Risk of Squamous Cell Carcinoma of the Skin in Relation to Plasma Selenium, α-Tocopherol, β-Carotene, and Retinol: A Nested Case-Control Study[1]

Margaret R. Karagas,[2] E. Robert Greenberg, David Nierenberg, Therese A. Stukel, J. Steven Morris, Marguerite M. Stevens, and John A. Baron

Section of Biostatistics and Epidemiology, Department of Community and Family Medicine and Norris Cotton Cancer Center [M. R. K., E. R. G., T. A. S., M. M. S., J. A. B.] and Department of Pharmacology and Toxicology [D. N.], Dartmouth Medical School, Hanover, New Hampshire 03755-3861; and Research Reactor Center, University of Missouri-Columbia, Columbia, Missouri 65211 [J. S. M.]

Abstract

We conducted a nested case-control study of squamous cell skin cancer (SCC) to determine whether risk was related to plasma concentrations of selenium, α-tocopherol, β-carotene, and retinol. We derived the study sample from participants in our Skin Cancer Prevention Study, all of whom had at least one basal cell or squamous cell skin cancer before study entry. Those who developed a new squamous cell skin cancer during the 3–5-year follow-up period were selected as cases (n = 132). Controls (n = 264) were chosen at random, with matching by age, sex, and study center, from among those who did not develop SCC but were being followed actively at the time the SCC case was diagnosed. Prediagnostic plasma samples were analyzed for α-tocopherol, β-carotene, and retinol using high-performance liquid chromatography. Selenium determinations were made using instrumental neutron activation analysis. Odds ratios were computed using conditional logistic regression for matched samples. We found no consistent pattern of SCC risk associated with any of the nutrients examined. The odds ratios (95% confidence intervals) for the highest *versus* the lowest quartiles of β-carotene, retinol, α-tocopherol, and selenium were 0.73 (0.38–1.41), 1.43 (0.77–2.64), 0.89 (0.43–1.85), and 0.86 (0.47–1.58), respectively. Thus, our data add to the growing body of evidence that these nutrients, at the concentrations we evaluated, are not related strongly to SCC risk.

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[2] To whom requests for reprints should be addressed, at the Section of Biostatistics and Epidemiology, Department of Community and Family Medicine, Dartmouth Medical School, Hinman Box 7927, Hanover, NH 03755-3861.

Introduction

There is experimental evidence that certain nutrients, including antioxidants, inhibit SCC[3] tumor formation and UV light carcinogenesis (1–7). The role of nutritional factors in human skin cancer is largely unknown, however, and few epidemiological studies of SCC have been conducted (8–9). Using data and stored plasma samples from a skin cancer prevention trial, we conducted a nested case-control study to the examine the risk of SCC in relation to four potentially anticarcinogenic nutrients: β-carotene, retinol, α-tocopherol, and selenium.

Materials and Methods

Skin Cancer Prevention Study. We derived our study sample from a multicenter, randomized trial designed to test the effectiveness of oral β-carotene supplementation in preventing nonmelanoma skin cancer (10–11). The trial patients were less than 85 years of age and had had at least one basal cell or squamous cell skin cancer removed since January 1, 1980. Of the 5232 potentially eligible patients identified, 1805 fulfilled study criteria and were enrolled (11).

At study entry, participants completed a questionnaire regarding personal characteristics and habits, such as age and smoking history. Questions relating to sun exposure included the proportion of time they worked outdoors during their longest-held job between the ages of 20 and 39 years, 40 and 59 years, and 60 and 79 years. They also had dermatological evaluation of skin type (ability to tan/burn) and extent of UV skin damage (classified as mild, moderate, or severe); at this time, the examining dermatologist recorded the number and histological type of previous nonmelanoma skin cancers and attempted to document each event from the patient's records.

Follow-up consisted of an interval questionnaire mailed every 4 months to the study participants and an annual dermatological examination. Microscopic slides of lesions suspected of being cancerous were re-reviewed by a dermatopathologist at the study coordinating center. Follow-up for each patient continued for 5 years or until September 30, 1989 when the treatment phase of the study ended. Approximately 4% of patients were lost to follow-up each study-year because of death or failure to return for an annual dermatological exam (11).

At enrollment and at each follow-up visit, we obtained a nonfasting, 20-ml blood specimen from each participant. Blood samples were collected in heparinized vacuum tubes and centrifuged. Plasma was stored in 3.5-ml aliquots in polypropylene tubes at −20°C or less until shipment to a central laboratory at Dartmouth Medical School, where specimens have been stored at −75°C.

[3] The abbreviations used are: SCC, squamous cell carcinoma; BCC, basal cell carcinoma; CI, confidence interval; OR, odds ratio.

Nested Case-Control Study Design. Of the 1805 patients enrolled in the intervention trial, 132 developed a new, nonrecurrent SCC during the follow-up period. For each of these "case" patients, we randomly selected two controls from among patients who, up until or during the study year the SCC case was diagnosed, (*a*) were actively being followed (had not died or been lost to follow-up) and (*b*) had not developed SCC during the study. Controls were then assigned the diagnosis date (reference date) of the cases to whom they were matched to ensure comparability with respect to time in the study. We also matched controls to the cases on sex, age (<45, 45–49, 50–54, 55–59, 60–64, 65–69, 70–74, and 75–84 years old) and study center (Hanover, NH; Minneapolis, MN; Los Angeles, CA; and San Francisco, CA). We attempted to locate and analyze a baseline (prerandomization) plasma sample for determination of α-tocopherol, selenium, cholesterol, and triglyceride for each case and control included in our sample. If the baseline sample was unavailable (either depleted from other analyses or not collected), we submitted the earliest blood sample collected provided that it was drawn before the diagnosis date for SCC cases or the reference date for controls. Baseline plasma concentrations of β-carotene and retinol were assessed as part of the original study (11).

Plasma Nutrient Determinations. We provided the laboratory with a list of patient identification numbers, blood-draw dates, and freezer locations of the subjects chosen for the study. Identification numbers were listed in sets of three (one case-control set) and scrambled so that laboratory personnel could not determine the case-control status of the subjects. Each set was processed on the same day to ensure that temporal variability in the laboratory assays would equally affect cases and controls. Blood samples were thawed at room temperature and mixed using a standard Vortex mixer. A 0.5-ml aliquot was prepared for analysis of α-tocopherol and 0.5 ml for cholesterol and triglyceride. A 1.5–2.0 ml aliquot was refrozen and shipped by overnight mail with dry ice to the University of Missouri for selenium analysis. If available, an additional 2.5–3.0-ml aliquot was refrozen and stored as a quality-control sample for selenium and α-tocopherol; these samples were assigned a similar appearing "dummy" identification number. Variability in β-carotene and retinol measurements were evaluated previously (12).

β-Carotene, α-tocopherol, and retinol concentrations were assayed using high-performance liquid chromatography on the same day bloods were thawed (13–14, 12). UV absorption was measured at 280 nm for retinol and α-tocopherol and 436 nm for β-carotene; peak areas were integrated and recorded. Sensitivity of the assay is approximately 60 ng/ml for retinol, 0.9 μg/ml for α-tocopherol, and 10 ng/ml for β-carotene. Laboratory quality control included participation in the National Institute of Standards and Technology (Gaithersburg, MD), and our results averaged within 1–2% of the "gold standard average" of participating laboratories.

Measurement of bound selenium concentrations were made using instrumental neutron activation analysis as described previously (15). The 162-KeV γ-ray of the radioactive decay (isomeric transition) of Se-77m (half-life, 17.4 s) was used for analysis. Peak areas were extracted using a computerized multielement analysis system, and concentrations were determined via standard comparison. Laboratory accuracy was monitored through comparisons of quality-control samples with accepted values. The sensitivity of the assay ranges from 0.1 to 0.02 ppm.

Triglyceride and cholesterol concentrations were measured using an Ektachem 700 XR Series C (Kodak Medical Instruments Inc., Rochester, NY).

Table 1 Distribution of selected characteristics among SCC cases and controls

Characteristic	SCC Cases		Controls	
	No.	%	No.	%
Extent of UV skin damage[a]				
Mild	11	8.4%	71	27.1%
Moderate	83	63.4%	156	59.5%
Severe	37	28.2%	35	13.4%
Smoking				
Never	34	25.8%	109	41.3%
Former	67	50.8%	125	47.4%
Current				
1–20 cigarettes/day	17	12.9%	19	7.2%
21–40 cigarettes/day	10	7.6%	8	3.0%
>40 cigarettes/day	4	3.0%	3	1.1%
Season of blood draw[b]				
Winter	28	21.4%	57	21.6%
Spring	27	20.6%	68	25.8%
Summer	36	27.5%	62	23.5%
Fall	40	30.5%	77	29.2%

[a] One case and two controls are missing information on UV skin damage.
[b] One case did not have an available blood sample.

Statistical Analysis. We assessed the relationship between risk of SCC and β-carotene, retinol, selenium, and α-tocopherol using stratified and conditional logistic regression analysis for matched data (16). The OR and 95% CI were computed for each nutrient of interest, modeled both as a continuous variable and in quartiles (based on the control distribution). To account for possible seasonal variation in food consumption, we evaluated whether cases and controls differed with respect to month of blood draw. We considered the potentially modifying or confounding effects of previously identified risk factors (occupational sun exposure, skin type, extent of UV skin damage, and smoking history; Ref. 17) as well as the other nutrients of interest and treatment assignment (β-carotene or placebo). When we examined the relation between α-tocopherol and SCC, we included total cholesterol and triglyceride as linear terms in our model, because adjustment for these factors has been found to improve the correlations between dietary intake and the plasma concentrations of vitamin E (18, 15). We computed ORs separately for those who did, and those who did not, have a prior SCC.

Results

Patients selected for the study ranged in age from 35 to 84 years (mean 67.1 years); 89% were men. Cases and controls were balanced with respect to age, sex, and study center through the matched design. Cases and controls also had a comparable follow-up interval from the time their blood was drawn to the diagnosis date of the SCC cases (30.0 months) or matched reference date for controls (28.7 months). The distribution of seasons in which the blood samples were collected were also roughly similar between cases and controls (Table 1). In comparison with controls, cases were more likely to be current and former smokers and to have more severe actinic damage reported by the examining dermatologist (Table 1).

We located a prediagnostic blood sample for α-tocopherol measurement and lipid analysis on all but one out of 132 SCC cases and all 264 controls. One control sample sent for selenium measurement was lost, leaving 131 matched sets for

selenium analysis. The samples analyzed were drawn at baseline on more than 90% of both the cases and controls. One hundred thirty cases and 254 controls had baseline measurements of β-carotene and retinol, providing complete data on 129 case-control sets.

The mean concentrations of retinol, α-tocopherol, and selenium were 723.7 ng/ml (SD, 164.6 ng/ml), 13.0 μg/ml (SD, 6.1 μg/ml), and 0.128 ppm (SD, 0.0196 ppm), respectively. β-Carotene levels were log transformed to produce more symmetrically distributed data. The geometric mean level of β-carotene was 162.9 ng/ml (SE, 5.8 ng/ml) for the group. The coefficients of variability (SD/mean) for replicate measurements of selenium and α-tocopherol (n = 13) were 9.5 and 14.5%, respectively.

There were no statistically significant differences between cases and controls with respect to the mean values of any of the nutrients examined (Table 2). For β-carotene, individuals in the highest quartile had a lower risk in comparison with the bottom quartile (OR, 0.73; 95% CI, 0.38–1.41), but there was no consistent trend from lowest to highest quartile (P for trend, 0.37; Table 3). Risk estimates for SCC did not differ appreciably by plasma levels of α-tocopherol, even after adjustment for smoking and lipid levels. Lipid levels themselves were not related to SCC risk (Table 3). Individuals in the second quartile of plasma selenium had less than half the estimated relative risk of SCC of those in the lowest quartile (odds ratio, 0.44; 95% CI, 0.23–0.84), but the ORs for the third and fourth quartiles were not reduced (P for trend, 0.89; Table 3). Adjusted ORs were slightly higher in the higher quartiles of retinol concentrations, but not significantly so (P for trend, 0.31). These findings were largely unchanged when we excluded cases with a prior SCC. Although an inverse association was observed for the second, third, and fourth quartiles of plasma selenium, compared with the lowest quartile, there was no consistent trend in risk (P for trend, 0.25; Table 3). Adjustment for other risk factors (*e.g.*, skin type, extent of UV skin damage, levels of other nutrients or treatment assignment) did not alter our relative risk estimates.

Table 2 Mean (SE) of prediagnostic plasma concentration levels by case-control status

	SCC cases Mean (SE)	Controls Mean (SE)	P value
β-Carotene (ng/ml)[a]	153.2 (8.7)	168.2 (7.6)	0.21
Retinol (ng/ml)	725.3 (15.7)	722.9 (9.84)	0.90
α-Tocopherol (μg/ml)	12.8 (0.58)	13.1 (0.36)	0.63
Selenium (ppm)	0.127 (0.0018)	0.128 (0.0012)	0.62
Total cholesterol (mg/dl)	180.7 (3.45)	185.5 (2.58)	0.28
Triglycerides (mg/dl)	156.3 (7.54)	156.6 (5.47)	0.97

[a] Geometric mean concentrations and SEs.

Discussion

Some epidemiological studies, but not all, have found a reduced risk for cancers at multiple sites associated with higher blood concentrations of selenium (19). Plasma selenium levels were inversely related to SCC and BCC in a clinic-based case-control study conducted by Clark *et al.* (8). A limitation of this study was that controls were "healthy" age- and sex-matched patients attending a dermatology clinic who may have had higher selenium levels than the general population. In a subsequent prospective study of 240 patients with a history of nonmelanoma skin cancer, Clark *et al.* (20) reported a higher rate of new occurrences of nonmelanoma skin cancer among patients with lower baseline plasma selenium levels; BCC and SCC were not reported separately. A smaller nested case-control study (based on 37 cases of SCC) found that individuals whose prediagnostic serum selenium levels were in the upper two tertiles had a lower SCC risk, but this finding could have been due to chance, and there was no apparent trend in risk (9). We similarly found only weak evidence of an inverse association with plasma selenium and no discernible trend.

It is possible that low selenium intake increases cancer risk only if intake of other nutrients is also low. Ip (1), for example, found that animals fed selenium along with vitamin E had a greater reduction in tumor formation than animals fed either nutrient alone. In a prospective epidemiological study (19), low concentrations of selenium were more strongly associated with cancer risk among persons with low concentrations of vitamin E or vitamin A. Clark *et al.* (20) reported similar results for selenium and carotenoids and risk of nonmelanoma skin cancer. In our data, we did not find a greater risk of SCC associated with lower selenium concentrations for those who also had low α-tocopherol, β-carotene, or retinol (data not shown); however, our study had limited power to detect such interactions.

The role of vitamin E in the pathogenesis of human malignancies is unknown. In a large, randomized trial conducted in Linxian, China, a lower cancer mortality rate was found among those supplemented with a combination of β-carotene, α-tocopherol, and selenium (21). Unfortunately, the individual effects of these nutrients could not be determined. In a Finnish trial conducted among white male smokers, the group supplemented with α-tocopherol had a lower incidence of prostate and colorectal cancer but a higher rate of stomach cancer (22). The incidence of skin cancer was not reported. The possibility that vitamin E might prevent human nonmelanoma skin cancer, especially SCC, has only been examined in one previous investigation (9). As in our study, no association was found with prediagnostic α-tocopherol levels and SCC.

The potential antioxidant and anticancer effects of β-carotene have received a great deal of attention and investigation in clinical trials (2). In a small clinical trial, 180 mg/day of β-carotene for 10 weeks increased the minimal erythema dose of UV radiation (2–3). In our Skin Cancer Prevention Study, we found that 50 mg/day of oral β-carotene supplementation did not reduce new nonmelanoma skin cancer, SCC, or BCC occurrence (11). However, our treatment period was limited to 5 years, so we cannot rule out the possibility that β-carotene would be effective if used over a longer period of time. Yet, in neither our study nor that of Breslow *et al.* (9) was there a relation between prediagnostic plasma levels of β-carotene and SCC observed, so such an association appears unlikely.

A relation between plasma retinol and SCC was not observed in our study or the one prior study that examined this (9). Nonetheless, plasma retinol concentrations do not reflect dietary intake in populations that are not vitamin A deficient (24), and so the absence of a reduced risk of SCC in relation to plasma retinol does not preclude the possibility of an anticancer effect of retinoids.

Prospective assessment of the relationship between disease outcomes and nutrient intake often requires a large sample size and a long follow-up period. Our study had the advantage of following a group of high-risk patients, so that a relatively large number of participants developed new skin cancers. Also, we were able to minimize the expense of the laboratory assays for plasma nutrients by a employing a nested case-control design. In other studies, blood levels of selenium and α-tocopherol were both found to be reasonably correlated over time (24),

Table 3 ORs (95% CIs)[a] for SCC and first SCC according to quartile of plasma β-carotene, retinol, α-tocopherol, selenium, triglyceride, and total cholesterol

	Quartile of plasma nutrient level				*P* for trend[b]
	1	2	3	4	
β-Carotene	≤100 ng/ml	101–175 ng/ml	176–265 ng/ml	>265 ng/ml	
Any SCC	1.0 (reference)	0.80 (0.42–1.54)	0.92 (0.50–1.69)	0.73 (0.38–1.41)	0.37
No. of cases	38	32	33	26	
No. of controls	63	62	63	62	
First SCC	1.0 (reference)	0.76 (0.38–1.53)	0.80 (0.41–1.54)	0.71 (0.34–1.47)	0.46
No. of cases	34	30	29	24	
No. of controls	52	56	58	54	
Retinol	≤610 ng/ml	611–710 ng/ml	711–830 ng/ml	>830 ng/ml	
Any SCC	1.0 (reference)	0.89 (0.47–1.68)	1.26 (0.69–2.30)	1.43 (0.77–2.64)	0.31
No. of cases	33	26	34	36	
No. of controls	64	62	63	61	
First SCC	1.0 (reference)	0.80 (0.41–1.57)	1.19 (0.63–2.25)	1.16 (0.60–2.23)	0.72
No. of cases	31	24	32	30	
No. of controls	54	55	56	55	
α-Tocopherol	≤9.5 μg/ml	9.6–12.0 μg/ml	12.1–15.5 μg/ml	>15.5 μg/ml	
Any SCC	1.0 (reference)	0.88 (0.48–1.64)	1.10 (0.57–2.10)	0.89 (0.43–1.85)	0.72
No. of cases	37	33	34	27	
No. of controls	66	69	63	64	
First SCC	1.0 (reference)	0.68 (0.34–1.34)	0.98 (0.49–1.96)	0.76 (0.35–1.67)	0.72
No. of cases	37	28	31	23	
No. of controls	58	61	56	56	
Selenium	≤0.12 ppm	0.121–0.130 ppm	0.131–0.140 ppm	>0.140 ppm	
Any SCC	1.0 (reference)	0.44 (0.23–0.84)	0.74 (0.38–1.44)	0.86 (0.47–1.58)	0.89
No. of cases	49	19	28	35	
No. of controls	69	65	63	64	
First SCC	1.0 (reference)	0.45 (0.23–0.87)	0.62 (0.31–1.24)	0.67 (0.35–1.29)	0.25
No. of cases	49	18	24	28	
No. of controls	62	55	57	56	
Triglyceride	≤98mg/dl	99–140mg/dl	141–185mg/dl	>185mg/dl	
Any SCC	1.0 (reference)	0.80 (0.43–1.51)	0.65 (0.34–1.26)	0.97 (0.50–1.88)	0.90
No. of cases	39	30	25	37	
No. of controls	66	65	67	64	
First SCC	1.0 (reference)	0.76 (0.42–1.38)	0.65 (0.35–1.23)	1.06 (0.57–1.96)	0.65
No. of cases	36	29	23	31	
No. of controls	59	57	59	56	
Total Cholesterol	≤160 mg/dl	161–180 mg/dl	181–210 mg/dl	>210 mg/dl	
Any SCC	1.0 (reference)	0.97 (0.51–1.87)	1.29 (0.71–2.34)	0.77 (0.39–1.53)	0.31
No. of cases	36	29	42	24	
No. of controls	70	62	66	64	
First SCC	1.0 (reference)	1.18 (0.58–2.39)	1.38 (0.72–2.65)	0.73 (0.35–1.54)	0.20
No. of cases	32	28	38	21	
No. of controls	61	53	59	58	

[a] ORs obtained from a conditional logistic regression analysis adjusted for cigarette smoking [never, former, current (1–20, 21–40, and >40 cigarettes/day). Models for α-tocopherol also include linear terms for cholesterol and triglyceride.
[b] Based on the plasma nutrient level modeled as a continuous variable.

although, for α-tocopherol, this appears to be partly due to the stability of cholesterol levels. Among placebo-treated controls in our β-carotene trial, plasma β-carotene remained relatively constant over the 5-year study period.[4] Skin cancer patients are particularly suitable for the investigation of nutritional factors, because they are often otherwise healthy, and the development of their condition is unlikely to affect their nutritional status.

One limitation of our study is that many of our controls had new BCC occurrences during or before the follow-up period. Thus, any effects of the nutrients we examined on risk of SCC might be masked if the same nutrients influenced occurrence of BCC. Hunter *et al.* (25) evaluated dietary factors in relation to BCC and found only a weak association with total energy intake and virtually no relation with other factors assessed by a dietary history. In a 10-year prospective study, there was no clear trend in the risk of BCC over quintiles of serum α-tocopherol (26). In the original skin cancer trial (11), baseline plasma β-carotene was not related to new nonmelanoma skin cancer occurrences, most of which were BCC. In our analyses, we included the occurrence of BCC before the diagnosis of SCC as a covariate, and our findings were essentially the same (data not shown). Nonetheless, the fact that our study group comprised participants in a skin cancer prevention trial may limit the generality of our results.

In conclusion, although dietary risk factor information on SCC are limited, our data as well as others, do not support a role of selenium, α-tocopherol, or β-carotene in the development of SCC of the skin. The effects of retinoids on SCC risk will need to be investigated using more relevant measures. Nutritional measures of preventing SCC could be extremely valuable, particularly in light of the rapid increase in the incidence rate of

[4] Unpublished data.

these malignancies (27–28). For example, Black *et al.* (29) found that a low-fat diet reduced the occurrence of actinic keratoses in nonmelanoma skin cancer patients. Our study was not designed to examine this issue; however, we did not find either cholesterol or triglyceride levels to be associated with SCC risk. Additional epidemiological studies of SCC could also provide valuable insights for the planning process of large-scale intervention trials.

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