

An Analysis of the Association Between Herpes Simplex Virus Type 2 Antibodies and Cervical Cancer

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OVERVIEW

A greater occurrence of antibodies to HSV 2 among cervical cancer cases than among control women has been found repeatedly. However, considerable variation in the proportions of cases and controls with HSV 2 antibodies has been found in different populations. Marked variation in the incidence of cervical cancer has also been observed. In the study described in this paper, the interrelatedness of cervical cancer risk factors, including HSV 2 antibodies, was examined in samples obtained from women living in populations with different cervical cancer rates. Cases differed significantly from controls, primarily in number of marriages, although differences in the occurrence of HSV 2 antibodies were also found. Cervical cancer rates were best predicted by the occurrence of HSV 2 antibodies among control women. The data are compatible with infection by HSV 2 being a covariable of venereal factors although a role of the virus in the genesis of a portion of cervical cancers cannot be excluded.

INTRODUCTION

Epidemiological studies of women with cervical cancer and control women have identified early age at first coitus and multiple sex partners as risk factors in the development of this cancer (Rotkin 1973; Hulka 1982). These and other observations support the concept that cervical cancer is caused by a venereal disease (Kessler 1981). Herpes simplex virus type 2 (HSV 2) is transmitted venereally and seroepidemiological investigations have repeatedly demonstrated an excess of antibodies to the virus among cases of cervical cancer when compared to control

women (Rawls et al. 1980). The association between cervical cancer and HSV 2 could represent one in which the virus is causally related to the development of the malignancy, or infection by the virus could be a covariable of sexual behavior associated with exposure to some other venereally transmitted factor that is carcinogenic. The studies described were undertaken to attempt to distinguish between these possibilities.

The incidence of cervical cancer varies considerably between populations as well as over time within the same population (Waterhouse et al. 1976). This observation suggests that differences in sexual behavior exist between populations and by comparing the occurrence of risk factors between populations with different cancer rates, the impact of the risk factors on disease rates can be appreciated. Thus, we conducted a two-part study in which attributes of sexual behavior were correlated with cervical cancer incidence. The first part consisted of obtaining sera and information regarding age at first marriage, age at first pregnancy, and number of marriages from cervical cancer cases and control women. Samples were obtained from six different populations for which cancer rates were monitored by cancer registries. The sera were analyzed for HSV 2 antibodies under codes using a micro-neutralization assay. The interrelatedness of cancer and the risk factors were determined from the data obtained from these samples. The second part of the study consisted of determining the occurrence of HSV 2 antibodies in an 0.8% random sample of females over 9 years of age and living in the Republic of Panama. The occurrence of antibodies was correlated with the incidence of cervical cancer by Province as determined by Reeves and coworkers (1984). Details of the sampling methods and serological analysis are described elsewhere (W. Rawls et al., in prep.).

RESULTS

Comparison of Different Populations

Two factors repeatedly shown to represent risk factors for cervical cancer include multiple sex partners and early age at first coitus. To reduce possible cultural bias in ascertaining sex partner numbers outside the marriage bond, the number of marriages was used as an indirect measure. Age at first pregnancy and age at first marriage were used as indirect measures of age at first coitus for the same reason. Initially, the data regarding these attributes were dichotomized and the proportions positive for the risk factors were compared with HSV 2 antibody occurrence and cervical cancer rates. The conclusions drawn from this analysis were confirmed using logistic regression analysis.

The distribution of cervical cancer rates as well as the risk factors among case and control women from six different populations is shown in Table 1. The age-adjusted cervical cancer rates varied from 85.1 per 100,000 for black women in Houston, Texas to 9.3 per 100,000 for women living in British Columbia, Canada.

Table 1
Distribution of Risk Factors in Different Populations

Population	Cancer rate ^b	Percent with risk factor											
		Married two or more times		First married before 20 yrs of age		First pregnant before 20 yrs of age		HSV 2 antibody positive ^c					
		Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases				
Houston, Texas ^a	85.1	39.4	45.6	68.5	71.4	73.2	82.7	60	63.3				
Herrera Province Panama	79.1	24.5	41.8	62.1	70.0	50.4	59.3	39.9	46.9				
San Juan, Puerto Rico	19.4	16.1	37.9	47.8	72.4	39.5	58.6	20.2	50				
Toronto, Ontario, Canada	13.4	15.4	25.2	21.7	32.7	12.2	21.2	15.1	30.4				
London, England	11.3	14.9	19.4	32.5	19.1	24	7.3	7.4	22.2				
Vancouver, B.C., Canada	9.3	14.8	28.4	20.5	39.7	16.8	33.3	11.5	27.8				

^aBlack women

^bAge adjusted to world population; cases per 100,000

^cRates age-adjusted to world population for control samples

The proportion of control women who had married more than once varied from 39.4% for black women living in Houston, Texas to 14.8% for women living in British Columbia and the rank order paralleled the cancer rates. In all populations, a greater percent of cancer cases than control women had married two or more times. The percent of control women who married and/or became pregnant before 20 years of age also varied considerably between populations and, except for the sample from London, England, a greater percent of cases than controls had these attributes (Table 1). The age-adjusted occurrence of HSV 2 antibodies among control women ranged from 60% for black women living in Houston, Texas to 7.4% for women living in London, England. As with the other attributes, greater proportions of cases had HSV 2 antibodies than controls and this was observed in all populations.

It is apparent from the data shown in Table 1 that the risk factors correlate with each other and with the cancer rates. This interrelatedness was examined for all women as well as separately for cases and controls. The relation between HSV 2

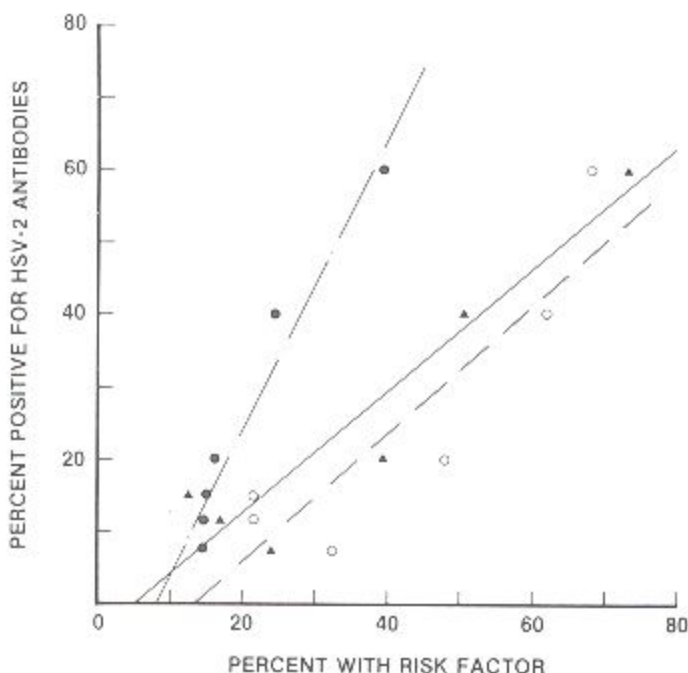


Figure 1

Scatter plot of the occurrence of HSV 2 antibodies and other cervical cancer risk factors. (●) Percent of women married two or more times; (○) the percent of women married before 20 years of age; (▲) the percent of women pregnant before 20 years of age. Regression lines for each factor are shown.

antibody occurrence and the other risk factors among control women is shown in Figure 1. The occurrence of HSV 2 antibodies correlated best with multiple marriages, and relatively small changes in the percent of a population with two or more marriages was reflected in a substantial increase in the percent with antibody. Correlations were also evident between HSV 2 antibody occurrence and the other factors examined.

The relation between the attributes of early coitus and multiple marriages among control women and cervical cancer rates is shown in Figure 2. The cervical cancer incidence in the different populations correlated significantly with the percent of women who married and/or were pregnant before 20 years of age and the percent of women who had multiple marriages. Of the attributes shown in Figure 2, the strongest correlation was found between cervical cancer rates and the percent of women with multiple marriages. A highly significant correlation was also found between cervical cancer incidence and the occurrence of HSV 2 antibodies (Fig. 3).

Analysis using the data shown in Table 1 revealed that for control women the occurrence of HSV 2 antibodies was best correlated with the percent of women

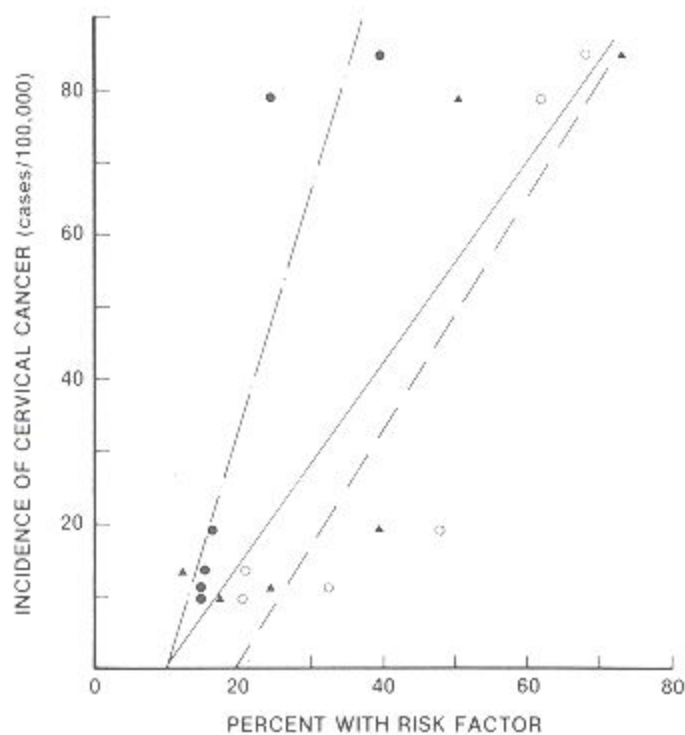


Figure 2

Scatter plot of cervical cancer rates and risk factors. See legend to Figure 1.

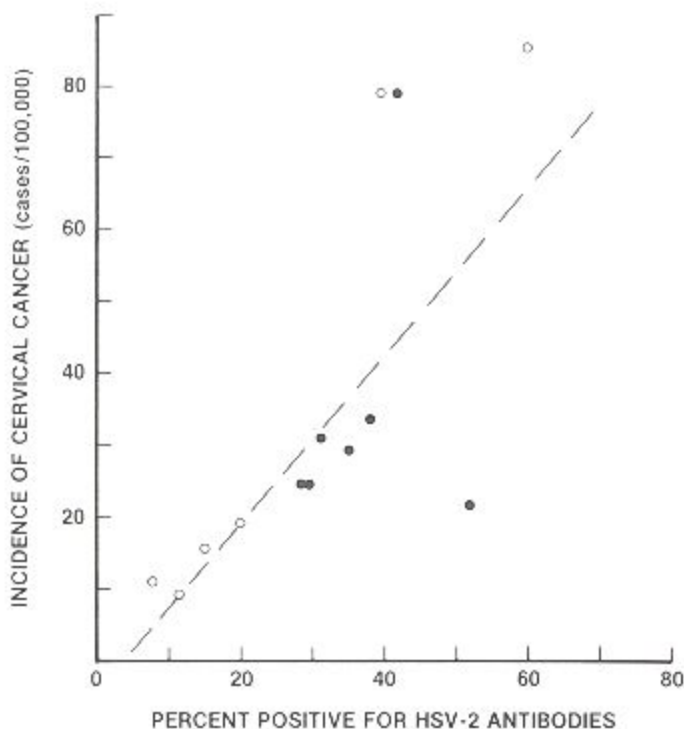


Figure 3

Scatter plot of cervical cancer rates and occurrence of HSV 2 antibodies. (○) Data obtained from case-control samples; (●) data from the random sample of Panamanian women.

with multiple marriages. The factor which best correlated with cervical cancer rates was the occurrence of HSV 2 antibodies among control women. When only attributes of women with cervical cancer were analyzed, cervical cancer rates best correlated with the percent with multiple marriages.

To augment the conclusions suggested by the analysis of proportions, the data on individuals were examined by logistic regression analysis. The results are summarized in Table 2. The samples were not homogeneous with respect to the relative distribution of the risk factors among cases and controls. Logistic regression analysis of each population revealed no significant differences in risk factors between cases and controls among women from the populations with the highest and lowest occurrence of HSV 2 antibodies, i.e., Houston, Texas and London, England, respectively. Cases differed from controls in number of marriages among women from Herrera Province. Differences in number of marriages and HSV 2 antibody were found between cases and controls from San Juan, Puerto Rico and from Vancouver, British Columbia. For the Toronto sample, age at marriage

Table 2
Summary of Logistic Regression Analysis of Case-control Samples

Dependent variable	Analyzed		Results contributing variables	X ²	P values
	Samples	Size			
Case control status	Total	1159	Number marriages	14,2	< 0,001
			HSV 2 antibody	4,8	0,029
HSV 2 antibody status	Controls	804	Number marriages	13,9	< 0,001
			Age	6,0	0,014
	Cases	473	Number marriages	5,8	0,016
			Age	4,7	0,030

differed significantly between cases and controls. Analysis of the total sample revealed differences between cases and controls in number of marriages and HSV 2 antibody occurrence (Table 2). Thus, by both analytic methods, number of marriages appeared to be the key risk factor distinguishing cases from controls. The presence of HSV 2 antibodies associated significantly with number of marriages and age.

Relation Between Cancer Rates and HSV 2 Antibodies

The data from the case-control samples indicated a correlation between the occurrence of HSV 2 antibodies and cervical cancer rates. However, as evident from the data presented in Table 1, the occurrence of HSV 2 antibodies (39.9%) among control women living in Herrera Province was much lower than expected since the cancer incidence in this province was high (79.1 per 100,000). To substantiate this observation, a random sample of sera from women living in the Republic of Panama, including Herrera Province, were analyzed for HSV 2 antibodies. The age-adjusted occurrence of antibodies in sera of the random sample from Herrera Province was 42.5%, confirming the validity of the measurement in our control sample.

The relation between HSV 2 antibody occurrence and cervical cancer rates by province in the Republic of Panama, along with the data from samples of control women in the different populations, is shown in Figure 3. There was a highly significant correlation ($r = 0.786$) between cancer incidence and the percent of women with HSV 2 antibodies.

Data points from two populations shown in Figure 3 deviate substantially from the linear regression best describing the association between antibody occurrence and cancer incidence. As indicated above, two samples from Herrera Province revealed a lower occurrence of HSV 2 antibodies than expected for the cervical cancer rates. In contrast, the occurrence of HSV 2 antibodies in sera from women living in Colon Province was higher (52%) than expected for the incidence of cervical cancer observed (21.7 per 100,000). Considerable variation in cancer rates

was found between districts of Colon Province (W.C. Reeves, unpubl.); thus this disparity was examined in greater detail by comparing cancer rates with the occurrence of HSV 2 antibody for eight districts of the country from which 25 or more women were sampled. Of the eight districts, one was in Herrera Province and one was in Colon Province. The disparity between cervical cancer rates and HSV 2 antibody occurrence was again observed for women living in the district of Herrera Province. However, for women residing in the district of Colon Province the cancer rate did not differ significantly from that predicted by the percent of women with HSV 2 antibodies. 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

The detection in cancer cells of viral DNA sequences coding for proteins with putative transforming functions, as well as quantifying the risk of cancer associated with infection by a virus, are requirements needed to evaluate the role of a virus in human cancer. Evidence suggesting the presence of HSV 2 protein (Aurelian et al. 1981; McDougall et al. 1982), mRNA (Eglin et al. 1981; McDougall et al. 1982), and DNA (Park et al. 1983; Prakash et al. 1985) in cervical cancer cells has been presented. However, these viral markers have been detected in only a portion of the cancer cases and the same regions of the viral genome have not been consistently found. These data lead to the hypothesis that transformation by HSV 2 may involve a "hit and run" mechanism (Galloway and McDougall 1983). Since the evidence currently available neither proves nor rejects this hypothesis, the involvement of HSV 2 in transformation of cervical epithelial cells is uncertain.

Risk estimates of cervical cancer among women infected with HSV 2 are also insecure. Marked variation was noted in relative risks estimated from seroepidemiological case-control studies (Melnick et al. 1974). More recently, prospective studies have failed to demonstrate a significant risk of intraepithelial and microinvasive neoplasia associated with HSV 2 antibodies (Vonka et al. 1984; Adam et al. 1985). A possible explanation for such discrepancies in risk estimates is that HSV 2 and other risk factors of cervical cancer vary in different culture settings. However, the results of our comparative study suggest that the key risk factors of cervical cancer interrelate with HSV 2 antibody occurrence in a fairly uniform way among women living in populations with different cervical cancer rates. We found a greater occurrence of HSV 2 antibodies among cases than among control women in the populations studied, but the importance of HSV 2 antibodies relative to the other risk factors in differentiating cases from controls varied between populations. A limited number of studies revealed an excess of HSV 2 antibodies among cases after controlling for sex partner number or age at first coitus (Adam et al. 1974; Graham

et al. 1982). Logistic regression analysis of cases and controls in our comparative studies revealed that number of marriages and occurrence of HSV 2 antibodies differentiated cases from controls with number of marriages being the major risk factor. These observations suggest that infection with HSV 2 is a covariable of venereal factors rather than being a major etiological agent in cervical cancer.

We found that the number of marriages best predicted the occurrence of HSV 2 antibodies, a finding in agreement with the known venereal mode of transmission of the virus. Interestingly, an excellent correlation was observed between cervical cancer rates and the occurrence of antibodies to HSV 2. This correlation implies that the major factor in cervical cancer risk is exposure to venereally transmitted agents. Other putative risk factors of cervical cancer, such as cigarette smoking, diet, and cervical cytology, varied considerably between the populations studied; and yet the incidence rates varied little from those predicted by HSV 2 antibody occurrence. The exception was women living in Herrera Province where the cancer rate was almost twice that predicted by HSV 2 antibody. The occurrence of HSV 2 antibodies among these women corresponded to that expected from the percent married two or more times (see Figure 1), supporting the findings of a case-control study in which no unusual attributes of sexual behavior could be found to account for the cancer rates (Reeves et al. 1985). Thus, cancer rates among women living in Herrera Province were disproportionately high in relation to exposure to venereally transmitted agents, and investigations of this population for unique risk factors is clearly indicated.

It is apparent that HSV 2 is not a necessary cause of cervical cancer since evidence of viral genetic information has been found in only a portion of the cases. However, the virus could be etiologically related to a portion of the cases, which is a possibility that cannot be excluded from the seroepidemiological data available. Human papillomavirus (HPV) types 16 or 18 DNA have been found in 45-80% of cervical cancers examined (Dürst et al. 1983; Boshart et al. 1984; Prakash et al. 1985; Scholl et al. 1985); and it has been postulated that HPV and HSV 2 may function synergistically (zur Hausen 1982). In a preliminary study of the distribution of risk factors, HSV 2 antibody and HPV 16 DNA in cervical cancer patients, the HPV 16 DNA was found more frequently than HSV 2 antibodies (Prakash et al. 1985). HSV 2 antibodies and HSV 16 DNA were found, respectively, 1.7 and 1.1 times more often among cases with early age at first coitus when compared with later ages at first coitus. In addition, HPV 16 DNA was found at equal frequency among cancer cases with and without HSV 2 antibodies. These preliminary observations do not support a synergism between the two viruses but suggest HPV may be a more important risk factor of cervical cancer than HSV 2. Well-designed epidemiological studies defining the interrelations between HSV 2 and HPV 16 infection and other risk factors of cervical cancer would be helpful in determining the roles of these viruses on the genesis of the cancer.

CONCLUSIONS

In this comparative study of the risk factors of cervical cancer among populations experiencing different cervical cancer rates, cases differed from controls in number of marriages and the occurrence of antibodies to HSV 2, with the former being most prominent. Cervical cancer rates best correlated with the occurrence of HSV 2 antibodies among control women, implying that venereally transmitted factor(s) account for most of the risk of acquiring the disease. An exception was observed for women living in Herrera Province, Republic of Panama, where the cancer rates were twice that predicted by sexual risk factors, including HSV 2 antibody. These seroepidemiological findings suggest that HSV 2 does not play a role in the genesis of most cases of cervical cancer.

ACKNOWLEDGMENTS

The contributions of Dr. Aileen Clark, Dr. Ervin Adam, Dr. Jennifer Best, Dr. Edmundo Kraiselburd, and Dr. Louis J. Benedet in collecting the samples are greatly acknowledged. Invaluable technical assistance was provided by Carol Lavery. The study presented in this paper was funded by a grant from the National Cancer Institute of Canada. L.D.M. is a National Health Research Scholar.

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