

COMPARISON OF RISK FACTORS FOR CERVICAL CANCER IN DIFFERENT POPULATIONS

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The incidence of cervical cancer has been found to vary between populations. Risk factors of cervical cancer include early age at first marriage, multiple marriages and antibodies to herpes simplex virus type 2 (HSV-2). The interrelatedness of these risk factors was examined by comparing data collected from 428 cancer cases and 947 control women selected from 6 populations having standardized cervical cancer incidence rates varying from 9.3 to 85.1 per 100,000. Logistic regression analysis revealed that multiple marriages, early age at first marriage or pregnancy and HSV-2 antibodies were all associated with significant risk when all 3 factors were entered into the model. Cervical cancer incidence rates were best predicted by the occurrence of HSV-2 antibodies among control women. To further assess the relation between cervical cancer rates and HSV-2 antibody, 2,306 additional sera representing an 0.8% random sample of females over 9 years of age residing in the Republic of Panama were assayed for antibodies to the virus, and the occurrence of antibodies was correlated with invasive cervical cancer rates specific to each Province. Data from both the random sample and the other study populations yielded a linear relation between the occurrence of HSV-2 antibodies and the incidence of cervical cancer. An exception was found for women living in Herrera Province, Republic of Panama, who had a higher cancer rate than predicted by HSV-2 antibody occurrence. The data suggested that infection with HSV-2 is a co-variable of venereal factors, although a role for the virus in the genesis of a certain proportion of cervical cancers is not excluded.

A number of case-control studies have identified early age at first coitus and multiple sex partners as risk factors in the development of cervical cancer (reviewed by Rotkin, 1973; Hulka, 1982; Singer, 1983). These and other findings strongly support the hypothesis that cervical cancer is caused by venereal factors (reviewed by Kessler, 1981). Interestingly, the incidence of cervical cancer varies considerably between populations as well as over time within the same population (Devesa and Diamond, 1980; Waterhouse *et al.*, 1976). The implications of these observations is that differences in sexual behavior, either between populations or between cohorts within the same population, account for variations in cervical cancer rates. The present studies were undertaken to examine this possibility.

Herpes simplex virus type 2 (HSV-2) is transmitted venereally and the immune responses associated with infections by the virus persist for many years. Thus, the presence of antibodies to HSV-2 in sera provides a stable biological marker for exposure to venereal diseases (reviewed by Rawls and Campione-Piccardo, 1981). In addition, seroepidemiological and experimental evidence suggests that HSV-2 may play a role in some cases of cervical cancer (reviewed by Galloway and McDougall, 1983; Aurelian, 1984; Rawls, 1984). To examine the interrelation between sexual behavior, HSV-2 antibodies and cervical cancer in greater detail, we conducted a two-part study. The first part consisted in sampling invasive cervical cancer cases and control women living in populations where cancer rates were monitored by cancer registries. Sera as well as information regarding sexual behavior were obtained from these women. The sera were assayed for HSV-2 antibodies and the occurrence of HSV-2 antibodies was correlated with aspects of sexual behavior and with cervical cancer rates.

The second part of the study consisted in determining the occurrence of HSV-2 antibodies in a random sample of sera from females living in the Republic of Panama and correlating antibody occurrence with Province-specific invasive cervical cancer rates. With the exception of one Province in Panama, a good correlation was found between cervical cancer rates and the prevalence of HSV-2 antibodies, suggesting that variations in cervical cancer rates primarily reflect differences in exposure to venereally transmitted factors.

MATERIAL AND METHODS

Case-control samples

The first component of the study consisted in sampling invasive cervical cancer cases and control women from 6 different populations. We assumed that control women would be representative, with respect to risk factors, of the populations from which the cases were drawn. The 6 populations were selected for study on

TABLE I - CASE-CONTROL SAMPLES

Study sites	Years	Source of cases	Source of controls	Incidence areas	Years
York County (Toronto), Ontario, Canada	1975-1977	Princess Margaret Hospital, Toronto	Neighborhood	Ontario, Canada	1975-1977
Herrera Province, Republic of Panama	1974-1980	All hospitals	Neighborhood	Herrera Province	1974-1980
Harris County (Houston), Texas ¹	1969-1977	Municipal Hospitals Houston, TX	Municipal Hospitals of Houston	Harris County	1962-1966
London, UK	1976-1983	St. Thomas's Hospital London, UK	St. Thomas's Hospital	St. Thomas Health District	1975-1979
San Juan, Puerto Rico	1981	University of Puerto Rico Medical Center, San Juan, P.R.	University of Puerto Rico Medical Center, San Juan, P.R.	Puerto Rico	1979
Vancouver, British Columbia, Canada	1981-1983	A. Maxwell Evans Cancer Clinic, Vancouver, BC.	A. Maxwell Evans Cancer Clinic Vancouver	British Columbia	1979-1981

¹The sample from Houston included black women only and cancer rates are for black women residing in Harris County.

the basis of well-documented invasive cervical cancer rates which covered a wide range. The characteristics of the samples collected from these populations are presented in Table I. In York County, Ontario, Canada, and Herrera Province, Republic of Panama, study subjects were chosen by population-based sampling, employing age-matched neighborhood controls (Clarke and Anderson, 1979; Reeves *et al.*, 1985). Standard questionnaires were administered to obtain information on a wide variety of putative cervical cancer risk factors. In the other areas, cases and controls were selected from the same health-care facility as previously described (Adam *et al.*, 1971; Ferrer *et al.*, 1984). Attempts were made to select about 30 control women in each of 4 age groups; 30-39, 40-49, 50-59 and 60 or more years. Women with a history of cervical cancer were excluded from control groups. Only black women were included in the sample from Houston, TX. In areas other than Toronto and Herrera Province, a brief questionnaire was used to elicit information concerning the major sexual risk factors; *i.e.* multiple sex partners and early age at first coitus. In order to keep the questions culturally non-biased and morally non-evaluative, we queried subjects regarding number of marriages and ages at first marriage and pregnancy. The minimum age at first coitus for each woman was taken as the lesser of the age at first marriage or age at first pregnancy. These risk factors were dichotomized for analysis into those with one marriage or less versus those with 2 or more marriages and those aged 20 or over at first marriage or pregnancy versus those under 20. Women who reported never having been married but having had one or more pregnancies were excluded (2.1%) from both "number of marriage" categories and from all subsequent analysis. Although some cases of dysplasia and carcinoma *in situ* were included in the samples from some populations, the analysis was based on those with histologically diagnosed invasive carcinoma.

Incidence rates

Invasive cervical cancer incidence rates in 4 of the 6 study areas (Vancouver, Toronto, London, and San

Juan) were obtained through population-based cancer registries (for British Columbia, Ontario, St. Thomas Health District, and Puerto Rico, respectively) which routinely monitor cancer incidence (Waterhouse *et al.*, 1976). Rates were selected to cover as nearly as possible the same time period as the study. In Houston, the rates for Harris County black women in 1962-66 were used; these years represented the last quinquennium of a period when a cancer surveillance program was in operation (MacDonald and Heinze, 1978). In Panama, a special population survey was undertaken to determine the incidence of cervical cancer for all Provinces between 1974 and 1979 (Reeves *et al.*, 1984). Rates have been age-adjusted to the World Standard population, using 5- or 10-year age groups, depending on available incidence data, up to the highest age group for which data could be obtained (ranging from 60 and over to 85 and over).

Random serologic survey in Panama

The second component of the study consisted in determining the occurrence of HSV-2 antibodies in sera from randomly selected females residing in the Republic of Panama. Information regarding sexual behavior was not available; however, the cervical cancer incidence rates of the populations from which these sera were taken were known (Reeves *et al.*, 1982). In June 1978 the Ministry of Health and Gorgas Memorial Laboratory conducted a random serologic survey of the Republic of Panama; the purpose was to assess immunity to diseases preventable by vaccination such as polio, measles, diphtheria, tetanus and pertussis. The survey encompassed every Province with the exception of isolated San Blas and Darien and used standard methods to select the population (Serfling and Sherman, 1965). At the Province level, a random selection of Corregimientos (boroughs) was made within each District; this included at least one urban and one rural Corregimiento. Within each selected Corregimiento, census tracts were randomly selected and then within the chosen census tracts households were randomly selected so that by surveying all household members approximately 1% of the total population

TABLE II - AGE-SPECIFIC CERVICAL CANCER RATES

Age (years)	Average annual incidence rates per 100,000 population					
	Harris County (1962-66)	Herrera Province (1974-80)	Puerto Rico (1979)	Ontario Province, Canada (1975-77)	St. Thomas district, London (1975-79)	British Columbia Province, Canada (1979-81)
30-39	196.9	142.6	20.4	20.9	12.4	16.6
40-49	119.8	119.7	51.8	26.1	17.0	14.8
50-59	131.3	170.8	44.6	35.5	25.3	16.9
60-69	159.0	183.4	71.8	34.7	43.8	19.1
Age-adjusted rates	85.1	79.1	19.4	13.4	11.3	9.3

would be included. The survey obtained sera from 0.8% of the 1978 population. Overall compliance varied from 36 to 80% and was lowest in higher socioeconomic census tracts; the lowest compliance rates were in adult males. In order to estimate the occurrence of HSV-2 antibodies, we selected sera from all females older than 9 years of age.

Assays for antibodies to herpes simplex virus type 2

Neutralizing antibodies were assayed by a previously described micro-neutralization test (Rawls *et al.*, 1980). Strain KOS of HSV-1 and strain 333 of HSV-2 were used and non-neutralized virus was detected in monolayers of Vero cells grown in microtiter plates. Antibody titers to HSV-1 and HSV-2 were recorded to the log₁₀ and a II/I index (Rawls *et al.*, 1970) of 85 or more was considered positive for HSV-2 antibodies. To reduce the effects of inter-test variability, single stocks of HSV-1 and HSV-2 were used to test sera from cases and controls. In addition, sera were tested under code and each test included sera from different populations. Exceptions to this were the sera from the random sample of Panamanian women which were tested with another set of virus stocks in separate assays, and sera from women residing in Toronto which had been previously tested (Rawls *et al.*, 1980). Adequate quantities were not available to retest all of the sera from Toronto women. In order to exploit the data from the original analysis of these sera, we used 77 sera (24%), of which ample quantities were available, to standardize the original data to the results obtained in the analysis of the sera from the other case-control samples. This was done by including the 77 sera in the microneutralization assays used to detect antibodies in sera from the other areas. Of the 77 sera, 22% were seropositive on reanalysis while 49% had been considered seropositive in the original assay. Comparable seropositivity rates for those 77 sera were obtained by using II/I indices of 97 or greater as criteria for HSV-2 antibodies in the original analysis. Thus, the original data set obtained for sera from Toronto women was standardized to the results of the remaining areas by applying the revised criteria of seropositivity.

For comparison of the occurrence of HSV-antibodies with cancer rates, percentages of positivity were age-adjusted (using 10-year age-groups) to the standard world population for women aged 30 through 69 years.

Statistical analysis

Stepwise linear regression was used to investigate the relationship between the prevalence of risk factors among cases or controls and the appropriate popula-

tion's cervical cancer rates. Case-control differences were examined by logistic regression using BMPD statistical software (University of California Press) with age (as a continuous variable) included in all models. For all 6 areas together, the 3 dichotomized risk factors of interest were entered into the logistic model in a stepwise fashion with "dummy" variables included for areas. The risk factors included age at first marriage or pregnancy of 20 or less, *versus* more than 20 years of age, less than 2 marriages *versus* 2 or more marriages, and HSV-2 antibody-positive status *versus* HSV-2 antibody-negative status. In addition, a stepwise model which included all meaningful 2-way interactions was examined. Since a significant interaction between area and one of the risk factors was apparent, regressions were performed for each area separately including all risk factors which were significant in the all-area analysis. Persons with missing values for any of the variables included in the models were excluded from all logistic regression analyses. The relationship between HSV-2 seropositivity and sexual variables in the controls was also examined by logistic regression. Once again, age and area variables were forced into the model, while the dichotomous variables for number of marriages and age at first marriage or pregnancy were added in a stepwise fashion. The association between HSV-2 seropositivity and cervical cancer rates was estimated by calculating the correlation coefficient.

RESULTS

Cervical cancer rates

The rates of cervical cancer for women living in the study areas are shown in Table II. The highest rates were observed among black women living in Harris County, TX, and among women in Herrera Province, Republic of Panama. The lowest rates were observed in women in St. Thomas Health District of London, UK, and in British Columbia. The age-specific rates were fairly constant for the 4 age groups in British Columbia; however, the incidence of invasive cervical cancer tended to increase with age in other populations. In addition, increased rates occurred in women of the 30-39 age group living in Herrera Province and Harris County. Thus, variation in the pattern of age-specific incidence as well as in the absolute incidence of cervical cancer was apparent in the populations studied.

Comparison of risk factors and cervical cancer incidence

Multiple sex partners and early age at first coitus have been repeatedly shown to be associated with an

TABLE III - OCCURRENCE OF RISK FACTORS AMONG CASES AND CONTROLS

Area	Risk factors			
	Two or more marriages		First marriage or pregnancy before 20 years of age ²	
	Controls	Cases	Controls	Cases
Houston	43/110 (39.1) ¹	26/56 (46.4)	96/120 (80)	49/57 (86.0)
Herrera Province	62/257 (24.1)	23/55 (41.8)	158/254 (62.2)	39/55 (70.9)
San Juan	19/126 (15.1)	23/60 (38.3)	60/126 (47.6)	46/62 (74.2)
Toronto	31/220 (14.1)	26/104 (25.0)	49/220 (22.2)	36/104 (34.6)
London	13/93 (14.0)	14/69 (20.3)	28/96 (29.2)	14/75 (18.7)
Vancouver	18/123 (14.6)	19/67 (28.4)	27/124 (21.8)	26/67 (38.8)

¹Number with attribute over number of subjects for which information was available (percent with risk factor). ²Estimated as lesser of age at first marriage or age at first pregnancy.

TABLE IV - AGE-SPECIFIC OCCURRENCE OF HSV-2 ANTIBODIES AMONG CONTROL WOMEN

Age (years)	Number positive/number tested (percent) with HSV-2 antibodies in:					
	Houston	Herrera Province	San Juan	Toronto	London	Vancouver
20-29	—	19/68 (27.9)	1/10 (10)	0/6	0/7	7/20 (35)
30-39	11/30 (36.7)	38/110 (34.5)	3/24 (12.5)	5/36 (13.9)	2/21 (9.5)	3/17 (17.6)
40-49	19/27 (70.3)	15/41 (36.6)	3/27 (11.1)	8/52 (15.4)	0/13 (0)	1/22 (4.5)
50-59	25/35 (71.4)	7/16 (43.8)	8/33 (24.2)	11/67 (16.4)	1/23 (4.3)	4/28 (14.3)
60-69	15/24 (62.5)	7/12 (58.3)	11/25 (44)	6/51 (11.8)	4/20 (20.0)	2/18 (11.1)
≥70	4/4	1/3	2/7	1/9	3/11 (27.3)	2/17 (11.8)
Age-adjusted ¹ % positive	(59.1)	(41.4)	(20.2)	(14.5)	(7.0)	(11.8)

¹Age-adjusted for women aged 30 to 69 as indicated in "Material and Methods".

TABLE V - AGE-SPECIFIC OCCURRENCE OF HSV-2 ANTIBODY AMONG WOMEN WITH CERVICAL CANCER

Age (years)	Number positive/number tested (percent) with HSV-2 antibodies in:					
	Houston	Herrera Province	San Juan	Toronto	London	Vancouver
20-29	3/7	7/14	3/4	0/1	0/5	2/9
30-39	5/12 (41.7)	9/24 (37.5)	4/10 (40)	2/8	3/13 (23.1)	5/21 (23.8)
40-49	6/12 (50)	5/8 (62.5)	9/18 (50)	8/29 (27.6)	2/8	4/8
50-59	10/13 (76.9)	4/7	5/14 (35.7)	11/39 (28.2)	5/22 (22.7)	5/12 (41.7)
≥60	14/16 (87.5)	1/2	10/16 (62.5)	9/27 (33.3)	6/25 (24.0)	4/22 (18.2)
All ages	38/60 (63.3)	26/55 (47.3)	31/62 (50)	30/104 (28.8)	16/75 (21.3)	20/72 (27.8)

increased risk of cervical cancer and this study measured these factors indirectly as number of marriages and age of first marriage or pregnancy. The distribution of these factors in our case and control groups is shown in Table III. Mean ages of case and control groups from each area were very similar (data not shown). The percentage of control women married 2 or more times ranged from 39.1 for black women in Houston, TX, to 14.0 for women in London, UK, and the rank order roughly paralleled that for the cancer rates. A greater proportion of cases than controls had married more than once in all populations. The rank order of the percentages of women who were first pregnant or married prior to 20 years of age also paralleled that for the cancer rates and, except for women in London, the proportion of cases with this attribute exceeded that of controls.

A third factor associated with cervical cancer is the presence of HSV-2 antibodies. The distribution of HSV-2 seropositivity according to age and area of residence for control women is shown in Table IV. The highest occurrences of antibodies were found among residents of Houston and Herrera Province. An

increase in prevalence of HSV-2 antibodies was associated with increased age in Houston, Herrera Province and San Juan. Among women in Vancouver, the reverse was observed, with the highest rate of seropositivity in women aged 20-29. The age-specific occurrences of HSV-2 antibodies among the women with cervical cancer are shown in Table V. The highest percentages of cases with HSV-2 antibodies was found in those living in Houston while the lowest percentage resided in London.

It is apparent from the data presented in Tables III to V that the risk factors are interrelated. The relation between the proportion of women married 2 or more times and women first married or pregnant before 20 years of age is depicted in Figure 1. These risk factors correlated poorly among control women from populations with the lower cancer rates, while among women from the populations with higher cancer rates there was an increasing percentage with multiple marriages as the percentage with early age at first marriage or pregnancy increased. Among cancer cases, early age at first coitus correlated with multiple marriages in all populations.

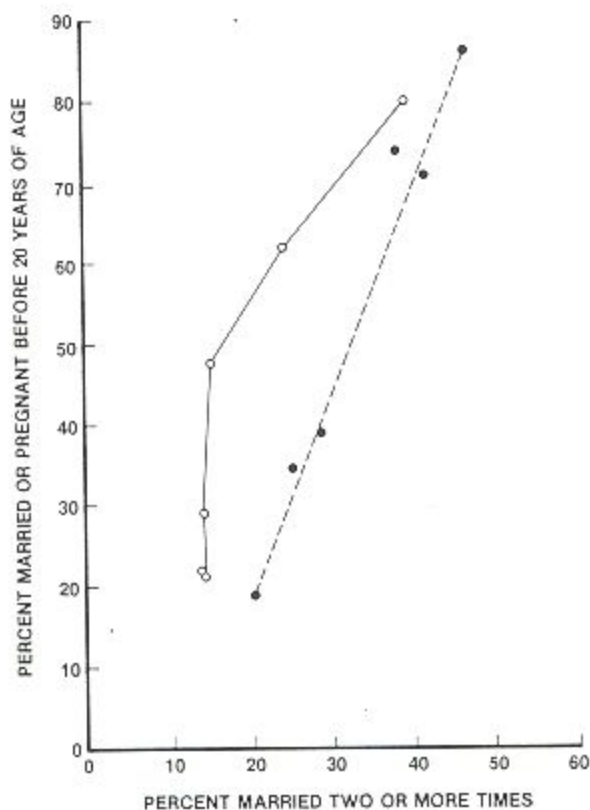


FIGURE 1 - Scatter plot of the percentage of women married or pregnant before 20 years of age and the percentage of women married 2 or more times. ●, cases; ○, controls.

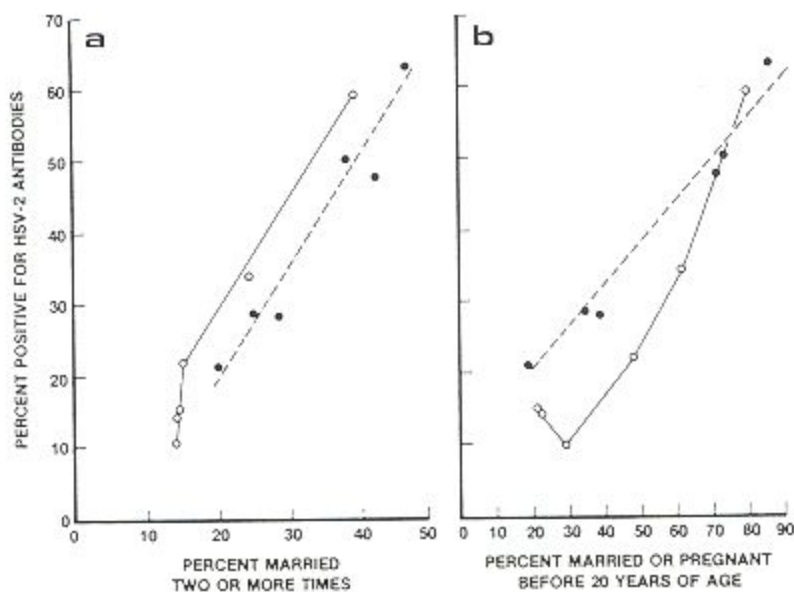


FIGURE 2 - Scatter plot of the percentage of women with HSV-2 antibodies and (a) percentage of women married 2 or more times or (b) percentage married or pregnant before 20 years of age. ●, cases; ○, controls.

Similar relationships were found when HSV-2 antibody occurrence was compared with the 2 markers of sexual behavior (Fig. 2). Both the percentage of women married 2 or more times (Fig. 2a) and the percentage

married or pregnant before 20 years of age (Fig. 2b) correlated well with the percentage having HSV-2 antibodies among cases from all populations. However, among control women these correlations were less

TABLE VI - ODDS RATIO ESTIMATES FROM LOGISTIC REGRESSION ANALYSIS OF CERVICAL CANCER CASES AND CONTROLS BY STUDY AREA¹

Area	Numbers analyzed		Risk factor		
	Number of cases	Number of controls	Number of marriages < 2 vs. > 2	HSV2 antibodies neg. vs. positive	Age 1st marriage/pregnancy > 20 yrs vs. < 20 yrs
Houston	53	110	1.30	1.18	1.18
Herrera Province	55	251	1.41 ²	1.22	1.13
San Juan	60	126	1.57 ²	1.61 ²	1.53 ²
Toronto	104	219	1.22	1.46 ²	1.29
London	67	88	1.39	1.47	0.70
Vancouver	64	123	1.38	1.45	1.42 ²
All	403	917	1.33 ³	1.38 ³	1.21 ³

¹ "All areas" model included terms for age and area while risk factor variables were added stepwise. Area-specific models included terms for age and all three risk factors. ² $p < 0.05$. ³ $p < 0.01$.

TABLE VII - AGE-SPECIFIC OCCURRENCE OF HSV-2 ANTIBODIES IN PANAMANIAN WOMEN ACCORDING TO PROVINCE OF RESIDENCE

Age (years)	Chiriqui		Cocle		Colon		Herrera		L. Santos		Panama		Veraguas	
	Number tested	% +	Number tested	% +	Number tested	% +	Number tested	% +	Number tested	% +	Number tested	% +	Number tested	% +
10-19	228	7.5	75	13.3	65	13.8	78	10.2	81	12.3	92	14.1	89	10.1
20-29	160	21.8	53	15.1	50	32.0	37	27.0	38	18.4	82	10.9	41	17.1
30-39	100	23.0	50	24.0	25	52.0	43	39.5	43	32.6	62	29.0	48	16.7
40-49	60	36.7	28	21.4	27	55.5	27	33.3	21	33.3	36	25.0	39	33.3
50-59	50	34.0	27	40.7	25	32.2	13	46.1	16	50.0	35	31.4	20	45.0
≥ 60	83	28.9	19	68.4	24	66.7	24	58.3	18	55.5	27	37.0	27	25.9
Age-adjusted ¹ % positive		30.6		35.1		52.0		42.5		37.9		29.8		29.7
Age-adjusted rates ²		30.9		28.8		21.7		79.1		33.7		24.6		24.5

¹See footnote to Table IV. ²Cervical cancer incidence per 100,000 population.

apparent in populations with low cancer rates. The incidence of cervical cancer in the different populations correlated with all of the risk factors; however, stepwise linear regression analysis suggested that the cancer rates were best predicted by the occurrence of HSV-2 antibodies among control women.

Logistic regression analysis of risk factors for HSV-2 seropositivity in control women indicated that only 2 or more marriages significantly increased the risk of having HSV-2 antibodies with an odds ratio of 1.42 ($p < 0.01$). The variable for age at first marriage or pregnancy never entered the model.

Logistic regression analysis of case-control differences

The results of the logistic regression analysis of case-control differences with no interaction terms in the model are shown in Table VI. These analyses were based on 1,320 of 1,375 subjects (96%) for whom complete data were available. For all areas together, all 3 risk variables entered the model, with coefficients corresponding to odds ratios that exceeded and differed significantly from 1 at the 0.01 level. These odds ratios were very similar for the 3 factors. In the model which included interactions, 2 two-way interactions were statistically significant at the 0.05 level. These included area by age at first marriage or pregnancy and age by number of marriages. The former was due almost entirely to London, since here more controls than cases had early age at first marriage or pregnancy (see Table III).

In other areas and for the other risk factors in London, odds ratio estimates were remarkably consistent from area to area (Table VI).

HSV-2 antibodies and cervical cancer rates

The incidence of cervical cancer was found to correlate with the occurrence of HSV-2 antibodies (see Tables II, IV, and V). However, an apparent disparity existed for women living in Herrera Province where the cervical cancer incidence was 79.1 per 100,000 while the antibody occurrence was only 41.4%. This disparity was investigated by using a random sample of sera from women living in the Republic of Panama. The age-specific occurrence of HSV-2 antibodies from the random sample along with cervical cancer incidence rates by Province are shown in Table VII. The age-specific occurrences of HSV-2 antibodies in the sample of randomly selected women residing in Herrera Province were similar to those obtained for neighborhood controls of the case-control study (see Table IV). Thus, the disparity between rate of cervical cancer and HSV-2 antibody occurrence in the study control from Herrera Province cannot be attributed to a spurious sampling error.

The relationship between the age-adjusted incidence of cervical cancer and the age-adjusted occurrence of HSV-2 antibodies for both the Provinces of the Republic of Panama and other areas sampled is shown in Figure 3. There was a highly significant correlation ($r = 0.786$) between cancer rates and the occurrence of HSV-2 antibodies.

Two data points shown in Figure 3 deviate substantially from the regression line best describing the correlation between antibody occurrence and cancer incidence. These include samples from Herrera Province and from Colon Province. As noted above, two sepa-

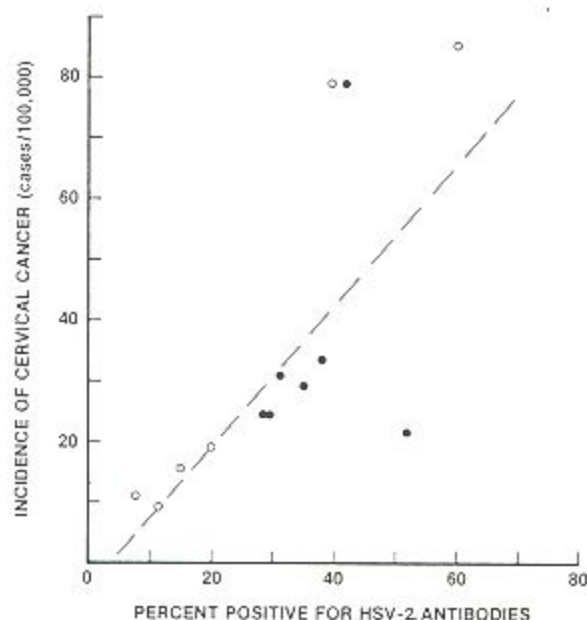


FIGURE 3 - Scatter plot of the incidence of cervical cancer and the percentage of control women with antibodies to HSV-2. ●, data from random samples; ○, data from case-control samples. Broken line represents regression line calculated from all points except 2 representing Herrera Province.

rate samples from Herrera Province revealed a lower occurrence of HSV-2 antibodies than expected on the basis of cervical cancer rate. In contrast, the occurrence of HSV-2 antibodies among women living in Colon Province was higher (52%) than expected from the cancer rate observed (21.7 per 100,000). Considerable variation in cervical cancer rates was found between districts of Colon Province (data not shown). Thus, attempts were made to confirm this disparity by comparing cancer rates with the occurrence of HSV-2 antibody using the data from Districts of the Republic of Panama from which 25 or more women were sampled. One such district was in Colon Province and one was in Herrera Province. The disparity between the cervical cancer rate and the occurrence of HSV-2 antibodies was again observed for women living in the Herrera Province District (data not shown). However, for women residing in the district in Colon Province the cervical cancer rate (51.4/100,000) was similar to that predicted by the finding of HSV-2 antibodies in 56.7% of women. Thus, for all samples except those from women living in Herrera Province, the occurrence of HSV-2 antibodies correlated well with the incidence of cervical cancer.

DISCUSSION

Women with cervical cancer have been found to differ from other women with respect to a number of social and sexual attributes. Women with the disease have been over-represented in lower socio-economic classes (Devesa and Diamond, 1980) and in some minority ethnic groups (Graham and Schotz, 1979; Jordan and Key, 1981). Cancer patients have reported more sexual partners, earlier ages at first coitus, marriage and pregnancy, and a higher prevalence of mari-

tal breakdown and venereal infections, especially infections with HSV-2, than control women (reviewed by Kessler, 1981; Hulka, 1982). More recently, cigarette smoking (Trevathan *et al.*, 1983; Williams and Horm, 1977; Winkelstein *et al.*, 1984; Stellman *et al.*, 1980; Marshall *et al.*, 1983; Vonka *et al.*, 1984a), the use of oral contraceptives (Stern *et al.*, 1970; Meisels *et al.*, 1977), and low dietary intake of beta-carotene (Romney *et al.*, 1981; Marshall *et al.*, 1983; LaVecchia *et al.*, 1984; Orr *et al.*, 1985) have also been associated with cervical neoplasia. The strongest associations have been with early age at first coitus, number of sexual partners and infections with HSV-2 (Kessler, 1981) while the weaker associations have not always been reproducibly found or have been thought to be secondary to the key factors. While the prominence of these 3 attributes among cancer cases strongly supports the hypothesis that cervical cancer is a late consequence of venereally transmitted carcinogens, the roles played by the individual risk factors in the genesis of the lesions are not well understood.

An approach to studying the relations between risk factors and cancer is to examine their interactions in populations with different cancer rates. Models of carcinogenesis predict a linear relation between cancer rates and the amount of exposure to a carcinogen (Crump *et al.*, 1976). For cancers thought to be induced by viruses, a relation should exist between the proportions of populations infected with the virus and the incidence of the cancer (Rawls, 1983). In the present study, we attempted to examine the relationships between the key sexual risk factors for cervical cancer, including infection with HSV-2, by comparing their occurrence in samples from populations with different cancer rates. We found a correlation between the proportions of women with early age at first marriage or pregnancy and the proportions married more than once (Fig. 1). A good correlation was evident for cases in all populations; however, this correlation was less apparent for control women in populations with lower cancer rates. We also observed a correlation between the proportions of women with early age at first marriage or pregnancy and the occurrence of HSV-2 antibodies (Fig. 2). Again, the correlation was most pronounced among cases in all populations and among control women in populations with high cancer rates. Not surprisingly, a similar pattern was observed when the proportions of women with multiple marriages were compared to those of women with HSV-2 antibodies. These findings suggest that, although the relations between sexual risk factors of cervical cancer are not uniform in all populations, they are similar in women who develop cervical cancer in all populations.

The role of the key risk factors in the genesis of cervical cancer is not clear. In the model incriminating a sexually transmitted agent, multiple sexual partners would increase the probability of being exposed to the agent, while early age at first coitus could represent an increased susceptibility of pubertal cervical epithelium to oncogenic insult (Coppleson *et al.*, 1967) or a covariable of sexual behavior associated with multiple sexual partners (Rawls *et al.*, 1976; Vonka *et al.*, 1984a). Infection of the cervical epithelium by HSV-2 could initiate or promote carcinogenesis, or be a covariable of sexual behavior accompanying the other 2 risk factors. A number of early studies suggested that

early age at first coitus may represent an independent risk factor for cervical cancer (reviewed by Rotkin, 1973), but several more recent studies have failed to demonstrate an excess of women with early age at first coitus among cervical cancer cases after controlling for numbers of sexual partners (Graham and Schotz, 1979; Harris *et al.*, 1980; Reeves *et al.*, 1985). Similarly, an excess of HSV-2 antibodies among cases, after controlling for numbers of sexual partners, has been reported in most but not all studies (Adam *et al.*, 1974; Graham *et al.*, 1982; Reeves *et al.*, 1985). We used logistic regression analysis to assess the interdependence of these 3 risk factors in the samples examined. Although there was a degree of interdependence, multiple marriages, early age at first marriage or pregnancy and HSV-2 seropositivity all represented significant risk factors after the other two factors had been entered into the model (Table VI). If two of the risk factors were covariable to the third, a dependence should have become apparent and adding the third factor into the model should have abolished the effects of the other two. Thus, our finding of a degree of independence of the key risk factors suggests that all 3 factors are covariable to yet another factor. In the case of HSV-2, this conclusion is in keeping with the results of prospective studies which have failed to demonstrate a significant risk of intraepithelial and microinvasive neoplasia associated with HSV-2 antibodies (Vonka *et al.*, 1984b; Adam *et al.*, 1985).

While our data suggest that HSV-2 is not a cause of most cases of cervical cancer, they do not exclude the virus being etiologically related to some of the cases. It could act in concert with other venereally transmitted agents such as human papillomavirus (HPV). DNA sequences of HPV 16 or 18 have been found in a majority of cervical cancer biopsies examined (Durst *et al.*, 1983; Boshart *et al.*, 1984; Prakash *et al.*, 1985; Scholl *et al.*, 1985; Schneider *et al.*, 1985) and it has been postulated that HSV-2 and HPV act synergistically (zur Hausen, 1982).

The sexual behavior of husbands of women who develop cervical neoplasia has been found to differ from that of husbands of control women (Buckley *et al.*, 1981). HSV-2 is venereally transmitted and the probability of a woman being infected with the virus reflects not only her sexual behavior but also that of her male partners. Interestingly, we found an excellent correlation between the occurrence of HSV-2 antibodies and cervical cancer rates. In fact, HSV-2 antibodies appeared to be a better predictor of cancer rates than the proportion of women with multiple sexual partners. This implies that the major risk factor in cervical cancer is exposure to venereally transmitted agents.

There are several limitations in evaluating and interpreting the results of our study. One was the use of age at first marriage or first pregnancy as an indirect measure of age at first coitus, and use of marriage number as an indirect means of counting sex partners. This could have introduced bias since the relation between age at first marriage and age at first coitus may vary in different cultural settings. However, responses to inquiry regarding age at first coitus or number of lifetime sex partners are not verifiable. The use of age at first coitus and numbers of sex partners in comparing cases and controls from the same cultural setting is

generally considered acceptable since no apparent reporting bias would be expected in relation to the development of the cancer. However, in comparing women from different cultural settings, responses to inquiry on verifiable and morally non-evaluative attributes would appear more reliable than responses to inquiry about non-verifiable attributes. Thus, we feel the use of age at first marriage or pregnancy and number of marriages to be valid.

Ideally, the relations between risk factors of cervical cancer and cancer rates should have been examined using randomly selected women from different populations. However, we chose to select women from the same health care facilities or from neighborhoods in which cancer cases were identified. The representativeness of these samples with respect to cervical cancer risk factors is not known. Data on the occurrence of HSV-2 antibodies in random samples representing two of the study populations are available. HSV-2 antibodies were found in 41.4% and 42.2% of neighbors of cancer cases and randomly selected women residing in Herrera Province, respectively (See Tables IV and VII). We found HSV-2 antibodies in 15.1% of neighbors of cancer cases in Toronto, which was similar to the 17.5% positive for antibodies among a stratified random sample of 35- to 50-year-old women living in Toronto (Stavraky *et al.*, 1983). These observations suggest that the samples were sufficiently representative for meaningful comparisons.

In addition, our incidence rates are subject to some inaccuracies. Firstly, the geographic areas of the case-control study do not coincide exactly with incidence rate areas except in Herrera Province (see Table I). In general, incidence rates for a larger geographic area than that under study had to be used. Therefore, the rates which we have used only approximate the incidence in the study population. However, it is unlikely that major differences exist between rates for areas used and rates for study areas. Secondly, some cases of carcinoma *in situ* are often included as invasive cancers in the registries and the magnitude of overestimation of invasive cervical cancer incidence is not known for all registries which supplied incidence data. The degree of overestimation due to inclusion of *in situ* cases has been determined for British Columbia (Husted *et al.*, 1983) and Ontario (Clarke and Hilditch, 1983). Ninety-eight percent of the cases from Herrera Province were verified histologically, and independent review of a 10% random sample of slides confirmed the diagnosis of invasive cancer in 90% of the sample, the remaining 10% being carcinoma *in situ* (data not shown), a degree of misclassification similar to that reported by the Canadian investigators. Although completeness of registration and accuracy of the denominators undoubtedly varies from registry to registry we feel the rates are sufficiently accurate for meaningful comparison.

Finally, a special problem exists for the cervical cancer rates used for black women residing in Houston which is not covered by a population-based registry. The incidence estimates for those women came from a special survey for which the most recent time period was 1962-1966 (MacDonald and Heinze, 1978). In order to assess the accuracy of these rates, other data sources have been used to provide independent esti-

mates of the incidence of cervical cancer in black women residing in Houston. In 1960-1969, the mortality rate from cervical cancer among Harris County (Houston) blacks was 16.8 per 100,000 (Riggin and Mason, 1983). The ratio of cervical cancer incidence to mortality rates for this cancer was 2.4 among black populations included in the Surveillance, Epidemiology and End Results (SEER) program of incidence reporting in the United States in 1973-77 (Young *et al.*, 1981). Thus, the incidence of cervical cancer in Harris County blacks in 1960-69 would be expected to be about 40.3 (16.8×2.4) per 100,000. This estimate is less than half the incidence rate for 1962-66 available to us from the survey. If indeed the incidence rate for Houston blacks were closer to 40, this area would fit more closely to the risk factor pattern observed in other areas with the notable exception of Herrera Province.

The exception to the correlation between cervical cancer rates and the occurrence of HSV-2 antibodies observed for women living in Herrera Province is of special interest. Two separate samples from women living in this Province yielded similar proportions with HSV-2 antibodies (see Tables IV and VII) and two separate surveys confirmed high cervical cancer rates (Reeves *et al.*, 1982, 1984). The cancer rate in the Province was twice that expected on the basis of HSV-

2 seropositivity. Nevertheless, the occurrence of HSV-2 antibodies among these women corresponded to that expected from the percentage of women married 2 or more times (Fig. 2a), supporting the findings of a case-control study in which no unusual attributes of sexual behavior could be found to account for the high cancer rates (Reeves *et al.*, 1985). Thus, cervical cancer rates among women living in Herrera Province appear disproportionately high in relation to exposure to sexually transmitted agents. Clearly, investigation of this unusual population for unique risk factors is indicated.

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