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Folate Is Associated with the Natural History of High-Risk Human Papillomaviruses

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ABSTRACT

Several micronutrients have been implicated in cervical carcinogenesis. However, their mode of action is still a matter of speculation. In particular, it is unclear whether certain nutrients reduce the probability of acquiring high-risk human papillomavirus (HPV) or whether they facilitate the clearance of high-risk HPV. We conducted a 24-month prospective follow-up study to test the hypothesis that systemic concentrations of folate are associated with the occurrence and duration of high-risk HPV infections after controlling for other micronutrients (vitamins B_{12}, A, E, and C, total carotene) and known risk factors for high-risk HPV infections and cervical cancer. Circulating concentrations of these micronutrients and risk factors for cervical cancer were determined in a cohort of 345 women who were at risk of developing cervical intraepithelial neoplasia. Using the hybrid capture 2 (HC-2) assay, high-risk HPV status was evaluated at 6-month intervals up to 24 months. All women had at least three consecutive visit high-risk HPV test results. Higher folate status was inversely associated with becoming HC-2 test-positive [odds ratio (OR): 0.27; 95% confidence interval (CI), 0.08–0.91; $P = 0.04$]. Women with higher folate status were significantly less likely to be repeatedly HC-2 test-positive (OR: 0.33; 95% CI, 0.13–0.86; $P = 0.02$) and more likely to become test-negative during the study (OR: 2.50; 95% CI, 1.18–5.30; $P = 0.02$). To our knowledge, this is the first long-term prospective follow-up study reporting an independent protective role of higher folate status on several aspects of the natural history of high-risk HPV after controlling for known risk factors and other micronutrients. Improving folate status in subjects at risk of getting infected or already infected with high-risk HPV may have a beneficial impact in the prevention of cervical cancer.

INTRODUCTION

Worldwide, cervical cancer is second only to breast cancer as the most common malignancy in both incidence and mortality (1, 2). In the United States, it accounted for an estimated 12,900 new cases and 4,400 deaths in 2001 (3). The introduction of the Pap smear test by George Papanicolaou in 1943 allowed for the study of precursor lesions of cervical cancer and has led to cervical dysplasia becoming one of the most widely studied precancers (4). In much of the world, cervical cancer is diagnosed in women in their thirties and has a major impact on the stability and social adhesion of families. The incidence of cervical cancer is likely to increase over the next several decades as women in the developing world begin to age and as the number of women infected with HIV increases (5). Cervical intraepithelial neoplasia (CIN), which precedes cervical cancer, have also reached epidemic proportions, with an estimate of at least 600,000 new cases per year in the United States (6).

Although an infection with high-risk human papillomavirus (HPV) is probably a necessary cause for CIN and cervical cancer (7–9), most of the epidemiologic evidence comes from retrospective, case-control studies, which do not shed light on the dynamics of cervical HPV infection and its progression to cancer (10). Recent interest in assessing the potential for screening high-risk HPV as a replacement or augmentation of cervical cytology and in developing HPV vaccines have compelled the initiation of longitudinal studies of the natural history of HPV infections and cervical lesions (11–17).

Factors that may play a role in HPV acquisition include a higher number of sexual partners, high frequencies of vaginal sex, alcohol consumption and certain characteristics of partners (12), sexual behavior (rate of new partners), non-white race and use of hormonal contraceptives (18), younger age, Hispanic or black ethnicity, history of herpes simplex virus infection, and history of vulvar warts (11). It is likely that many of these factors are indicators of exposure to HPV-infected partners or of increased susceptibility to transmission. Most high-risk HPV infections are transient because of its clearance from the affected tissues. Although the factors that may enhance or influence the clearance of high-risk HPV are important in reducing the risk associated with this virus, lifestyle factors that govern HPV clearance are poorly understood.

Infection with high-risk and multiple types of HPV and older age have been shown to be risk factors for HPV persistence (18). In turn, persistent HPV infection is a likely mechanism of persistence of cervical dysplasia (19–21) and the progression of HPV-infected lesions to cervical cancer (15). The associations with persistent infection and multiple types of infection could be associated with certain characteristics of these subjects, which predispose them to persistent infection. Women who are immunosuppressed by infection with the HIV are at risk for infection with multiple types of HPV (22). It is also possible that the reduced immunocompetence associated with deficiencies of micronutrients, including folate (23, 24), is likely to modify the natural history of HPV infections.

Several short-term studies assessing limited numbers of nutrients to evaluate the significance of micronutrients in the natural history of HPV have resulted in inconsistent results. Effects of higher concentrations of *trans*- and *cis*-lycopene in reducing the time to clearance of oncogenic HPV infections in United States women (25), an association between lower serum β-carotene, β-cryptoxanthin, and lutein concentrations and HPV persistence among United States Hispanics (26), and an association between low plasma vitamin B_{12} concentrations and persistent HPV infection among Hispanic women (27) have been reported. A small study among Hispanic women, however, did not support a role for folate, vitamin B_{12}, or homocysteine in HPV persistence or cervical dysplasia (28). Palan *et al.* (29) found no associations among circulating concentrations of retinal, α- and β-carotene, or lycopene and persistent HPV infections. The aforementioned studies were shorter term (3 to 10 months) than our study (24 months), only tested their study subjects for high-risk HPV at two time points, and focused on one aspect of the natural history of high-risk HPV (persistence or clearance) in a given study. These individual studies were also not designed to investigate a combination of antioxidants,

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folate, and vitamin B_{12} in order test the relationship between specific micronutrients and the natural history of high-risk HPV after controlling for confounding by other important micronutrients.

We undertook a study intended to be the first comprehensive long-term (24-month) prospective follow-up evaluation of the influence of folate on the natural history of HPV. The study used the hybrid capture 2 (HC-2) assay to classify subjects as positive or negative for high-risk HPV. The study was designed to evaluate the associations between folate and the (*a*) likelihood of becoming HC-2 test-positive (incidence of high-risk HPV), (*b*) repeated HC-2–positive test (persistence of high-risk HPV), and (*c*) likelihood of becoming test-negative after an infection (clearance of high-risk HPV) after controlling for other specific micronutrients (vitamins B_{12}, C, A, and E and carotenoids).

Study Design. The subjects were a partial cohort from a total of 1549 women recruited for the Atypical Squamous Cells of Undetermined Significance–Low-Grade Squamous Intraepithelial Lesion Triage Study at the clinical center at Birmingham, Alabama, who also agreed to participate in an ancillary study of nutrient interactions and risk of developing CIN. The Atypical Squamous Cells of Undetermined Significance–Low-Grade Squamous Intraepithelial Lesion Triage Study was designed to determine the optimal clinical management for low-grade cervical cytological abnormalities. Clinical centers located in Birmingham, Alabama, Oklahoma City, Oklahoma, Pittsburgh, Pennsylvania, and Seattle, Washington, were established to enroll and provide follow-up care to women with cytology diagnoses of atypical squamous cells of undetermined significance and low-grade squamous intraepithelial lesion from referring community laboratories. Recruitment began in October 1996 and ended in December 1998. Subjects were followed every 6 months for 2 years from the date of recruitment through December 2000. A more detailed description of the Atypical Squamous Cells of Undetermined Significance–Low-Grade Squamous Intraepithelial Lesion Triage Study is published elsewhere (30).

Our study was performed after approval by the Institutional Review Board at the University of Alabama at Birmingham and the Atypical Squamous Cells of Undetermined Significance–Low-Grade Squamous Intraepithelial Lesion Triage Study Ancillary Studies Committee. Informed consent was obtained from each subject. At the enrollment visit, blood samples (5 mL, EDTA) were obtained from 720 women who consented to participate in the ancillary study, and a brief questionnaire was administered to assess the use of vitamin supplements. The administration of a risk-factor questionnaire, pelvic examinations, collection of specimens for cytology and high-risk HPV testing by HC-2 assay, and cervicography were carried out by the main Atypical Squamous Cells of Undetermined Significance–Low-Grade Squamous Intraepithelial Lesion Triage Study personnel, and the results were made available for the ancillary study. All women were examined every 6 months for a total of 24 months. HPV testing was planned at each visit. One subject was excluded because of incorrect referral diagnosis. Ten subjects of races other than whites and African Americans were excluded to reduce potential variations in results by races other than the two main races. Eighty-one women who were treated for CIN2/3 at the initial visit and 91 women treated for similar lesions during the follow-up were also excluded. Of the remaining 537 women, 345 women had at least three consecutive visit high-risk HPV test results. Among these 345 women, 70 women were high-risk HPV test positive at all visits, whereas 50 subjects were high-risk HPV negative at all visits. A total of 114 subjects was high-risk HPV test-positive at more than or equal to two consecutive visits (Fig. 1).

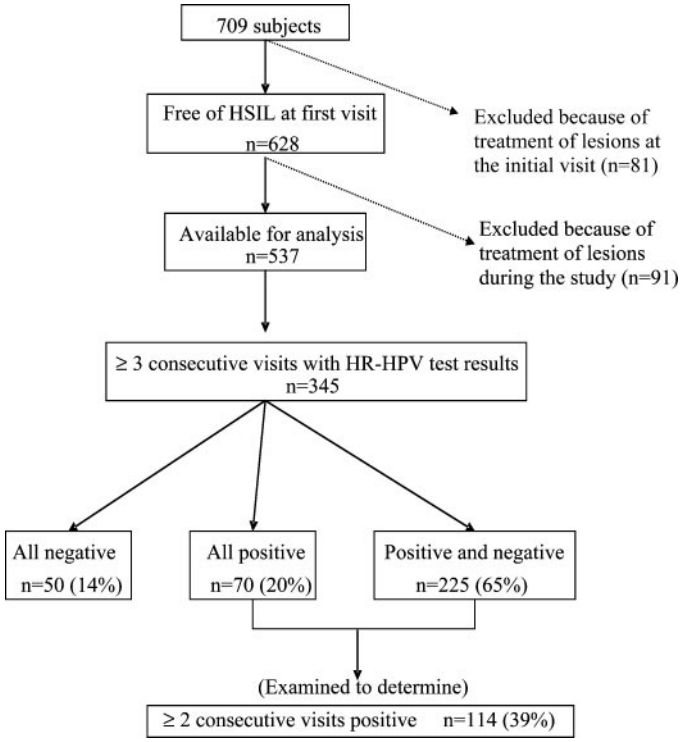

Fig. 1. Distribution of study subjects by CIN and high-risk (HR)-HPV status.

Outcome Classifications. Three different outcomes were compared during the follow-up period: (*a*) becoming test-positive *versus* remaining test-negative (incident infection); (*b*) being repeatedly test-positive *versus* remaining test-negative; and (*c*) becoming test-negative *versus* remaining test-positive during the study period (clearing infection). The outcomes were determined as follows: (*a*) becoming HC-2 test-positive (acquisition of high-risk HPV infection) was classified as having at least one negative test result (at enrollment) or several negative test results (at enrollment and 6-, 12-, and 18-month visit) and then becoming test-positive at least once thereafter and remained positive for the remainder of the study; (*b*) repeatedly test-positive status was classified as having a positive test result at all visits or at more than equal to two consecutive visits during the 24-month follow-up; and (*c*) becoming test-negative (clearance of high-risk HPV infection) was classified as having a positive test result at enrollment or at enrollment and 6, 12, and 18 months and then become test-negative for the remainder of the study.

Twenty-five individuals who became test-positive also had subsequent HPV test-positive results for more than or equal to two consecutive visits. These twenty-five individuals were included in analyses for both becoming test-positive and being repeatedly test-positive. Thirty-six individuals who were HPV positive for more than or equal to two consecutive visits and then became test-negative at subsequent visits were included in analyses for both being repeatedly test-positive and becoming test-negative. Thirty-four individuals who had more than or equal to two consecutive visits test-positive were also in the all visits test-positive group and were included in both analyses.

Laboratory Methods. After collection, blood samples were stored in a refrigerator until transported on ice for processing in our laboratory. All samples were processed for specific assays within 2 hours of sample collection and were stored at −80°C until assays were performed. Concentrations of plasma and RBC folate, vitamins B_{12} (radio-binding assays), A, E, and C (high-performance liquid chromatography), and total carotene (spectrophotometric) were measured with protocols routinely used in our laboratory (31, 32). To monitor the reproducibility of these assays, two pooled samples (low and high)

prepared from plasma obtained from the American Red Cross were assayed at least 30 times, and the means and SDs were determined. These served as the basis for the quality control for the assays (the study sample assays were repeated if the values of the control samples in each run were >2 SDs from the mean). The low and high controls were included in every run of the assays. The coefficient of variation and recovery of these assays were 5 to 7% and 96 to 105%, respectively. All assays were performed within a month of sample collection. HPV test results or the diagnoses of the study participants were unknown at the time of vitamin assays performed. The HC-2 assay (Digene Corporation, Gaithersburg, MD), selected by the Atypical Squamous Cells of Undetermined Significance–Low-Grade Squamous Intraepithelial Lesion Triage Study investigators, detected 13 high-risk HPV subtypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and classified patients as positive or negative for high-risk HPV.

Statistical Analysis. The analyses were limited to 345 subjects who had at least three consecutive visits with complete HPV test results because it was only in this population that we could be sure of an accurate outcome classification. In individuals with incomplete information, it is impossible to determine the meaning of a missing visit with regard to HPV status, and this uncertainty could lead to misclassification of the outcome. To address the issue of potential bias introduced by inclusion of 345 women with at least three consecutive visits, we compared the group included with the group excluded ($n = 192$) with regard to demographic, risk and behavioral factors and plasma concentrations of micronutrients. Descriptive statistics such as mean, median, SD of the mean, and range were computed to examine the differences between these variables. Frequency distributions, χ^2 test, Kruskal-Wallis test, and other univariate analysis methods were used to carry out comparisons within the study groups. The cutoffs used in specific analyses were based on the values for the entire study population. Unconditional logistic regression models with a binary indicator of outcome (became test-positive yes/no, repeatedly test-positive yes/no, and became test-negative yes/no) as the dependent variables and folate concentrations as independent predictors were fit while adjusting for age, total number of lifetime sexual partners, smoking status (current *versus* noncurrent), and other micronutrient concentrations. Preliminary analyses assessed the relation of the outcomes with categories indicating the tertiles of the concentration of folate (plasma and RBC), vitamins B_{12}, C, A, and E, and total carotene. We determined that a more parsimonious binary categorization of micronutrient concentrations (more than or equal to the population median *versus* being below the median) was equivalent to the tertiles for the purpose of adjustment and more precise for estimation of odds ratios (ORs). We also computed a combined binary indicator of folate status (more than or equal to median concentrations of both plasma and RBC folate *versus* below the median for either). Separate regression models were run, including alternative measures of blood folate as independent variables: we evaluated models with plasma folate, RBC folate, and a combined measure of RBC and plasma folate. The decision to include other variables as potential confounders in regression models was based on our review of the literature regarding natural history of high-risk HPV infections and included factors that have been reported as potential confounders in other studies. Because the literature is richer in evaluations of potential risk factors for CIN and cervical cancer, we also assessed whether known risk factors for cervical cancer were associated with micronutrient concentrations, and if so, we included them in the main analysis. All statistical analyses were conducted using the SAS version 8.2 (SAS Institute, Cary, NC).

RESULTS

As shown in Table 1, there were significantly more African Americans among the subjects included in the analyses *versus* those excluded. Adjustment by race, however, did not appreciably change the reported point estimates. The subjects included in the study also had more pregnancies that ended in live births. No differences in distributions of plasma folate, vitamin A, vitamin E, or total carotene were found between the subjects included in the analysis ($n = 345$) and the subjects excluded from analysis ($n = 192$; Table 2). A difference between these groups was found, however, in the median concentration of vitamin C ($P = 0.04$). Vitamin C is not considered a significant predictor of any of the three outcomes studied, but because of this difference, we controlled for it in the analyses.

Alternative regression models for plasma folate, RBC folate, and a combined measure of RBC and plasma folate yielded similar results, but the combined measure of folate status was a stronger predictor of the natural history of high-risk HPV, and these results are presented in

Table 1 *Descriptive statistics of study participants with three or more consecutive visits and fewer than three consecutive visits*

Variables	Study participants with three or more consecutive visits*N* (%)	Study participants with fewer than three consecutive visits*N* (%)	*P* *
Total	345	192	
Age group			
<20 y	52 (15.1)	32 (16.7)	
20–29 y	114 (35.0)	68 (35.4)	
30–39 y	66 (19.1)	41 (21.4)	
40–49 y	41 (11.9)	26 (13.5)	
≥50 y	72 (20.9)	25 (13.0)	0.27
Mean ± SD (median)	27.6 ± 8.4 (25.0)	26.4 ± 7.5 (24.0)	0.17
African American	240 (69.6)	108 (56.2)	0.002
Current smoker	90 (26.1)	58 (30.2)	0.31
Number of pregnancies that ended in live births			
None	50 (14.5)	48 (25.0)	
One	120 (34.8)	62 (32.3)	
Two or more	175 (50.7)	82 (42.7)	0.009
Used hormonal contraceptives	238 (69.0)	138 (71.9)	0.48
Ever had a sexually transmitted disease	161 (46.7)	82 (42.7)	0.32
Under age 18 at first intercourse	304 (88.1)	175 (91.2)	0.28
Estimated lifetime number of sexual partners			
Not reported	1 (0.3)		
<10	272 (79.1)	160 (83.3)	
10–20	65 (18.8)	28 (14.6)	
>20	7 (2.0)	4 (2.1)	0.45
Used vitamin supplements	116 (33.6)	51 (26.6)	0.09

NOTE. * Two-sided *P* for Kruskal-Wallis test when comparing medians and two-sided *P* for χ^2 when comparing proportions. Statistically significant at $\alpha = 0.05$.

Table 2 *Micronutrient concentrations of the study populations with three or more consecutive visits and fewer than three consecutive visits*

Micronutrients	Study participants with three or more consecutive visits (n = 345) Mean ± SD (median)	Study participants with fewer than three consecutive visits (n = 192) Mean ± SD (median)	P *
Plasma folate (nmol/L)	22.2±10.4 (20.4)	23.8±18.9 (21.1)	0.19
RBC folate (nmol/L)	894.2±265.1 (847.5)	914.6±283.7 (858.4)	0.57
Vitamin B-12 (pmol/L)	383.5±176.8 (355.1)	356.4±151.3 (321.0)	0.08
Vitamin C (μmol/L)	62.5±62.5 (51.1)	56.8±68.1 (45.4)	0.04
Vitamin A (μmol/L)	1.4±0.5 (1.3)	1.4±0.6 (1.3)	0.29
Vitamin E (μmol/L)	18.6±39.5 (16.3)	23.2±97.5 (16.3)	0.83
Total carotene (μmol/L)	1.4±0.5 (1.3)	1.4±0.5 (1.3)	0.97

* Two-sided P for Kruskal-Wallis test when comparing medians. Statistically significant at $\alpha = 0.05$.

Table 3 *Associations between micronutrient concentrations and becoming HC-2 test–positive during the study*

	Becoming test-positive	
	Adjusted OR *	95% CI
Plasma folate (≥ 20.4 nmol/L) and RBC folate (≥847.5 nmol/L)	0.27	0.08–0.91
Vitamin B-12 (> 341.5 pmol/L)	1.46	0.51–4.18
Vitamin C (>45.4 μmol/L)	3.23	(0.93–11.16)
Vitamin A (>1.3 μmol/L)	0.48	0.16–1.46
Vitamin E (>16.3 μmol/L)	0.78	0.24–2.57
Total carotene (> 1.3 μmol/L)	1.43	0.44–4.66
Having more than three sexual partners (lifetime)	1.64	0.43–6.26
Current smoker	0.96	0.26–3.58
Age at enrollment (y)	0.96	0.90–1.02

NOTE. Subjects who were test-negative at the first visit and became test–positive during the follow-up period (n = 37) were compared with women who remained test–negative during the follow-up period (n = 50).

* Multivariable adjustment for age, number of lifetime partners, smoking status, and micronutrient concentrations.

detail here. The population median concentrations for plasma and RBC folate were 20.4 nmol/L (9 ng/mL) and 847.5 nmol/L (374 ng/mL), respectively. The estimates for folate status were similar in models, including alternative categorization schemes for other micronutrients.

The Association between Folate and Becoming Test-Positive. In this analysis, subjects who were test-negative at the first visit and became test-positive during the follow-up period (n = 37) were compared with women who remained test-negative during the follow-up period (n = 50). The results are shown in Table 3. Subjects with higher folate status were 73% less likely to become test-positive during the study period [OR: 0.27, 95% confidence interval (CI), 0.08–0.91; P = 0.04].

The Association between Folate and Being Repeatedly Test-Positive. The association between folate and being repeatedly test positive was examined by looking at women who were test-positive for all visits (n = 70) and women who were test positive for two or more consecutive visits (n = 114). The comparison group for both analyses consisted of women who were repeatedly negative for the study period (n = 50). Subjects higher folate status were at lower risk of being test-positive at all visits (OR: 0.33; 95% CI, 0.13–0.86; P = 0.02; Table 4). When repeatedly test-positive status was defined as more than or equal to two consecutive visits test-positive, subjects with higher folate status were less likely to be repeatedly test-positive when compared with lower folate status during the study period (OR: 0.39; 95% CI, 0.17–0.89; P = 0.02).

The Association between Folate and Becoming Test-Negative. In this analysis, all subjects who were test-positive initially and who became and remained test-negative during follow-up (n = 75) were compared with subjects who remained test-positive during the follow-up period (n = 70; Table 5). Women with higher folate status were more likely to become test-negative (OR: 2.50; 95% CI, 1.18–5.30; P = 0.02).

DISCUSSION

Although HPV testing by the HC-2 assay is approved for routine care in the United States, there are no treatments currently available to treat HPV itself, causing anxiety for both patients and clinicians. Our study was designed to evaluate whether folate is associated with a lower likelihood of a woman becoming high-risk HPV positive by the HC-2 assay, and of having a repeated positive test, and a greater likelihood of becoming test-negative.

There have not been carefully conducted epidemiologic studies designed to examine where in the cervical carcinogenesis continuum nutrients such as folate and vitamin B_{12} may influence the natural history of the disease. Incorporation of reliable high-risk HPV assessments, comprehensive risk factor information, including smoking histories and oral contraceptive use, and biomarkers of disease with a prospective study design are critical for the success of future nutrient-based chemoprevention trials of cervical cancer. We demonstrated that higher folate status is significantly and inversely associated with becoming positive for high-risk HPV and with positive test status. Higher folate status is also positively associated with becoming test-negative. These associations hold after controlling for other micronutrients and other known risk factors for cervical cancer.

Folate could modify the risk associated with high-risk HPV in

Table 4 *Associations between micronutrient concentrations and being repeatedly HC-2 test-positive at all visits during the study*

	All visits positive	
	Adjusted OR *	95% CI
Plasma folate (≥20.4 nmol/L) and RBC folate (≥847.5 nmol/L)	0.33	0.13–0.86
Vitamin B-12 (>341.5 pmol/L)	1.79	0.70–4.54
Vitamin C (>45.4 μmol/L)	2.29	0.81–6.53
Vitamin A (>1.3 μmol/L)	0.83	0.32–2.13
Vitamin E (>16.3 μmol/L)	0.85	0.29–2.47
Total carotene (>1.3 μmol/L)	0.82	0.29–2.34
Having more than three sexual partners (lifetime)	2.27	0.75–6.84
Current smoker	2.43	0.77–7.64
Age at enrollment (y)	0.89	0.84–0.95

NOTE. Subjects who were test-positive for all visits (n = 70) were compared with subjects who were repeatedly test-negative during the study period (n = 50).

* Multivariable adjustment for age, number of lifetime partners, smoking status, and micronutrient concentrations.

Table 5 *Associations between micronutrient concentrations and becoming HC-2 test-negative during the study period*

	Becoming test-negative	
	Adjusted OR *	95% CI
Plasma folate (≥20.4 nmol/L) and RBC folate (≥847.5 nmol/L)	2.50	1.18–5.30
Vitamin B-12 (>341.5 pmol/L)	1.14	0.53–2.46
Vitamin C (>45.4 μmol/L)	1.12	0.53–2.38
Vitamin A (>1.3 μmol/L)	1.22	0.58–2.60
Vitamin E (>16.3 μmol/L)	1.85	0.82–4.14
Total carotene (>1.3 μmol/L)	0.96	0.44–2.09
Having more than three sexual partners (lifetime)	1.04	0.39–2.76
Current smoker	0.98	0.44–2.17
Age at enrollment (y)	0.97	0.92–1.03

NOTE. Subjects who were test-positive initially and became and remained test negative during follow-up (n = 75) were compared with subjects who remained test-positive during the follow-up period (n = 70)

* Multivariable adjustment for age, number of lifetime partners, smoking status, and micronutrient concentrations.

several ways. Reduced immunocompetence associated with folate deficiency (23, 24) could increase the risk of infection with multiple types or higher viral loads of high-risk HPV. This is likely to increase the acquisition of high-risk HPV types, their persistence, and their integration into the host genome. A common chromosomal fragile site that is sensitive to folate deficiency has been shown to coincide with a site of HPV-16 integration in the tissues of primary cervical carcinomas (33) and three of the four sites at which HPV-18 integrates its DNA into the host (34), suggesting a plausible mechanism through which suboptimal folate concentrations could increase the risk for cervical cancer (35). In contrast to advanced lesions and cervical cancer (36), integration of HPV DNA into the host genome has been considered a rare event in early preneoplastic lesions of the cervix. Recent studies that used novel quantitative real-time PCR techniques, however, have demonstrated that integration of HPV may occur in early CIN lesions (37) and even in those containing HPV but no CIN (38). Also, rapid progression, in 1 to 2 years, from non-CIN lesions or CIN 2 to CIN 3 was shown to be associated with a heavy load of integrated HPV. Although the mechanisms of HPV integration and cervical carcinogenesis are poorly understood, it is possible that HPV DNA that remains episomal would be more likely expelled, resulting in clearance of HPV infection. A decrease in persistent HPV infection and increased clearance of HPV in subjects with high folate may result from preventing its integration.

Cigarette smoking is an independent risk factor for infection with high-risk HPV (39) and enhances the progression of HPV-infected cervical cells toward neoplasia (40). It has been suggested that smoking induces suppression of local immune response and facilitates persistent HPV infection (41). Smoking status was accounted for in the analysis of this study. However, smoking may have contributed indirectly by creating localized folate deficiency in the cervix due to biological inactivation of folate by exposure to cigarette smoke. We have previously documented that buccal mucosal concentrations of folate are significantly lower in cigarette smokers compared with nonsmokers and that smoking-related cancerous tissues of the lung have significantly lower folate concentrations compared with normal lung tissues (31, 32).

This study demonstrated that higher folate status may reduce the risk of cervical cancer by influencing the natural history of high-risk HPV infection. The validity of these results is enhanced by a sensitive assessment of HPV status, measurement of circulating concentrations of multiple cancer-protective micronutrients and potential confounding factors such as smoking, oral contraceptive, and other risk factors for cervical cancer, and vitamin supplement use in a prospective follow-up design. Since food fortification with folate became mandatory in 1998 in the United States, one could argue that adverse effects of low folate on cancer risk may not be relevant in the future. The median plasma folate in this population was 20.4 nmol/L, reflecting a postfortification level of folate status (42). Because higher than median postfortification plasma concentrations of folate were associated with the natural history of high-risk HPV, our data suggest that the current level of folate fortification is inadequate to provide an optimally protective effect against cervical cancer. Other results from our ancillary study demonstrated that high folate concentrations are also independently protective against the development of CIN 2 and 3 (35). The details of these associations will be published in a separate article after the main Atypical Squamous Cells of Undetermined Significance–Low-Grade Squamous Intraepithelial Lesion Triage Study reports the incidence of CIN.

A limitation of our study is that the analysis was limited to a subset of the Atypical Squamous Cells of Undetermined Significance–Low-Grade Squamous Intraepithelial Lesion Triage Study group. The risk factors and outcomes in the subjects included in the study, however, were not different from those in the subject excluded from the study in a way that should cast a doubt on the internal validity of the study results. The risk factors in which the included and excluded groups differed (*i.e.,* median concentrations of vitamin C, ethnicity, and having one or more pregnancies that ended in a live birth) were not independent predictors of the outcomes. With respect to external validity, our study results should be generalizable to high-risk populations similar to those recruited for the Birmingham arm of the Atypical Squamous Cells of Undetermined Significance–Low-Grade Squamous Intraepithelial Lesion Triage Study trial, but additional studies may be needed before they can be generalized to a broader population. Additional studies are also needed to test whether there are similar associations between folate and specific types of high-risk HPV, and this will enhance our understanding of the role of micronutrients in cervical carcinogenesis. A documentation of the associations between folate and the natural history of specific high-risk HPV subtypes may also strengthen the argument for folate supplementation to control high-risk HPV related risk of cervical cancer.

In conclusion, our findings suggest that improving folate status in subjects at risk of getting infected or infected with high-risk HPV may reduce the risk of cervical cancer. To our knowledge, this is the first long-term prospective follow-up study reporting independent associations between folate and several aspects of the natural history of high-risk HPV.

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