ORIGINAL PAPER

Inverse association between serum 25(OH) vitamin D levels and non-melanoma skin cancer in elderly men

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Abstract To determine the relationship between 25(OH) vitamin D levels and non-melanoma skin cancer (NMSC), we performed a nested case–control study in ambulatory, elderly men enrolled in the Osteoporotic Fractures in Men (MrOS) Study. Health habit and medical history, including self-reported history of NMSC were recorded and 25(OH)D levels were measured on serum collected at baseline from a random sample of Caucasian MrOS subjects. Mean age (73 ± 5), BMI, daily vitamin D and calcium intake were similar in the men with ($n = 178$) and without NMSC ($n = 930$), but higher levels of 25(OH)D were associated with a decreased risk of having a history of NMSC ($P_{trend} = 0.04$). Men in the highest quintile of 25(OH)D (>30 ng/mL) had 47% lower odds of NMSC (95% CI: 0.30–0.93, $p = 0.026$) compared to those in the lowest quintile. Our results suggest that a diagnosis of NMSC is not a surrogate for adequate 25(OH)D levels or increased UV exposure, and high 25(OH)D levels may be associated with a reduced risk of NMSC.

Keywords Skin cancer · Vitamin D · Prevention

Introduction

Non-melanoma skin cancer (NMSC) affects close to 1 million Americans annually. Although metastases are rare, their high incidence and frequent recurrence in affected individuals make NMSCs the 4th most costly cancers of the Medicare population [1]. NSMC is comprised of two subtypes—squamous cell carcinomas (SCC) and basal cell carcinomas (BCC). The majority of NMSCs are of the BCC subtype in older, white subjects (4:1 ratio of BCC:SCC) [2]. Public health education campaigns advocate sunscreen use and sun avoidance for prevention, but the incidence of NMSC continue to rise [2, 3].

NMSC is caused at least in part by sunlight [2] and sunlight is also the primary source of vitamin D [4]. The level of 25(OH)D, the major circulating form of vitamin D, is widely accepted as the best indicator of vitamin D status and vitamin D deficiency occurs if 25(OH)D levels are <20 ng/mL and insufficiency if <30 ng/mL. There is increasing evidence that vitamin D may be protective against cancers of the colon, breast, and prostate [5]. Observational data and one interventional clinical trial suggest that higher levels of 25(OH)D are associated with reduced visceral cancer risk [4, 6, 7]. Thus, based on the idea that the risk of NMSC is related to the amount of ultraviolet (UV) exposure, a diagnosis of NMSC has been used as a surrogate for high 25(OH)D levels [8–10, 11]. NMSC subjects have been hypothesized to have a reduced risk of visceral cancers, but the data are inconsistent. Two studies showed that NMSC subjects have a *reduced* risk

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of colorectal cancer [8, 10] while other studies show an *increased* risk for colorectal and visceral cancers [11, 12]. There has been no study comparing 25(OH)D levels and risk for NMSC. We initially hypothesized that NMSC subjects would have *higher* 25(OH)D levels given their chronic exposure to UV. To determine the association 25(OH)D levels and risk for NMSC, we conducted a nested case-control study in a cohort of elderly men.

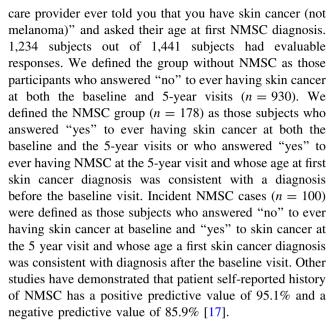## Methods

Study participants

Subjects were participants in the Osteoporotic Fractures in Men Study (MrOS), a prospective cohort study of risk factors for fracture. During the baseline examination from March 2000 to April 2002, 5,995 community-dwelling men 65 years or older were recruited from population-based listings at six clinical centers in the United States: Birmingham, AL; Minneapolis, MN; Palo Alto, CA; Portland, OR; Pittsburgh, PA; and San Diego, CA. MrOS enrollees represent a relatively healthy and highly educated population [13, 14].

Analysis of serum 25(OH)D

At the baseline visit, 1,608 randomly selected MrOS participants had fasting morning blood collected, and serum was prepared immediately and stored at $-70°C$ in vials foil-wrapped to prevent light exposure. Measures for $25(OH)D_2$ (derived from ergocalciferol and obtained from nutritional supplements and plants such as sun-dried mushrooms) and $25(OH)D_3$ (derived from cholecalciferol and obtained primarily from UV irradiation, fortified foods, and supplements) were performed at the Mayo Clinic Labs using LC-mass spectroscopy[15]. Duplicate pooled serum controls were included. The inter-assay coefficient of variation was 4.4% and the intra-assay coefficient of variation was 4.9%. Almost all circulating total 25(OH)D levels measured were due to $25(OH)D_3$ [16] and we define 25(OH)D as $25(OH)D_3$ in our analysis as we sought to measure endogenous vitamin D3 produced from UV irradiated skin. Levels of $25(OH)D_2$ were detected in 26% of samples and contributed to less than 10% of the total 25(OH) [16]. Because levels of 25(OH)D vary by race/ethnicity, we limited our analysis to Caucasian men ($n = 1,441$).

Non-melanoma skin cancer outcome and covariates

At the baseline and the 5-year follow-up visit, MrOS participants responded to a questionnaire item that inquired about having a history of NMSC ("Has a doctor or health care provider ever told you that you have skin cancer (not melanoma)" and asked their age at first NMSC diagnosis. 1,234 subjects out of 1,441 subjects had evaluable responses. We defined the group without NMSC as those participants who answered "no" to ever having skin cancer at both the baseline and 5-year visits ($n = 930$). We defined the NMSC group ($n = 178$) as those subjects who answered "yes" to ever having skin cancer at both the baseline and the 5-year visits or who answered "yes" to ever having NMSC at the 5-year visit and whose age at first skin cancer diagnosis was consistent with a diagnosis before the baseline visit. Incident NMSC cases ($n = 100$) were defined as those subjects who answered "no" to ever having skin cancer at baseline and "yes" to skin cancer at the 5 year visit and whose age a first skin cancer diagnosis was consistent with diagnosis after the baseline visit. Other studies have demonstrated that patient self-reported history of NMSC has a positive predictive value of 95.1% and a negative predictive value of 85.9% [17].

Covariates were obtained by self-administered questionnaire at the baseline visit [13, 14]. Physical activity was quantified using the physical activity scale for the elderly (PASE) and "walking outdoors" for exercise was used as a proxy for outdoor activity [18]. Total calcium and vitamin D intake (from foods and supplements) in the past 12 months were estimated using a modified block food frequency questionnaire (Block Dietary Data Systems, Berkeley, CA). UV exposure history of each subject was estimated by participant's clinic site (to determine latitude) and walking outdoors physical activity [6]. UV exposure is a major confounder as increased UV exposure contributes to NMSC risk and raises 25(OH)D levels.

Statistical analysis

We used univariate and multivariable logistic regression to determine a dose-response association between serum 25(OH)D and odds of NMSC. Quintiles of 25(OH)D were based on the distribution of 25(OH)D in the NMSC and no NMSC groups. Odds of NMSC for quintiles Q2 through Q5 were compared with the lowest quintile. We calculated 95% confidence intervals and used the Mantel extension test to evaluate the presence of linear trend across quintiles. We tested for an interaction between 25(OH)D per ng/mL and each of the covariates; age, PASE walking score and clinic site for effect modification on the association of 25(OH)D3 with NMSC and reported the p value for interaction.

Results

Baseline characteristics for men with and without NSMC were similar in mean age (73.4 ± 5.5 years), BMI, smoking,

education, calcium intake, and in their general health (Table 1). The mean value of 25(OH)D for the entire cohort was 23 ng/mL and consistent with reported values in the persons >70 years of age in the US population[16, 19]. Subjects with NMSC diagnosis had slightly lower levels of outdoor walking activity ($p = 0.02$) and minimally higher (22 IU) daily vitamin D intake ($p = 0.05$). The average age of skin cancer development was 61 ± 12 years, approximately 10 years prior to the median age at which blood for assessing vitamin D status was collected.

As a measure of consistency, we investigated the association of known predictors to serum 25(OH)D levels in univariate analyses. As expected, increasing age was significantly associated with decreasing 25(OH)D levels ($p < 0.0001$) [16] likely due to the age-related decline in vitamin D status due to decreasing amounts of the vitamin D precursor, 7-dehydrocholesterol in the skin [4]. Participants who resided near a clinic with higher UV exposure

(San Diego) had higher levels of 25(OH)D compared to participants residing in clinics in Minnesota and Portland ($p < 0.0001$) as previously reported [16].

As a measure of internal validity, we examined the effect of a number of known risk factors for NMSC (Table 2). Subjects residing in Minneapolis or Portland versus San Diego had a reduced odds of NMSC ($p < 0.004$), consistent with other reports showing a lower incidence of NMSC in areas of low UV exposure [20]. Subject age was not associated with prevalent history of NMSC, perhaps because our subjects were of an older age range (65–78 years). BMI, cigarette smoking, and outdoor walking activity were not significantly associated with history of NSMC [2].

We found that higher quintiles of serum 25(OH)D levels are associated with *decreased* odds of NMSC when adjusting for age, BMI, season of blood draw, and clinic site ($P_{trend} = 0.032$) (Table 3). This inverse association persisted in the multivariable model when cigarette smoking and outdoor walking activity were adjusted ($P_{trend} = 0.044$). The majority of the effect was observed among men in the highest quintile of 25(OH)D who had a 47% lower odds of NMSC (OR: 0.53, 95% CI: 0.30–0.93, $p = 0.026$) compared to those in the lowest quintile in both multivariable models. A 40% reduction in the odds of NMSC was also detected when comparing Q5 versus Q1-4 (≥ 29.9 ng/mL) in both models. Findings were similar in analysis expressing 25(OH)D levels using standard clinical definitions [21, 22, 23]. Subjects who had sufficient levels of 25(OH)D (>32 ng/mL) had a 43% lower odds compared to subjects with insufficient levels (OR: 0.57, 95% CI: 0.33–0.98, $p = 0.045$) in the base model, and the association approached but did not reach statistical significance after further adjustment for walking and cigarette use. The association between 25(OH)D and NMSC risk was not significantly modified by age, clinic site, or walking activity ($P_{interaction} > 0.05$).

Table 1 Baseline characteristics of men with and without non-melanoma skin cancer (NMSC)

Characteristics	NMSC ($n = 178$)	No NMSC ($n = 930$)	p-value
Mean (SD)			
Age, years	73.6 (5.7)	73.2 (5.6)	0.35
BMI, kg/m^2	27.2 (3.6)	27.6 (3.7)	0.13
Daily vitamin D intake[a], IU	182 (125)	160 (114)	0.05
Daily calcium intake[a], IU	821 (415)	807 (385)	0.66
Outdoor walking activity	14.3 (13.1)	17.1 (18.8)	0.02
Number (%)			
Current smokers	2 (1.1%)	31 (3.3%)	0.11
Spent >1 week confined to bed	8 (4.5%)	37 (3.9%)	0.75
Season of blood draw			
Winter	33 (19%)	202 (22%)	0.58
Spring	47 (26%)	244 (26%)	
Summer	49 (27%)	268 (29%)	
Fall	49 (27%)	216 (23%)	
Clinic			
Minneapolis	21 (12%)	164 (17%)	0.03
Pittsburgh	28 (16%)	199 (21%)	
Portland	29 (16%)	140 (15%)	
Birmingham	33 (18%)	134 (14%)	
Palo Alto	25 (14%)	143 (15%)	
San Diego	42 (24%)	150 (16%)	
Education			
Elementary	2 (1.1%)	15 (1.6%)	0.54
High school	35 (20%)	212 (23%)	
College	70 (39%)	381 (41%)	

[a] Combined intake from diet and supplements

Table 2 Selected factors and the risk of non-melanoma skin cancer (multivariable model)

Baseline covariates	Odds ratio (OR)[a] and 95% CI	p-value
Age (per 1 year)	1.01 (0.98–1.04)	0.50
BMI (per 1 kg/m^2)	0.86 (0.73–1.02)	0.09
Current cigarette smoking (yes/no)	0.22 (.029–1.65)	0.14
Outdoor walking	0.99 (0.98–1.001)	0.08
Clinic site		
Minneapolis vs. San Diego	0.41 (0.23–0.73)	0.003
Portland vs. San Diego	0.46 (0.27–0.78)	0.004

[a] OR are for each covariate in a multivariable model adjusted for quintiles of 25(OH)D, age, BMI, season of blood draw, clinic site, outdoor walking activity (continuous variable), and cigarette smoking (yes/no)

Table 3 Association of increasing serum 25(OH)D levels with non-melanoma skin cancer

25(OH)D (ng/mL)	Odds ratio (OR) and 95% CI	
	Base model[a]	Fully adjusted model[b]
Q1 (<16)	1.00 (referant)	1.00 (referant)
Q2 (16–20.8)	0.94 (0.56–1.55)	0.94 (0.56–1.55)
Q3 (20.9–25.1)	0.91 (0.55–1.55)	0.93 (0.56–1.54)
Q4 (25.2–29.8)	0.84 (0.50–1.42)	0.86 (0.51–1.45)
Q5 (29.9–58.3)	**0.53 (0.30–0.93)**	**0.54 (0.31–0.96)**
P_{trend}	*0.032*	*0.044*
≥29.9 ng/mL (Q5 vs. Q1–Q4)	**0.57 (0.35–0.93)**	**0.60 (0.37–0.98)**
≥32.0 ng/mL	**0.57 (0.33–0.98)**	0.59 (0.34–1.01)

[a] Adjusted for age (continuous variable), BMI (continuous variable), season of blood draw, and clinic site

[b] Adjusted for age, BMI, season of blood draw, clinic site, outdoor walking activity (continuous variable), and cigarette smoking (yes/no)

There were relatively few incident NMSC cases ($n = 100$) during the 5 year follow-up period. When we compared subjects with 25(OH)D levels ≥32 ng/mL versus those with levels <32 ng/mL, we found trends suggesting a reduction of NMSC risk (OR: 0.72; 95% CI: 0.38–1.38, $p = 0.33$) in both base and multivariable models although this did not reach statistical significance.

Discussion

We have found that higher serum 25(OH)D levels are associated with a decreased risk of NMSC in older Caucasian men. To our knowledge, this is the first study to examine this association, and our data argue against the assumption that a NMSC diagnosis is a surrogate for increased UV exposure or elevated 25(OH)D levels. Studies in NMSC are difficult as these cancers are not reported in US national registries. One prior prospective cohort study on vitamin D intake from diet found no association between vitamin D and BCC risk [24]. One explanation for these differences is that our study measured 25(OH)D$_3$ levels which primarily reflect vitamin D obtained from UV exposure and a smaller contribution from diet and supplements.

A major strength of this study is that we are using a large cohort of community dwelling men from diverse geographical regions for which we have information on season and lifestyle factors that may modify 25(OH)D levels and NMSC risk. In addition, 25(OH)D measurements were performed using mass spectroscopy, a more precise assay compared to other techniques [16, 25]. Our study had several limitations. NMSC outcome was

ascertained through subject self-report and not histological confirmation, although most (but not all) previous investigators have reported a positive predictive value of 80–95% [17, 24, 26]. Our analyses were adjusted for several skin cancer risk factors such as smoking, amount of outdoor activity, and geographic residence but we did not have information on other skin cancer risk factors such as subject skin type, hair color, or eye color [2]. Differences in baseline pigmentation among case and non-case groups might affect skin cancer development and 25(OH)D as subjects with lighter skin color would have an increased risk for skin cancer but also an increased ability for cutaneous synthesis of vitamin D. Given the observational design, the possibility of residual confounding cannot be eliminated and we are unable to determine a causal inference between 25(OH)D levels and NMSC risk.

Our results suggest that subjects with 25(OH)D >32 ng/ml appear to have a 40% lower risk of NMSC and perhaps raising the 25(OH)D levels may be protective. Laboratory studies have also shown that vitamin D may be protective as 1.25(OH)D$_2$ inhibits SCC cell lines, and mice deleted for the vitamin D receptor exhibit SCC and BCC tumors [27, 28]. Consistent with this hypothesis, we detected a trend of reduced risk of incident NMSC among subjects with higher 25(OH)D levels. Another possible explanation is that NMSC subjects may have lower 25(OH)D levels due to sun avoidance behaviors after their NMSC diagnosis. A prospective study of 25(OH)D and incident NMSC cases is needed to further explore the possible protective effect of vitamin D on non-melanoma skin cancer.

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