Serum Micronutrients and Cervical Dysplasia in Southwestern American Indian Women

By

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CERTIFICATE OF APPROVAL

This is to certify that the M.P.H. thesis of

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PRECIS

My interest in special population groups was sparked during my undergraduate years at Biola University. At that time, I had the opportunity to study abroad both in Europe and Israel. In addition, I had the privilege of working with underserved populations such as the villagers in Honduras and the orphans of Romania and Moldova. All these were enriching and humbling experiences that have contributed to my desire to gain training in public health as I pursue a degree in medicine.

I was particularly excited when Dr. Thomas Becker mentioned a potential thesis topic on serum micronutrients and the risk of cervical dysplasia in Southwestern American Indian women during one of the faculty-student dinner forums. He stated that a major Indian health study had been completed and secondary data analysis could be done using the completed database. As it is generally known, American Indians have a lower socioeconomic status as measured by income and education. They are also a minority population with inadequate health care and access to care. In my mind, I reasoned that this would be a potential thesis project that could positively affect this disenfranchised population and I would gladly invest my time doing that. Hence, my involvement in this thesis topic.

I hope the ultimate outcome of this major Indian health study, including my thesis project, is the improvement in the quality of life for Southwestern American Indians. If I can even contribute modestly to this end, I would be content.

Abstract

We carried out a clinic-based case-control study to assess serum micronutrients as risk factors for cervical dysplasia among southwestern American Indian women, a group with high rates of pre-invasive cervical lesions. Cases were American Indian women with biopsy-proven cervical intraepithelial neoplasia (CIN) I or II/III. Controls were from the same Indian Health Service clinics with normal cervical epithelium. We interviewed women about histories of sexually transmitted diseases, sexual behavior, diet, hygienic practices, cigarette smoking, and reproductive factors. Laboratory assays included serum for retinol (vitamin A), ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), and red blood cell (RBC) folate levels, DNA for human papillomavirus (HPV) typing, and tests for other sexually transmitted diseases (STDs). The strongest risks for cervical dysplasia were associated with cervical HPV infection [odds ratio 3.2 (95% Confidence Interval 2.2-4.6) and 7.9 (95% CI 4.8-13.1) for CIN I and CIN II/III, respectively]. With adjustments made for HPV infection and other relevant confounders, subjects in the lowest serum retinol quartile were at increased risk of CIN I as compared with women in the highest quartile (OR 2.3, 95% CI 1.3-4.1). The data suggest that low serum alpha-tocopherol was associated with CIN II/III, although the adjusted odds ratio was not statistically significant (OR 2.0, 95% CI 0.9-4.8). Low serum ascorbic acid and RBC folate were not associated with cervical dysplasia.

Introduction

The role of serum or dietary micronutrients is controversial in the development of pre-invasive cervical dysplastic lesions or cervical cancer, also known as cervical intraepithelial neoplasia (CIN). Several studies have shown that low serum levels or dietary intake of vitamin A (1,2), C (3-8), E (3,5,7) and folate (4,7-9) are associated with increased risk of cervical neoplasia. However, other epidemiologic studies have shown no association between serum or dietary intake of vitamin A (10-13), C (11-13), E (2,10,12), folate (2,11,12,14) and cervical neoplasia.

Historically, American Indian women in the Southwest had high rates of invasive cervical cancer (15-17). Although rates for invasive cancer and carcinoma-in-situ (CIS) have been decreasing in recent years (18), American Indian women continue to experience high rates of pre-invasive cervical neoplastic lesions (18,19). In addition to having higher rates of cervical neoplasia compared to non-Indians, American Indians in New Mexico have a diet that differs substantially from diets of non-Indians in the Southwest (20). However, discrepant findings concerning the role of diet and serum micronutrients in cervical dysplasia have been reported in southwestern American Indian women (7,12). To further evaluate the role of serum micronutrients in the development of cervical dysplasia, The investigators conducted a comprehensive, clinic based, case-control study of cervical dysplasia in southwestern American Indian women. Using established laboratory techniques, the investigators quantified the levels of serum retinol (vitamin A), serum ascorbic acid (vitamin C), serum alpha-tocopherol (vitamin E) and red blood cell (RBC) folate and evaluated their potential role in the development of CIN. Our analyses included other confounders associated with cervical dysplasia.

Methods

Subjects

Subjects were enrolled in this study through the Indian Health Service (IHS) hospitals and clinics in New Mexico. The IHS system serves approximately 95% of all American Indian women in the state as well as the Navajo Indian reservation. The case women in this study were enrolled through the colposcopy clinics at IHS facilities before their clinical evaluation for CIN diagnosed on routine Papanicolaou tests. Colposcopy clinic visits were scheduled within a month of the diagnosis of cervical dysplasia on the routine screening Papanicolaou test. All case women were American Indian (by self report and validated through medical record reviews, which include tribal enrollment information), aged 18 to 45 years, and not pregnant. Subjects were contacted on presentation to the colposcopy clinic and invited to participate in the interviews as an additional part of their clinic visit. Subjects were asked to sign informed consent forms, to participate in interviews, to provide 20 ml of blood for micronutrient and serological assays, and to undergo cervical cultures as described below.

Control women with normal cervical epithelium were selected from the same clinics through which case women were referred for their colposcopic examinations. Controls were also American Indian women aged 18 to 45 years, and not pregnant. In addition, controls were required to have had histories of all normal Papanicolaou tests. The investigators enrolled control subjects from women who presented to the IHS hospitals and clinics who required a pelvic examination for any reason, including family planning-related visits, annual examinations, and other reasons listed in Table 1. Medical records were reviewed to ensure that potential study controls had documented normal Papanicolaou

tests in addition to meeting the other study enrollment criteria. The investigators enrolled subjects during a 3 year period of the study, beginning November 1994 and extending through October 1997. Study participation among eligible study subjects was very high, with a total of 48 refusals (6.6% of the total eligible subjects who were invited into the study). Of the 48 refusals, 45% were case women. The relatively small numbers of refusals were related primarily to lack of available time to undergo the interviews (See Figure 1).

Figure 1. Selection of cases and controls

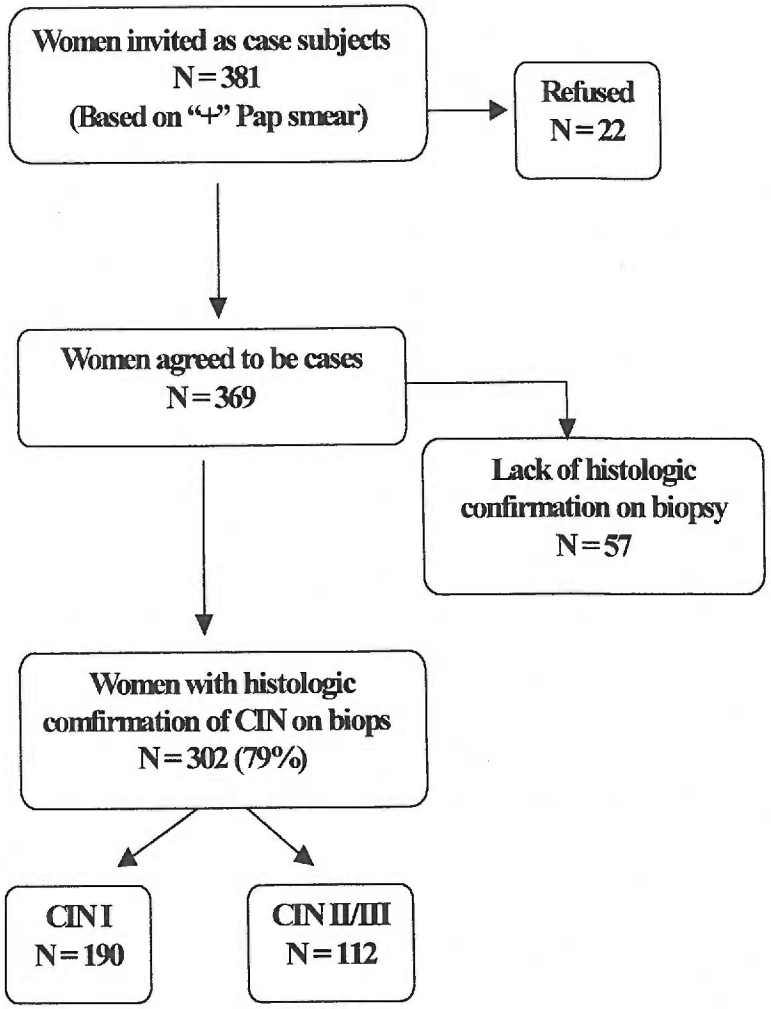
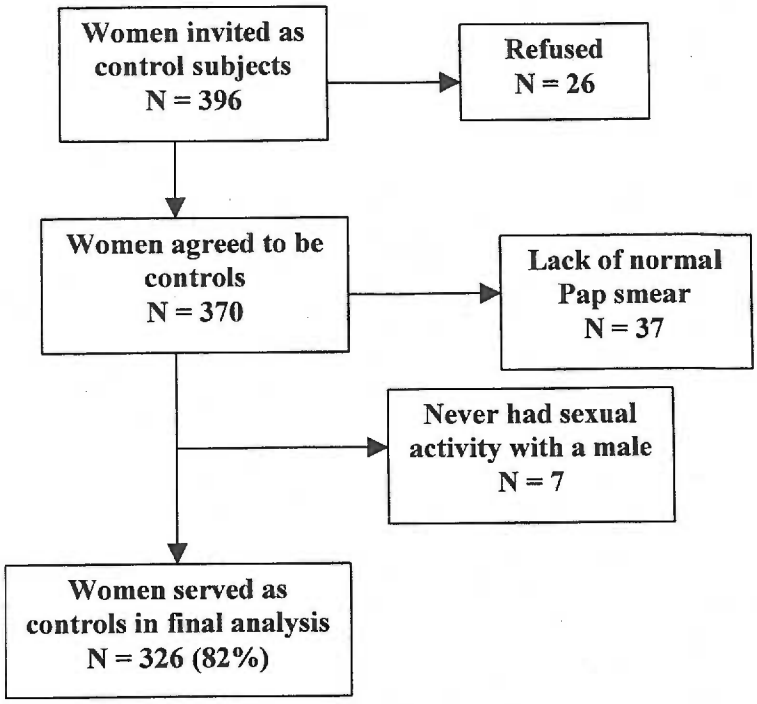


Figure 1. Selection of cases and controls, continued



Although case women with cervical dysplasia were invited into the study based on Papanicolaou tests showing squamous intraepithelial lesions, the presence of dysplasia on histologic examination of cervical tissue taken on the day of study entry was necessary for subjects to remain in the analysis. Of the case women enrolled, on the basis of Papanicolaou tests, 57 did not have histologic confirmation of dysplasia on biopsy and were dropped from the final analysis. Of these subjects, 56 had normal or nondiagnostic biopsy results and 1 subject did not have a biopsy performed. No study subject had invasive cervical carcinoma on biopsy. Most (94.6%) of the cervical smears from the clinic sites were interpreted by the same reference pathology laboratory under contract to the IHS facilities in New Mexico. The remaining 5.4% of all study Pap smears were processed by a separate laboratory that was under a contract to the IHS facility in Albuquerque. To determine inter-laboratory comparisons in cytologic diagnoses before the study began, IHS clinicians examined a sample of smears from the alternate laboratory and found that their

interpretations correlated highly with the main laboratory's interpretation (Jill Miller, MD, personal communication).

For control women, biopsy specimens were not obtained; the investigators required that the Papanicolaou test on the day of study entry be negative for subjects to remain in the analysis. Women who were selected as controls but who had atypia or dysplasia on the Papanicolaou test on the day of study entry were excluded from the analysis (n=37 [5.1%]). The investigators also excluded 7 women from the final analysis, who reported never having sexual activity with a male partner, as these subjects would not have the opportunity to be exposed to some of the primary risk factors (sexually transmitted diseases) of interest to this study. All study subjects were paid $20 for their time for participation in this investigation.

This protocol was approved by the National IHS Human Research Review Board as well as by the appropriate IHS hospital health boards.

Interviews

Trained interviewers interviewed all study subjects. After informed consent was obtained from study subjects, the interviewers asked participants about reproductive and sexual histories, sexually transmitted diseases (STDs), hygienic practices, cervical cancer screening practices, cigarette use, and dietary intake. Demographic data were also collected. Interviews were carried out in English and lasted from 60 to 90 minutes. The colposcopy clinic was scheduled to meet on specific days of the week; thus, due to logistical problems associated with the clinic's schedules, the interviewers were not blinded

to case or control status of the study subjects. Medical records were examined to validate responses about episodes of STDs, Papanicolaou test screening, and contraceptive use. Dietary intake data were collected using a food frequency interview based on a questionnaire developed from pilot data that described usual food items included in diets of American Indian women in the Southwest (7).

Pelvic Examination and Specimen Collection

Pelvic examinations and cervical specimen collection preceded colposcopic examination of the cervix for case women. Control women had cervical specimens taken at the time of pelvic examination. Specimens were collected on all women in the following order: Papanicolaou tests, fixed and air dried; dacron cervical swab of the endocervix and ectocervix, placed in transport media (Digene Diagnostics, Silver Spring, Md.) for later identification of HPV genome; endocervical swab for identification of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* using Genprobe (Genprobe Inc., San Diego, CA) test kits; vaginal pool swab for wet mount identification of trichomonads, yeast, and clue cells under light microscopy; and vaginal pool swab placed on glass slide and air-dried for later microscopic identification of organisms that are associated with bacterial vaginosis (21).Twenty milliliters of blood was collected, allowed to coagulate in a light-protected environment and serum was then stored at -70°C for later analysis. Laboratory testing included identification of antibodies to HSV-1 and HSV-2 using purified glycoprotein assays (22, 23) and analysis for micronutrients including hematocrit, RBC folate, serum retinol (vitamins A), ascorbic acid (vitamin C), and alpha-tocopherol (vitamin E). The investigators also carried out microhemagglutination assays (Sera-tek treponemal antibody test [MHA-TP], Fujirebio, Inc, Tokyo, Japan) to identify antibodies to *Treponema pallidum*,

and commercial assays to identify antibodies to hepatitis B (Corzyme, Abbott Laboratories, Abbott Park, Ill) and hepatitis C (Abbott HCV EIA2.0, Abbott Laboratories, Abbott Park, Ill). Laboratory personnel were not informed of the case or control status of the subjects. Due to the very low prevalence of HIV infection in American Indian communities in the Southwest (24), the investigators did not test for HIV antibodies.

Following cervical and blood specimen collection, case subjects underwent colposcopic examination of the cervix using application of 5% acetic acid with cotton swabs. All case women underwent cervical biopsy and endocervical curettage. Treatment for any abnormal conditions was provided by gynecologists and staff at the IHS.

Identification of HPV

HPV genome was identified from cervical specimens using a newly developed reverse blot method that employs a biotin-labeled PCR product hybridized to an array of immobilized oligonucleotide probes. Using the reverse blot strip test, genotype discrimination of multiple HPV types can be accomplished in a single hybridization and wash cycle (25). The genotypes discriminated on this strip included types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 68 (ME180), MM4 (W13B), MM& (P291), MM8 (P155), and MM9 (PAP 238A). Two concentrations of B-globin probes allowed for assessment of individual specimen adequacy following amplification. This method of HPV detection has shown high accuracy compared to other dot blot methods (25). The newly developed strip test does not provide type-specific identification of 11 HPV types that are usually identified with more commonly used PCR-based assays for HPV infection. HPV types included in the strip test were: HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58,

59, 68, mm4, mm7, and mm9. The test also identified low risk types, including HPV 6, 11, 40, 42, 53, 54, 57, 66, and mm8. In our HPV analysis, we compared subjects with any HPV types, and with HPV 16/18 to subjects with no HPV as detected by the strip test.

Serum Micronutrient and RBC Folate Measurements:

Ascorbic acid (vitamin C):

Ascorbic acid (vitamin C) levels were determined on our samples utilizing the procedure described by Garry et al (26). Briefly, serum ascorbic acid was stabilized by diluting (1:1) with 10% meta-phosphoric acid and frozen at -70°C. Using an automated AutoAnalyzer II system (Bran and Luebbe, Chicago, IL), the samples were diluted with 0.9% NaCl and mixed with 3% meta-phosphoric acid, pH 3.5. The buffered ascorbic acid was then mixed with a 2,6-dichloroindophenol reagent and measured spectrophotometrically. Serum ascorbic acid concentrations were determined from the standard curve derived from working standards of ascorbic acid. The normal range for this procedure is 0.5 to 1.5 mg/dl and the assay sensitivity is 0.25 mg/dl. Control samples containing high (1.5 mg/dl) and low (0.25 mg/dl) levels of ascorbic acid were used to determine the inter-assay precision. The coefficient of variation for these samples was determined to be 1.5% and 9.6% (n=36 assays), respectively.

Retinol (vitamin A) and alpha-tocopherol (vitamin E):

Utilizing the HPLC procedure described by Catignani et al, retinol (vitamin A) and alpha-tocopherol (vitamin E) levels were determined simultaneously (27). Serum samples, maintained under protected light conditions, were extracted with hexane and redissolved in ethanol. Using an automated Spectra-Physics HPLC System (San Jose, CA), the samples

were chromatographed on a reverse phase C-18, 5 micron, HPLC column and methanol was used as the mobile phase. Retinol and alpha-tocopherol concentrations were automatically determined by extrapolating their concentrations from standard curves, which were derived from working standard solutions of retinol and alpha-tocopherol, respectively. To ensure sample-to-sample quality control, an internal standard of retinol acetate was included in each sample. The normal range for these micronutrients as determined by this procedure are 0.1-0.5 mg/L for retinol and 7.8-12.5 mg/L for alpha_tocopherol, with the detection limits being 0.02 mg/L for retinol and 0.8 mg/L for alpha-tocopherol. Control samples containing low (0.133 mg/L) and high (0.798 mg/L) retinol concentrations and low (2.95 mg/L) and high (12.78mg/L) alpha-tocopherol levels were used to determine the inter-assay precision. The coefficients of variation for the retinol control samples were determined to be 9.6% and 6.8% (n=10 runs), respectively and 8.6% and 7.9% (n=10 runs), respectively, for alpha-tocopherol.

RBC Folate:

A Quantaphase II Folate Radioassay kit, Cat# 191-1045, from Bio-Rad Corporation, was used to determine RBC folate levels (28). A blood specimen, collected in the presence of EDTA, was diluted 1:11 with an ascorbic acid solution (0.4%) to allow hemolysis of the red cells and stabilization of the released folate. Following a further incubation, the endogenous folate conjugases hydrolyze the conjugated pteroylpolyglutamates to pteroylmonoglutamates. The resulting sample was then incubated with I^{125} labeled folate where the endogenous and labeled vitamins compete for the limited number of binding sites based on their relative concentrations. A standard curve was prepared using folate standards and the RBC folate levels were calculated based on the results from this curve

and the following formula: $\frac{(22 \times \text{lysate folate})}{(\text{hematocrit}/100)}$. The inter-assay precision was determined using control samples from Bio Rad Corporation containing high (639 ng/ml) vs low (81 ng/ml) folate levels. The coefficient of variation for these concentrations was 6.3% and 4.1% (n=22 assays), respectively. The normal range for this analyte is 95-570 ng/ml and the sensitivity of this assay is <0.1 ng/ml.

Statistical Analysis

We used SPSS software version number 8.0 for our analyses (29). We used logistic regression to model case-control status and separated case groups according to level of severity of the histological findings into CIN I and CIN II/III. Descriptive analyses of the demographic variables, sexual, behavioral and medical history of the study subjects were performed (Table 1). Most of the predictor variables were coded as categorical variables; other variables were recoded into categories for ease of interpretation or to diminish the effects of outliers. Estimates of the crude odds ratios and their 95% confidence intervals were calculated for potential confounding variables for cervical dysplasia. Analysis of specific HPV types was compared to subjects with no HPV infection. Means of serum micronutrients among cases and controls were calculated and examined with the student's t-test. Quartiles for the serum micronutrient variables were defined from the distribution of the controls and the highest quartile of each was utilized as the referent group. Crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for the association between each serum micronutrient and cervical dysplasia, using simple and multiple logistic regression, respectively. Test for effect of incremental serum micronutrient change upon case-control status were obtained by entering the serum micronutrient as continuous, normalized variable in the multiple logistic regression. In our final logistic

regression model, we included variables that were biologically plausible and variables that were statistically significant in our crude analyses. The adjusted odds ratios associating serum micronutrients with cervical dysplasia among cases and controls who are tested negative for HPV infection typed 16, 18, 31 and 35 are also analyzed to assess whether HPV type 16, 18, 31 or 35 infection status significantly affects the odds ratios.

Results

Demographic data are presented in Table 1.

Table 1: Demographic characteristics of the southwestern American Indian subjects, case-control study of cervical dysplasia,1994-97.

	Controls (n =326) No.	(%)	CIN I (n = 190) No.	(%)	CINII/III (n=112) No.	(%)
Reason for screening visit						
Annual exam	294	(90.2%)	108	(56.8%)	67	(59.8%)
Planned follow-up	2	(0.6%)	23	(12.1%)	11	(9.8%)
Suspected STD	2	(0.6%)	4	(2.1%)	2	(1.8%)
Other	28	(8.6%)	55	(28.9%)	32	(28.6%)
Age at study entry (years)						
Mean	28.57		28.46		27.63	
Median	28.5		27.0		27.0	
Marital Status						
Single	98	(30.1%)	56	(29.5%)	33	(29.5%)
Married/living with partner	203	(62.3%)	117	(61.6%)	63	(56.3%)
Divorced/Separated/Widowed	25	(7.7%)	17	(8.9%)	16	(14.3%)
Tribal affiliation						
Southwestern	291	(89.3%)	171	(90.0%)	101	(90.2%)
Other	35	(10.7%)	19	(10.0%)	11	(9.8%)
Years of education						
>12	231	(70.9%)	112	(58.9%)	63	(56.3%)
12	66	(20.2%)	41	(21.6%)	33	(29.5%)
<12	29	(8.9%)	37	(19.5%)	16	(14.3%)
Annual family income						
>$30,000	87	(26.7%)	33	(17.4%)	13	(11.6%)
$20,000-$29,999	69	(21.2%)	36	(18.9%)	19	(17.0%)
$10,000-$19,999	90	(27.6%)	48	(25.3%)	33	(29.5%)
<$10,000	80	(24.5%)	73	(38.4%)	47	(42.0%)

Table 1: Demographic characteristics of the southwestern American Indian subjects, case-control study of cervical dysplasia,1994-97 (continued).

Current Residence						
Urban Albuquerque	198	(60.7%)	80	(42.1%)	35	(31.3%)
Other Urban	55	(16.9%)	29	(15.3%)	32	(28.6%)
Reservation	62	(19.0%)	70	(36.8%)	42	(37.5%)
Other	11	(3.4%)	11	(5.8%)	3	(2.7%)
Usual Residence						
Urban Albuquerque	77	(23.6%)	19	(10.0%)	14	(12.5%)
Other Urban	73	(22.4%)	54	(28.4%)	31	(27.7%)
Reservation	160	(49.1%)	109	(57.4%)	62	(55.4%)
Other	16	(4.9%)	8	(4.2%)	5	(4.5%)

Approximately 90% of the study participants were from southwestern tribes with comparable mean ages among controls (28.6 years) and cases (28.5 years for CIN I and 27.6 years for CIN II/III). The majority of study subjects were either married or living with a partner (Table 1). In crude analysis, women with the lowest levels of education, lowest annual family income and those who resided outside of urban Albuquerque had significantly increased risk of CIN (Table 2). However, smoking status, past or current, was not associated with risk of CIN (Table 2).

HPV infection was present in 30.4% of controls, 58.4% of CIN I cases and 77.7% of CIN II/III cases. Women with DNA-based evidence of any HPV infection detected by our strip test had an increased risk of CIN (ORs of 3.2 (95% CI 2.2-4.6) and 7.9 (4.8-13.1) for CIN I and CIN II/III, respectively). The data showed a highly significant association between presence of specific HPV types 16 and/or 18 with CIN (Table 2)

Table 2: Odds ratios associating non-dietary risk factors with cervical dysplasia among southwestern American Indian cases and controls, 1994-97.

	Controls (n=326)		CIN I (190)		Crude OR (95%CI)*	CIN II/III (n=112)		Crude OR (95%CI)
Variable	No.	(%)	No.	(%)		No.	(%)	
Years of education								
>12	231	(70.9%)	112	(58.9%)	1.0	63	(56.3%)	1.0
12	66	(20.2%)	41	(21.6%)	1.3 (0.8-2.0)	33	(29.5%)	1.8 (1.1-3.0)
<12	29	(8.9%)	37	(19.5%)	2.6 (1.5-4.5)	16	(14.3%)	2.0 (1.0-4.0)
Annual family income								
>$30,000	87	(26.7%)	33	(17.4%)	1.0	13	(11.6%)	1.0
$20,000-$29,999	69	(21.2%)	36	(18.9%)	1.4 (0.8-2.4)	19	(17.0%)	1.8 (0.9-4.0)
$10,000-$19,999	90	(27.6%)	48	(25.3%)	1.4 (0.8-2.4)	33	(29.5%)	2.4 (1.2-5.0)
<$10,000	80	(24.5%)	73	(38.4%)	2.4 (1.4-4.0)	47	(42.0%)	3.9 (2.0-7.8)
Current Residence								
Urban Albuquerque	198	(60.7%)	80	(42.1%)	1.0	35	(31.3%)	1.0
Other Urban	55	(16.9%)	29	(15.3%)	1.3 (0.8-2.2)	32	(28.6%)	3.3 (1.9-5.8)
Reservation	62	(19.0%)	70	(36.8%)	2.8 (1.8-4.3)	42	(37.5%)	3.8 (2.3-6.5)
Other	11	(3.4%)	11	(5.8%)	2.5 (1.0-5.9)	3	(2.7%)	1.5 (0.4-5.8)
Smoking								
Never	222	(68.1%)	137	(72.1%)	1.0	80	(71.4%)	1.0
Ever	104	(31.9%)	53	(27.9%)	0.8 (0.6-1.2)	32	(28.6%)	0.8 (0.5-1.4)
Past	57	(17.5%)	23	(12.1%)	0.6 (0.4-1.1)	20	(17.9%)	1.0 (0.5-1.7)
Current	47	(14.4%)	30	(15.8%)	1.0 (0.6-1.7)	12	(10.7%)	0.7 (0.4-1.4)

Tabled percentiles may not consistently add to 100% due to missing values

* Odds ratio, 95% confidence interval

Table 2: Odds ratios associating non-dietary risk factors with cervical dysplasia among southwestern American Indian cases and controls, 1994-97 (continued).

	Controls (n=326)		CIN I (190)		Crude OR (95%CI)*	CIN II/III (n=112)		Crude OR (95%CI)
	No.	(%)	No.	(%)		No.	(%)	
Variable								
Age 1st intercourse								
> 20	62	(19.0%)	29	(15.3%)	1.0	14	(12.5%)	1.0
16 - 19	193	(59.2%)	126	(66.3%)	1.4 (0.8-2.3)	72	(64.3%)	1.6 (0.9-3.1)
< 16	71	(21.8%)	35	(18.4%)	1.0 (0.6-1.9)	26	(23.2%)	1.6 (0.8-3.4)
Lifetime number of sex partners								
1	76	(23.3%)	31	(16.3%)	1.0	15	(13.4%)	1.0
2 - 5	171	(52.5%)	101	(53.2%)	1.4 (0.9-2.3)	66	(58.9%)	2.0 (1.0-3.6)
> 5	79	(24.2%)	58	(30.5%)	1.8 (1.0-3.1)	31	(27.7%)	2.0 (1.0-4.0)
Number of sex partners before age 20 years (Limited to those age 20 and over)								
≤ 1	165	(50.6%)	93	(48.9%)	1.0	56	(50.0%)	1.0
2 - 4	108	(33.1%)	60	(31.6%)	1.0 (0.7-1.5)	37	(33.0%)	1.0 (0.6-1.6)
≥ 5	34	(10.4%)	23	(12.1%)	1.2 (0.7-2.2)	12	(10.7%)	1.0 (0.5-2.1)
Presence of Human Papillomavirus								
Negative	225	(69.0%)	79	(41.6%)	1.0	25	(22.3%)	1.0
Positive	99	(30.4%)	111	(58.4%)	3.2 (2.2-4.6)	87	(77.7%)	7.9 (4.8-13.1)
- HPV16/18	8	(2.5%)	18	(9.5%)	6.4 (2.7-15.3)	23	(20.5%)	25.9 (10.5-63.9)

Tabled percentiles may not consistently add to 100% due to missing values

* Odds ratio, 95% confidence interval

Mean serum micronutrient levels of cases compared with controls are shown in Table 3. All mean levels of serum micronutrients of cases were lower compared to controls. However, the difference was significant only for serum retinol among CIN I cases versus controls ($p = 0.001$) and serum alpha-tocopherol among women with CIN II/III versus controls ($p = 0.03$).

Table 3. Mean serum micronutrients for cases compared with controls in southwestern American Indians cervical dysplasia study, 1994-97.

	Controls (n = 326)	CIN I (n = 190)	p value	CIN II/III (n = 112)	p value
Mean serum levels					
Retinol (mg/L)	0.397	0.364	0.001	0.375	0.06
Ascorbic Acid (mg/dL)	1.12	1.10	0.68	1.07	0.19
α-tocopherol (mg/L)	8.32	8.18	0.55	7.81	0.03
Red blood cells Folate (ng/ml)	189.1	188.5	0.94	186.9	0.83

Crude and adjusted odds ratios associating serum micronutrients with cervical dysplasia among cases and controls are presented in Table 4. The data show that CIN I was associated with low serum retinol. Test for effect of incremental serum retinol change upon case-control status was also significant for women with CIN I, compared to controls ($p = 0.002$). In crude analyses, subjects in the lowest serum alpha-tocopherol quartile showed a higher risk for CIN II/III as compared with those of the highest quartile (Table 4), although this association was not significant in adjusted analyses. Both serum ascorbic acid and red blood cell folate showed no significant association with cervical dysplasia. The adjusted odds ratios associating serum micronutrients with cervical dysplasia among cases and controls who tested negative for HPV infection types 16, 18, 31 and 35 are presented in Table 5. The adjusted odds ratios are found to be comparable (table 5).

Table 4. Odds ratios associating serum micronutrients with cervical dysplasia among southwestern American Indians, 1994-97.

	Controls (n = 326)	CIN I (n = 190)	Crude OR (95%CI)	Adjusted OR[a] (95%CI)	p[a$]	CIN II/III (n = 112)	Crude OR (95%CI)	Adjusted OR[a] (95%CI)	p[a$]
Serum Micronutrient levels									
Retinol (mg/L)									
> 0.4465	80	29	1.0	1.0	0.002	23	1.0	1.0	0.69
0.385-0.4465	77	33	1.2 (0.7-2.1)	1.1 (0.6-2.1)		20	0.9 (0.5-1.8)	0.7 (0.3-1.6)	
0.3186-0.384	81	58	2.0 (1.1-3.4)	1.9 (1.1-3.5)		30	1.3 (0.7-2.4)	1.0 (0.5-2.1)	
< 0.3186	80	67	2.3 (1.4-3.9)	2.3 (1.3-4.1)		34	1.5 (0.8-2.7)	1.1 (0.5-2.3)	
Ascorbic Acid (mg/dL)									
> 1.30	73	46	1.0	1.0	0.74	26	1.0	1.0	0.43
1.10-1.30	85	38	0.7 (0.4-1.2)	0.6 (0.3-1.0)		22	0.7 (0.4-1.4)	0.5 (0.2-1.2)	
0.93-1.09	76	36	0.8 (0.4-1.3)	0.6 (0.3-1.1)		19	0.7 (0.4-1.4)	0.7 (0.3-1.5)	
< 0.93	83	65	1.2 (0.8-2.0)	1.1 (0.7-1.9)		40	1.4 (0.8-2.4)	1.1 (0.5-2.3)	
α-tocopherol (mg/L)									
> 9.59	80	37	1.0	1.0	0.42	16	1.0	1.0	0.19
7.982-9.59	79	50	1.4 (0.8-2.3)	1.2 (0.7-2.1)		29	1.8 (0.9-3.6)	1.8 (0.8-4.2)	
6.836-7.981	79	45	1.2 (0.7-2.1)	1.1 (0.6-1.9)		26	1.6 (0.8-3.3)	1.6 (0.7-3.8)	
< 6.836	80	55	1.5 (0.9-2.5)	1.5 (0.8-2.6)		36	2.2 (1.2-4.4)	2.0 (0.9-4.8)	
Red blood cells folate (ng/ml)									
> 231	78	49	1.0	1.0	0.75	24	1.0	1.0	0.35
165-231	79	36	0.7 (0.4-1.2)	0.8 (0.4-1.4)		26	1.1 (0.6-2.0)	1.1 (0.5-2.4)	
124.6-164	80	58	1.2 (0.7-1.9)	1.1 (0.6-1.8)		32	1.3 (0.7-2.4)	1.0 (0.5-2.1)	
<124.6	80	43	0.9 (0.5-1.4)	0.7 (0.4-1.3)		25	1.0 (0.5-1.9)	0.8 (0.4-1.7)	

Tabled controls and cases may not consistently add to 100% of study subjects due to missing values

[a] Adjusted for human Papillomavirus status, age, annual family income, current residence, and lifetime number of sex partners.
[$] Tests for effect of incremental serum micronutrient change upon disease status achieved by entering the serum micronutrient as continuous, normalized variable in the multiple logistic regression.

Table 5. Odds ratios associating serum micronutrients with cervical dysplasia among all subjects versus subjects testing negative for HPV type 16, 18, 31 and 35.

	CIN I		CIN II/III	
	Adjusted OR[a] (95%CI)	Adjusted OR[b] (95%CI)	Adjusted OR[a] (95%CI)	Adjusted OR[b] (95%CI)
Serum Micronutrient levels				
Retinol (mg/L)				
> 0.4465	1.0	1.0	1.0	1.0
0.385-0.4465	1.1 (0.6-2.1)	1.3 (0.7-2.7)	0.7 (0.3-1.6)	0.7 (0.3-1.8)
0.3186-0.384	1.9 (1.1-3.5)	2.2 (1.1-4.2)	1.0 (0.5-2.1)	0.8 (0.3-2.0)
< 0.3186	2.3 (1.3-4.1)	2.5 (1.3-4.8)	1.1 (0.5-2.3)	0.8 (0.3-2.1)
Ascorbic Acid (mg/dL)				
> 1.30	1.0	1.0	1.0	1.0
1.10-1.30	0.6 (0.3-1.0)	0.6 (0.3-1.2)	0.5 (0.2-1.2)	0.9 (0.4-2.4)
0.93-1.09	0.6 (0.3-1.1)	0.6 (0.3-1.2)	0.7 (0.3-1.5)	0.8 (0.3-2.2)
< 0.93	1.1 (0.7-1.9)	1.4 (0.8-2.6)	1.1 (0.5-2.3)	1.3 (0.5-3.3)
α-tocopherol (mg/L)				
> 9.59	1.0	1.0	1.0	1.0
7.982-9.59	1.2 (0.7-2.1)	1.2 (0.6-2.2)	1.8 (0.8-4.2)	1.6 (0.6-4.2)
6.836-7.981	1.1 (0.6-1.9)	1.3 (0.7-2.5)	1.6 (0.7-3.8)	1.3 (0.5-3.6)
< 6.836	1.5 (0.8-2.6)	1.7 (0.9-3.3)	2.0 (0.9-4.8)	1.2 (0.4-3.4)
Red blood cells folate (ng/ml)				
> 231	1.0	1.0	1.0	1.0
165-231	0.8 (0.4-1.4)	0.7 (0.4-1.3)	1.1 (0.5-2.4)	0.7 (0.3-1.8)
124.6-164	1.1 (0.6-1.8)	1.0 (0.5-1.7)	1.0 (0.5-2.1)	0.8 (0.3-1.9)
<124.6	0.7 (0.4-1.3)	0.7 (0.4-1.3)	0.8 (0.4-1.7)	0.5 (0.2-1.4)

[a] Adjusted for human Papillomavirus status, age, annual family income, current residence, and lifetime number of sex partners.

[b] Adjusted as above. In addition, subjects are excluded from analysis who tested positive for HPV 16, 18, 31 or 35

Discussion

The most important findings from our study included: (1) the strong association between HPV infection and CIN, especially HPV types 16 and 18; (2) the lack of significant associations between CIN and other previously reported risk factors such as cigarette smoking, early age at first intercourse, and number of sex partners before the age of 20 years; (3) the significant association of low serum retinol with CIN I; (4) the possible association of low serum alpha-tocopherol with CIN II/III.

Previous studies have documented different levels of serum micronutrients among women with normal Papanicolaou smears and those with CIN. Our study found that women in the lowest quartile of serum retinol had over a two-fold increase in risk of CIN I, as compared to women in the highest quartile. Although crude and adjusted odds ratios showed no significant association between CIN II/III and serum retinol, the data suggest mean serum level of retinol of women with CIN II/III was lower than controls ($p = 0.06$). Most published dietary and serologic studies have shown no association between retinol (vitamin A) and CIN (3,13,16,30-32). Similarly, our earlier pilot studies performed in the southwestern American Indian tribes reported no association between dietary and serologic levels of retinol and CIN (7,12). Contrary to most studies, Liu et al, reported low dietary intake of vitamin A to be associated with increased risk of CIN, after adjustment for HPV infection status and other confounders (33). A significant trend of increasing risk was also observed with lower intake of vitamin A (33). Moreover, a subsequent longitudinal analysis of the effect of HPV 16 infection, and nutritional status on CIN progression done by the same investigators showed high serum levels of retinol were related to the regression of CIN (1). Finally, data from a large randomized trial showed that topical application of beta-*trans*-

retinoic acid was effective in reversing moderate but not severe CIN (34). The lack of significant association of low serum retinol in women with CIN II/III in our study suggests a less important role in higher grade cervical lesions in this population.

Our data also suggest that low serum alpha-tocopherol may be associated with a higher risk of CIN. The crude odds ratio showed a significantly lower mean value for serum alpha-tocopherol found in CIN II/III cases as compared to controls. When the analysis was restricted to women with CIN I or women who are tested negative for HPV infection typed 16,18,31 or 35, the association with low serum alpha-tocopherol was not apparent, however. The role of alpha-tocopherol in the development of CIN has not been as extensively studied as other micronutrients. Most published dietary or serologic studies were conflicting as to its etiologic importance with regard to the development of CIN (2,3,13,16,30,32). Our pilot study among American Indians showed no effect of low serum levels of alpha-tocopherol in CIN (12). Prospective studies by Knekt et al and Batieha et al have shown a weak negative association or no association between serum alpha-tocopherol levels and risk of developing cervical cancer, respectively (35,36). In contrast, three other studies done in different populations demonstrated decreasing trend in serum levels of alpha-tocopherol with more advanced cervical lesions (30,37). Our data suggest a negative association of serum alpha-tocopherol with CIN II/III, implying a role for alpha-tocopherol in more advanced cervical lesions. The potential physiological interactions between HPV infection and serum alpha-tocopherol levels have not been delineated. Additional confounding between serum alpha-tocopherol and high risk HPV infection status was observed in our analysis (table 5). This relationship warrants additional investigation. Further studies would be required to determine if the results were due to chance or reflect

some biologic relation.

Most serologic and dietary studies suggest a negative association of ascorbic acid with all stages of CIN (3,4,6,14,16,29,32). Two of the dietary studies that did not find an overall negative association of ascorbic acid did find it to be protective among smokers (11,38). Liu et al reported low dietary intake of ascorbate to be associated with an increased risk of CIN (33). However, a negative association between serum ascorbic acid and CIN was not observed in the subsequent published study by the same investigators (1). The two pilot studies done on southwestern American Indians have inconsistent findings. We found ascorbic acid as assessed by dietary intake to be negatively associated with CIN but did not find any significant serologic associations (7,12). Our data from the current study did not show serum ascorbic acid to be associated with CIN I or CIN II/III. Furthermore, analysis by smoking status indicated no significant associations.

Several epidemiological studies reported an association of CIN with low dietary, serum or RBC folate status (7-9). However, the same relationship is often not found in most studies where cases have in-situ or invasive carcinoma of the cervix (5,11,14). The data from our current study do not show any significant differences between mean RBC folate in case and control subjects, and no suggestion of a protective effect of high RBC folate level against CIN I or CIN II/III. These results supported the findings from the original pilot study done in this population where RBC folate level was not found to be associated with CIN (12). In addition, two large, randomized controlled trials have shown that folic acid is ineffective in reversing low to moderate grade CIN (34).

Previous studies have consistently shown moderate to strong risks associating cervical HPV infection with CIN (3,12,16,33,39-41). Our study indicates that women with cervical HPV infection had an elevated risk of CIN I and CIN II/III, compared to women without HPV infection. Our data also showed that women with HPV type-16 and/or 18 had very high risk of CIN I and CIN II/III compared to women without evidence of HPV infection. This finding is consistent with many other studies that have shown strong effect of HPV types 16 and 18 infection as a risk for CIN (3,16,33,39-41).

In a number of investigations, cigarette smoking, early age of first intercourse, and total number of sexual partners before 20 years of age, have been associated with CIN and/or cervical cancer (9,16, 40,42,43). However, the findings have not been consistent across all studies. This lack of consistency in identification of risk factors for CIN may be related to lack of control for important variables such as HPV infection in the analysis (16,32). In our study, after adjustment for HPV infection using highly sensitive tests, the data showed that cigarette smoking (whether past or current), early age at first intercourse and high number of sexual partners before the age of 20 years were not significantly associated with cervical dysplasia. In our earlier report on southwestern Hispanic and non-Hispanic white women, we did not find age at first intercourse and number of sex partners before age 20 years to be significantly associated with CIN (40). However, cigarette smoking at the time of diagnosis was associated with CIN in Hispanic and non-Hispanic whites in New Mexico (40). Although our current findings showed no significant associations among American Indian women, different patterns of cigarette smoking (such as length of inhalation) may be related to this inconsistency among groups. The investigators did not collect such detailed data related to patterns of use of cigarettes.

Limitation

This study has several limitations that must be addressed. First, interviewers were not blinded to case or control status. However, strict adherence to protocol and structured interviews should minimize any interviewer bias. In addition, frequent monitoring of the interviews by the Principal Investigator and the reinterviewing of a 5% sample of the study participants showed no systematic biases in data collection. Another potential limitation of this research is that the role of male sexual behavior was not examined and thus, the impact of this variable could not be evaluated. Recall bias and information bias are always potential problems when dealing with sensitive issue such as sexual behavior and dietary histories. To minimize bias, study participants were frequently reassured of the confidentiality of their responses and the need for accurate responses. Although state-of-the-art laboratory tests were used to detect cervical infections, the investigators only assessed the status of cervical HPV infection at study entry. This may not have accurately reflected the true status of cervical HPV infection as studies have shown that sequential testing in the same women increases measures of prevalence of cervical HPV infection, even in the absence of new sex partners (44). Although control subjects were not randomly selected from tribal rosters or other IHS rosters, our approach in sampling eligible women who presented to the IHS clinics requiring a pelvic examination for any reason should not have introduced systematic error into the study design or analysis of the results. The inherent weakness of case-control studies is their inability to establish temporal relationship between exposure and disease. Reverse causality could be an explanation to the association found between cervical HPV infection and cervical dysplasia if HPV detection is enhanced in dysplastic cells or if risk of infection is greater in dysplastic cells.

Conclusions

Despite the potential limitations of this study, we found that cervical dysplasia, especially CIN II/III is strongly associated with HPV infection, particularly HPV type 16 and 18. In addition, our data indicated that low serum level of retinol (vitamin A) was significantly associated with an increased risk of CIN I, with and without adjustments made for relevant confounders. This is further supported by a significant difference in mean serum retinol among CIN I cases versus controls. Our data also suggested that low serum alpha-tocopherol (vitamin E) may be associated with CIN II/III while low serum ascorbic acid and RBC folate were not associated with cervical dysplasia. Analysis of our dietary data based on food frequency questionnaires will help with our understanding of the associations among these micronutrients and CIN lesions.

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APPENDIX A

Case-control Study / Cervical Dysplasia in American Indian Women

Questionnaires for demographics, social history, sexual history, and gynecological history.

CASE-CONTROL STUDY/CERVICAL DYSPLASIA IN AMERICAN INDIAN WOMEN

DEMOGRAPHICS, SOCIAL HISTORY, SEXUAL HISTORY, GYN HISTORY

SUBJECT ____ ____ ____ ____ ____
RID #

(USE RED PEN -- fill in all leading "zero" values: i.e., 01 or 02, etc.)

_____ Pap smear diagnosis at study entry point
1. normal/no significant pathology (for controls)
2. slight dysplasia (CIN I)
3. moderate dysplasia (CIN II)
4. severe dysplasia/carcinoma-in-situ (CIN III)
5. ASCUS/atypia/slight dysplasia/HPV (CIN I) for women who enter the study with biopsy already completed that shows dysplasia
6. Other, specify _____

_____ Interviewer Number
1.
2.
3.
4.

Interviewer questions/instructions

(Fill in your assigned number)

Date of Interview

(Fill in today's date)

__ __/__ __/__ __
M M D D Y Y

_____ Location of Interview
1. Albuquerque IHS Hospital
2. Gallup Indian Medical Center
3. Santa Fe IHS
4. Other, specify_____

(Fill in location of interview)

Why did you have (are you having) your pap done? In other words, why did you come into the clinic the day your Pap smear was taken?

___ ___ Reason for screening visit which led to diagnosis of dysplasia, or reason control women have presented for their pap screening

01 family planning advice

02 annual exam

03 suspected STD

04 pregnancy testing

05 abnormal menstrual flow

06 missed period or reduced menstrual flow

07 acyclical or irregular bleeding

08 dysmenorrhea (painful menstruation)

09 abdominal pain

10 infertility

11 suspected vulvar lesion

12 planned follow-up

13 other; specify _____

The next few questions are asked to help us understand a little more about characteristics of women who have pap smear abnormalities.

Birthdate

__ __/__ __/__ __
M M D D Y Y

When were you born?

Age

How old are you now?

__ __ years

Marital status

What is your marital status? Are you...

_____

1. single, never married
2. married or living with partner
3. divorced
4. separated
5. widowed

Years of Education

How many years of classroom education did you complete? ***(for example, completing high-school would be 12 years; 2 additional years of Tech school or college- would be 14 years, etc.)***

___ ___ years

Annual family income

What is your family's annual income before taxes? ***(Show the subject these catagories).***

_____

1. $ 9,999 or less
2. $10,000 to $19,999
3. $20,000 to $29,999
4. greater than or equal to $30,000

Tribal affiliation _____	1. Pueblo Specify: _____ 2. Navajo 3. Other Specify: _____	**What is your tribal affiliation or heritage? (Where are you registered?)**
Blood Quantum _____	1. Full 2. 3/4 3. 1/2 4. 1/4 5. Less than 1/4	**What is your blood quantum, or degree of Indian blood?**
Consistent Blood Quantum _____ If no, specify: _____	1. Yes 2. No	**Is your blood quantum within the same tribe?** **If no, what is your heritage?**
Current residence _____	1. Urban Albuquerque 2. Other urban area (Santa Fe, Gallup, Bernalillo, etc.) 3. Pueblo/reservation area 4. Other Specify: _____	**Where do you live now?**
Usual residence _____	1. Urban Albuquerque 2. Other urban area (Santa Fe, Gallup, Bernalillo, etc.) 3. Pueblo/reservation area 4. Other Specify: _____	**Sometimes people live in many different places during their lives. Where have you lived most of your life?**

MENSTRUAL HISTORY

Age 1st period

___ ___ years

Last Menstrual Period

___ ___ ___ ___ days ago

The next few questions concern your menstrual history. How old were you when you had your first period?

When did you begin your <u>last</u> menstrual period?

BIRTH CONTROL HISTORY

Contraception- ever

____

Code

1. Yes
2. No

The next questions are about contraception. Have you ever used any form of contraception or family planning method?

(If no, fill in all "2's" below and skip to question on douching.)

Types of contraception

What kinds of contraception or family planning methods have you used (ever)? *(Code yes or no for each item.)*

1. Yes
2. No

_____ BC pills

_____ IUD

_____ Condoms

_____ Foam

_____ Diaphragm

_____ Rhythm method

_____ Sterilization (tubes tied)

_____ Sterilization of sex partner (vasectomy)

_____ Norplant

_____ Depo Provera shots

_____ Sponge

_____ Other: specify _____

Present contraception Code

____ 1. Yes

2. No

Are you currently using any form of contraception or family planning method? *("Currently " means contraception in the past month)*

What form of contraception or family planning method do you use now? *(Code yes or no for each item.) {If subject uses no birth control, all responses should be coded "no" (2)). (If the subject is not sexually active, and is not using birth control code all answers as "no".)*

Types of contraception now 1. Yes

2. No

_____ BC pills

_____ IUD

_____ Condoms

_____ Foam

_____ Diaphragm

_____ Rhythm method

_____ Sterilization (tubes tied)

_____ Sterilization of sex partner (vasectomy)

_____ Norplant

_____ Depo Provera shots

_____ Sponge

_____ Other: specify _____

(If birth control pill use was coded:)

BIRTH CONTROL PILL HISTORY

Age 1st started B.C.P.

___ ___ years

If you have used birth control pills, how old were you when you started using them? ***(If not applicable, code 99)***

Birth control pills years of use

___ ___ years

For how many years total have you used birth control pills? ***(Some addition or subtraction may be needed to calculate this number. Be prepared to assist the study subject). (If not applicable, code 99)***

DOUCHING HISTORY

Douche ever	Code	**The next questions are about douching. Have you ever douched?**
____	1. Yes	
	2. No	
		(If no, fill in "9's" below and skip to questions on GYN history)
Douche frequency	1. less than 1/month	**How frequently have you douched in the past year?**
____	2. 1-2 times/month	
	3. 3-4 times/month	
	4. > 4 times/month	
Douche preparation	1. Yes	**What have you used to douche with (ever)?** ***(Code yes or no for each item)***
	2. No	

_____ Store-bought preparation other than water and vinegar or water and baking soda.

_____ Water

_____ Water + vinegar

_____ Water + baking soda

_____ Other - specify _____

MEDICAL PROBLEMS

Medical problems	Code	The next questions are about general medical problems. Do you have any major medical problems that required you to see a health professional at least twice during the past year?
____	1. Yes	
	2. No	
Problem list:		If so, what are they?
_____		
_____		
_____		
Diabetes	1. Yes	Have you ever been diagnosed with diabetes, other than the type that sometimes occurs with pregnancy?
_____	2. No	
	8. Don't know	
Years with diabetes		If yes, how long have you had diabetes? *(If no, code 99)*
___ ___ years		
Cold sores	1. Yes	Have you ever had cold sores or fever blisters?
_____	2. No	
	8. Don't know	
Current Medications	1. Yes	Are you taking any prescription medicines now (besides birth controls pills)? If so, what are they? *(If no, skip to question below on pregnancy.)*
_____	2. No	

Medicines list

_____

_____

_____

PREGNANCY HISTORY

Pregnant ever?

____

Code

1. Yes

2. No

The next questions are about pregnancy. Have you ever been pregnant? ***(If no, skip to question on failed pregnancy attempts. and list 99 or 9 where applicable)***

Pregnant no. times

___ ___ times

If yes, how many times have you been pregnant?

Can you tell me what happened with each pregnancy? ***(Interviewer will need to refer to the medical chart for accuracy of this information. Go by the charted information if there is any discrepancy.)***

OUTCOME	Code
Pregnancy 1	1. live birth, vaginal delivery
____	2. live birth, cesarean section
Pregnancy 2	3. induced abortion
____	4. miscarriage < 20 weeks
Pregnancy 3	5. still birth > 20 weeks
____	6. neonatal death < 28 days after birth
Pregnancy 4	7. ectopic pregnancy
____	8. other (specify)
Pregnancy 5	
____	
Pregnancy 6	
____	
Pregnancy 7	
____	
Pregnancy 8	
____	
Pregnancy 9	
____	

Failed pregnancy attempts

_____

1. Yes
2. No
8. Don't know

Have you ever tried to become pregnant, but were unable to do so after a year or more?

Told infertile

1. Yes
2. No
8. Don't know

Have you ever been told by a doctor that you were infertile, or underwent evaluation for infertility?

Reasons infertile

Code

1. blocked tubes after laparoscopic exam
2. blocked tubes-- no laparoscopic confirmation
3. problem ovulating
4. uterine problems
5. infertile husband/ boyfriend
6. Other, specify

_____

If you have been diagnosed as infertile, can you tell me why or what the reason was?
(Interviewer will need to check chart.)

PAP SMEAR HISTORY

Last Pap

___ months ago

The next questions are about previous Pap smears. Before the most recent Pap smear that led to this visit, how long ago had it been since your last Pap? ***(Interviewer will need to check the chart)***

Abnormal Pap smear

____

Code

1. Yes
2. No
8. Don't know

Before the most recent Pap smear that led to this visit, have you ever had an abnormal smear? ***(If no, skip to questions on family history)***

Cervix treatment

____

1. Yes
2. No
8. Don't know

Have you ever been treated for any previous abnormal Pap smear results?

Diagnosis

____

1. atypia
2. slight dysplasia (CIN I)
3. moderate dysplasia (CIN II)
4. severe dysplasia (CIN III)
8. don't know and not charted

What was the diagnosis or report on the "worst" or most abnormal Pap smear that you've ever had? ***(This response will need chart verification. For controls, who will have had all negative paps, code 9).***

FAMILY HISTORY

Fam His CD

____

1. Yes
2. No
8. Don't know

The next questions are about dysplasia and cancer among family members. Has anyone in your family had cervical dysplasia, or been sent to colposcopy clinic for an abnormal pap?

Fam Rel CD

____

____

____

1. mother
2. sister
3. grandmother, maternal
4. grandmother, paternal
5. aunt, maternal
6. aunt, paternal
7. other, specify_____
8. don't know

If yes, what was her (or their) relationship to you? ***(Fill in 9's if not applicable)***

Fam His CC

____

Code

1. Yes
2. No
8. Don't know

Has anyone in your family had cervical cancer?

Fam Rel CC

____

____

____

1. mother
2. sister
3. grandmother, maternal
4. grandmother, paternal
5. aunt, maternal
6. aunt, paternal
7. other, specify_____
8. don't know

If yes, what was her (or their) relationship to you?

SMOKING HISTORY

Smoke ever	Code	**The next questions are about cigarette smoking. Have you ever smoked cigarettes on a regular basis?** ***(Regular means a half pack per week for 3 or more months.) (If no, skip to sexual history and code "9" or "99" where applicable.)***
____	1. Yes	
	2. No	
Smoke now	1. Yes	**If yes,**
____	2. No	**Do you still smoke regularly?**
Smoke period		**How long have you (had you) smoked (years)?**
__ __ years		
Smoke start age		**How old were you when you started smoking regularly?**
__ __ years		
Quit smoking		**If you were a former smoker but quit, how long ago did you quit smoking?**
__ __ years ago		
No. cigarettes		**What is/was your average number of cigarettes smoked per day?**
__ __ per day		

SEXUAL HISTORY

Explain to the subject that you are about to ask a series of personal questions, but re-state that all of the information is important and will be kept strictly confidential.

Age first intercourse

__ __ years old

How old were you the first time you had sex? By this, I mean the first time you had vaginal intercourse with a male partner?

Partner number

__ __ __ partners

How many different male sex partners have you had in the last month?

Partners before 20

__ __ __ partners

How many different male sex partners have you had before age 20 years?

Total partner No.

__ __ __ partners

How many different male sex partners have you had in your entire life?

STD HISTORY

We're almost done with this questionnaire. Now I am going to ask you if you have had any sexually transmitted infections from a long list of infections.

Warts	Code	Have you ever had: **A. Genital warts?** *(Describe these as fleshy wart-appearing growths on the external genitalia or vagina.)*
____	1. Yes	
	2. No	
	8. Don't know	
Herpes	1. Yes	**B. Genital herpes?** *(Describe these lesions as recurrent blisters on the vulva, lower abdomen, buttocks, or inner thigh).*
____	2. No	
	8. Don't know	
Yeast	1. Yes	**C. Vaginal yeast infection?**
____	2. No	
	8. Don't know	
Trich	1. Yes	**D. Trichomonas infection?**
____	2. No	
	8. Don't know	

NSV ____	Code 1. Yes 2. No 8. Don't know	**E. Non-specific vaginitis, or Gardnerella, also known as BV or bacterial vaginosis?**
Syphilis ____	 1. Yes 2. No 8. Don't know	**F. Syphilis?**
GC ____	1. Yes 2. No 8. Don't know	**G. Gonorrhea?**
Chlamy ____	1. Yes 2. No 8. Don't know	**H. Chlamydia?**
PID ____	Code 1. Yes 2. No 8. Don't know	**I. Pelvic Inflammatory Disease or PID ? (This term refers to an infection of your tubes).**

Hep

____

1. Yes
2. No
8. Don't know

J. Hepatitis? (We just want to know about hepatitis you may have acquired since you became sexually active).

M.C.

____

1. Yes
2. No
8. Don't know

K. Molluscum contagiosum?
These are tiny, solid bumps on the skin near your vulva.

The interviewer will need to check the chart to verify all responses. When conflicting information becomes apparent, use the charted information and make not of the inconsistencies on the next page under 'comments.'

INTERVIEWER COMMENTS

How cooperative was the respondent?

____ Code

1. very cooperative
2. somewhat cooperative
3. not very cooperative
4. somewhat hostile
5. very hostile

Was a translator necessary for this interview?

____ Code

1. Yes
2. No

Was the quality of the interview diminished due to lack of understanding and/or lack of ability of the respondent?

____ Code

1. Yes, somewhat diminished
2. No
3. Yes, greatly diminished

Comments (include items that were not consistent between interview and chart):

_____

_____

_____

APPENDIX B

Nutrient Status as Associated with Cervical Neoplasia: Case-control Studies

Nutrient status as associated with cervical neoplasia from case-control studies

Researchers	Exposures	Outcome studied	Odds Ratio / p value
De Vet, 1991	Retinol (diet)	CIN	p = 0.38 <NS>
	β-carotene (diet)	CIN	2.31 high intake associated w/ dysplasia
	Vitamin C (diet)	CIN	p = 0.17 <NS>
	Use of Vit. C supplements	CIN	p = 0.06
Butterworth Jr. 1992	Retinol (serum)	CIN	0.8 to 1.0 low vs. high levels
	Carotenoids (serum)	CIN	0.8-1.3 low vs. high level
	Ascorbate (serum)	CIN	0.9 low vs. high level
	RBC folate	CIN	1.4 low vs. high level
			5.1 in HPV+ women with ≤ 660nmol/L
Slattery, 1989	Vitamin A (diet)	234 CIS + 36 Invasive	0.8 to 0.94 high vs. low intake
	Carotene (diet)	234 CIS + 36 Invasive	0.99 to 1.21 high vs. low intake
	Vitamin C (diet)	234 CIS + 36 Invasive	0.68 to 1.06 high vs. low intake
	Vitamin E (diet)	234 CIS + 36 Invasive	0.65 to 1.27 high vs. low intake
VanEenwyk, 1991	α-carotene (diet)	CIN	0.15 to 0.26 low vs. high intake
	Cryptoxanthin (diet)	CIN	1.9 to 2.7 low vs. high intake
	Lycopene (diet)	CIN	4.6 to 5.8 low vs. high intake
	Lycopene (serum)	CIN	3.5 to 4.7 low vs. high intake

Nutrient status as associated with cervical neoplasia from case-control studies (continued)

Researchers	Exposures	Outcome studied	Odds Ratio / p value
Buckley, 1992	Retinol (diet)	CIN	0.7 low vs. high intake
	Carotene (diet)	CIN	0.98 low vs. high intake
	Vitamin C (diet)	CIN	3.03 low vs. high intake
	Vitamin E (diet)	CIN	1.65 low vs. high intake
	Folacin (diet)	CIN	3.31 low vs. high intake
Brock, 1988	Retinol (diet)	CIN III – In situ	1.2 to 1.7 high vs. low intake
	Retinol (serum)	CIN III – In situ	0.4 to 1.3 high vs. low intake
	Carotene (diet)	CIN III – In situ	1.0 to 1.2 high vs. low intake
	Carotenoids (serum)	CIN III – In situ	0.4 to 0.9 high vs. low intake
	β-carotene (serum)	CIN III – In situ	0.2 to 0.5 high vs. low intake
	Vitamin C (diet)	CIN III – In situ	0.5 to 0.7 high vs. low intake
	Folate (diet)	CIN III – In situ	0.9 to 1.8 high vs. low intake
Verreault, 1989	Retinol (diet)	Invasive	0.6 to 1.1 high vs. low intake
	Carotene (diet)	Invasive	0.6 to 0.8 high vs. low intake
	Vitamin C (diet)	Invasive	0.5 to 1.2 high vs. low intake
	Vitamin E (diet)	Invasive	0.4 to 0.5 high vs. low intake
	Folate (diet)	Invasive	0.8 to 1.3 high vs. low intake

Nutrient status as associated with cervical neoplasia from case-control studies (continued)

Researchers	Exposures	Outcome studied	Odds Ratio / p value
Herrero, 1991	Retinol (diet)	Invasive	p = 0.9 <NS>
	β-carotene (diet)	Invasive	p = 0.01 high levels protective
	Other carotenoids (diet)	Invasive	p = 0.003 high levels protective
	Vitamin C (diet)	Invasive	p = 0.003 high levels protective
Potischman, 1991	Retinol (serum)	Invasive	p = 0.39
	β-carotene (serum)	Invasive	p = 0.05 high levels protective
	α-tocopherol (serum)	Invasive	p = 0.63
	γ-tocopherol (serum)	Invasive	p = 0.03 high level associated w/ cancer
McPherson, unpublished	Vitamin A (diet)	CIN	0.93 to 1.19 low vs. high intake
	Retinol (diet)	CIN	0.96 to 1.50 low vs. high intake
	Carotene (diet)	CIN	0.85 to 1.37 low vs. high intake
	Vitamin C (diet)	CIN	1.10 to 2.07 low vs. high intake
	Vitamin E (diet)	CIN	0.76 to 0.77 low vs. high intake
	Folate (diet)	CIN	1.66 to 2.25 low vs. high intake
Palan, 1991	Retinol (serum)	CIN & Invasive	p = 0.7976
	β-carotene (serum)	CIN & Invasive	p < 0.0001
	α-tocopherol (serum)	CIN & Invasive	p < 0.005

Nutrient status as associated with cervical neoplasia from case-control studies (continued)

Researchers	Exposures	Outcome studied	Odds Ratio / p value
Orr, 1985	Vitamin A (serum)	Invasive	<NS>
	β-carotene (serum)	Invasive	$p < 0.0005$ cases lower than controls
	Vitamin C (serum)	Invasive	$p < 0.0005$ cases lower than controls
	Folate (plasma)	Invasive	$p < 0.005$ cases lower than controls
	RBC Folate	Invasive	<NS>
Romney, 1985	Vitamin C (serum)	CIN	$p < 0.0001$
Ziegler, 1990	Vitamin A (diet)	Invasive	0.89 to 1.25 low vs. high intake
	Carotenoids (diet)	Invasive	0.7 to 1.02 low vs. high intake
	Vitamin C (diet)	Invasive	0.88 to 1.14 low vs. high intake
	Folate (diet)	Invasive	0.73 to 0.87 low vs. high intake
Shimizu, 1996	Retinol (diet)	CIN	1.98 to 2.08 high vs. low intake
	Retinol (serum)	CIN	0.56 to 0.61 high vs. low intake
	Carotene (diet)	CIN	1.39 to 1.74 high vs. low intake
Ramaswamy, 1996	Vitamin A (serum)	Invasive	<NS>
	Carotenoids (serum)	Invasive	$p < 0.01$ cases lower than controls
	Vitamin C (serum)	Invasive	$p < 0.01$ cases lower than controls
Potischman, 1991	Folate (plasma)	Invasive	1.01 to 1.06 high vs. low intake

Nutrient status as associated with cervical neoplasia from case-control studies (continued)

Researchers	Exposures	Outcome studied	Odds Ratio / p value
Kwasniewska, 1997	α-tocopherol (serum)	CIN	p < 0.05 cases lower than controls
Bhuvarahamurthy, 1996	Vitamin C (serum)	Invasive	p < 0.05 cases lower than controls
	Vitamin E (serum)	Invasive	p < 0.01 cases lower than controls
Wassertheil-Smoller, 1981	Vitamin C (diet)	CIN	p < 0.01 cases lower than controls
Maclehose, unpublished	Vitamin A (serum)	CIN II/III	p = 0.35
	Vitamin C (serum)	CIN II/III	p = 0.34
	Vitamin E (serum)	CIN II/III	p = 0.87
	RBC folate	CIN II/III	p = 0.19
Becker, 1993	Retinol (serum)	CIN	<NS>
	Vitamin C (serum)	CIN	<NS>
	Vitamin E (serum)	CIN	<NS>
	RBC folate	CIN	<NS>
Cuzick, 1990	Vitamin A (serum)	CIN I	p = 0.1
		CIN III	p = 0.3
	Vitamin E (serum)	CIN I	p < 0.05 cases lower than controls
		CIN III	p < 0.01 cases lower than controls

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