

Dietary Supplements Reduce the Risk of Cervical Intraepithelial Neoplasia

Jong Ha Hwang,* Mi Kyung Kim,† and Jae Kwan Lee*

Objective: To examine the effects of dietary supplements on high-risk human papillomavirus (HPV) infection and cervical carcinogenesis.

Methods: A multi-institutional cross-sectional study was carried out to examine whether dietary supplements were associated with the risk of cervical intraepithelial neoplasia (CIN). We enrolled 1096 women aged 18 to 65 years to participate in an HPV cohort study from March 2006 up to present. For this analysis, we included 328 HPV-positive women (166 controls; 90 CIN I and 72 CIN II/III). The details of each participant's routine dietary intake during the prior year were collected. Specific dietary supplements were classified into 5 categories, namely, multivitamins, multinutrients, vitamin C, calcium, and miscellaneous.

Results: A higher HPV viral load was associated with an increased risk of CIN II/III (odds ratio [OR], 3.32; 95% confidence interval [CI], 1.54–7.16; *P* for trend 0.002). Dietary supplement use including multivitamins (OR, 0.21; 95% CI, 0.09–0.48), vitamins A (OR, 0.19; 95% CI, 0.07–0.53), C (OR, 0.24; 95% CI, 0.10–0.56), E (OR, 0.20; 95% CI, 0.07–0.53), and calcium (OR, 0.21; 95% CI, 0.08–0.50) was significantly associated with a lower risk of CIN II/III. The patients who took multivitamins and had a lower HPV viral load (<15.5 relative light units/positive control) had a significantly decreased frequency of CIN I (OR, 0.35; 95% CI, 0.14–0.87; interaction *P* = 0.925) and CIN II/III (OR, 0.11; 95% CI, 0.04–0.37; interaction *P* = 0.304).

Conclusions: The findings of this study suggest that dietary supplements may reduce the risk of CINs in women with high-risk HPV infection.

Key Words: Dietary supplements, Multivitamins, HPV, Viral load, CIN

Received September 17, 2009, and in revised form December 1, 2009.

Accepted for publication December 17, 2009.

(*Int J Gynecol Cancer* 2010;20: 398–403)

Human papillomavirus (HPV) infection has been shown to be a causal agent involved in cervical carcinogenesis.¹ However, not all women infected with HPV develop cervical

neoplasia. Only persistent and high-risk HPV infection increases the risk of cervical carcinogenesis when compared with transient infections.² Although a woman's risk for cervical neoplasia is significantly higher if she has an HPV infection, HPV infection alone may not be sufficient to cause cervical neoplasia. Factors known to contribute to the progression of HPV infection to neoplasia include oral contraceptive use, cigarette smoking, as well as infection with other sexually transmitted diseases, immunosuppression, and nutritional status.³ The relationship between cervical carcinogenesis and diet has received much attention in the last 2 decades. Antioxidant micronutrients (eg, carotenoids and tocopherols) may affect the progression of HPV infection to cervical intraepithelial neoplasia (CIN). The results of epidemiological studies have suggested that specific dietary or circulating micronutrients, especially vitamin A and the carotenoids, in particular lycopene^{4,5} and tocopherols,⁶ may be protective against cervical neoplasia. Some studies have

*Department of Obstetrics and Gynecology, Korea University Guro Hospital, Korea University College of Medicine, Seoul; and †Carcinogenesis Branch, National Cancer Center, Kyunggi-do, Korea.

Address correspondence and reprint requests to Mi Kyung Kim, Carcinogenesis Branch, Division of Basic Sciences, National Cancer Center, 809 Madu-dong, Ilsan-gu, Goyang-si, Gyeonggi-do, 411-769, Korea. E-mail: alrud@ncc.re.kr. This study was supported by a Korea Science and Engineering Foundation grant funded by the Korean government (R01-2006-000-10621-0).

The authors declare that there are no conflicts of interest.

Copyright © 2010 by IGCS and ESGO

ISSN: 1048-891X

DOI: 10.1111/IGC.0b013e3181d02ff2

shown an association between nutritional status, HPV seropositivity,⁷ and the persistence of infection.⁸ In addition, an association between low serum levels of retinol,⁷ β -carotene, β -cryptoxanthin, lutein, lycopene,⁸ and HPV persistence has been demonstrated. However, no previous studies have reported on the association between dietary supplements and high-risk HPV status on the HPV DNA load. Therefore, we evaluated the role of dietary supplements on high-risk HPV and cervical carcinogenesis.

PATIENTS AND METHODS

Subject Recruitment

The National Cancer Center of Korea recruited 1096 women, aged 18 to 65 years, to participate in an HPV cohort study from March 2006 up to present. The study was approved by the ethics committees of the National Cancer Center of Korea and each study center. The women were randomly selected from the Gynecologic Oncology clinics of 6 university hospitals in the Republic of Korea. Women were eligible to participate if they were currently sexually active or seeking birth control, were not currently pregnant, had an intact uterus, and had no history of hysterectomy or treatment of cervical dysplasia within the previous 18 months. Exclusion criteria included a history of gynecologic cancers such as cervical cancer, endometrial cancer, or ovarian cancer, insufficient data on the questionnaire, drug dependency, or psychological problems, a chronic disease such as liver cirrhosis, cardiovascular disease, and renal failure. All study participants signed an informed consent form.

Upon study entry, participants filled out a questionnaire on risk factors for cervical cancer and underwent a gynecologic examination, Hybrid Capture II (HC II) testing, and Papanicolaou tests. The Cervex-Brush (Rovers Medical Devices, Oss, The Netherlands) was used to obtain samples from the cervical os. The brush was immediately rinsed in a vial of PreservCyt solution (Cytoc Corporation, Marlborough, MA), and the vial was placed in the ThinPrep (Cytoc Corporation) processor. The grade for the cytological diagnosis was based on the Bethesda classification system⁹ for Papanicolaou test reports. The HC II assay was used to identify patients who were HPV DNA positive or negative, and the viral load (relative light units/positive control [RLU/PC]) was also determined.

Follow-up visits were scheduled every 4 months during the first year and every 6 months thereafter. At each visit, the patients filled out a questionnaire on lifestyle and dietary habits; a pelvic examination was performed, and cervical specimens were collected. Colposcopic examinations and histological verification were performed at baseline and during the follow-up visits in all women with a persistent diagnosis of atypical squamous cells of undetermined significance or with cytological evidence of atypical squamous cells excluding high-grade lesions, low-grade squamous intraepithelial lesions, and high-grade squamous intraepithelial lesions. For this analysis, we only included women that had positive oncogenic HPV testing results (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68).

The present analysis included 328 HPV-positive women. Among the 1487 women enrolled, we excluded 391 with a history of gynecologic cancers such as cervical cancer, ovarian cancer, or endometrial cancer, and 354 with insufficient questionnaire data. These exclusions left 742 patients that completed all measurements. Among the 742 women, HPV testing with HC II was performed in 635 women, and the 328 HPV-positive women (51.7%) were included in this analysis.

Liquid-Based Cytology

The Cervex-Brush (Rovers Medical Devices) was used to obtain samples from the cervical os. The brush was immediately rinsed in a vial of PreservCyt solution (Cytoc Corporation), and the vial was placed in the ThinPrep (Cytoc Corporation) processor.

Detection of HPV DNA

We used the HC II system (Digene) for detection of HPV. This technology is a signal-amplified hybridization antibody capture assay that uses chemiluminescent detection, with a specific HPV RNA assay probe cocktail for carcinogenic high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Cervical sampling for HPV DNA was performed with the Digene Cervical Sampler, and the HPV test was performed according to the manufacturer's instructions. Relative light units measured on a luminometer denoted the presence or absence of HPV DNA sequences in the specimen. The sample was classified as positive when the RLU/PC ratio (RLU of specimen/mean RLU of 3 controls) was 1 pg/mL or greater. When the RLU measurement was less than the cutoff value, the sample was considered to be negative for specific HPV DNA sequences or to have HPV DNA levels below the detection limit of the assay. The RLU/PC ratios also provide an approximation of the amount of HPV DNA and the HPV DNA load in the sample. The positive HPV cases were categorized into 3 equal groups according to their percentile distribution. The 3 groups were defined as follows: low, HPV DNA less than 8.76; medium, 8.76 less than or equal to HPV DNA less than 114.8; and high, 114.8 greater than or equal to HPV DNA to investigate the association between the viral load and cervical dysplasia. The positive HPV cases were also categorized into 2 equal groups according to their percentile distribution, low (1~15.5 RLU/PC) or high (≥ 15.5 RLU/PC), to evaluate the relationship between dietary supplements and the risk of cervical dysplasia and determine whether this association was modified by the HPV viral load.

Data Collection

After the clinical visit, participants were scheduled for a personal interview to be carried out at the outpatient department of obstetrics and gynecology. At the enrollment, the women were interviewed by a trained interviewer, who was blinded to each woman's disease status, using a questionnaire. The survey included demographic and background information, history of sexual activity, and reproductive and medical history. Lifestyle history such as diet, smoking, alcohol, and exercise was also included. The study

TABLE 1. Demographic data and cervical dysplasia in HPV-positive women

Characteristics	Normal (n = 166)	CIN I (n = 90)	CIN II/III (n = 72)	P
	n (%)	n (%)	n (%)	
Age, yr				
≤29	16 (9.7)	19 (21.1)	9 (12.50)	0.005
30–39	47 (28.3)	33 (36.7)	33 (45.83)	
40–49	41 (24.7)	23 (25.6)	16 (22.22)	
50–59	49 (29.5)	11 (12.2)	11 (15.28)	
≥60	13 (7.8)	4 (4.4)	3 (4.17)	
Body mass index, kg/m ²				
<18.5	12 (7.2)	9 (10.2)	6 (8.3)	0.915
18.5–23	104 (62.7)	51 (58.0)	44 (61.1)	
23–25	30 (18.1)	20 (22.7)	15 (20.8)	
>25	20 (12.0)	8 (9.1)	7 (9.7)	
Education level				
≤Middle school	29 (17.8)	13 (15.3)	14 (19.4)	0.530
High school	66 (40.5)	42 (49.4)	35 (48.6)	
≥University	68 (41.7)	30 (35.3)	23 (31.9)	
Monthly household income, USD				
<2000	34 (21.3)	23 (26.7)	18 (26.47)	0.088
2000–3999	48 (30.0)	28 (32.6)	30 (44.12)	
≥4000 USD	78 (48.7)	35 (40.7)	20 (29.41)	
Cigarette smoking				
Nonsmoker	147 (88.6)	69 (80.2)	62 (86.1)	0.214
Smoker	19 (11.6)	17 (19.8)	10 (13.9)	
Passive smoking				
No	97 (56.9)	41 (48.2)	41 (56.9)	0.334
Yes	69 (42.1)	44 (51.8)	31 (43.0)	
Alcohol consumption				
Nondrinker	74 (44.58)	17 (19.8)	29 (40.3)	
Drinker	92 (55.4)	69 (80.2)	43 (59.7)	0.001
Alcohol consumption frequency				
Nondrinker	72 (43.9)	17 (20.0)	28 (39.4)	0.004
≤1–3 times/mo	55 (33.5)	47 (55.3)	22 (31.0)	
1–2 times/wk	31 (18.9)	15 (17.7)	16 (22.5)	
≥3–4 times/wk	6 (3.7)	6 (7.0)	5 (7.0)	
Ever use oral contraceptive				
Never	136 (81.9)	74 (86.1)	52 (72.2)	0.072
Current/former	30 (18.1)	12 (13.9)	20 (27.8)	
No. childbirth				
1	19 (18.3)	13 (23.6)	12 (24.0)	0.785
2	77 (74.0)	40 (72.7)	35 (70.0)	
≥3	8 (7.7)	2 (3.7)	3 (6.0)	

participants were asked whether they had used any vitamin or mineral supplements. We also recorded each participant's routine dietary intake, with details of food habits during the year before enrollment for each participant. We used a 95-item semiquantitative food frequency questionnaire,¹⁰ including the routine frequency of consumption and typical portion sizes. The frequency of intake in the FFQ was classified into 9 categories as follows: almost never, once per month, 2 to 3 times per month, 1 to 2 times per week, 3 to 4 times per week, 5 to 6 times per week, once per day, twice per day, and 3 times per day. The standard portion size of each dish item per meal was determined using the mean amount, the typical or standard value, or the natural unit according to the Korean Ministry of Health and Welfare portion size booklet.¹¹ Specific dietary supplements were classified into 5 categories, namely, multivitamins, multinutrients, vitamin C, calcium, and miscellaneous. The brand name, frequency, and dosage of all supplements consumed by each of the subjects were reported, and the consumption level of each of the vitamins and minerals (mainly vitamins A, C, and E, and calcium) was calculated based on the composition of the supplements provided by the manufacturers. The patients that used dietary supplements were defined as subjects that used at least 1 category of a dietary supplement for 3 months or longer during the past year.

Statistical Analysis

The χ^2 test was used for the analysis of differences in the distribution of the categorical demographic variables. Multinomial logistic regression models were used to estimate the crude and multivariate odds ratios (ORs) and corresponding 95% confidence intervals (CIs) to assess whether dietary intake of dietary supplements and the HPV viral load were associated with the risk of CINs and cervical cancer. In this multinomial logistic model, women with normal cytology were the base category for the dependent variable. The model estimated 2 ORs simultaneously for each risk factor evaluated: the OR for CIN I versus normal, and CIN II/III versus normal. Potential confounders were evaluated, and these confounders were left in the multivariate analysis. The potentially confounding variables that were adjusted included age, socioeconomic status, menopausal status, parity, oral contraceptive, smoking habits, and alcohol consumption. Values for all tests were considered to be significant when $P < 0.05$.

We evaluated the association between dietary intake of multivitamins and HPV using multinomial logistic regression models with joint categories of HPV viral load (high [≥ 15.5 RLU/PC] and low [< 15.5 RLU/PC]) for multivitamins. We evaluated whether the association of dietary intake of multivitamins with the risk of CINs could be modified by the HPV viral load. Variables for joint effects were coded using a higher level of HPV viral load (≥ 15.5 RLU/PC) with no use of multivitamins as a reference group. Tests of interaction were performed using multiplicative interaction terms of the ordinal score for each genotype and multivitamin use in the model. Interactions were tested by using the log likelihood ratio test, in which the model that includes the interaction term was compared with that without the term. All

TABLE 2. Association between vitamin use and cervical dysplasia in HPV-positive women

Characteristics	Normal (n = 166)		CIN I (n = 90)		CIN II/III (n = 72)	
	n (%)	n (%)	Multivariate OR (95% CI)*	n (%)	Multivariate OR (95% CI)*	
Multivitamins						
No	89 (53.6)	60 (66.7)	1.0 (ref)	61 (84.7)	1.0 (ref)	
Yes	77 (46.4)	30 (33.3)	0.58 (0.31–1.09)	11 (15.3)	0.21 (0.09–0.48)*	
Vitamin A						
No	122 (73.5)	72 (80.0)	1 (ref)	66 (91.7)	1 (ref)	
Yes	44 (26.5)	18 (20.0)	0.67 (0.32–1.37)	6 (8.33)	0.19 (0.07–0.53)	
<i>P</i> for linear trend			0.26		0.0014*	
Vitamin C						
No	107 (64.4)	65 (72.2)	1 (ref)	62 (86.1)	1 (ref)	
Yes	59 (35.6)	25 (27.8)	0.73 (0.38–1.41)	10 (13.9)	0.24 (0.10–0.56)	
<i>P</i> for linear trend			0.34		0.0009*	
Vitamin E						
No	122 (73.5)	72 (80.0)	1 (ref)	66 (91.7)	1 (ref)	
Yes	44 (26.5)	18 (20.0)	0.66 (0.32–1.36)	6 (8.3)	0.20 (0.07–0.53)	
<i>P</i> for linear trend			0.26		0.0014*	
Calcium						
No	110 (66.3)	69 (76.7)	1 (ref)	64 (88.9)	1 (ref)	
Yes	56 (33.7)	21 (23.3)	0.62 (0.31–1.20)	8 (11.1)	0.21 (0.08–0.50)	
<i>P</i> for linear trend			0.15		0.0005*	

*Adjusted for age, socioeconomic status, smoking habit status, oral contraceptive use, alcohol consumption status, menopausal status, and number of parity.

analyses were conducted using Intercooled Stata/SE (Stata-Corp 2001, Stata Corporation, Stata Statistical Software: Release 10.0. College Station, TX).

RESULTS

Compared with the controls, the patients with cervical dysplasia tended to be younger; however, this difference was not significant. Most women did not smoke tobacco or use oral contraceptives. More than half of the participants had a positive pregnancy history. There was no significant association observed between cervical dysplasia and smoking, use of oral

contraceptives, parity, or menarche. The demographic variables associated with the HPV-positive women with cervical dysplasia (n = 328) are shown in Table 1.

The ORs for dietary supplements and cervical dysplasia (CIN I, II, and III) in HPV-positive women by the daily nutrient intake, including multivitamin use, are shown in Table 2. The analysis was based on the total dietary intake plus supplements. The findings illustrate that use of dietary supplements was associated with a reduced severity of cervical dysplasia. There was a significant association between no intake of multivitamins and more severe cervical dysplasia

TABLE 3. Association between viral load and cervical dysplasia in HPV-positive women

Characteristics	Normal (n = 166)		CIN I (n = 90)		CIN II/III (n = 72)	
	n (%)	n (%)	Multivariate OR (95% CI)*	n (%)	Multivariate OR (95% CI)*	
Viral load (RLU/PC)						
<8.76	71 (42.8)	28 (31.1)	1.0 (ref)	14 (19.4)	1.0 (ref)	
8.76–114.8	54 (32.5)	25 (27.8)	1.18 (0.60–2.31)	27 (37.5)	2.57 (1.20–5.51)	
≥114.8	41 (24.7)	37 (41.1)	1.83 (0.95–3.54)	31 (43.1)	3.32 (1.54–7.16)	
<i>P</i> for trend			0.07		0.002	

*Adjusted for age, socioeconomic status, smoking habit status, oral contraceptive use, alcohol consumption status, menopausal status, and number of parity.

TABLE 4. Association of cervical dysplasia with viral load and multivitamin use in HPV-positive women

Viral Load		CIN I		CIN II/III	
		Multivitamin Use		Multivitamin Use	
		No	Yes	No	Yes
High (≥ 15.5)	n (control/case)	(40/36)	(33/15)	(40/43)	(33/6)
	OR (95% CI)*	1.0 (ref)	0.53 (0.25–1.16)	1.0 (ref)	0.17 (0.07–0.46)
	Multivariate OR (95% CI) [†]	1.0 (ref)	0.49 (0.21–1.13)	1.0 (ref)	0.15 (0.05–0.43)
Low (< 15.5)	n	(39/15)	(35/10)	(39/10)	(35/4)
	OR (95% CI)*	0.44 (0.20–0.94)	0.34 (0.15–0.81)	0.24 (0.11–0.55)	0.12 (0.04–0.37)
	Multivariate OR (95% CI) [†]	0.53 (0.24–1.19)	0.35 (0.14–0.87)	0.27 (0.11–0.66)	0.11 (0.04–0.37)
Interaction <i>P</i>			0.925		0.304

*Adjusted for age.

[†]Adjusted for age, socioeconomic status, smoking habit status, oral contraceptive use, alcohol consumption status, menopausal status, and number of parity.

(CIN II/III) (multivariate adjusted OR of 0.21 and 95% CI of 0.09–0.48). An inverse association was observed between vitamin A, vitamin C, vitamin E, and calcium and CIN II/III, with a multivariate adjusted OR of 0.19 (95% CI, 0.07–0.53; $P_{\text{trend}} = 0.0014$), 0.24 (95% CI, 0.10–0.56; $P_{\text{trend}} = 0.0009$), 0.20 (95% CI, 0.07–0.53; $P_{\text{trend}} = 0.0014$), and 0.21 (95% CI, 0.08–0.50; $P_{\text{trend}} = 0.0005$), respectively.

When the HPV DNA load and the diagnosis of cervical dysplasia were considered together in the HPV DNA-positive women, there was a significant association between the HPV DNA load and the grade of cervical dysplasia (Table 3). Compared with the subjects who did not take multivitamins and had a higher HPV viral load (≥ 15.5 RLU/PC), those who did use multivitamins and had a lower HPV viral load (< 15.5 RLU/PC) had a significantly decreased frequency of CIN I (OR, 0.35; 95% CI, 0.14–0.87; interaction $P = 0.925$) and CIN II/III (OR, 0.11; 95% CI, 0.04–0.37; interaction $P = 0.304$). Similarly, we observed a significantly decreased risk of CIN II/III (OR, 0.15; 95% CI, 0.05–0.43; interaction $P = 0.304$) among subjects that used multivitamins and had a higher HPV viral load (≥ 15.5 RLU/PC). However, the association of dietary intake and multivitamin use with HPV viral load and the risks for CINs was not statistically significant in patients with CIN I and CIN II/III (Table 4).

DISCUSSION

HPV infection alone does not lead to cervical neoplasia; other factors, such as the patient's nutritional status, play a role in cervical carcinogenesis.³ Dietary guidelines for the prevention of cervical cancer recognize the importance of antioxidants and have recommended an increase in the consumption of fruits and vegetables as good sources of dietary antioxidants.¹² However, to date, diet has not been consistently observed to play a role in the development of cervical cancer and its precursors. Several bioactive food components, including vitamins and minerals, have been investigated for their ability to affect cancer risk. However, a few large, randomized, placebo-controlled clinical trials of dietary supplements and cancer have been performed. The re-

sults of most large-scale trials of dietary supplements used to prevent cancer have been mixed.¹³ Most of the studies reporting on dietary supplements included breast cancer, colorectal cancer, and prostate cancer.¹⁴ One case-control study showed that the duration of dietary supplements was strongly correlated with a reduced risk of carcinoma in situ of cervical cancer.¹⁵

In our study, we concentrated on the effects of diet supplements, especially intake of multivitamins, vitamin A, vitamin C, vitamin E, and calcium on high-risk HPV infections and cervical carcinogenesis. Prior studies have reported on the association between the high-risk HPV DNA viral load and degree of cervical dysplasia.^{16,17} A persistently high viral load was correlated with the progression of cervical dysplasia.¹⁷ The dose-response relationship between the viral load and degree of cervical dysplasia has also been reported in another study.¹⁸ However, there are studies that have not shown an association between the viral load and the severity of the cervical dysplasia.¹⁹

Overall, HPV 16, 31, 52, and 58 have been associated with the highest viral load. Women with single infections had higher viral loads than those with multiple-type infections. The viral load was independently associated with the severity of the cervical lesions.²⁰ Recent studies suggest that the HPV 16 viral load may be a possible molecular marker for the course of HPV infections. Using semiquantitative measurements of HPV 16 DNA levels, several observational studies have found a high correlation between a high viral load and high-grade intraepithelial lesions.¹⁶ One cohort study showed that a persistent infection with HPV 16 along with high viral load values was a risk factor for the progression to cervical cancer.²¹ However, the underlying mechanism explaining the association of viral load to cervical lesion remains obscure. The findings of this study also show a strong association between high-risk HPV infection and CIN. We observed that the HPV viral load was associated with the severity of the cervical dysplasia. The intake of dietary supplements such as multivitamins, vitamin A, vitamin C, vitamin E, and calcium was inversely associated with cervical dysplasia.

No prior study has shown a correlation between HPV viral load with cervical dysplasia and dietary supplements. Protective effects have been reported for specific dietary and plasma nutrients, including tocopherols, carotenoids, vitamin C, and folate.²² However, the results of epidemiological studies have been inconsistent with respect to the relationship between specific nutrients and cervical neoplasia. We explored the relationship of cervical dysplasia with the viral load and dietary supplements. Our findings showed that oral intake of dietary supplements seemed to have a protective effect in preventing the progression of cervical dysplasia in women with HPV, especially in patients with a higher viral load. However, a significant association between dietary supplements and viral load was not observed. Our results suggest that the dietary supplements and HPV are thought to affect cervical carcinogenesis independently.

Although HPV infection is necessary for carcinogenesis, cofactors such as long-term use of oral contraceptives and smoking increase the risk of progression from infection to cancer. It is interesting that most women in this study did not smoke or have a history of oral contraceptive use, both of which are known cofactors in the epidemiology of cervical cancer. In Korean women, the frequency of smoking and oral contraceptive use is low compared with women in western countries. This is different from studies conducted in other parts of the world.

This is the first study to report on an association between cervical dysplasia in women with high-risk HPV, the HPV DNA load, and dietary supplements. A history of dietary supplements was associated with a decreased risk of CIN I and CIN II/III in women with a higher viral load. However, the results of the present investigation should be considered preliminary as they are limited by the relatively small sample size. HPV is also a transient infection in most women; only a minority will go on to develop persistent infections. Cervical intraepithelial neoplasia I in particular can spontaneously regress. Larger studies are needed for confirmation of these findings before the results can be generalized to a broader population. Although we found a strong association between cervical dysplasia and high-risk HPV, DNA load, and dietary supplements, there were limitations with regard to the evaluation of the HPV status. Additional studies are needed to test whether there are similar associations between dietary supplements and specific types of high-risk HPV.

REFERENCES

1. Franco EL, Rohan TE, Villa LL. Epidemiologic evidence and human papillomavirus infection as a necessary cause of cervical cancer. *J Natl Cancer Inst.* 1999;91:506–511.
2. Koutsky LA, Holmes KK, Critchlow CW, et al. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. *N Engl J Med.* 1992;327:1272–1278.
3. Schiffman MH, Brinton LA. The epidemiology of cervical carcinogenesis. *Cancer.* 1995;76:1888–1901.
4. Nagata C, Shimizu H, Yoshikawa H, et al. Serum carotenoids and vitamins and risk of cervical dysplasia from a case-control study in Japan. *Br J Cancer.* 1999;81:1234–1237.
5. Schiff MA, Patterson RE, Baumgartner RN, et al. Serum carotenoids and risk of cervical intraepithelial neoplasia in southwestern American Indian women. *Cancer Epidemiol Biomarkers Prev.* 2001;10:1219–1222.
6. Goodman MT, Kiviat N, McDuffie K, et al. The association of plasma micronutrients with the risk of cervical dysplasia in Hawaii. *Cancer Epidemiol Biomarkers Prev.* 1998;7:537–544.
7. Lehtinen M, Luostarinen T, Youngman LD, et al. Low levels of serum vitamins A and E in blood and subsequent risk for cervical cancer: interaction with HPV seropositivity. *Nutr Cancer.* 1999;34:229–234.
8. Sedjo RL, Roe DJ, Abrahamsen M, et al. Vitamin A, carotenoids, and risk of persistent oncogenic human papillomavirus infection. *Cancer Epidemiol Biomarkers Prev.* 2002;11:876–884.
9. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA.* 2002;287:2114–2119.
10. Kim YO, Kim MK, Lee SA, et al. A study testing the usefulness of a dish-based food-frequency questionnaire developed for epidemiological studies in Korea. *Br J Nutr.* 2009;101:1218–1227.
11. Korea Health Industry Development Institute, Ministry of Health and Welfare. *Development of Nutrient Database, Recipe, and Portion Size* [In Korean]. Seoul, Korea: Korea Health Industry Development Institute, Ministry of Health and Welfare; 2000.
12. Norman HA, Butrum RR, Feldman E, et al. The role of dietary supplements during cancer therapy. *J Nutr.* 2003;133:3794S–3799S.
13. Greenwald P, Anderson D, Nelson SA, et al. Clinical trials of vitamin and mineral supplements for cancer prevention. *Am J Clin Nutr.* 2007;85:314S–317S.
14. Velicer CM, Ulrich CM. Vitamin and mineral supplement use among US adults after cancer diagnosis: a systematic review. *J Clin Oncol.* 2008;26:665–673.
15. Ziegler RG, Jones CJ, Brinton LA, et al. Diet and the risk of in situ cervical cancer among white women in the United States. *Cancer Causes Control.* 1991;2:17–29.
16. Sun CA, Liu JF, Wu DM, et al. Viral load of high-risk human papillomavirus in cervical squamous intraepithelial lesions. *Int J Gynaecol Obstet.* 2002;76:41–47.
17. Ylitalo N, Sorensen P, Josefsson AM, et al. Consistent high viral load of human papillomavirus 16 and risk of cervical carcinoma in situ: a nested case-control study. *Lancet.* 2000;355:2194–2198.
18. Schlecht NF, Trevisan A, Duarte-Franco E, et al. Viral load as a predictor of the risk of cervical intraepithelial neoplasia. *Int J Cancer.* 2003;103:519–524.
19. Lorincz AT, Castle PE, Sherman ME, et al. Viral load of human papillomavirus and risk of CIN3 or cervical cancer. *Lancet.* 2002;360:228–229.
20. Flores R, Papenfuss M, Klimecki WT, et al. Cross-sectional analysis of oncogenic HPV viral load and cervical intraepithelial neoplasia. *Int J Cancer.* 2006;118:1187–1193.
21. Josefsson AM, Magnusson PK, Ylitalo N, et al. Viral load of human papilloma virus 16 as a determinant for development of cervical carcinoma in situ: a nested case-control study. *Lancet.* 2000;355:2189–2193.
22. Hernandez BY, McDuffie K, Wilkens LR, et al. Diet and premalignant lesions of the cervix: evidence of a protective role for folate, riboflavin, thiamin, and vitamin B12. *Cancer Causes Control.* 2003;14:859–870.