ORIGINAL ARTICLE

A population based study of herpes simplex virus 2 seroprevalence in rural Costa Rica

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Objectives: To determine seroprevalence and determinants of herpes simplex virus 2 (HSV-2) seropositivity, in a random sample of a population based cohort of 10 049 women of Guanacaste, Costa Rica, using a highly sensitive and specific serological assay.

Methods: Seroprevalence was determined by a type specific HSV-2 ELISA assay in an age stratified random sample of 1100 women. Univariate and multivariate logistic regression was used to calculate odds ratios and 95% confidence intervals for risk factors of seropositivity.

Results: Overall age adjusted HSV-2 seroprevalence was 38.5% (95% CI, 37.5 to 39.5), and it was strongly associated with increasing age ($p_{Trend<0.0001}$), both among monogamous women and women with multiple sexual partners. A greater number of lifetime sexual partners increased the risk of seropositivity, with a 28.2% (95% CI, 24.4 to 32.2) seroprevalence among monogamous women and 75% (95% CI, 65.6 to 83.0) seroprevalence for those with four or more partners (OR = 7.6 95% CI, 4.7 to 12.4 $p_{Trend<0.0001}$). Barrier contraceptive use was negatively associated with HSV-2 seropositivity (OR 0.54, 95% CI, 0.31 to 0.94). Women with antibodies against HPV 16, 18, or 31 were 1.6 times more likely to be HSV-2 seropositive (OR 1.6, 95% CI, 1.2 to 2.1).

Conclusions: HSV-2 infection is highly endemic in Guanacaste, even among lifetime monogamous women, suggesting a role of male behaviour in the transmission of the infection. Until vaccination against HSV-2 is available, education to prevent high risk sexual behaviour and the use of condoms appear as preventive measures against HSV-2.

Herpes simplex virus type 2 (HSV-2) infection has increased in the past few decades in some geographical areas,[1–4] and has become more relevant given its possible association with risk of acquisition of HIV infection.[3 5 6] Seroprevalence in women is age dependent, and varies from under 20% to over 40%[7–10] in population based studies and up to 90% in populations with high risk sexual behaviour.[8] In Latin America, seroprevalences among women has been reported to be 35.8% in Mexico City[10] and 42% in Sao Paulo, Brazil.[9] In Costa Rica, a study of women aged 25–59 reported a seroprevalence of 39.4%.[11] By contrast, seroprevalence in men is ~25% lower than in women.[2 8]

The most consistent risk factors for HSV-2 seropositivity are female sex and cumulative risk of exposure including age, years of sexual activity, and multiple lifetime sexual partners.[1 2 11–13] Some studies report associations with early sexual debut,[9 12 13] parity,[13] and multiple sexual partners before the first pregnancy.[10] Others report marital status, lower socioeconomic status,[2 13] limited education,[2] smoking, vaginal douching,[10] cocaine use,[13] and sexually transmitted infections (STI).[8 12 13] Conversely, condom use has been found to be protective in a few cross sectional[9 11] and one prospective study.[14]

True population based surveys describing prevalence and risk factors for HSV-2 antibodies using highly sensitive and specific assays, are rare in developing areas. As part of a worldwide survey of the International Agency for Research on Cancer, we present data from an age stratified sample selected within a population based cohort in Guanacaste (adult female population in 1993 was 66 289), a rural province of Costa Rica with low HIV incidence rates.

METHODS

Study population and sample design

One sixth of 1038 censal segments (comprising 40–60 households each) of Guanacaste were randomly selected and, between June 1993 and December 1994, all resident women were invited to participate in a cohort study of the natural history of cervical neoplasia. The study enrolled 10 049 women with 93.6% participation. Details have been described elsewhere.[15] Briefly, after signing informed consent, participating women were given a questionnaire on sociodemographic, reproductive, contraceptive, sexual, and smoking history. A pelvic examination was performed including collection of exfoliated cervical cells with a Cervex brush (Unimar, CT, USA) for conventional and liquid based cytology (ThinPrep, Cytyc Corporation, MA, USA). Additional cells were collected with a Dacron swab and stored in a DNA transport medium (Virapap, Digene Corporation) for human papillomavirus (HPV) testing. Immediately afterwards the cervix was rinsed twice with 5% acetic acid and two images of the cervix were taken (Cervigrams, National Testing Laboratories, MI, USA). A ~15 ml heparinised blood sample was drawn, and plasma aliquots were frozen at −70°C. Plasma was shipped to the United States on dry ice and stored in liquid nitrogen. Of the 10 049 women, 9967 (99.2%) donated blood and 9175 (96.9% of sexually active women) underwent a pelvic examination. This analysis is based on an age stratified random sample of 1100 women participating in the enrolment visit. A total of 100 women were randomly selected from each of 11 age strata (18–19 years, 5 year groups to 74 years, and 75 years or older). Plasma samples

Table 1 Univariate analysis of HSV-2 antibody seroprevalence by selected reproductive and sociodemographic risk factors

Factor		Number*	% Positive	OR	95% CI
Age (years)					
	18–24	141	17.7	1	
	25–34	183	25.1	1.6	0.9 to 2.7
	35–44	195	47.7	4.2	2.5 to 7.1
	45–54	193	53.9	5.4	3.2 to 9.1
	55–64	194	55.2	5.7	3.4 to 9.6
	65+	97	58.8	6.6	3.7 to 11.9
				Trend p<0.001	
No of lifetime sexual partners					
	1	543	28.2	1	
	2–3	356	56.5	3.3	2.5 to 4.4
	4+	104	75.0	7.6	4.7 to 12.4
				Trend p<0.001	
Education					
	None	116	61.2	1	
	Primary	592	44.6	0.5	0.3 to 0.8
	Lower secondary	109	29.4	0.3	0.2 to 0.5
	Upper secondary	73	34.2	0.3	0.2 to 0.6
	Post secondary	113	35.4	0.4	0.2 to 0.6
				Trend p<0.001	
Marital status					
	Married	732	39.8	1	
	Separated/ divorced	71	63.4	2.6	1.6 to 4.3
	Widowed	60	55.0	1.8	1.1 to 3.1
	Single	140	45.0	1.2	0.9 to 1.8
Smoking					
	Never	875	40.5	1	
	Former	78	59.0	2.1	1.3 to 3.4
	Current	50	64.0	2.6	1.4 to 4.7
				Trend p<0.001	
Socioeconomic status†					
	Low	146	49.3	1	
	Intermediate	168	37.5	0.6	0.4 to 1.0
	High	688	43.2	0.8	0.5 to 1.1
				Trend p=0.108	
Age at first intercourse (years)					
	17	668	38.5	1	
	16	335	52.2	1.7	1.3 to 2.3
Number of live births					
	0–2	293	35.2	1	
	3–4	253	43.9	1.4	1.0 to 2.0
	5–7	246	50.8	1.9	1.3 to 2.7
	8+	152	54.6	2.2	1.5 to 3.3
				Trend p<0.001	
Abortion					
	Never	637	40.0	1	
	Ever	307	54.4	1.8	1.4 to 2.4
Cytology					
	Never	150	37.3	1	
	Ever	852	44.0	1.3	0.9 to 1.9
Hysterectomy†					
	Never	335	51.9	1	
	Ever	85	62.4	1.5	0.9 to 2.5
Vaginal pH					
	4	28	32.1	1	
	4.5	538	37.6	1.3	0.6 to 2.9
	5	261	53.3	2.4	1.0 to 5.5
	5.5	145	46.9	1.9	0.8 to 4.4
				Trend p<0.001	
HPV 16, 18, or 31 serological status					
	Negative	518	35.9	1	
	Positive	485	50.7	1.8	1.4 to 2.4
Oral/injectable contraceptive use					
	Never	448	46.9	1	
	Former	416	42.6	0.9	0.6 to 1.1
	Current	139	32.4	0.5	0.4 to 0.8
				Trend p=0.01	
HPV DNA‡					
	Negative	755	42.0	1	
	Positive	214	46.3	1.2	0.9 to 1.6
Barrier method use					
	Never	619	50.1	1	
	Former	285	34.0	0.5	0.4 to 0.7
	Current	99	25.2	0.3	0.2 to 0.5
				Trend p<0.001	

*Women without sexual experience or with missing HSV-2 results are excluded.
†One missing case.
‡34 missing HPV DNA results.

were selected irrespective of sexual experience and cervical diagnosis.

HSV-2 serological testing

Plasma samples were shipped to Dr Rhoda Ashley's laboratory for HSV-2 serological testing using type specific HSV-2 and HSV-1 ELISA assays (Focus Technologies, CA, USA). All testing was conducted blinded to other data. All HSV-2 positive sera were retested for confirmation with another Focus Technologies HSV-2 ELISA; 18 specimens without valid result were excluded from analyses.

As a validation of the ELISA, 50 random positive specimens and 50 negative specimens by the HSV-2 ELISA were tested with a western blot test, the current reference standard for HSV-2 serological testing.[16] The kappa was 0.88 (95% CI 0.68 to 1.0), after exclusion of three pairs with missing results. The ELISA had a sensitivity of 100%, a specificity of 89.5%, a positive predictive value (PPV) of 87%, and a negative predictive value (NPV) of 100% compared to western blot.

Human papillomavirus (HPV) testing

VLP based ELISA

VLPs were prepared in Trichoplusia *ni (*High five) cells (Invitrogen, Carlsbad, CA, USA) from recombinant baculoviruses expressing L1 and L2 genes of HPV 16 or 31 or L1 gene alone of HPV 18 and purified by density gradient ultracentrifugation and column chromatography techniques. HPV-16, 18, and 31 specific ELISAs were performed as previously described,[17] except for the use of an automatic plate washer (Skanwasher 300, Skatron, Norway) and a MultiProbe II robotic liquid handling system (Packard Instruments, CT, USA) to dilute serum samples in 1:10 in 0.5% polyvinyl alcohol (PVA, MW 30 000–70 000, Sigma, St Louis, MO, USA) and 10 µl of the diluted serum sample were added to antigen coated plates containing 100 µl per well of 0.5% PVA.

The cut-off point for positive results was determined from the reactivity of concurrently tested plasma samples from self reported virgins. The mean and standard deviation (SD) of optical density (OD) values for controls were calculated and values greater than the mean plus 3 SD were excluded. The analysis was repeated until no further OD values could be excluded by this criterion. A positive cut-off point of 3 SD above the mean of this distribution was chosen. Using this threshold of seropositivity and three HPV types combined (HPV 16, 18, and 31), 97% of seropositive women were HPV DNA positive for either of the types and among those HPV DNA positive 78% were seropositive.

Polymerase chain reaction (PCR)

Details of the MY09/11 L1 consensus primer PCR with AmpliTaq Gold have been previously reported.[18–20] After an aliquot of the DNA transport medium (Virapap, Digene Corporation) specimen was lysed, the DNA was precipitated by ammonium acetate/ethanol solution, by centrifugation, resuspended in 10 mM TRIS, pH 7.5, 0.1 mM EDTA, and stored frozen until used. Quality control, amplification, and thermocycling conditions were as previously described.[18–20]

PCR products were analysed by gel electrophoresis and then transferred to Nylon filters. The filters were hybridised overnight with radiolabelled generic probes for HPV (HPV 11, 16, 18, 51, 73, and 81 combined) as has been described previously. Dot-blot hybridisation for HPV type specific detection (HPV types 2, 6, 11, 13, 16, 18, 26, 31–35, 39, 40, 42–45, 51–59, 61, 62, 64, 66–70, 71 (AE8), AE2 (IS39), AE9, AE10, and the combination of 2, 13, 34, 42–4, 57, 62, 64, 69, 74, 82 (W13B), and AE9) was conducted on specimens that were positive for the generic probes. Three experienced investigators interpreted each dot-blot result, and discrepancies were resolved by consensus.

Statistical analysis

Overall prevalence in the cohort was estimated using the direct standardisation method with the cohort and the world standard population as reference populations. Univariate and age stratified analyses (18–24, 25–34, 35–44, 55–64, 65+ years) were carried out to examine associations between HSV-2 seropositivity and the different variables. Odds ratios (ORs) with 95% exact binomial confidence intervals were calculated and Pearson χ^2 test ($p<0.001$) was used for significance (STATA 7.0, Stata Press, TX, USA). Continuous variables were categorised.

Multivariate logistic regression models, adjusting for lifetime number of sexual partners and age, were then constructed for each risk factor identified in the univariate analysis. To test for statistical significance of dose-response relations (p_{Trend}), categorical variables were treated as continuous in the logistic models. The risk factor analysis was restricted to sexually active women with valid HSV-2 results (n = 1003). In accordance with the sexual mode of transmission, seroprevalence among virgins was low (n = 80, 79 with valid HSV-2 result; 2.5%).

RESULTS

The mean age of the entire cohort was 41.1 years (range 18–97). Among sexually active women, 54% reported having had only one lifetime sexual partner and 76% had fewer than three partners. Elementary schooling is common (89%), but few (27%) received 9 or more years of education. Guanacaste is a rural, agricultural province with relatively good public services; 50% of the women reported to have all household amenities (refrigerator, running water, in-house toilet, electricity, television) and only 9% declared having none.

The age standardised HSV-2 antibody prevalence for the entire Guanacaste cohort was estimated to be 38.5% (95% CI, 37.5 to 39.5). The prevalence adjusted for the world standard population was 38.4% (95% CI, 38.1 to 38.7).

In univariate analysis, age and number of lifetime sexual partners were the major predictors of HSV-2 seropositivity (table 1). Seroprevalence ranged from 17.7% (95% CI, 11.8 to 25.0) among women 18–24 years old to 58.8% (95% CI, 48.3 to 68.7) among those 65 years or older with a 6.6 (95% CI, 3.7 to 11.9) fold increase in risk ($p_{Trend}<0.0001$). A strong trend of increasing prevalence was observed with increasing number of sexual partners ($p_{Trend}<0.0001$). Sexually inexperienced women had a seropositivity of 2.5% (95% CI, 0.3 to 8.8), while women reporting four or more partners had a seroprevalence of 75% (95% CI, 65.6 to 83.0) with a 115.5-fold (95% CI, 26.5–503.4) increase in risk compared to virgins and 7.6 (95% CI, 4.7 to 12.4) compared to monogamous women. Only two HSV-2 seropositive women (0.5%) reported a history of genital herpetic ulcers.

Additional univariate determinants of HSV-2 seropositivity include limited education, separation or divorce, smoking, low socioeconomic status, sexual initiation before age 17, number of live births, abortion, higher vaginal pH, and seropositivity for HPV 16, 18, or 31. Use of oral/injectable contraceptive methods, having had a cytology, hysterectomy, or being positive for HPV DNA were not significantly associated, but use of barrier methods showed a significant decrease in risk that was even stronger for current users compared to never users (OR 0.3, 95% CI, 0.2–0.5).

In the multivariate logistic regression, women who were 65 years of age or older were approximately eight times more likely to be HSV-2 seropositive compared to women under 25 years ($p_{trend}<0.0001$) (table 2). A clear trend of increasing risk with increasing number of partners was observed. The age adjusted OR associated with having had four or more sexual partners was 21.4 (95% CI 7.2 to 63.6) compared to

Table 2 HSV-2 antibodies adjusted odds ratios (OR) for seropositivity among sexually active women by selected reproductive and sociodemographic risk factors

Factor		OR*†	95% CI
Age (years)			
	18–24	1	
	25–34	1.5	0.8 to 2.7
	35–44	3.8	2.2 to 6.6
	45–54	5.6	3.2 to 9.6
	55–64	6.4	3.7 to 11.0
	65+	7.9	4.2 to 14.9
		Trend: p<0.0001	
No of lifetime sexual partners			
	1	1	
	2–3	3.6	2.7 to 4.9
	4–5	6.0	3.4 to 10.7
	6+	21.4	7.2 to 63.6
		Trend: p<0.001	
Marital status			
	Married	1	
	Separated/divorced	1.3	0.8 to 2.3
	Widowed	1.1	0.6 to 2.0
	Single	.84	0.5 to 1.3
Smoking			
	Never	1	
	Former	1.4	0.9 to 2.4
	Current	1.4	0.7 to 2.7
		Trend: p=0.14	
Education			
	None	1	
	Elementary	0.86	0.6 to 1.4
	High school	0.81	0.4 to 1.5
	Technical/university	0.86	0.5 to 1.6
		Trend: p=0.67	
Socioeconomic status‡			
	Low	1	
	Intermediate	0.68	0.4 to 1.1
	High	0.98	0.6 to 1.5
		Trend: p=0.61	
Age at first intercourse (years)			
	17	1	
	16	1.6	1.1, 2.1
Ever pregnant			
	Never	1	
	Ever	2.2	1.0 to 4.6
Number of live births			
	0–2	1	
	3–4	0.99	0.7 to 1.5
	5–7	0.73	0.5 to 1.1
	8+	0.77	0.5 to 1.3
		Trend: p<0.001	
Hysterectomy‡			
	Never	1	
	Ever	1.4	0.9 to 2.4
Oral/injectable contraceptive use			
	Never	1	
	Former	1.2	0.9 to 1.7
	Current	1.6	0.9 to 2.6
		Trend: p=0.09	
Barrier method use			
	Never	1	
	Former	0.64	0.4 to 0.9
	Current	0.54	0.3 to 0.9
		Trend: p=0.003	
IUD use			
	Never	1	
	Former	1.7	1.1 to 2.6
	Current	1.5	0.8 to 3.1
		Trend: p=0.01	
HPV 16, 18, or 31 serological status			
	Negative	1	
	Positive	1.6	1.2 to 2.1
HPV DNA§			
	Negative	1	
	Positive	1.1	0.8 to 1.5

*Women with missing HSV-2 results are excluded.
†ORs adjusted for age and number of sexual partners.
‡One missing case.
§34 missing HPV DNA results.

women with one lifetime partner ($p_{Trend}<0.0001$). Only 10 women reported more than one recent partner and thus this variable could not be evaluated.

The only additional factors that remained statistically significant were age at first intercourse before or at 16 years (OR = 1.6, 95% CI, 1.1 to 2.1, compared to those initiating at age 17 or older). Similarly, former intrauterine device users (OR = 1.7, 95% CI, 1.1 to 2.6, compared to never users) and HPV 16, 18, or 31 seropositivity (OR = 1.6; 95% CI 1.2 to 2.1) were significant after adjustment. Barrier methods were protective with a 50% reduction in detection of HSV-2 antibodies for current (last month) versus never users (OR = 0.54; 95% CI, 0.31 to 0.94).

Among monogamous women, those separated or divorced had an age adjusted 3.4 (95% CI 1.4 to 8.2) increase in risk of HSV-2 detection.

No statistically significant difference in risk was seen for smoking, education, and socioeconomic status (number of household amenities). Similarly, number of pregnancies, use of oral/injectable contraceptives or positivity for HPV DNA status were not associated with HSV-2 seropositivity.

DISCUSSION

HSV-2 antibody seroprevalence in Guanacaste (38.5%) is relatively high for women when compared to other population based studies like the United States (25.6%; aged 12+ years) and Spain (3.6%; age range 5–59).[2 21] However, it is consistent with reported seroprevalences from other Latin American countries, 42% in Brazil[9] and 35.8% in Mexico,[10] although the mean age in this cohort (41 years) was younger than in those two studies. Women from Guanacaste have a low risk self reported sexual behaviour, with only one fourth reporting more than two lifetime sexual partners and only 4.2% referring having had an STD. The high seroprevalence in the context of low risk female behaviour is probably explained by high risk behaviour of their male partners, as suggested by Oberle *et al*.[11] HSV-2 seroprevalence is generally more frequent among women than men, but it is unclear whether women are more susceptible, men are more efficient as vectors, or infected men are less likely to seroconvert. Only one study in Jordan reported higher prevalence in males[22] and another in Spain reported no difference by sex.[21] There are no reported HSV-2 studies in Costa Rican men and we had no concurrent information on partners' sexual behaviour.

The previous HSV-2 study in Costa Rican women reported a seroprevalence of 39.4% in a population with a similar mean age. The provinces of Limón and Puntarenas, which are similar to Guanacaste with regard to rurality and sexual behaviour,[23] had higher HSV-2 seroprevalence (~48.7%) than the rest of the country and also a significant association of HSV-2 seropositivity and being positive for another STD was found. We found no association with a history of STDs or current HPV DNA positivity. The negative association of barrier methods (mainly condoms, other methods are rare in Guanacaste) with HSV-2 seropositivity was consistent in both studies, suggesting that condoms may protect against HSV-2 contagion.

Age and lifetime sexual partners were the major determinants of HSV-2 seropositivity. As expected for any STD, high risk sexual behaviour increases the probability of being exposed. The finding of two HSV-2 seropositive virgins (2.5%) may reflect non-sexual transmission (that is, perinatal infection, oral) or false positivity of the ELISA used, which was 90% specific. Among women with multiple sexual partners, HSV-2 prevalence was higher than among monogamous women in all age groups, particularly in the young, and the prevalence peak occurred at earlier ages. In the monogamous group, seroprevalence continued to rise with age, although less steeply for the older age groups (data not shown). The possibility of HSV-2 being acquired among older age women, an age-cohort effect, or both cannot be ruled out.

Key messages

- HSV-2 seropositivity is high in Guanacaste, Costa Rica (38.5%, 95% CI 37.5 to 39.5).
- HSV-2 seropositivity in Guanacaste is only a modest sexual behaviour marker for women, given the high seroprevalence in monogamous women (28.2%, 95% CI 24.4 to 32.2).
- Use of barrier contraceptives was negatively associated with HSV-2 seropositivity (OR 0.54, 95% CI 0.31 to 0.94 compared to never users).
- Until vaccination against HSV-2 is available, education to prevent high risk sexual behaviour and the use of condoms appear to be as preventive measures against HSV-2.

In contrast with other studies, education, socioeconomic indicators, number of live births, ever being pregnant, use of oral and injectable hormonal contraceptives were not associated with HSV seropositivity. Although hysterectomy was not associated with an increased risk in the general population, menopausal hysterectomised women had higher HSV-2 seropositivity (data not shown). Although smoking is considered as a possible contributor to viral diseases by altering immune function,[24] we found no significant association with smoking.

The risk of HSV-2 seropositivity was higher in women with antibodies against a subgroup of high risk HPV types, a partial indicator of HPV exposure. Whether this reflects an interaction that facilitates co-infection or just represents a subgroup at higher risk for both infections is unclear. However, detection of HPV DNA was not associated with HSV-2 seropositivity, probably indicating that HPV DNA represents current infection, which is usually transient and occurs more commonly in younger women.

HSV-2 is an endemic but largely asymptomatic infection in Costa Rican women. Although it is commonly recognised that HSV-2 infection is generally asymptomatic, under-reporting cannot be ruled out. HSV-2 serological screening programmes should be evaluated in selected population groups at especially high risk, either of becoming reservoirs with potential public health impact or in groups at risk of more serious forms of the disease. Identification of antibodies is a reliable method for identifying asymptomatic, never diagnosed population reservoirs for future infections. Pregnant women could be a target group for screening given the silent nature of an infection that can result in neonatal disease.

Control of this viral infection by vaccination may be possible in the future, although many difficulties have to be overcome[25] in the interim. Use of condoms and education of adolescents, women of reproductive age, and health related personnel on the importance of the diagnosis, treatment, and prevention may help control the spread of HSV-2 infections.

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CONTRIBUTORS

ACR was involved in the statistical analysis, interpretation, and manuscript preparation of the HSV-2 study, also in the overall coordination of the cohort follow up; PEC was involved in the initial idea, study design and overall coordination of the HSV-2 study,

statistical analysis, and manuscript review; JSS was involved in the initial idea, study design, and manuscript review of the HSV-2 study; CB was involved in the design and overall coordination of the cohort and the interpretation and manuscript review of the HSV-2 study; AH was involved in the design and overall coordination of the cohort, as well as in the initial idea, interpretation, and manuscript review of the HSV-2 study; MS was involved in the design and overall coordination of the cohort, as well as in the initial idea, interpretation, and manuscript review; RV did the HPV serology and manuscript review; RDB did the HPV PCR determination and manuscript review; RA did the HSV-2 serology and manuscript review; XC was involved in the statistical analysis and interpretation and manuscript review; RH was involved in the design and overall coordination of the cohort, as well as in the initial idea, study design, statistical analysis, interpretation and manuscript review of the HSV-2 study.

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