Short Communication

The Risk of Cervical Cancer in Relation to Serum Concentrations of Folate, Vitamin B_{12}, and Homocysteine[1]

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Abstract

Due to its role in the synthesis and repair of DNA, folate may protect against the development of cervical cancer. Prospective data on the possible association between folate and cervical cancer have been lacking. There is also a paucity of prospective evidence concerning the possible associations between cervical cancer and vitamin B_{12}, which shares pathways with folate, and homocysteine, a marker of low B vitamin concentrations. A nested case-control study was conducted to prospectively evaluate the associations between cervical cancer and serum concentrations of folate, vitamin B_{12}, and homocysteine. Among a community-based cohort of women who donated blood in 1974 for a serum bank in Washington County, Maryland, 39 cases of cervical cancer diagnosed between 1975 and mid-1990 were included in the study (13 cases of invasive cervical cancer and 26 cases of carcinoma *in situ*). Two controls were matched to each case by age, race, and sex. Stored serum from the cases and controls was assayed for folate, B_{12}, and homocysteine concentrations. For folate, adjusted odds ratios were 1.0, 0.62, and 0.60 for the low to high thirds of the serum concentrations, respectively, a trend in the protective direction that was not statistically significant (P for trend = 0.42). Overall, the results for vitamin B_{12} tended to mimic those for folate, whereas the associations for homocysteine tended to be in the opposite direction. None of the results of this study were statistically significant, but patterns of the associations are in accord with hypothesized mechanistic pathways concerning B vitamins and cervical cancer.

Received 12/7/98; revised 3/22/00; accepted 4/12/00.

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[1] Supported by National Institute of Environmental Health Sciences Grant ES08030. A. J. A. is a recipient of a Preventive Oncology Academic Award (CA73790) from the National Cancer Institute. G. W. C. is a recipient of a Research Career Award from the National Heart, Lung, and Blood Institute (HL21670).

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Introduction

One interesting lead to understanding the etiology of cervical cancer has been the possible protective role of folate, one of the B vitamins whose primary sources are fruits and vegetables. The rationale for folate's possible protection against cervical cancer is based on its roles in DNA synthesis and repairing damaged DNA (1, 2). Folate is involved in DNA methylation, through which it may influence gene stability and expression (2).

Interest in the relationship between folate and pathogenesis of cervical cancer extends at least as far back as 1973, when Whitehead *et al.* (3) reported that administering folic acid reversed megaloblastic changes in the cervical epithelium that were associated with oral contraceptive use, a finding that Butterworth *et al.* (4) extended to cervical dysplasia in 1982. Preliminary observations such as these, along with an improved understanding of the role of folate, led to the hypothesis that women with low folate levels may have increased susceptibility to cervical cancer.

Several case-control studies have been conducted to investigate the relation between folate and cervical dysplasia or cervical cancer. These studies have measured folate in different ways, including dietary intake and serum or erythrocyte folate concentrations. The findings are summarized in Table 1. Two of the four studies of dietary intake and cervical dysplasia found a dose-response relationship, as did one of the two carcinoma *in situ* studies. None of the three investigations of diet and invasive cervical cancer showed a dose-response association with folate intake. Those studies of folate levels in serum, plasma, or RBCs have also had equivocal results. Only one of the five found a protective association, which was an inverse association between RBC folate and cervical dysplasia.

Inspection of Table 1 suggests that if it were possible to properly combine the results of all of the studies, the net result would be a slight inverse association of dietary and circulating folate concentrations with cervical dysplasia and neoplasia. Although encouragement from these findings is minimal, there are reasons for additional studies of the folate-cervical cancer relationship. First, none of the previous studies has been prospective. More importantly, folate may inhibit the carcinogenic potential of HPVs,[3] possibly by keeping the virus from becoming integrated into genomic DNA (1). In most of the studies, most of the major cervical cancer risk factors were accounted for; however, women were tested for the presence of HPV infection in only five of the studies. The results of the studies with no measure of HPV infection are thus difficult to interpret clearly.

Although the focus has been on folate, the close interrelationship of folate and vitamin B_{12} in the synthesis, repair, and methylation of DNA suggests that insights might be gained by

[3] The abbreviations used are: HPV, human papillomavirus; OR, odds ratio, CL, confidence limits.

Table 1 Summary of the dose-response associations (ORs) observed between folate and cervical dysplasia, cervical carcinoma *in situ*, and invasive cervical carcinoma in case-control studies

First author (year) (ref. no.)	No. of cases/ no. of controls	Categories of dietary intake				Categories of circulating concentrations				Adjusted for[b]
		1 = low[a]	2	3	4	1 = low[a]	2	3	4	
Dysplasia										
Van Eenwyk (1992) (5)	100/100	1.0	0.8	0.7	0.4	1.0	0.9	1.1	0.3 (serum)	A–G, J, K
						1.0	0.5	0.6	0.1 (RBC)	
Liu (1993) (6)	257/133	1.0	0.93	1.33	0.67					A–D, H–J
Buckley (1992) (7)	42/58	1.0	0.59	0.30						Nothing
Butterworth (1992) (8)	294/170					1.0	1.0	0.71	— (RBC)	B–D, H, I
Kanetsky (1998) (9)	32/113	1.0	1.40	0.28		1.0	0.13	1.50	— (serum)	A, C, E, G, I, L
						1.0	1.3	0.7	— (RBC)	
Carcinoma *in situ*										
Brock (1988) (10)	117/196	1.0	1.8	0.9	1.3					A–E, L
Ziegler (1991) (11)	229/502	1.0	0.93	0.79	0.73					A–G
Invasive cervical carcinoma										
Ziegler (1990) (12)	271/502	1.0	0.86	1.02	1.18					A–D, F, G
Verreault (1989) (13)	189/227	1.0	0.6	1.3	0.8					A–G, L
Herrero (1991) (14)	748/1411	1.0	0.99	1.00	0.95					A, B, E, G–I
Potischman (1991) (15)	330/565					1.0	1.01	1.06	1.05 (serum)	A, B, E, G–I

[a] Referent category.
[b] A, age; B, sexual activity; C, smoking; D, contraceptive use; E, socioeconomic status; F, history of genital infection; G, time since last Pap smear or frequency of Pap smears; H, parity; I, HPV infection (HPV-16 and/or HPV-18); J, race; K, body mass index; L, total energy.

considering both of these B vitamins. B_{12} is a cofactor for methionine synthase, which catalyzes the conversion of homocysteine to methionine and also controls cellular folate uptake (16). Folate also acts as a cofactor for methionine synthase (17), so both folate and B_{12} are inversely associated with homocysteine concentrations (17). Homocysteine, which is produced during methionine metabolism (16), could be associated with increased risk of cervical cancer as a marker of low folate and B_{12} levels (18). The present study was carried out to prospectively assess the risk of cervical cancer in relation to serum folate, vitamin B_{12}, and homocysteine using a nested case-control study design.

Materials and Methods

The resources used to implement this study were the Washington County Serum Bank and the Washington County Cancer Registry. The serum bank was established in 1974, when a community-wide campaign was conducted to collect blood samples for use in biomedical research from adult volunteers in Washington County, Maryland. Serum from these samples has been stored at −70°C. A brief interview at the time of blood donation included sociodemographic information and health-related practices such as cigarette smoking.

This study is based on the cohort of 11,009 female Washington County residents who were older than 18 years when they donated blood. Fifty of these women were diagnosed with cervical cancer, either *in situ* or invasive, for the first time between January 1, 1975 and June 1, 1990, slightly more than the number expected from the age- and race-specific rates published by the Surveillance, Epidemiology and End Results registries for 1978–1981 (19).

Earlier studies of cervical cancer based on this population concerned other micronutrients (20) and antibodies to the HPV (21). The sera that remained after completion of these two studies were assayed for folate, vitamin B_{12}, and homocysteine. Of the 50 initial cases, sera were available for 39 cases, 13 cases with invasive cervical carcinoma and 26 cases with carcinoma *in situ*. The inability to include 11 of the 50 cervical cancer cases introduces the potential for bias. Compared with the 39 cases included in the present study, the 11 excluded cases were similar with respect to age, race, education, oral contraceptive use, and hypertensive medication use but were 29% less likely to be cigarette smokers and 73% less likely to be HPV-16 positive at the time of blood donation.

For each case, two controls were identified from cohort members who were not in the cancer register and were not known to have died at the time the case was diagnosed. Controls were matched to cases with respect to age, race (only one case was black), date of blood collection, time between blood drawing and previous meal, and time since last menstrual period. Of the 39 case-control sets included in the present study, 7 had only one matched control with sufficient serum for the assays.

Laboratory Assays. Aliquots of serum were packed with dry ice in a styrofoam container and sent to the Human Nutrition Research Center, Tufts University (Boston, Massachusetts), where they were assayed in case-control sets. Five sets of quality control specimens from pooled serum were also inserted at irregular intervals. The following assays were used: (*a*) microbiological assay for folate (22); (*b*) radioimmunoassay using the Bio-Rad Quantaphase II kit for B_{12}; and (*c*) the assay method of Araki and Sato (23) for homocysteine. Laboratory personnel did not know the source of the individual serum specimens (case, control, or quality control). The interset coefficients of variation ranged from 6.8–8.8%, and the intraset coefficients of variation ranged from 2.4–6.3%.

The assays for HPV-16 antibodies have been reported in detail elsewhere (21). Briefly, IgG antibodies to virus-like particles of HPV-16 were measured by ELISA. Seropositivity, indicating current or prior infection with HPV-16, was taken to be an absorbance value of 0.20 or more.

Statistical Analysis. Serum concentrations were categorized by thirds of the distribution among the controls. Matched ORs for the middle and high thirds relative to the low third were estimated using conditional logistic regression (24). The test for dose-response trend was based on the Likelihood Ratio Test from a conditional logistic regression model with a single quantitative variable entered, coded as the controls' median

Table 2 Relative odds (and 95% CLs) of cervical cancer according to thirds of serum concentrations of folate, B_{12}, and homocysteine, Washington County, Maryland, 1975–1990

Serum concentration[a]	Folate		B_{12}		Homocysteine	
	Matched[b]	Additional adjustments[c]	Matched[b]	Additional adjustments[c]	Matched[b]	Additional adjustments[c]
Low[d]	1.0	1.0	1.0	1.0	1.0	1.0
Middle	0.86 (0.31, 2.39)	0.62 (0.19, 1.98)	1.12 (0.46, 2.73)	0.97 (0.33, 2.84)	0.64 (0.21, 1.98)	0.92 (0.24, 3.53)
High	0.61 (0.24, 1.60)	0.60 (0.19, 1.88)	0.47 (0.16, 1.40)	0.53 (0.16, 1.75)	2.17 (0.82, 5.77)	2.67 (0.81, 8.82)
P for trend	0.30	0.42	0.18	0.28	0.03	0.05

[a] The tertiles were 2.9 and 4.8 ng/ml for folate, 441.0 and 655.0 pg/ml for B_{12}, and 8.6 and 10.4 nmol/ml for homocysteine.
[b] Adjusted for matching factors (age, race, and date of blood donation).
[c] Adjusted for matching factors plus education, current smoking, current oral contraceptive use, and HPV-16 positivity.
[d] Referent category.

serum folate concentration within each third of the distribution. To assess the joint relationship of folate and B_{12} on the risk of cervical cancer, each micronutrient concentration was first classified as high or low based on whether it was at or above the controls' median serum concentration or below the controls' median serum concentration. Then, based on the results for both folate and B_{12}, the results were categorized as low/low, low/high, high/low, and high/high.

Results

Average age at study baseline was 38 years for the cases and 37 years for the controls; the average interval from blood donation to diagnosis of the cases was 6 years. Compared with the controls, cases at baseline were more likely to have had less than 12 years of education (OR, 2.5; 95% CL, 1.0, 6.0), to have been current smokers (OR, 2.1; 95% CL, 0.9, 4.8), to have been using oral contraceptives (OR, 2.4; 95% CL, 0.7, 8.4), and to test positive for HPV-16 antibodies (OR, 4.3; 95% CL, 1.4, 13.7). Adjustment for the other factors made no more than trivial changes in any of the associations.

As expected, serum concentrations of folate and B_{12} were positively correlated ($r = 0.25$; $P = 0.03$), and both folate and B_{12} were negatively correlated with serum homocysteine concentrations (folate, $r = -0.32$ and $P < 0.01$; B_{12}, $r = -0.21$ and $P = 0.08$). Compared with the controls, the mean serum concentrations of the cases were 2.3% lower for folate ($P = 0.76$), 10.3% lower for B_{12} ($P = 0.11$), and 10.1% higher for homocysteine ($P = 0.18$). Adjusted ORs for folate and B_{12} were consistent with an inverse linear dose-response trend, but neither was statistically significant (Table 2). When folate and B_{12} were jointly assessed with respect to cervical cancer risk due to their shared metabolic pathway, the results were similar to when either micronutrient was considered singly [matched ORs, 1.0 (low/low), 0.92 (high/low or low/high), and 0.58 (high/high)]. Those with the highest serum homocysteine concentrations had increased risk of cervical cancer, but no monotonic dose-response trend was present despite the statistical significance of the trend.

Accounting for the follow-up time to diagnosis could be important if occult disease affects B vitamin concentrations. The results of analyses that were limited to the 25 case-control sets diagnosed 5 or more years after donating blood were consistent with the findings for the total study population (data not shown). The data were also analyzed separately for the cases of invasive cervical cancer and the cases of cervical carcinoma *in situ*. The results for both groups tended to be similar; the lone exception was that the protective association observed for folate in the total study population was present only among the carcinoma *in situ* cases, but this was consistent with chance variation (P for interaction = 0.60).

Discussion

Despite being a small study, the evidence provided does support, albeit to a modest degree, a role for B vitamins in the etiology of cervical cancer. The observed pattern of associations tended to be in accord with the hypothesized mechanistic pathways concerning B vitamins and cervical cancer. The ORs in the protective direction provide some support for folate protecting against cervical cancer. The OR for the highest third of serum B_{12} was also less than unity, as expected based on its shared role with folate in DNA repair and synthesis. Conversely, the highest levels of serum homocysteine, a marker of low B vitamin concentrations, were associated with a nonsignificantly increased risk of cervical cancer. None of these associations except the trend for homocysteine was statistically significant, but the results for homocysteine were more compatible with a threshold than a dose-response association.

Measurement error needs to be considered in interpreting our results. Oral contraceptive use was measured based on current use at the time of blood donation. HPV infection was determined by serology in the distant past, rather than genotyping HPV types in the cervix, and was limited to HPV-16. Thus, although oral contraceptive use and HPV status were accounted for to some degree, residual confounding due to the misclassification of these factors could still play a role in the observed associations. The likelihood of the limitations in these measures leading to erroneous conclusions is slight, however, given that the associations observed for oral contraceptive use, education, smoking, and HPV-16 infection were in the direction expected based on previous research. Another limitation of the study is that, compared with serum concentrations, measurement of RBC folate is considered a better marker of long-term folate levels (25). The nondifferential misclassification of folate that could result from the use of serum rather than erythrocyte concentrations would be expected to result in more conservative estimates of the association between folate and cervical cancer than may actually exist. Nevertheless, serum and erythrocyte folate concentrations are highly correlated (7), and serum folate concentrations are correlated with concentrations in cervical tissue (26).

Placed in context of the existing evidence, this study adds a piece to the overall puzzle concerning the B vitamins and cervical cancer. This was a prospective investigation, allowing assessment of serum folate, B_{12}, and homocysteine status before the development of cervical cancer. Such prospective data are essential for discerning whether a true association exists and

are needed to augment the results of case-control studies in which exposures have been measured after the diagnosis of cervical cancer. A measure of the study's internal validity is that the observed associations for smoking, oral contraceptive use, education, and HPV-16 infection were in the direction expected based on previous research. Clearly, however, more prospective studies are needed to supply the additional pieces to the puzzle that will eventually resolve the role, if any, of the B vitamins in the etiology of cervical cancer.

Acknowledgments

We thank the anonymous reviewers who provided helpful comments on an earlier version of the manuscript.

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Cancer Epidemiol Biomarkers Prev 2000;9:761-764.