Association of Prediagnostic Serum Vitamin D Levels with the Development of Basal Cell Carcinoma

Maryam M. Asgari[1,2], Jean Tang[3], Margaret E. Warton[1], Mary-Margaret Chren[2,4], Charles P. Quesenberry Jr[1], Dan Bikle[2], Ronald L. Horst[5], Norman Orentreich[6], Joseph H. Vogelman[6] and Gary D. Friedman[1,7]

We investigated the association between serum 25-hydroxyvitamin D (25(OH)D) levels and basal cell carcinoma (BCC) risk in a nested case–control study at Kaiser Permanente Northern California (KPNC). A total of 220 case patients with BCC diagnosed after serum collection were matched to 220 control subjects. We estimated odds ratios (ORs) and 95% confidence intervals (CIs) using conditional logistic regression. Fully adjusted models included body mass index (BMI), smoking, education, sun-exposure variables, X-ray exposure, and personal history of cancer. For each measure of serum 25(OH)D (continuous, clinically relevant tertiles, quintiles), we found an increased risk of BCC in unadjusted models (OR = 1.03, 95% CI 1.00–1.05, $P < 0.05$; OR = 3.98, 95% CI: 1.31–12.31, deficient vs. sufficient, test for trend P-value < 0.01; OR = 2.32, 95% CI: 1.20–4.50, 1st vs. 5th quintile, test for trend P-value 0.03). In fully adjusted models, the values attenuated slightly (OR = 1.02, 95% CI 1.00–1.05, $P < 0.05$; OR = 3.61, 95% CI: 1.00–13.10, deficient vs. sufficient, t-trend $P = 0.03$; OR = 2.09 1st vs. 5th quintile, 95% CI: 0.95–4.58, t-trend $P = 0.11$). Our findings suggest that higher prediagnostic serum 25(OH)D levels may be associated with increased risk of subsequent BCC. Further studies to evaluate the effect of sun exposure on BCC and serum 25(OH)D levels may be warranted.

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INTRODUCTION

Basal cell carcinoma (BCC) is the most common cancer in the United States affecting nearly one million Americans annually (Jemal *et al*, 2009) and its incidence is rising (Miller and Weinstock, 1994; Christenson *et al*, 2005). Although BCCs are rarely fatal, their high incidence and the frequent occurrence of new primary BCCs in affected individuals (Karagas *et al*, 1992) can cause significant morbidity. BCCs also pose a substantial financial impact and are among the most costly cancers to treat in the Medicare population (Housman *et al*, 2003). The main known risk factors for BCC are sun sensitivity and exposure to ultraviolet (UV) radiation. The same spectrum of UV radiation (280–320 nm) causes both DNA damage to keratinocytes and vitamin D synthesis by these cells (Freeman *et al*, 1989). This has led some investigators to propose that vitamin D formation in keratinocytes may be an innate protective mechanism against UV damage (Bikle, 2008).

Recent studies have shown that vitamin D can regulate differentiation of normal skin cells (Bikle, 2004) and can reduce hedgehog signaling in and proliferation of murine BCC cell lines (Xiao *et al*, 2009). In mice, vitamin D receptor knockouts are more susceptible to chemically induced and UVR-induced skin tumors, suggesting that disruption of vitamin D receptor signaling predisposes to skin cancer (Zinser *et al*, 2002). Vitamin D receptor polymorphisms have been associated with increased BCC risk (Ramachandran *et al*. 2003). Immunohistochemical studies of human BCCs suggest that the vitamin D pathway may be important for the growth behavior of BCCs (Kamradt *et al*, 2003; Mitschele *et al*, 2004). Despite mounting evidence that vitamin D and its receptor are involved in cutaneous carcinogenesis, no studies to date have examined the association of serum vitamin D levels with BCC risk in humans.

The epidemiology of BCCs has been difficult to characterize because most cancer registries, such as the Surveillance, Epidemiology, and End Results program exclude non-melanoma skin cancers. However, the Kaiser Permanente Northern California (KPNC) setting includes electronic databases that capture information on all pathology specimens received for examination, allowing for accurate

[1]*Division of Research, Kaiser Permanente Northern California, Oakland, California, USA;* [2]*Department of Dermatology, University of California, San Francisco, San Francisco, California, USA;* [3]*Department of Dermatology, Stanford University School of Medicine, Stanford, California, USA;* [4]*Health Services Research Enhancement Award Program (REAP), San Francisco Veterans Affairs Medical Center, San Francisco, California, USA;* [5]*Heartland Assays, Ames, Iowa, USA;* [6]*Orentreich Foundation for the Advancement of Science Inc., Cold Spring-on-Hudson, New York, USA and* [7]*Department of Health Research and Policy, Stanford University School of Medicine, Stanford, California, USA*

Correspondence: Maryam M. Asgari, Kaiser Permanente Division of Research, Kaiser Permanente Northern California, 2000 Broadway, Oakland, California, 94612, USA. E-mail: Maryam.m.asgari@kp.org

Abbreviations: BCC, basal cell carcinoma; OR, odds ratio; CI, confidence interval; KPNC, Kaiser Permanente Northern California; MHC, Multiphasic Health Checkup; MHCQ, MHC questionnaire

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recording of BCC. Using an established cohort of KPNC members with data on self-reported cancer risk factors and prediagnostic serum vitamin D levels, we performed a nested case–control analysis to study the association between prediagnostic serum vitamin D levels and subsequent BCC risk.

RESULTS

The 440 study participants included 228 men and 212 women with a mean age of 54.9 years (SD 10.1, range 28–78). The mean years to BCC among cases was 8.74 years (SD 1.28, min = 6.14, max = 11.33). Cases were more likely than controls to report a personal history of cancer and slightly more likely than control subjects to have lower BMI and to have never smoked (Table 1). Cases and controls did not differ with regard to education, any of the sun exposure variables, or history of X-ray exposure. Serum 25(OH)D levels were slightly higher among the cases than among the controls.

The mean 25(OH)D concentration in all study subjects did not differ by baseline characteristics including age at cohort entry, eye color, occupational sun exposure, time spent in leisure activities, smoking status, personal history of cancer, or history of X-ray exposure (Table 2). Subjects with higher 25(OH)D levels were more likely to be male, more educated, and to report no exposure to occupational UV 1 year before taking the Multiphasic Health Checkup (MHC). Those who exercised 2–4 h per day had higher levels than those who exercised less (0–1 h day^{-1}) whereas obese patients (BMI $\geqslant 30\,kg\,m^{-2}$) had lower 25(OH)D levels compared with non-obese patients (BMI <25).

In the unadjusted model, a positive association was noted between higher 25(OH)D levels and increased BCC risk whether assessing 25(OH)D as a continuous variable, a categorical variable based on clinical cutoffs, or a categorical variable based on the distribution of 25(OH)D levels among controls divided into quintiles (Table 3). For every 1 ng ml^{-1} rise in serum 25(OH)D levels, there was a 3% increase in BCC risk. An added quadratic term for continuous 25(OH)D levels in the unadjusted model was not significant, indicating that a linear model better approximated the relationship between 25(OH)D and BCC risk (data not shown). Individuals who had clinically sufficient 25(OH)D levels ($\geqslant 30\,ng\,ml^{-1}$) were at increased risk of BCC compared with those who were 25(OH)D deficient ($<10\,ng\,ml^{-1}$). Similarly, those in the highest quintile had increased risk compared with those in the lowest quintile.

The results did not substantially change when analyzed using the parsimonious model (model 1) or the fully adjusted model (model 2). In both models, each 1 ng ml^{-1} increase in serum 25(OH)D levels was associated with a corresponding 2% rise in BCC risk ($P<0.05$). Subjects with sufficient 25(OH)D levels were still at increased risk of BCC compared to those with deficient levels using the parsimonious and fully adjusted models. The association between serum 25(OH)D divided into quintiles and BCC risk was significant in the parsimonious model ($P=0.05$, test for trend). Risk estimates in the fully adjusted model using quintiles were slightly

Table 1. Selected characteristics of BCC case and control subjects nested within the KPNC Multiphasic Health Checkup cohort

Characteristic	BCC *n*=220	Control *n*=220[1]	*P*-value[2]
Demographic			
Education, n *(%)*			
High school or less	94 (42.7)	105 (47.7)	
Any college	126 (57.3)	115 (52.3)	0.29
Sun exposure surrogate variables			
Occupational UV (self-reported), n *(%)*			
No	212 (96.4)	214 (97.3)	0.76
Yes	6 (2.7)	5 (2.3)	
Occupational sun (assigned), n *(%)*			
High	7 (3.2)	6 (2.7)	0.96
Moderate	118 (53.6)	119 (54.1)	
Low	95 (43.2)	95 (43.2)	
Leisure time activities (no. of hours/day), n *(%)*[3]			
0–1	27 (12.3)	25 (11.4)	0.90
2–4	151 (68.6)	153 (69.6)	
>5	26 (11.8)	29 (13.2)	
Missing	16 (7.3)	13 (5.9)	
Exercise (no. of hours/day), n *(%)*[4]			
0–1	140 (63.6)	138 (63.7)	0.96
2–4	55 (25.0)	59 (26.8)	
>5	11 (5.0)	11 (5.0)	
Missing	14 (6.4)	12 (5.5)	
Other variables			
BMI ($kg\,m^{-2}$), mean (SD)	24.9 (3.3)	25.5 (3.7)	0.07
Smoking status (cigarettes), n *(%)*			
Never	103 (46.8)	86 (39.1)	0.17
Former	49 (22.3)	61 (27.7)	
Current	57 (25.9)	54 (24.6)	
Missing	11 (5.0)	19 (8.6)	
Personal history of cancer, n *(%)*			
No	169 (76.8)	200 (90.9)	
Yes	44 (20.0)	12 (5.5)	<0.001
Missing	7 (3.2)	8 (3.6)	
X-ray exposure, n *(%)*			
No	212 (96.4)	215 (97.7)	0.40
Yes	8 (3.64)	5 (2.3)	
Serum 25(OH)D, mean (SD), ng ml^{-1}	25.5 (11.4)	23.3 (10.1)	0.03

[1]Controls individually matched by age (±1 years), skin color (white), eye color, sex, date of serum collection (±1 month to control for seasonality), Multiphasic location and length of KPNC membership.
[2]*P*-values are derived from χ^2-test for categorical and *t*-test for continuous variables.
[3]Defined as "hobby, TV, etc."
[4]Defined as "walking, sports, etc."

Table 2. Mean serum 25-hydroxyvitamin D according to subject characteristics

Characteristic	*n*	Mean 25(OH)D (SD)	*P*-value[1]
Demographics			
Age in years (at study entry)			
28–44	78	25.1 (12.1)	0.31
45–54	129	25.6 (10.3)	
55–64	156	23.7 (10.5)	
⩾ 65	77	23.2 (10.7)	
Gender			
Male	228	26.3 (10.4)	<0.001
Female	212	22.4 (10.9)	
Eye color			
Brown	74	23.2 (8.4)	0.30
Green/Gray	24	27.7 (7.9)	
Blue	216	24.2 (11.0)	
Other	126	24.8 (12.2)	
Education (years)			
High school or less	199	22.7 (10.1)	<0.01
Any college	241	25.8 (11.2)	
Sun exposure surrogate variables			
Occupational UV (self-reported)			
No	429	24.6 (10.8)	0.07
Yes	11	18.6 (7.6)	
Occupational sun (assigned)			
High	13	27.0 (7.8)	0.68
Moderate	237	24.2 (11.1)	
Low	190	24.5 (10.6)	
Leisure time activities (no. of hours/day)[2]			
0–1	52	25.9 (11.1)	0.24
2–4	304	24.6 (10.8)	
⩾5	55	21.9 (10.4)	
Missing	29	24.6 (11.1)	
Exercise (no. of hours/day)[3]			
0–1	278	23.3 (10.3)	0.04
2–4	114	26.7 (11.8)	
⩾5	22	25.3 (9.0)	
Missing	26	24.7 (11.5)	
Other variables			
Body mass index (kg m^{-2})			
< 25	221	25.9 (12.1)	
25–29.9	168	23.7 (9.2)	0.004
⩾30	31	19.5 (8.5)	
Missing	20	21.3 (8.4)	
Smoking status (cigarettes)			
Never	189	23.8 (9.6)	0.08
Former	110	26.7 (11.1)	
Current	111	23.5 (12.5)	
Missing	30	23.2 (9.3)	
Personal history of cancer			
No	369	24.7 (11.0)	0.32
Yes	56	23.8 (9.7)	
Missing	15	20.5 (9.8)	
X-ray exposure			
No	427	24.4 (10.8)	0.71
Yes	13	25.5 (11.3)	

[1]For categorical variables, *P*-values calculated for evidence of dissimilar mean values among groups using PROC ANOVA.
[2]Defined as ''hobby, TV, etc.''
[3]Defined as ''walking, sports, etc.''

attenuated, bordering on statistical significance ($P = 0.11$, test for trend).

DISCUSSION

The findings from this nested case–control study suggest that higher prediagnostic serum 25(OH)D levels may be associated with increased risk of subsequent BCC. For every 1 ng ml^{-1} increase in serum 25(OH)D levels, there was a corresponding 2% increased adjusted risk of BCC. Our data do not support *in vitro* evidence which suggests that vitamin D may inhibit BCC cell growth (Xiao *et al*, 2009). However, that growth is inhibited by vitamin D3, and our measurements were of 25(OH)D, which is ineffective in the *in vitro* assays. To our knowledge, there have been no published papers comparing serum 25(OH)D levels in a population-based sample of individuals with BCC to controls.

UV exposure is a known risk factor for BCCs and is the most readily available source of vitamin D in sunny climates, such as that in the San Francisco Bay Area. A possible explanation of our finding is that the carcinogenic effects of the amount of UV exposure that leads to high serum 25(OH)D levels may overwhelm any possible protective effect of vitamin D noted *in vitro*. In addition, residual confounding by

Table 3. ORs and 95% CIs for the association between serum 25-hydroxyvitamin D and BCC risk (unadjusted, parsimonious model, fully adjusted)

Vitamin D	Unadjusted OR (95% CI)	Model 1 (parsimonious) OR (95% CI)[1]	Model 2 (fully adjusted) OR (95% CI)[2]
Continuous (ng ml^{-1})	1.03 (1.00, 1.05)	1.02 (1.00, 1.05)	1.02 (1.00, 1.05)
Clinical tertiles (ng ml^{-1})			
Deficient (<10)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Insufficient (10–<30)	2.41 (0.86, 6.80)	2.65 (0.89,7.95)	2.30 (0.70, 7.60)
Sufficient (⩾30)	3.98 (1.31, 12.13)	4.07 (1.26, 13.13)	3.61 (1.00, 13.10)
P-value (trend)	< 0.01	0.02	0.03
Quintiles (ng ml^{-1})			
1 (<14.70)	1.00 (referent)	1.00 (referent)	1.00 (referent)
2 (14.70–20.06)	1.70 (0.94, 3.09)	1.76 (0.94, 3.33)	1.67 (0.84, 3.34)
3 (20.07–24.67)	1.10 (0.56, 2.13)	1.13 (0.56, 2.30)	1.11 (0.51, 2.43)
4 (24.68–29.78)	1.64 (0.83, 3.24)	1.66 (0.81, 3.40)	1.54 (0.70, 3.37)
5 (>29.78)	2.32 (1.20, 4.50)	2.20 (1.10, 4.40)	2.09 (0.95, 4.58)
P-value (trend)	0.03	0.05	0.11

Abbreviations: BCC, basal cell carcinoma; BMI, body mass index; CI, confidence interval; OR, odds ratio.
[1]Adjusted for smoking status and continuous BMI with imputed values for missing BMI (*n*=20).
[2]Adjusted for continuous BMI (with imputed values for *n*=20 missing), education, sun-exposure surrogates (hours of exercise and leisure activities, occupational UV, occupational sun exposure level), X-ray exposure, smoking status, and personal history of cancer.

UV exposure is possible in this analysis. We attempted to control for sun exposure by reasoning that the exposure would come from two primary sources: time spent in the sun for leisure and exercise and time spent in the sun related to one's occupation. We therefore used occupational UV and occupational sun exposure as well as time spent in leisure activities and exercise as surrogate markers for sun exposure. However, none of our sun exposure surrogate variables was a significant risk factor for BCC, suggesting that our surrogates did not adequately capture the type of sun exposure (acute, intermittent) that has been reported to be associated with BCC risk (Kricker *et al*, 1995; Rosso *et al*, 1998). With the exception of exercise, the sun exposure surrogate variables also were not associated with levels of serum 25(OH)D. In fact, subjects who reported higher occupational UV exposure paradoxically had lower serum 25(OH)D levels. One explanation for this finding may relate to the way the question was phrased, which asked subjects to report whether they worked in a place where they were often or daily exposed to ultraviolet radiation before 1 year before taking the MHC. UV exposure in this time period may not have been relevant to serum 25(OH)D levels over a year later. Also subjects may not have comprehended that ultraviolet radiation is primarily derived from the sun. This lack of comprehension is supported by the fact that the same individuals who reported in the affirmative about ''UV exposure'' did not have occupational codes that matched high sun-exposure occupations.

Another potential limitation of our study is the length of storage of the samples. However, these samples have been used in the past for multiple serum vitamin D metabolite studies, which have documented levels in the expected normal range (Corder *et al*, 1993, 1995; Hiatt *et al*, 1998). Indeed, our overall mean serum 25(OH)D level of 24.4 does not differ substantially from the mean serum 25(OH)D level in the US population between 1988 and 2004 (24–30 ng ml^{-1}) as reported by NHANES (Ginde *et al*, 2009). Also, any systematic bias in the storage of the specimens between cases and controls is unlikely, as cases and controls were matched by serum date.

Attenuation of risk estimates may have been possible if some control subjects had BCC diagnosed outside of the KPNC system. All cases and controls were members during each year of follow-up, and so we believe this is unlikely, as KPNC is a comprehensive healthcare system and members would have had to pay out-of-pocket for services received outside the health plan. It should be noted that the exposures that we studied were obtained at a single point in time and were not measured over the entire study follow-up period. Also, subsequent BCC diagnoses as captured through pathology records were not necessarily incident cases.

The strength of this study is that vitamin D status was assessed up to 11 years before the diagnosis of BCC, thereby reducing the likelihood of reverse causality. Our study also has internal validity because both cases and controls were derived from the same prospective cohort. The measurement of serum 25(OH)D levels reflects internal vitamin D status and is considered superior to measures of vitamin D intake by dietary questionnaires alone or predictors of vitamin D status. It is interesting to note that previous reports of dietary intake of vitamin D and BCC risk have found no association (van Dam *et al*, 2000; Gandini *et al*, 2009). Also,

serum 25(OH)D is a measure of vitamin D levels over the past several weeks to several months and is a valid measure of steady-state levels (Holick 1990). Furthermore, to minimize misclassification of vitamin D status due to seasonal variation, we matched cases and controls by season of blood draw (±1 month). Finally, men and women with a diverse age range (28–78 years) were included in the analysis, making our results more generalizable to whites in the US population.

In summary, we observed an increased BCC risk with higher prediagnostic serum vitamin D levels. It is likely that sun exposure, especially acute intermittent exposure, confounds this association. Our findings may also have been influenced by other variables not ascertained in our study, such as supplemental vitamin D use or healthcare screening bias. Future studies that can accurately measure acute intermittent sun exposure, supplemental vitamin D use, and other potential confounding factors may be warranted.

MATERIALS AND METHODS

Study population

The source population consisted of members of KPNC who had completed a MHC between August 1968 and January 1970. The MHC was a voluntary, comprehensive health evaluation that included a detailed self-administered MHC questionnaire (MHCQ), a standardized physical examination, and a group of specialty examinations and laboratory tests administered to a total of 206,974 KPNC members between 1964 and 1973. MHC participants were instructed to fast overnight before a blood collection that was used for routine screening. Details about the MHC have been previously published (Collen and Davis 1969; Cutler *et al*, 1973) and the cohort has been used for numerous risk factor studies (Hiatt and Friedman 1982; Selby *et al*, 1988; Alexander *et al*, 1995; Corley *et al*, 2008). The sera from the collections were stored at −23 °C or colder until 1980, when they were shipped to a frozen storage facility (−40 °C) at the Orentreich Foundation for the Advancement of Science, 25(OH)D levels in these stored sera have been shown to be stable (Corder *et al*, 1993, 1995; Hiatt *et al*, 1998). Pathology records for BCC diagnosis were from KPNC's medical center in Oakland, CA, which had computer-stored pathology records starting in 1974.

Ascertainment of cases and control selection

A total of 3,164 subjects with histologically confirmed BCCs diagnosed between 1974 and 1989 have been previously identified (Friedman and Tekawa, 2000). These individuals were identified by examining pathology records classified using Systematized Nomenclature of Human and Veterinary Medicine codes (Cote, 1993). We limited our time period for case selection to those individuals who completed the MHCQ between 1968 and 1970 to minimize the time interval between serum draw and BCC development. Power calculations indicated that a sample size of 220 cases and 220 controls would result in a minimum detectable pattern of odds ratios of 1.0, 1.21, 1.47, 1.78, and 2.16 for Q1 (quintile 1; referent), Q2, Q3, Q4, and Q5, respectively, (test for trend, two-sided test; $\alpha = 0.05$; power = 0.80).

Our inclusion criteria for cases ($n = 220$) were: (1) ''white'' skin color, as determined by MHC staff (categories of ''yellow'' and ''brown'' excluded), (2) previously unused serum sample associated with a specific MHC visit between 1968 and 1970, (3) BCC diagnosed based on Systematized Nomenclature of Human and Veterinary Medicine morphology code between January 1974 and 31 December 1979 and (4) active KPNC membership each year during the entire follow-up period.

Using incidence density sampling (Rothman and Greenwald, 1998) control subjects were matched 1:1 to cases by age (±1 year), skin color (white), sex, eye color, date of serum collection (±1 month to control for seasonality), MHC location and length of KPNC membership. All control subjects also had at least one unused serum sample available for analysis with a specific MHC visit between 1968 and 1970 and had active KPNC membership each year during the follow-up period. The study was approved by the Institutional Review Board of KPNC and the Declaration of Helskinki protocols were followed. The requirement for informed consent was waived.

Serum vitamin D levels

De-identified aliquoted frozen samples were sent from Orentreich Foundation for the Advancement of Science (Cold Spring-on-Hudson, New York) to Heartland Assays (Ames, IA) and analyzed using the DiaSorin LIAISION 25(OH) Vitamin D Total Assay, which includes 25(OH)D metabolites of vitamin D2 and D3 from plant and animal foods, as well as that synthesized endogenously (Wagner *et al*, 2009). Case and control specimens were handled in the same standard manner, and laboratory personnel were blinded to case–control status. All assays were repeated for reliability. The coefficients of variation for 25(OH)D samples were intra-assay 8.4% and inter-assay 11.4%.

We examined serum 25(OH)D levels as: (1) a continuous variable, (2) a categorical variable divided into quintiles based on distribution among controls, and (3) a categorical variable divided into clinically accepted cutoffs (deficient: $<10\,ng\,ml^{-1}$; insufficient: 10 to $<30\,ng\,ml^{-1}$, sufficient: $\geqslant 30\,ng\,ml^{-1}$) (Kricker *et al*, 1995); http://ods.od.nih.gov/factsheets/vitamind.asp, accessed 20 July 2009.

Covariates

All participants in the MHC had completed the MHCQ that included information on age, education, current and past smoking behavior, history of cancer, occupation, and occupational exposures. Information on possible BCC risk factors was obtained from each subject's MHCQ and from information recorded by the MHC staff (height, weight, eye color, skin color). When BMI data were missing from the MHCQ administered on the serum draw date ($n = 20$), we imputed a value based on the cohort mean value and included a missing data flag in all models. When smoking information was missing, we obtained smoking status from previous prediagnostic MHCQs, if available. When data on education were missing, we used the highest education level recorded among all available MHCQ, hypothesizing that educational status would not have changed significantly in the cohort between 1968 and 1970. We found education level differences between questionnaires in only 2 cases and 4 controls.

We analyzed four variables which served as surrogates of sun exposure: time spent in leisure activities (ordered categorical variable), time spent in exercise (ordered categorical variable), previous occupational exposure to ultraviolet radiation (yes/no), and occupational sun exposure derived from self-reported occupation (categorical). For each reported occupation, we assigned a sun exposure level (low, moderate, high) based on the standard duties of

that occupation. Thus, for example, a mail carrier was deemed to have high occupational sun exposure whereas an office clerk was deemed to have low exposure. Sun exposure levels were reviewed and agreed by the co-authors (MA, JT, MW, GF) and outside investigators with relevant expertise.

Statistical analysis

Our overall analytic strategy was to: (1) compare baseline characteristics of cases and controls (Table 1), (2) determine the association between covariables and 25(OH)D levels in the entire sample (Table 2), (3) examine the association between 25(OH)D levels and BCC risk, controlling for identified confounding variables, and (4) determine the association of serum 25(OH)D levels to BCC risk, controlling for hypothesized potentially confounding variables. We used conditional logistic regression to estimate odds ratios and 95% confidence intervals for BCC risk. We developed a multivariate model that included only the variables associated with both 25(OH)D levels and BCC risk at the $P<0.2$ level; that is, BMI, and smoking status (parsimonious multivariable model, model (1)). We also developed a multivariate model in which all potentially confounding variables were included (fully adjusted multivariate model, model (2)). Tests for linear trend (1 d.f.) were conducted by treating the ordered categorical values of the exposure categories as continuous variables. Statistical analyses were performed using SAS, version 9.1 (SAS Institute, Cary, NC).

CONFLICT OF INTEREST

Dr Bikle is a consultant for Chugai Pharmaceutical.

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