Serum Micronutrients and the Subsequent Risk of Cervical Cancer in a Population-based Nested Case-Control Study[1]

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Abstract

A nested case-control study was conducted in Washington County, MD, to determine whether low serum micronutrients are related to the subsequent risk of cervical cancer. Among the 15,161 women who donated blood for future cancer research during a serum collection campaign in 1974, 18 developed invasive cervical cancer and 32 developed carcinoma *in situ* during the period January 1975 through May 1990. For each of these 50 cases, two matched controls were selected from the same cohort. The frozen sera of the cases and their matched controls were analyzed for a number of nutrients. The mean serum levels of total carotenoids, α-carotene, β-carotene, cryptoxanthin, and lycopene were lower among cases than they were among controls. When examined by tertiles, the risk of cervical cancer was significantly higher among women in the lower tertiles of total carotenoids (odds ratio 2.7; 95% confidence limit, 1.1–6.4), α-carotene (odds ratio, 3.1; 95% confidence limit, 1.3–7.6), and β-carotene (odds ratio, 3.1; 95% confidence limit, 1.2–8.1) as compared to women in the upper tertiles and the trends were statistically significant. Cryptoxanthin was significantly associated with a lower risk of cervical cancer when examined as a continuous variable. Retinol, lutein, α- and γ-tocopherol, and selenium were not related to cervical cancer risk. Smoking was also strongly associated with cervical cancer. These findings are suggestive of a protective role for total carotenoids, α-carotene and β-carotene in cervical carcinogenesis and possibly for cryptoxanthin and lycopene as well.

Introduction

Cervical cancer is the third most common cancer among Latinos in the United States and ranks as the sixth most common cancer among other white women (1). In developing countries, it is a leading cause of death from cancer and the most common cancer among women (2). The evidence of a relationship between cancer and diet has received much attention in the last decade. Retinol, β-carotene, ascorbic acid, α-tocopherol, and selenium are among the most widely studied dietary factors in cancer. For cervical cancer, the epidemiological evidence of a relationship with these micronutrients is still limited. A number of epidemiological studies examined the relationship of cervical neoplasia with the serum level of retinol (3–10), β-carotene (3, 5, 7–9, 11–13, 14), α-carotene, lutein, cryptoxanthin, lycopene (14), folates (15–17), α-tocopherol (6, 8, 18), and selenium (10, 19). Most of these studies were cross-sectional case-control studies in which sera were obtained from the cases after the development of the disease so that a potential disease effect on nutrient levels could not be ruled out. Prediagnostic sera were used only in the studies by Coates *et al.* (10) and Knekt *et al.* (9,18). However, the number of cervical cancer cases was too small (12 cases in the study by Coates *et al.* and 23 cases in the studies by Knekt *et al.*) to permit reliable case-control comparisons. The overall evidence is only suggestive of a protective effect of β-carotene against cervical cancer.

In the present nested case-control study, we examined the relationship of the prediagnostic serum levels of retinol, α-tocopherol, selenium, and a number of carotenoids with the subsequent risk of cervical cancer. In addition, we explored the relationship of cervical cancer to γ-tocopherol. We were not able to study ascorbic acid because of its complete disappearance in serum stored without special preservatives.

Methods

This study was conducted in Washington County, MD. In August through November of 1974, 15,161 females participated in a project to collect blood for a serum bank. The project involved obtaining 15 ml of blood from as many adults as possible to be used for future cancer research. In addition, participants answered a short questionnaire. Sera were stored at −73°C. Information derived from the questionnaire and relevant for this study include birth date, years of schooling, marital status, oral contraceptive use, and smoking history.

The representativeness of the participants in this project was assessed by matching this cohort to the nonofficial census conducted in the county in 1975. More than 33% of the eligible female residents participated in the project (20). The participation rate was better among whites and the better educated.

Cases with a first diagnosis of invasive cervical cancer or carcinoma *in situ* among the participants in this project (the study cohort) during the period January 1, 1975, and

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May 31, 1990 were ascertained from the cancer register maintained by The Johns Hopkins Training Center For Public Health Research. Abstracts of hospital records were reviewed for all cases. Those with a histologically confirmed diagnosis of invasive cancer or carcinoma *in situ* were eligible for inclusion in the study. However, five cases of invasive cancer with no histological report were included because their hospital record abstracts furnished good evidence on which to base the diagnosis. These five cases were included before their exposure status was determined. The completeness of coverage of the cancer register was assessed by comparing the observed numbers of cancer cases to the expected numbers based on the 1978-1981 rates in Surveillance Epidemiology End Results registries.[3] The observed/expected ratio for invasive cancer was 1.26. With respect to carcinoma *in situ* and because of the asymptomatic nature of the disease, it is difficult to assess its completeness of coverage.

Controls were selected from a birth date list of the study cohort. To be eligible, controls had to be alive and free of cancer (with the exception of nonmelanoma skin cancer) at the time of diagnosis of the case. For each case the next younger and the next older woman who matched the case on race, age within 1 year, time of blood collection within 1 month of the case, hours since last meal at the time of blood collection in 2-h intervals, and time since last menstrual period on the collection day were selected. Matching for this last item was done because the sera were to be used for hormone assays in a subsequent study.

Forty-nine cases, 17 with invasive cancer and 32 with carcinoma *in situ*, were successfully matched to two controls each. One additional case with invasive cancer could be matched only to one control. Ten *in situ* cases were excluded because they could not be matched to controls. The final study population consisted of 49 matched triplets and one matched pair.

The sera of the cases and controls were thawed at room temperature under dim yellow light and the required serum aliquots were pipetted into Nunc tubes. Sera comprising a set were refrozen and subsequently assayed for micronutrients on the same day, with the same reagent, and by the same technician. The laboratory did not know whether a sample belonged to a case or a control. Selenium was assayed by neutron activation analysis at the Research Reactor Facility of the University of Missouri (21). Retinol and total carotenoids, α-carotene, [beta]-carotene, cryptoxanthin, lutein, lycopene, [alpha]-tocopherol, and γ-tocopherol, were measured by reversed-phase high pressure liquid chromatography at Our Lady of Mercy Medical Center in New York (22).

Serum nutrients of the cases and controls were compared using a paired *t* test (23) to account for the matched design of the study. In addition, the risk of cervical cancer as a function of serum nutrient levels was estimated using conditional logistic regression. Nutrients were examined both as continuous variables and as tertiles. Categorization into tertiles was based on the serum concentrations among controls. The significance of interactions between nutrients and between nutrients and other study variables was tested by adding interaction terms to the regression models.

[3] Unpublished data.

Table 1 Comparison of mean serum micronutrient levels in cervical cancer cases and controls in the study cohort in Washington County, MD

Nutrient	Mean		% of difference[a]	*P* value[b]
	Cases	Controls		
Retinol (μg/dl)	49.2	48.2	2.0	0.67
Total carotenoids (μg/dl)	79.1	93.2	−15.2	0.05
α-carotene (μg/dl)	1.4	2.5	−41.7	0.02
β-carotene (μg/dl)	10.8	14.1	−23.1	0.07
Cryptoxanthin (μg/dl)	6.2	8.6	−27.1	0.03
Lutein (μg/dl)	18.2	19.3	−5.6	0.55
Lycopene (μg/dl)	30.4	34.6	−12.1	0.16
α-tocopherol (mg/dl)	1.0	0.97	3.1	0.61
γ-tocopherol (mg/dl)	0.19	0.20	−6.5	0.45
Selenium (ppm)	0.12	0.12	−1.7	0.64

[a] 100 × mean difference/mean level of controls, based on mean values to 3 decimal places.
[b] Based on paired *t* tests.

Results

Mean serum nutrient levels in cases and controls are shown in Table 1. The table also shows the percentage of difference (100 × mean difference/mean control level) for each nutrient. Total carotenoids, α-carotene, and cryptoxanthin were significantly lower among cases as compared to their matched controls. β-carotene was substantially lower in cases than controls but the difference (23.1%) was of marginal significance ($P = 0.07$). Lycopene was also lower among cases than controls but the difference (12.1%) was not statistically significant ($P = 0.16$). There were no significant differences between cases of cervical cancer and controls in levels of retinol, lutein, α-tocopherol, γ-tocopherol, and selenium.

The risk of cervical cancer for tertiles of serum nutrients is shown in Table 2. In general, total carotenoids and individual carotenoids measured in this study except lutein were uniformly associated with a 2-3-fold increase in the risk of cervical cancer for women in the lower tertile compared with women in the upper tertile. The dose-response relationship was highly significant for total carotenoids ($P = 0.02$), α-carotene ($P = 0.01$), and β-carotene ($P = 0.02$) and marginally significant with lycopene ($P = 0.08$) and cryptoxanthin ($P = 0.07$). On the other hand, retinol, lutein, α- and γ-tocopherol, and selenium were not significantly related to the risk of cervical cancer. Similar results were observed when nutrients were examined as continuous variables except that the association between cryptoxanthin and cervical cancer became statistically significant ($P = 0.05$).

The average interval between donation of blood and the diagnosis of disease was about 6 years for carcinoma *in situ* and 7.5 years for invasive cancer. Because of the possibility that some of the cases had unrecognized diseases at the time they donated blood, we evaluated the association of cervical cancer with carotenoids after exclusion of the seven matched sets in which the case was diagnosed in the early period of follow-up, before 1976. No major differences were observed from the above analyses based on the whole sample. Potential confounders on which data were available in this study include education, marital status, oral contraceptive use,

Table 2 Odds ratios of cervical cancer for the lower and middle tertiles of nutrients compared to the upper tertile, and significance of trends, based on the study cohort in Washington County, Maryland

Micronutrient	Serum level	Odds ratio	95% confidence limit	*P* value for trend
Retinol				
Upper	>53.50 μg/dl	1		
Middle	41.90-53.50 μg/dl	0.84	0.33-2.14	
Lower	<41.90 μg/dl	1.22	0.44-3.39	0.67
Total carotenoids				
Upper	>100.80 μg/dl	1		
Middle	74.8-100.80 μg/dl	1.61	0.64-4.04	
Lower	<74.80 μg/dl	2.68	1.12-6.40	0.02
α-carotene				
Upper	>2.70 μg/dl	1		
Middle	1.20-2.70 μg/dl	1.26	0.47-3.37	
Lower	<1.20 μg/dl	3.09	1.26-7.61	0.01
β-carotene				
Upper	>13.80 μg/dl	1		
Middle	8.80-13.80 μg/dl	1.45	0.58-3.63	
Lower	<8.80 μg/dl	3.06	1.16-8.10	0.02
Cryptoxanthin				
Upper	>9.00 μg/dl	1		
Middle	4.50-9.00 μg/dl	1.75	0.71-4.34	
Lower	<4.50 μg/dl	2.34	0.93-5.91	0.07
Lutein				
Upper	>19.10 μg/dl	1		
Middle	13.90-19.10 μg/dl	0.98	0.44-2.16	
Lower	<13.90 μg/dl	1.17	0.52-2.63	0.71
Lycopene				
Upper	>41.80 μg/dl	1		
Middle	24.90-41.80 μg/dl	2.42	0.94-6.28	
Lower	<24.90 μg/dl	2.52	0.96-6.62	0.08
α-tocopherol				
Upper	>1.04 (mg/dl	1		
Middle	0.80-1.04 mg/dl	0.58	0.22-1.51	
Lower	<0.80 mg/dl	0.98	0.34-2.83	1.00
γ-tocopherol				
Upper	>0.21 mg/dl	1		
Middle	0.15-0.21 mg/dl	0.87	0.36-2.14	
Lower	<0.15 mg/dl	1.08	0.45-2.58	0.85
Selenium				
Upper	>0.123 ppm	1		
Middle	0.109-0.123 ppm	1.06	0.46-2.45	
Lower	<0.109 ppm	1.12	0.50-2.53	0.78

and smoking. The mean number of years of schooling completed by the cases and controls was, respectively, 10.7 and 11.1 years. Ever married women (married, separated, widowed, and divorced) comprised 80% of the cases and controls. Twenty % of the cases and 12% of the controls were current users of oral contraceptive in 1974. Only smoking was significantly associated with cervical cancer. None of the other three variables showed a significant association with cervical cancer in the univariate analysis. However, each micronutrient measured in this study (including those which showed no association with cervical cancer in the univariate analysis) was evaluated separately in the presence of these factors by conditional logistic regression. There was no significant association between cervical cancer and any of the following nutrients after adjustment for education, contraceptive use, marital status, and smoking habits: retinol; lutein; α-tocopherol; γ-tocopherol; and selenium. Because education, marital status, and oral contraceptive use were not significantly associated with cervical cancer in any of these analyses and their inclusion did not alter the effects of other variables, these factors and the above-mentioned nutrients were excluded from further analyses. Because members of the carotenoid family were correlated with each other and with the total carotenoid levels, they were incorporated one at a time in conditional logistic regression models in the presence of smoking. The adjusted odds ratios for women in the lower tertile as compared to women in the upper tertile for carotenoids were similar to the unadjusted ratios. The data were also examined for differences in serum levels of nutrients among smokers and nonsmokers in the control group. The significance of the differences was evaluated by the two-sample *t* test. As seen in Table 3, smokers showed significantly lower levels of γ-tocopherol, cryptoxanthin, and lutein and

Table 3 Mean levels of serum micronutrients among smokers and nonsmokers in the control group

Micronutrient	Smokers (n = 27) Mean	Nonsmokers (n = 72) Mean	Difference		P value
			Mean	%[a]	
Retinol (μg/dl)	45.2	49.5	−4.21	−8.5	0.16
Total carotenoids (μg/dl)	88.9	94.3	−5.43	−5.8	0.50
α-carotene (μg/dl)	1.95	2.70	−0.75	−27.7	0.16
β-carotene	12.1	14.8	−2.74	−18.5	0.27
Cryptoxanthin (μg/dl)	5.13	9.63	−4.5	−46.7	0.00
Lutein (μg/dl)	15.5	20.3	−4.76	−23.5	0.02
Lycopene (μg/dl)	40.2	32.7	7.45	22.8	0.05
α-tocopherol	0.98	0.97	0.01	1.2	0.87
γ-tocopherol (mg/dl)	0.98	0.22	−0.04	−20.4	0.03
Selenium (ppm)	0.12	0.12	0.002	1.7	0.65

[a] 100 × difference in means/mean of nonsmokers.
[b] P values are based on the two sample t tests. Inconsistencies are due to rounding.

higher levels of lycopene. Retinol, total carotenoids, α-carotene, and β-carotene were also lower among smokers than they were among nonsmokers but the differences were not statistically significant. α-tocopherol and selenium levels were not associated with smoking.

Separate analyses were carried out for invasive cancer and carcinoma *in situ*. Similar patterns of risk were observed with both types of disease, although the associations with nutrients were generally of smaller magnitude with invasive cancer.

The data were evaluated for the following potential interactions: (*a*) interactions between nutrients; (*b*) interactions between nutrients and smoking; (*c*) interactions between nutrients and age (a matching variable); and (*d*) interactions between nutrients and oral contraceptive use. None of these interactions were statistically significant or sufficiently large to be important.

Discussion

In the present study, a strong association was observed between cervical cancer and the prediagnostic serum levels of total carotenoids and other members of the carotenoid family, *i.e.*, α- and β-carotene, cryptoxanthin, and lycopene. Previous reports on the relationship of cervical cancer and carotenoids were concerned largely with β-carotene. Our results with respect to β-carotene are in agreement with the majority of previous cross-sectional serum studies (7–8, 11–13). They are also in agreement with the only previous report from a nested case-control study (9), although in that report the association failed to attain statistical significance probably due to the small number of cases of cervical cancer (23 cases). With respect to α-carotene, cryptoxanthin, and lycopene, our data are in agreement with a previous report from a cross-sectional case-control serum study of cervical intraepithelial neoplasia (14).

Lycopene and cryptoxanthin are among the hydrocarbon carotenoids which do not convert to retinol in mammals (24). The presence of such an association between cervical cancer and carotenoids including carotenoids with no pro-vitamin A activity (lycopene and cryptoxanthin) indicates that the antitumorigenic activity of carotenoids is mediated by mechanisms other than their role as precursors of retinol. Carotenoids, by virtue of their ability to quench free radicals and singlet oxygen, may protect DNA from oxidative damage, an important event in cancer initiation (25). In addition, several aspects of immune function were reported to be enhanced by carotenoids including increased helper T-lymphocytes in humans (26), increased proliferation of T- and B-lymphocytes in rats (27), increased cytotoxic activities in tumor models (28), and increased killer cell function (29). However, much remains to be elucidated about the availability of carotenoids and other antioxidants in different body tissues and within the cells, the factors influencing their concentrations in different tissues and the relation between serum levels and tissue levels. This information may be necessary to understand the observed site-specific antitumorigenic effects of antioxidants and may have future implications in cancer prevention and treatment.

In our data, there was no difference between women who subsequently developed cervical cancer and their matched controls with respect to serum selenium level. There are two previous reports concerning the relationship between serum selenium and cervical neoplasia (10, 19). The first came from a cross-sectional case-control study of 36 cases of cervical dysplasia and 36 controls (19). The author reported a significantly lower mean level of selenium among cases with dysplasia than controls. In the present study, our cases were women with carcinoma *in situ* or invasive cancer rather than dysplasia. In addition, the sera were taken before diagnosis of the disease, thus avoiding a possible disease-related effect on the serum selenium level. The second came from a nested case-control study which evaluated the risk of all-site cancer in relation to the prediagnostic levels of selenium (10). Similar to our findings, they found no relationship between selenium level and cervical cancer.

Neither α-tocopherol nor γ-tocopherol were related to cervical cancer in the present study. Previous studies dealt only with α-tocopherol. Two previous serum studies (6, 8) found evidence of a significant protective effect for α-tocopherol against cervical neoplasia while another investigator found no significant association with cervical cancer (18). The first two reports came from cross-sectional case-control studies while the last report came from a prospective study utilizing the nested case-control design. The finding of a lower level of α-tocopherol among cases in the first two studies could be a disease effect. In the study by Knekt *et al.* (18), as well as in the present study, the possibility of this disease effect was avoided by utilizing prediagnostic sera.

Despite the advantages of the prospective approach adopted in the present study, certain limitations have to be considered in interpreting the study findings. First,

micronutrient levels measured at one point in time (as in the present study) are subject to random fluctuations and may not reflect the long-term levels of these micronutrients. Measurement errors and temporary deviations from the usual "long-term" levels may result in what is known as the "regression dilution bias" (30). This bias systematically underestimates the true association between the disease and the nutrient of interest. Consequently, the associations we observed between cervical cancer and a number of carotenoids may be lower than the true associations. Likewise, the lack of association with other micronutrients examined in this study may be due to dilution of their true effects by this type of bias. Second, despite the screening efforts in Washington County before and during the study period, some of our cases might have had subclinical cancer at the time of blood donation. Although unlikely, it is still theoretically possible that subclinical disease may lead to lowering of serum nutrient levels. To account for this possibility, we performed the analysis after exclusion of the seven matched sets in which the cases were diagnosed early in the follow-up period (before 1976). Results were in agreement with our analysis based on the whole sample. Third, because of lack of data on sexual behavior, we were unable to directly control for this risk factor which serves as a proxy for the risk of infection with the human papilloma virus. Adjustment for factors which may be related to sexual behavior, *i.e.*, marital status, education, oral contraceptive use, and smoking, did not affect our results. In addition, as mentioned earlier, race and age were controlled by matching in the design stage of this study. However, possible residual confounding by sexual behavior cannot be completely ruled out, although it is unlikely to explain the magnitude of risks we observed in this study.

In conclusion, our findings suggest a protective role for total carotenoids, α-carotene, β-carotene, cryptoxanthin, and lycopene in cervical carcinogenesis. This role of carotenoids is likely to be mediated by functions other than their conversion to retinol. Replication of this study in different populations may give further credence to its findings. The implication of this study in terms of prevention is to encourage increased intake of fresh vegetables and fruits.

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