7.0 to 10.4)	8.6 (10.4 to 6.9)	0.61 (0.44 to 0.84)	0.61 (0.49 to 0.75)
Indigenous status		$\chi^2(1) = 7.8\ p=0.005$			$\chi^2(1) = 4.3\ p=0.038$
Non-Indigenous	3885	12.8 (11.8 to 13.9)	12.0 (11.0 to 3.0)	1.00	1.00
Indigenous	115	21.7 (14.2 to 29.3)	17.7 (10.7 to 4.6)	1.69 (0.94 to 3.05)	1.51 (1.05 to 2.18)
Australia	4000	13.1 (12.1 to 14.2)	12.1 (11.1 to 3.1)		

95% confidence interval (CI) of rates calculated by the normal approximation of the binomial; CIs for weighted rates are based on weighted proportions but actual n (given in the table). Relative risk (RR) calculated from a Poisson regression analysis. 95% CIs of RR calculated from the Wald method using SAS.
*Age group, sex, geography, Indigenous status.
†Weighted to the age, sex, geographic, and Indigenous structure of the 2001 Australian census population.
$\chi^2(x)$, chi square at x degrees of freedom.

Australian population, and a mean age in the 1996 census over 25 years of 48.1 years.[25] Ninety five per cent of the people were of white European origin.

From the AusDiab survey, a stratified random sample was obtained, that involved oversampling some demographic groups in order to ensure sufficient numbers to address the analytic hypotheses of the study. Sample size calculations used 95% significance and 90% power for finding hypothesised differences in HSV-2 prevalence, based on previous studies. As a consequence, all Indigenous subjects were included, and subjects resident in metropolitan regions other than capital cities, were oversampled; this provided more than sufficient numbers for precision of overall estimates and age group and sex comparisons. Although 3300 were indicated by sample size calculations, 4000 sera were randomly selected, yielding a sampling fraction of 40% for HSV-2 determinations. In seven instances where sera were not available, additional subjects were randomly selected. For HSV-1 analysis, 1000 serum samples were randomly selected from the 4000 sera used for HSV-2 determination. This is a 10% sampling fraction of the total survey.

Ethical approval for the study was obtained from the International Diabetes Institute ethics committee.

Analysis

In AusDiab, the proportion of participants living in state capitals, other metropolitan, and non-metropolitan areas
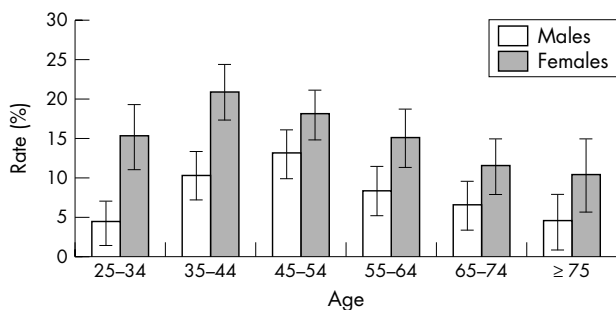
Figure 1 Seroprevalence of HSV-2 in women and men in the Australian population according to age.

were 65%, 7%, and 28% respectively, whereas in this study, the proportions were 54%, 20%, and 26% and the Indigenous proportion was 1%; in comparison, in this study, it is 3%. In addition, the age/sex distribution of the sample was found to differ marginally from the 1996 census because of differential response rates by age and sex.

For production of prevalence rates of HSV-1 and HSV-2, weights were calculated to adjust for: (a) purposeful oversampling of Indigenous and ''other metropolitan'' groups, and (b) the skewed age/sex distribution in AusDiab (compared to the census) from differential response by age and sex.[24] Weight factors were calculated as the ratio of the proportion of a stratum in the 2001 Australian census to the proportion in the same stratum in the study population of sera. For the non-Indigenous population, the strata were age group (6), sex (2), and geographic area (3). For the Indigenous population, because of the small numbers, the strata were age group (6) and sex (2). Strata specific weights were applied to cases before tabulations of weighted prevalences.

Analysis commenced by cross tabulation of HSV-2 and HSV-1 seroprevalence with age group, sex, geography of residence, and Indigenous status. Confidence intervals (95%) were calculated using the normal approximation of the binomial, except for instances where numerators were small (<30) and the exact binomial was used. The χ^2 test was used to assess heterogeneity between categories of variables. p Values are provided, and $p<0.05$ used to establish statistical significance. Prevalences weighted to the 2001 census are given for comparison. In this instance, 95% confidence intervals were calculated using the weighted proportions, but the actual number in each stratum (given in the tables) was given to estimate the standard error.

To examine the effects of possible confounding, Poisson regression was undertaken with each variable separately, then together in a model. Poisson regression, rather than logistic regression, was used because prevalences were considerable and the odds ratio (OR) from logistic regression only approximates the relative risk (RR) when proportions are small. In this analysis, the RR derives from the ratio of prevalences.[26] RRs are given with 95% confidence intervals. Statistical analyses were carried out in SPSS, SAS, and Stata.

Table 2 Demographic variation in HSV-1 seroprevalence in Australia

HSV-1	No	Rate (%) and 95% CI	Weighted†	Univariate RR	Multivariate RR*
Age		$\chi^2(5)=14.8$ p=0.011			$\chi^2(5)=16.0$ p=0.007
25–34	124	68.5 (60.3 to 76.7)	67.0 (58.7 to 75.3)	1.00	1.00
35–44	233	77.3 (71.9 to 82.7)	75.1 (69.6 to 80.7)	1.13 (0.98 to 1.29)	1.12 (1.00 to 1.26)
45–54	232	81.0 (76.0 to 86.1)	80.1 (75.0 to 85.2)	1.18 (1.03 to 1.35)	1.19 (1.06 to 1.34)
55–64	176	75.6 (69.3 to 82.0)	75.5 (69.2 to 81.9)	1.10 (0.95 to 1.27)	1.11 (0.98 to 1.25)
65–74	170	85.9 (80.7 to 91.1)	85.2 (79.9 to 90.5)	1.25 (1.09 to 1.44)	1.25 (1.11 to 1.41)
≥75	65	75.4 (64.9 to 85.9)	76.6 (66.3 to 86.9)	1.10 (1.10 to 1.33)	1.09 (0.93 to 1.28)
Sex		$\chi^2(1)=11.2$ p=0.001			$\chi^2(1)=11.4$ p=0.001
Male	516	71.3 (67.4 to 75.2)	71.3 (67.4 to 75.2)	1.00	1.00
Female	480	80.4 (76.9 to 84.0)	80.4 (76.9 to 84.0)	1.13 (1.05 to 1.21)	1.12 (1.05 to 1.19)
Geography		$\chi^2(2)=4.2$ p=0.122			$\chi^2(2)=3.9$ p=0.141
Capital city	536	75.7 (72.1 to 79.3)	73.6 (69.9 to 77.3)	1.00	1.00
Other metropolitan	193	82.4 (77.0 to 87.8)	78.9 (73.1 to 84.7)	1.09 (0.98 to 1.20)	1.08 (1.00 to 1.18)
Rural and remote	271	79.7 (74.9 to 84.5)	78.6 (73.7 to 83.5)	1.05 (0.96 to 1.15)	1.04 (0.97 to 1.12)
Indigenous status		$\chi^2(1)=5.7$ p=0.017			$\chi^2(1)=5.4$ p=0.020
Non-Indigenous	972	77.6 (75.0 to 80.2)	75.4 (72.7 to 78.1)	1.00	1.00
Indigenous	28	96.4 (81.7 to 99.9)¶	100.0 (87.7 to 100.0)‡¶	1.24 (1.01 to 1.53)	1.24 (1.04 to 1.47)
Australia	1000	78.1 (75.5 to 80.1)	75.7 (73.0 to 78.4)		

95% confidence interval (CI) of rates calculated by the normal approximation of the binomial except for those indicated. CIs for weighted rates are based on weighted proportions but actual n (given in the table). 95% CI of rates calculated by the normal approximation of the binomial. Relative risk (RR) calculated from a Poisson regression analysis. 95% CIs of RR calculated from the Wald method using SAS.
*Age group, sex, geography, Indigenous status.
†Weighted to the age, sex, geographic, and Indigenous structure of the 2001 Australian census population.
‡One sided test. ¶Exact binomial.
$\chi^2(x)$, chi square at x degrees of freedom.

HSV antibody testing

All sera were tested for antibody to HSV-2 using an indirect enzyme linked immunosorbent assay (ELISA), specifically the HerpeSelect 2 ELISA IgG (Focus Technologies). The manufacturer recommends that an index value of >1.10 is presumptive for the presence of antibody to HSV-2. However, we have found that using this cut-off value yields a high rate of false positive results (unpublished). To overcome this problem, a cut-off value of 3.5 was used to determine HSV-2 seropositive sera. Sera that gave an equivocal result in the HerpeSelect 2 ELISA (index values 0.9–3.5) were resolved using western blot.[27]

A subset of the sera (see above) was tested for antibodies to HSV-1. Depending on the HSV-2 serostatus of these sera, they were tested by one of two strategies as previously described:[14] If HSV-2 antibody was negative, serum was tested using the Enzygnost anti-HSV IgG ELISA (Behring) for detection of total antibody to HSV. If HSV-2 antibody was positive, serum was tested using HSV-1 western blot. This strategy was used owing to the lack of a low cost, sufficiently sensitive ELISA for antibody to HSV-1.

RESULTS
HSV-2 seroprevalence

The prevalence of HSV-2 in this large Australian population was 12%, and there were statistically significant differences by age group, sex, geography, and Indigenous status (table 1). Prevalence of HSV-2 was highest in the 35–44 year age range (14–19%) and in comparison with the youngest age group (25–34 years), the RR of HSV-2 seropositivity was 1.5 (p<0.001), adjusted for sex, geography, and Indigenous status (table 1). Women (16%) had a significantly higher prevalence of HSV-2 than men (8%) (adjusted RR 1.8). The age specific pattern of HSV-2 prevalence was similar for women and men, although the excess prevalence in women was greatest in the youngest age group (fig 1). Rural and remote populations had lower HSV-2 prevalence (9%) than capital city or other metropolitan populations (adjusted RR 0.6 compared to the capital city referent group). The Indigenous population had a significantly higher prevalence of HSV-2 (18%) than the non-Indigenous (12%) (the RR was 1.5 adjusted for age, sex, and geography).
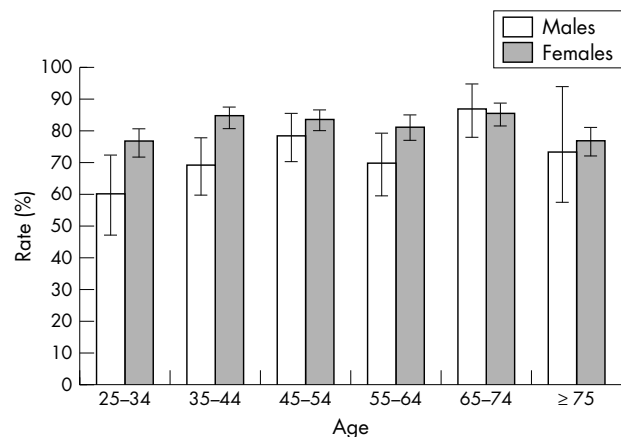
Figure 2 Seroprevalence of HSV-1 in women and men in the Australian population according to age.

HSV-1 seroprevalence

The prevalence of HSV-1 was 76%, and there were statistically significant differences by age group, sex, and Indigenous status (table 2). Prevalence of HSV-1 was highest in the 65–74 year age range (85%) and in comparison with the youngest age group (25–34 years), the RR of HSV-1 seropositivity was 1.25, adjusted for sex, geography, and Indigenous status. Women (80%) had significantly higher prevalence of HSV-1 than men (71%) (adjusted RR 1.12). The age specific pattern of HSV-1 prevalence was broadly similar for women and men (fig 2). There were no statistically significant differences in HSV-1 prevalence by geography. However, the Indigenous population had a higher prevalence of HSV-1 (close to 100%) than the non-Indigenous population (75%) (RR 1.24 adjusted for age, sex and geography) (table 2).

DISCUSSION

This is the first representative, nationwide, population based survey of HSV-1 and HSV-2 seroprevalence undertaken

outside the United States and showed that the prevalence of HSV-1 and HIV-2 antibodies in Australia were 76% and 12%, (at a mean age of 45–50). Analysis of HSV-1 and HSV-2 seroprevalence for available demographic factors; age, geography, sex, and Indigenous status revealed marked and significant differences for HSV-2, but much smaller differences for HSV-1 seroprevalence. Thus, the HSV-2 seroprevalence increased to a peak of 16% at ages between 35 and 44, and declined thereafter to ~8% in those over 65 years. Females had a significantly higher seroprevalence than males, again peaking at 35–44 and subjects in metropolitan areas, especially capital cities, had a significantly higher seroprevalence compared with those in rural areas. There was also a significantly higher HSV-1 (100% *v* 75%) and HSV-2 seroprevalence (18% *v* 12%), comparing Indigenous and non-Indigenous Australians.

The higher prevalence of HSV-2 antibody in Indigenous populations is consistent with a relatively high incidence of sexually transmitted infections in some of these populations.[28] The relatively small number of Indigenous people in our survey did not allow further distinction between rural and metropolitan communities. Further studies should focus specifically on Indigenous people, particularly in census districts where they predominate.

This study and our previous studies in pregnant women in western Sydney confirm that Australia has a relatively low HSV-2 seroprevalence compared with most other countries.[1 5 29–30] Conversely, HSV-1 seroprevalence in Australia is higher than in the United States and many parts of Europe.[1] In some developed countries, there has been a reduction in overall HSV-1 seroprevalence over recent decades as a result of decreased acquisition in infancy.[31] In contrast, worldwide, there is a trend towards increasing sexual acquisition of HSV-1 in adolescence and early adulthood,[32 33] and this trend is also apparent in Australia.[34] Therefore, HSV-2 seroprevalence is now less accurate as a predictor of the overall burden of genital herpes. This is offset by the fact that genital HSV-1 recurs much less frequently than HSV-2.[35] HSV-1 seroprevalence and early infant acquisition is much higher in many developing countries, where adult seroprevalence nears 100%.[1] This is similar to the HSV-1 seroprevalence documented in the Indigenous people in our survey. Such high levels of HSV-1 acquisition probably reflect extensive infant salivary spread in poorer hygienic conditions. Nevertheless, the reasons for higher overall HSV-1 seroprevalence in Australia, compared with the United States, require further studies of acquisition in infancy and in adolescence.

Unfortunately, this study lacked data from people aged 25 or younger. Information from NHANES II and III and other studies in high risk individuals, or selected populations around the world, have shown that HSV-2 seroprevalence increases rapidly from the mid-teens to the mid-twenties and then continues to slowly increase in the older age groups.[1–3 5 6 12 15 16 18 21 22] The few studies that have looked at HSV-2 seroprevalence in younger adolescents and children, have shown that this infection is uncommon in people below the age of 15. In Australia, further studies will be required to determine HSV-2 seroprevalence in these younger age groups.

Many studies[7] and a meta-analysis of them,[36] have indicated that previous genital HSV-2 infection is a risk factor for spread of HIV. The contribution to spread (attributable risk) is proportional to seroprevalence. Thus, in some African countries, where HSV-2 seroprevalence is more than 80%, the attributable risk may rise to 40%. This provides a rationale for the current trials of suppression of recurrences of genital HSV-2 with aciclovir in an attempt to reduce HIV transmission. Knowledge of variations of HSV-2 seroprevalence and hence susceptibility to HIV between populations or countries, should allow closer monitoring and early detection of HIV in these populations. Spread of HIV from Papua New Guinea to populations in northern Australia could be facilitated by previous HSV-2 infection among high risk (Indigenous) Australians.

As previous HSV-1 infection reduces the severity of disease and may or may not protect against HSV-2 infection, differences in seroprevalence between countries may alter the patterns of genital HSV-2 disease or even infection. Differences in HSV-1 seroprevalence are likely to influence the efficacy of the HSV-2 glycoprotein D2-dMPL vaccine manufactured by GSK (Simplirix), as efficacy has only been demonstrated in women with no previous antibodies to HSV-1 or HSV-2.[23] Therefore, in preparing for potential deployment of the vaccine in each country, it is important that there is an accurate estimate of HSV-1 seroprevalence in adolescent and adult populations. In some countries, or even some populations in other countries, very high HSV-1 seroprevalence might make deployment very difficult.

CONTRIBUTORS
ALC, RT, and AM, concept and design; ALC, AM, RT,and JT, mauscript writing; JS, epidemiological data collection; JT, HIV serological testing; RT, CM, data analysis; all authors, manuscript review.

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