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CASE-CONTROL STUDY OF CERVICAL CANCER IN HERRERA PROVINCE, REPUBLIC OF PANAMA

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A previous survey found the average annual age-adjusted incidence of cervical cancer in Herrera Province, Panama, to be 79/100,000, exceeding any other reported world rate. In an effort to clarify the reasons for this excessive occurrence, a case-control study was conducted among patients diagnosed between 1974-1980. Sixty-six percent of cervical cancer patients from Herrera Province were alive and were contacted by the study team; of these 91% were successfully interviewed and provided serum specimens. The total study encompassed 156/169 surviving patients and 309 age-neighborhood matched controls. Sexual promiscuity was uncommon, but it exerted a major effect, with those reporting 4 or more life-time sex partners being at a 4-fold excess risk compared to those reporting only one partner. First intercourse at a young age was common (21% began sexual activity prior to age 16) but it failed to alter risk once number of partners was taken into account. Oral contraceptive use was associated with a 2-fold excess risk and this was not substantially affected by controlling for sexual parameters. Thirty-three percent of the study subjects had anti-herpes-simplex type-2 antibody as measured by both neutralization and radioimmunoassays. Although results of the neutralization test were not predictive of risk, women with a radioimmunoassay indicative of HSV-2 infection were at a 40% excess risk for cervical cancer after adjustment for sexual characteristics.

Although the incidence of invasive cervical cancer has declined significantly in the United States, Canada and other industrialized countries, it remains a serious public health problem in most of Latin America. Cervical cancer is a leading cause of mortality throughout Latin America and in high-risk areas accounts for between 15 to 39% of all female cancer deaths (Pan American Health Organization, 1982). Cancer registries in Colombia, Jamaica, Panama, Brazil, Cuba, Chile and Peru have documented invasive cervical cancer rates considerably higher than those of other areas in the world; approximately one in every 1,000 women between ages 30-55 develops invasive cervical cancer each year (Rios Dalenz *et al.*, 1981; Persaud, 1976; Hensen, 1983; Reeves *et al.*, 1982). In addition, cancer registries in Los Angeles, CA and in New Mexico show that "Hispanic" women have higher cervical cancer rates than either Black or Indian women and have more than twice the risk of developing the disease than White women (Waterhouse *et al.*, 1982).

The reasons for such high cervical cancer incidence rates in Latin American women remain obscure. In other areas, multiple sexual partners, early age at first intercourse and infection with herpes-simplex-virus type 2 (HSV-2) have been identified as major cervical cancer risk factors (Kessler, 1976; Hulka, 1982; Rawls *et al.*, 1980). Recently, genital papilloma virus (HPV) infection has been implicated in the development of cervical cancer (zur Hausen, 1982; Reid *et al.*, 1982; Durst *et al.*, 1983; Prakash *et al.*, 1985). Finally, evidence has emerged regarding a possible etiologic role for cigarette smoking, oral contraceptive use (Stellman *et al.*, 1980; Swan and Petitti, 1982), "high-risk" male sex partners (Skegg *et al.*, 1982), low socio-economic status, poor personal hygiene and limited access to preventive measures such as Pap-smear screening (Clarke and Anderson, 1979).

In an effort to clarify the reasons for the high rates of cervical cancer in Latin America, we undertook a case-control study in Herrera Province, Republic of Panama. Herrera was chosen for study since a previous survey (Reeves *et al.*, 1984) showed an annual age-adjusted invasive cervical cancer incidence rate of 79/100,000 which is higher than any previously reported world rate.

METHODS

Case selection

This study included as cases all female residents of Herrera Province, Republic of Panama with a diagnosis of *in situ* or invasive cervical cancer made between 1974 and 1980. Cases were identified from the National Cervical Cancer Registry (Reeves *et al.*, 1984). The Registry had enumerated 255 Herrera residents with cervical cancer; 161 had invasive cancer and 94 *in situ* disease. Between April and June 1981 we attempted to locate and interview all patients at their place of residence. Of the cases, 169 (66%) were alive and available for interview. Of the 86 women unavailable for interview, 37 had died, 28 had no specific address other than Herrera Province, 11 had a street address but were unknown by neighbors and 10 had moved to unknown locales. We successfully contacted and interviewed 153/169 available women (91%); 3 previously unknown patients were identified during the course of field work and included as study subjects. The 16 available non-interviewed cases had moved to other parts of the country and we chose not to inter-

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view them. There was no difference in the distribution of diagnoses, age or other relevant factors among these 16 women compared with the women interviewed.

Controls

Control subjects were identified by asking cases to provide names of 4 women within 5 years of their own age who were residing in the same neighborhood at the time of diagnosis. Interviews were almost always obtained with the 2 closest neighbors; in the rare instances where these women were unavailable, interviews were obtained from the remaining potential subjects. Three eligible control subjects refused to participate and 10 women included as controls were subsequently found to have had a hysterectomy prior to the age at which their matched case developed cervical cancer. Thus, a total of 299 valid control interviews were obtained, nearly all occurring within one or two days of the time when the matched case was interviewed.

Field studies

Five female Panamanian interviewers conducted the case-control study. All 5 had collaborated in preparing the questionnaire and in preliminary field testing. Three interview teams worked simultaneously, using 4-wheel-drive vehicles or other forms of local transportation, to contact subjects at their residence. Patients were approached, the purpose of the study was explained, informed consent was obtained and they were asked to provide names of controls. Following this, a standardized 20-60 min interview was administered.

Following the interview, 10 ml of venous blood were obtained and held on wet ice until the end of the working day. Each evening, serum was separated, aliquoted into vials and frozen in liquid nitrogen. Serum was maintained frozen until it was tested for anti-HSV antibody. Control subjects were treated similarly to cases with respect to informed consent, interview and phlebotomy.

Laboratory methods

Neutralizing antibodies were assayed by a microneutralization test previously described by Rawls (1979). Strain KOS of HSV-1 and strain 333 of HSV-2 were used and non-neutralized virus was detected in monolayers of Vero cells. The titers of antibodies to HSV-1 and HSV-2 were estimated to $\log_{10}$ and a II/I index of 85 or more was taken as evidence of past HSV-2 infection (Rawls *et al.*, 1970). In addition, anti-HSV-2 antibodies were measured by a solid-phase radioimmunoassay (Graham *et al.*, 1982). Briefly, sera were initially adsorbed with HSV-1 antigen to remove cross-reacting antibodies, and then reacted with plastic-coated beads sensitized with HSV-1 and HSV-2 antigens. Antibodies bound to the beads were detected with ^{125}I-labelled goat anti-human IgG. Sera from which the amount of label bound to HSV-2-sensitized beads exceeded by 500 cpm or more the amount bound to HSV-1-sensitized beads were considered to possess HSV-2-specific antibodies.

Statistical methods

We used the relative risk (RR) as approximated by the odds ratio to measure associations and evaluate effects of exposure factors. Confounding variables were evaluated by stratified techniques, with maximum likelihood estimates of combined ratios and 95% confidence intervals (CI) derived (Gart, 1970). For multiple levels of exposure, significance was assessed using a one-tailed linear trend test (Mantel, 1963). Multivariate analyses using a disease probability logistic model were also employed to simultaneously control for a variety of potential confounding variables. Since matching was employed in the study design, analyses were also conducted using a logistic approach for matched data (Lubin, 1981). The results were similar to those derived from the unmatched stratified analyses, and thus unmatched estimates were chosen for presentation.

RESULTS

The distribution of selected demographic variables among cases and controls is shown in Table I. Most of the women were under 40 years of age and mestizo, over half lived in urban locales, and the majority of both cases and controls subsisted on monthly incomes of $200 or less. Cases were more likely than controls to be illiterate (19% *vs.* 11%), and conversely controls were more likely than cases to have a secondary or university level education (25% *vs.* 17%), but these differences were not statistically significant. In addition, more *in situ* than invasive cases reported monthly incomes greater than $200 (25% *vs.* 12%) and education beyond primary school (24% *vs.* 6%).

TABLE I - DISTRIBUTION OF SELECTED DEMOGRAPHIC VARIABLES AMONG CASES (INVASIVE + *IN SITU*) AND CONTROLS

	Cases (n = 156)		Controls (n = 299)	
	Number	%	Number	%
Age				
< 30	39	25.0	82	27.4
30–39	63	40.4	127	42.5
40–49	32	20.5	46	15.4
50–59	10	6.4	20	6.7
60+	10	6.4	18	6.0
Unknown	2	1.3	6	2.0
Race				
Mestizo	123	78.8	233	77.9
White	30	19.2	59	19.7
Black	2	1.3	6	2.0
Oriental	1	0.6	1	0.3
Monthly income				
Subsistence agriculture	21	13.5	48	16.0
< $70	48	30.8	96	32.1
$70–200	55	35.3	85	28.4
$201–500	28	18.0	51	17.1
$501–1,000	3	1.9	13	4.4
$1,000+	1	0.6	3	1.0
Unknown	0	—	3	1.0
Education				
Illiterate	30	19.2	32	10.7
No school, but literate	2	1.3	7	2.3
Primary school	98	62.8	189	63.2
Secondary school	25	16.0	63	21.1
University	1	0.6	8	2.7
Residence				
Rural	52	33.3	98	32.8
Semi-rural	25	16.0	50	16.7
Urban	79	50.6	151	50.5

Risk of both *in situ* and invasive cervical cancer was assessed in relation to several reproductive factors (Table II). Women with their first pregnancy prior to 18 years of age were at highest risk but there was no relation between risk and age at first pregnancy. Women with 0 or 1 pregnancy were at a low risk but

TABLE II - RELATIVE RISKS OF *IN SITU* AND INVASIVE CERVICAL CANCER BY REPRODUCTIVE FACTORS

	In situ (n = 82)	Invasive (n = 67)	Total[1] (n = 156)
Age at first pregnancy			
< 18	1.00 (31)[2]	1.00 (26)	1.00 (60)
18–19	0.93 (23)	0.76 (12)	0.85 (36)
20–21	0.24 (6)	0.90 (13)	0.49 (21)
22–23	0.58 (9)	0.83 (8)	0.67 (18)
24 +	0.88 (13)	0.83 (6)	0.81 (19)
Never pregnant	0.00 (0)	0.55 (1)	0.30 (1)
Number of pregnancies			
0–1	1.00 (3)	1.00 (1)	1.00 (4)
2–3	1.58 (25)	4.20 (21)	2.42 (46)
4–5	1.80 (26)	5.25 (18)	2.87 (47)
6 +	2.77 (28)	3.20 (27)	2.92 (59)
Number of abortions			
0	1.00 (48)	1.00 (48)	1.00 (98)
1	1.50 (17)	1.16 (13)	1.43 (34)
2 +	5.13 (16)	0.57 (6)	1.83 (23)

[1]Total includes 1 adenosquamous carcinoma and 6 with unknown extent of invasion. [2]Number of cases shown in parentheses.

multiparous women showed little difference in risk according to number of pregnancies. There was a 5-fold excess risk of *in situ* cancer associated with 2 or more abortions. Interviews did not differentiate between spontaneous and induced abortion.

We also ascertained menstrual history. Age at menarche was not associated with *in situ* or invasive cancer. Whether medical attention had ever been sought for menstrual problems was not predictive of risk, nor was the type of product used to control menstrual flow. Only 3% of study subjects reported ever having used vaginal tampons.

We also questioned study subjects concerning Pap-smear history. Sixty percent had never been screened, but this was not associated with an excess risk for invasive cervical cancer. In addition, there was no apparent relationship of risk with years since first Pap, frequency of Paps or reason for first Pap.

As expected, sexual behavior was highly associated with cervical cancer. Risk was significantly influenced by early age at first intercourse and multiple sexual partners (Table III). Twenty-one percent of women reported first intercourse before 16 years of age and this carried a 2-fold increased risk relative to those with first intercourse after 21 years. The excess risk associated with early age at first intercourse and the significant inverse linear relationship of this factor with risk were restricted to *in situ* cancer. Number of sexual partners, however, was predictive of both *in situ* and invasive cancer; women reporting 4 or more lifetime sex partners had a 3- to 6-fold excess risk compared to those with one partner.

TABLE III - RELATIVE RISKS OF *IN SITU* AND INVASIVE CERVICAL CANCER BY AGE AT FIRST INTERCOURSE AND NUMBER OF SEXUAL PARTNERS

	In situ (n = 82)	Invasive (n = 67)	Total (n = 156)
Age at first intercourse			
> 22	1.00 (9)[1]	1.00 (9)	1.00 (18)
20–21	0.58 (7)	1.24 (9)	0.87 (17)
18–19	1.37 (21)	1.12 (11)	1.27 (34)
16–17	1.33 (21)	1.46 (20)	1.48 (43)
< 16	2.92 (23)	1.31 (18)	2.08 (43)
Chi for trend	2.80	0.64	2.72
Number of sexual partners			
1	1.00 (40)	1.00 (35)	1.00 (77)
2	2.45 (18)	1.16 (9)	1.83 (30)
3	3.21 (15)	2.46 (11)	2.84 (28)
4 +	6.00 (8)	3.28 (12)	3.65 (20)
Chi for trend	4.14	2.87	4.78

[1]Number of cases shown in parentheses.

Since age at first intercourse was associated with number of sex partners, we cross-classified these 2 factors to determine the independence of effects (Table IV). Age at first intercourse did not affect cervical cancer risk after adjustment for number of sexual partners. However, the association with number of sexual partners persisted after adjustment for age at first intercourse: the relative risk reached 3.4 for women with 4 or more partners compared to those with only 1 partner.

The relationships between cervical cancer, age at first intercourse and number of sex partners differed between women with *in situ* and invasive disease (Table V). Both factors showed an apparent effect, although the relationship was strongest according to number of sexual partners (RR=5.0 for 4 or more partners after adjustment for age at first intercourse).

Cervical cancer risk was further evaluated in relation to contraceptive method. Overall, 75% of the women had used some form of contraception and relative risks were close to unity for those who had never used any contraception, those who had used intrauterine devices, barrier methods (diaphragm or condom), rhythm, and intravaginal preparations (commercial or home-made). However, women who had ever used oral contraceptives had nearly a 2-fold excess risk. These estimates remained elevated even after adjustment for number of sexual partners (RRs of 1.8, 1.8, and 1.9 for all cancers, *in situ* and invasive disease, respectively). Most women could not provide information concerning type of oral contraceptive or duration of use.

Cervical cancer risk was also related to previous herpes group viruses and sexually transmitted diseases

TABLE IV - RELATIVE RISKS OF CERVICAL CANCER (*IN SITU* AND INVASIVE) BY AGE AT FIRST INTERCOURSE AND NUMBER OF SEXUAL PARTNERS

Age at first intercourse	Number of sexual partners				All (adjusted)
	1	2	3	4 +	
> 20	1.00[1] (26)+	1.85 (6)	0.51 (1)	3.08 (2)	1.00 (35)
18–19	1.26 (23)	4.31 (7)	1.54 (3)	1.03 (1)	1.38 (34)
16–17	1.31 (20)	0.68 (4)	4.84 (11)	5.38 (7)	1.30 (42)
< 16	0.88 (8)	3.33 (13)	5.00 (13)	4.62 (9)	1.46 (43)
All (adjusted)	1.00 (77)	1.82 (30)	2.60 (28)	3.45 (19)	

[1]Referent group.
Chi for linear trend:
Age at first intercourse (adjusted for number of partners) = 1.36 (p = .09).
Number of partners (adjusted for age at first intercourse) = 3.93 (p < .001).

TABLE V – RELATIVE RISKS OF *IN SITU* CERVICAL CANCER BY AGE AT FIRST INTERCOURSE AND NUMBER OF SEXUAL PARTNERS

Age at first intercourse	Number of sexual partners				All (adjusted)
	1	2	3	4[2]	
> 20	1.00[1] (11)	2.67 (4)	— (0)	4.00 (1)	1.00 (16)
18–19	1.67 (15)	5.33 (4)	4.00 (1)	4.00 (1)	1.86 (21)
16–17	1.43 (10)	0.89 (2)	6.00 (6)	8.00 (2)	1.50 (20)
< 16	1.33 (4)	8.00 (8)	8.00 (8)	12.00 (3)	2.70 (23)
All (adjusted)	1.00 (40)	2.34 (18)	2.82 (15)	5.03 (7)	

[1]Referent group.–[2]Number of cases shown in parentheses.
Chi for linear trend:
Age at first intercourse (adjusted for number of partners) = 1.92 (p = .03).
Number of partners (adjusted for age at first intercourse) = 3.25 (p = .001).

(Table VI). None of the study subjects reported a history of genital herpes, but prior oral herpes and herpes zoster were associated with excess risks. Only a few patients reported a history of gonorrhea or syphilis, and neither disease was associated with increased cervical cancer risk.

TABLE VI – RELATIVE RISKS OF CERVICAL CANCER (*IN SITU* AND INVASIVE) BY HISTORY OF SELECTED MEDICAL EVENTS

	Cases	Controls	RR (95% CI)
Herpes			
No history of herpes	104	227	1.00 —
Oral	36	55	1.43 (0.9–2.3)
Zoster	10	9	2.43 (0.9–6.2)
Oral and Zoster	1	2	1.09 (0.1–12.2)
Unspecified	3	3	2.18 (0.4–11.0)
Unknown	2	3	1.46 (0.2–8.8)
Treatment for herpes			
No treatment	7	13	1.18 (0.5–3.0)
Home remedy	24	30	1.75 (0.9–3.1)
Pharmacy	14	18	1.70 (0.8–3.5)
Doctor	3	4	1.64 (0.4–7.4)
Unknown	4	7	1.25 (0.4–4.4)
Gonorrhea			
No	149	293	1.00 —
Yes	7	6	2.29 (0.8–7.0)
Syphilis			
No	154	297	1.00 —
Yes	2	2	1.93 (0.3–13.8)

We used 2 different serologic assays, micro-neutralization and a solid-phase radioimmunoassay, to examine the effects of prior genital HSV-2 infection. Thirty-two percent of the subjects were defined as having had HSV-2 on the basis of the neutralization assay and 33% on the basis of the radioimmunoassay. Anti-HSV-2 antibody as detected by either assay was not predictive of *in situ* or invasive cervical cancer at statistically significant levels, although the total sample size was small. No consistent pattern of risk associated with herpes infection was observed according to number of sexual partners. In addition, adjustment for evidence of prior herpes infection did not appreciably alter the risks associated with any of the identified risk factors, including number of sexual partners.

DISCUSSION

Multiple sex partners was the strongest risk factor for cervical cancer in our study, a finding consistent with other investigations outside of Latin America (Kessler, 1976; Aurelian, 1976). Women who reported having 4 or more sex partners had a 4- to 6-fold excess cervical cancer risk. In addition, those with first intercourse prior to age 16 had a 2-fold elevated risk compared to those with first intercourse after age 22. However, the observed relationship between early age at first intercourse and invasive cervical cancer was largely due to number of sex partners. The lack of association with age at first intercourse after adjustment for number of sex partners agrees with the study of Harris *et al.* (1980) and argues against adolescence being a period when the cervix is especially vulnerable to the effects of sexual behavior. The fact that independent effects of age at first intercourse were not apparent after adjustment for number of sex partners is particularly noteworthy, given that 21% of women in our study reported having had first intercourse before 16 years of age.

In spite of the fact that multiple sex partners comprised the strongest risk factor, only 8% of study subjects reported 4 or more partners, and this would argue against female promiscuity being primarily responsible for the high rates of cervical cancer in Herrera Province. Other findings support the notion that Panamanian women initiate stable sexual relationships at a young age and that those who have multiple partners generally have serial monogamous relationships. Skegg *et al.* (1982) have hypothesized that male associated risk factors relate to the occurrence of cervical cancer in Latin American women and this is supported by several lines of evidence: (1) both invasive cervical cancer and cancer of the penis occur with unusually high frequency in Latin America (Waterhouse *et al.*, 1982), particularly in Herrera (Reeves *et al.*, 1982), and some studies have found an association between the two diseases (Martinez, 1969; Graham *et al.*, 1979; Smith *et al.*, 1980); (2) Kessler (1976) has found that women married to men whose previous wives had cervical cancer have significantly elevated rates of cervical cancer; (3) Buckley *et al.* (1981) showed that husbands of cervical cancer patients were more promiscuous than husbands of controls.

Previous studies have suggested that the relationship of cervical cancer with sexual activity may reflect the effects of sexually transmitted agents, in particular infection with HSV-2 (Rawls *et al.*, 1980). Although 32% of women in this study showed evidence of prior HSV-2 infection, antibody was not predictive of risk. Our observations are in accord with a recent prospective study by Vonka *et al.* (1984) which controlled for effects of sexual risk factors and failed to show any relationship between cervical cancer risk and HSV-2 antibody (determined by both micro-neutralization and solid-phase radioimmunoassay). The lack of correlation between HSV-2 exposure and female sexual experience could be explained by male promiscuity and

is consistent with the hypothesis that male risk factors are major invasive cervical cancer determinants in Latin American populations.

The lack of association between HSV-2 infection and invasive cervical cancer in our study indicates that other sexually transmitted agents such as HPV may be the primary infectious risk factor. We recently completed a pilot study of genital HPV infection in Panamanian women with varying degrees of cervical disease (Prakash *et al.*, 1985). Cervical biopsies were tested for HPV type-16 DNA under stringent Southern blot hybridization conditions. HPV-16 DNA sequences were detected in 0/17 cervicitis patients, 3/12 (25%) with dysplasia and 12/20 (65%) invasive cervical cancer patients. Only 3 biopsies had HSV-2 DNA sequences and there was no evidence of an association between HSV-2 and HPV-16 among invasive cervical cancer cases. Durst *et al.* (1983) have reported that 53% of invasive cancer biopsies contained HPV-16 DNA. Crum *et al.* (1984) studied HPV in preneoplastic cervical lesions and their findings are compatible with the hypothesis that such lesions due to HPV-16 progress to invasive cancers. Current immunologic methods are not specific for anti-HPV-16 antibody and an important priority for future studies is to define genital infection with specific HPV strains in cases, controls and their male sex partners.

Two other sexual risk factors, a history of 2 or more abortions and the use of oral contraceptives, were associated with cervical cancer in our study. We do not know the proportion of induced abortions compared to those provoked by genital tract infection. It is noteworthy that a larger proportion of *in situ* than invasive cases reported prior abortion (40 *vs.* 28%) and that the excess risk associated with multiple prior abortions was restricted to *in situ* cancer.

We also found oral contraceptive use associated with a 2-fold excess risk of both *in situ* and invasive cervical cancer and this effect persisted after adjustment for number of sex partners. Studies by Meisels *et al.* (1977) and Vessey *et al.* (1983) presented similar results but did not control for possible confounding influences of sexual activity. A number of factors complicate evaluation of oral contraceptive use and cervical neoplasia; for example, Stern *et al.* (1970) found that women who choose oral contraceptives often have pre-existing cervical dysplasia. In addition, oral contraceptives may cause eversion of the endocervix, making cervical abnormalities easier to detect.

We were surprised that previous participation in Pap smear programs was equally common in cases and controls. Clarke and Anderson (1979) have speculated that lack of access to such programs might partly account for the high cervical cancer incidence rates in Latin America. A recent case control study by Aristizibal *et al.* (1984) in Cali, Colombia showed that women who had never been screened had a relative risk of 9.4 for invasive cervical cancer. The Panamanian Ministry of Health operates a well-organized cervical cytology screening program throughout Herrera Province and approximately 40% of cases and controls had had at least one Pap-smear, which is comparable to the screening rate in controls from Cali. Unfortunately, we did not obtain information concerning the time interval between the last Pap smear and onset of disease.

We were also surprised that smoking history was not associated with cervical cancer, which is in contrast with several recent studies of North American populations (Stellman *et al.*, 1980; Trevathan *et al.*, 1983). Only 38% of cases and 31% of controls had ever smoked, and 77% of them smoked cigarettes. On the average, cases and controls smoked for 11 years and 45% had smoked for 5 years or less. Finally, 52% of cases and controls smoked a maximum of one or two cigarettes per day.

In conclusion, our findings must be qualified by discussing an unavoidable methodological difficulty. The study was conducted in 1981 and involved cervical cancer cases diagnosed between 1974 and 1980. Thirty-four percent of the known cases were no longer available for interview. Thirty-seven patients had died and although we did not ascertain cause of death, women with advanced invasive disease were more likely to have died; 3% of *in situ* or microinvasive patients, 11% with stage I, 36% with stage II and 59% with stages III or IV had died. The same trend occurred with respect to women who were unknown by neighbors or had moved to unknown locales, and undoubtedly many of these had also died. The study successfully recruited 78% of women known to have *in situ* disease, 57% of those with microinvasive, 74% with stage I invasive disease, 33% with stage II and 20% with stage III or IV. Thus risk factors for higher stage invasive cervical disease may be under-represented in the analysis.

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