Dermatology Practical & Conceptual

Serum Total 25-Hydroxyvitamin D Levels in Patients With Cutaneous Malignant Melanoma: A Case-Control Study in a Low-Risk Southern European Population

Angeliki Befon,[1] Alexander C. Katoulis,[2] Sofia Georgala,[1] Andreas Katsampas,[1] Vasiliki Chardalia,[1] Aggeliki Melpidou,[3] Vasiliki Tzanetakou,[1] Vasiliki Chasapi,[1] Dorothea Polydorou,[1] Clio Desinioti,[1] Micaela Plaka,[1] Dimitris Rigopoulos,[1] Alexandros J. Stratigos[1]

1 First Department of Dermatology and Venereology, National and Kapodistrian University of Athens, Medical School, Andreas Syggros Hospital for Skin and Venereal Diseases, Athens, Greece

2 Second Department of Dermatology and Venereology, Attikon General University Hospital, National and Kapodistrian University of Athens, Greece

3 Biochemistry Laboratory, Evangelismos General Hospital of Athens, Greece

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Corresponding author: Dr. Angeliki Befon, First Department of Dermatology and Venereology, University of Athens, Andreas Syggros Hospital, 5 Dragoumi str, Athens 16121, Greece. E-mail: drbefon@yahoo.gr

ABSTRACT

Background: Recent data have shown an inverse association between serum 25-hydroxyvitamin D concentration and incidence of several cancers, including cutaneous malignant melanoma (CMM). In addition, lower serum 25-hydroxyvitamin D levels have been associated with thicker or higher stage melanomas and worse survival in observational studies.

Materials and Methods: Ninety-nine patients diagnosed with primary CMM and 97 matched healthy controls entered the study. Demographic characteristics, risk factors for CMM, and clinical and histological characteristics were recorded for patients with primary CMM. Total serum 25-hydroxyvitamin D levels of melanoma patients measured by fully automated chemiluminescent vitamin D total immunoassay (Elecsys vitamin D total, Roche) at the time of diagnosis were compared with those of healthy controls. In addition, we tested the association of serum total 25-hydroxyvitamin D levels at melanoma diagnosis with known risk and prognostic factors for CMM.

Results: Of the melanoma patients, 49 (49.49%) had deficient serum total 25-hydroxyvitamin D levels (<20 ng/mL), 23 (23.23%) had insufficient levels (21-29 ng/mL), and 27 (27.27%) had adequate

ABSTRACT levels (>30 ng/mL). The median serum total 25-hydroxyvitamin D levels were significantly lower in melanoma patients (20.62 ng/mL) compared with healthy controls (24.71 ng/mL), but statistical significance was not reached (chi-square test, P = 0.051) No statistically significant association was found between serum total 25-hydroxyvitamin D levels and demographic characteristics; risk factors for CMM; prognostic factors, such as Breslow thickness and ulceration; as well as clinical characteristics, such as melanoma stage, clinical type, and location.

Conclusions: Lower serum 25-hydroxyvitamin D levels were found in our Greek cohort of melanoma patients compared with healthy controls, without reaching, however, statistical significance; these levels were not statistically associated with established risk and prognostic factors for CMM.

Introduction

The incidence of cutaneous malignant melanoma (CMM) has increased steeply over recent decades and continues to grow worldwide despite intense efforts at primary prevention. CMM results from complex interactions between genetic and environmental factors. Excessive intermittent ultraviolet radiation (UVR) exposure and sunburns during childhood are considered as the principal causes of CMM insurgence in adults, with double the risk relative to a nonexposed population [1,2].

On the other hand, UVR stimulates the endogenous production of pre-vitamin D_3 in the skin, which supplies >90% of the body's requirements [3]. In vivo vitamin D demonstrates pleiotropic effects. Beyond its role in homeostasis of calcium and phosphorus, it modulates cellular functions, such as innate and adaptive immunity through the suppression of inflammation, cell proliferation, differentiation, apoptosis, and metastatic potential [4-8]. Recent data have linked low serum vitamin D levels to a wide range of diseases, including cardiovascular disease, insulin resistance, autoimmune disease, and infection. Most importantly, they have been linked with increased incidence of several malignancies, such as breast, colorectal, kidney, lung, and pancreatic cancer [6-10].

The relationship between vitamin D levels and CMM seems to be more intricate, compared with other malignancies [1]. Vitamin D receptor is present in normal melanocytes and in certain cell lines of melanoma [11]. In vitro studies have shown that a proportion of melanoma cell lines in culture respond to the antiproliferative effect of active vitamin D analogs [12,13]. Serum vitamin D levels have been examined as a marker for increased risk for CMM development. Lower serum vitamin D levels have been associated with thicker or higher stage melanomas [14-20]. Two recent large cohort studies have shown that vitamin D levels were significantly associated with overall survival, melanoma-specific survival, and disease-free survival [21,22].

In the present study, we sought to investigate serum total 25-hydroxyvitamin D levels in a cohort of patients diagnosed with primary CMM and compare them with those of matched healthy controls. In addition, we tested the association of serum 25-hydroxyvitamin D levels at the time of melanoma diagnosis with known risk and prognostic factors for CMM, in an attempt to assess their prognostic value.

Materials and Methods

Study Design

This prospective cohort study was conducted at the Melanoma Unit of the First Department of Dermatology and Venereology, Andreas Syggros Hospital, Athens, Greece. The study was approved by the ethics committee of the hospital. Written informed consent was obtained for all entered patients and controls. Patient recruitment took place from April 2011 to March 2014. Consecutive patients diagnosed with primary invasive CMM of any stage were enrolled. The diagnosis of CMM was made on histological grounds. Exclusion criteria included the following: patients with noncutaneous melanoma or metastatic melanoma of unknown primary; patients with self-reported preexisting conditions that could interfere with vitamin D metabolism, such as chronic liver or kidney disease; transplant recipients or those with other causes of immunosuppression; patients receiving high-dose calcium therapy; and patients receiving vitamin D supplementation during the previous 6 months.

For all recruited melanoma patients, complete personal and family history was obtained and total body skin examination was performed. Demographic characteristics such as age, sex, race, occupation; body measurements, ie, height and weight to calculate body mass index (BMI); risk factors for CMM including hair color, skin color, skin phototype, occupational and recreational sun exposure, sunburns during childhood; presence of dysplastic nevi, number of melanocytic nevi, personal or family history of CMM, have all been recorded. Clinical type of CMM and localization in sun-exposed or sun-protected areas were assessed. Breslow thickness and presence or absence of ulceration were obtained from the histology report at the time of diagnosis. For CMM staging, the seventh edition of the American Joint Committee on Can-

cer (AJCC) classification system (2010) was used. Patients were followed for 42-78 months. During follow-up, recurrences and melanoma-related deaths were recorded.

Age- and sex-matched healthy subjects with nonrelated minor skin conditions from the outpatient dermatology service were recruited as healthy controls after they had given their informed consent. The same exclusion criteria as for melanoma patients were used.

Assessment of Serum Total 25-Hydroxyvitamin D

For all recruited melanoma patients, whole blood was sampled within 1 month from the time of diagnosis of CMM. In healthy controls, blood sampling was performed at the time of recruitment. To avoid the effect of seasonal variation of vitamin D levels, for each entered patient, a control subject was recruited at the same time. Of our melanoma patients, 61 (61.61%) were diagnosed during spring and summer when sun exposure is high, and this is in accordance with other reports worldwide on the seasonality of melanoma diagnosis [20]. All blood samples were immediately processed and centrifuged. Serum 25-hydroxyvitamin D levels were measured using a commercial, fully automated chemiluminescent vitamin D total immunoassay (Elecsys vitamin D total, Roche). Measurements were performed at the Biochemistry Department of Evangelismos General Hospital of Athens, Greece. According to the Endocrine Society's Clinical Guidelines, reference serum values for vitamin D were classified as follows: levels greater than 30 ng/mL were considered adequate; values between 21 and 29 ng/mL, insufficient; and levels lower than 20 ng/mL, deficient.

Statistical Analysis

Median values and interquartile ranges for continuous variables, or number and proportions for categorical variables, were used to describe the data. To test the relationship between serum levels of vitamin D and various demographic and prognostic factors, the chi-square test or the Fisher exact test was used. In all the above comparisons vitamin D was used as a categorical variable with 3 levels. Comparisons of baseline characteristics between the study groups were performed by using the chi-square test. For the comparison of serum vitamin D median between patients and controls, the nonparametric Mann-Whitney U test was used as 25-hydroxyvitamin D serum concentrations could not be assumed to be normally distributed (demonstrated by Kolmogorov-Smirnov test). The level of significance was set at 5% ($P < 0.05$). All statistical analyses were performed using the statistical package SPSS Version 18 (IBM, USA).

Table 1. Demographic Characteristics and Serum 25(OH)D Levels for Patients and Controls

	Melanoma Patients	Controls	P Value
	N (%)	N (%)	
Sex			0.780[a]
Male	46 (46.46)	47 (48.45)	
Female	53 (53.54)	50 (51.55)	
Age			0.780[a]
≤40 years	31 (31.31)	34 (35.05)	
>40 years	68 (68.69)	63 (64.95)	
Age, median (IQR)	50 (37-63.5)	51 (36-63)	
Residence			0.493[a]
Urban	67 (67.68)	70 (72.2)	
Rural	32 (32.32)	27 (27.8)	
Serum 25(OH)D	**Median (IQR)**	**Median (IQR)**	0.051[b]
	20.62 (13.22-29.67)	24.71 (15.37-34.65)	
	N (%)	**N (%)**	0.260[a]
<20 ng/mL	49 (49.49)	42 (43.30)	
21-29 ng/mL	23 (23.23)	18 (18.56)	
>30 ng/mL	27(27.27)	37 (38.14)	
BMI			
≤25	42 (42.42)	NA	
>25	57 (57.58)	NA	

[a]Chi-square test.
[b]Mann-Whitney U test.
N (%) = frequency (percentage); 25(OH)D = 25-hydroxyvitamin D; BMI = body mass index; IQR = interquartile range; NA = not available.

Results

A total of 105 patients were diagnosed with primary CMM at our academic department during the study period. Six of them were excluded because they had been using oral vitamin D supplements; thus 99 patients were included in the study. Of the 99 patients, 53 were female (53.54%). Their age ranged from 37 to 64 years (median 50 years). The demographic characteristics of melanoma patients (n = 99) and healthy controls (n = 97) are presented in Table 1.

Regarding risk factors for CMM among melanoma patients, there were 7 patients with skin phototype I (7.07%), 43 with type II (43.43%), 34 with type III (34.34%), and 15 with type IV (15.15%). Twenty patients (20.20%) reported excessive nonintentional (occu-

Table 2. Descriptive Statistics of Risk Factors and Association With Serum 25-Hydroxyvitamin D

	N (%)	P Value[a]
Eye color		0.120
Light[b]	77 (77.78)	
Dark	22 (22.22)	
Hair color		0.948
Light[c]	52 (52.53)	
Dark	47 (47.47)	
Skin color		0.910
White	58 (58.59)	
Light brown	38 (38.38)	
Dark	3 (3.03)	
Phototype		0.813
Type I	7 (7.07)	
Type II	43 (43.43)	
Type III	34 (34.34)	
Type IV	15 (15.15)	
Occupational sun exposure		0.077
Yes	20 (20.20)	
No	79 (79.80)	
Recreational sun exposure		0.104
Yes	52 (52.53)	
No	47 (47.47)	
Photoaging		0.903
Yes	46 (46.46)	
No	53 (53.54)	
Sunburn during childhood (<20 years)		0.356
No	60 (60.61)	
Yes	39 (39.39)	
Sunburn during adulthood (>20 years)		0.408
No	64 (64.65)	
Yes	35 (35.35)	
Sunscreen use		0.845
Never	32 (32.32)	
Rarely	29 (29.29)	
During summer vacation	31 (31.31)	
Usually throughout year	7 (7.07)	
Nevi count		0.982
<10	41 (41.41)	
10-50	40 (40.40)	
>50	18 (18.18)	
Atypical mole		0.648
Yes	32 (32.32)	
No	67 (67.68)	
Family history of melanoma		0.977
Yes	8 (8.08)	
No	91 (91.92)	
Personal history of melanoma		0.999[d]
Yes	1 (1.01)	
No	98 (98.99)	

[a]Chi-square test (unless specified otherwise).
[b]Blue, green, and light brown eyes.
[c]Blonde, red, and light brown hair.
[d]Fisher exact test.
N (%) = frequency (percentage).

(data continues next column)

pational) sun exposure, while 52 (52.53%) reported excessive intentional (recreational) sun exposure. Thirty-nine patients (39.39%) had experienced blistering sunburns during childhood and adolescence and 35 (35.35%) during adulthood. Signs of photo-damaged skin—presence of elastosis, solar lentigines, actinic keratoses—were observed in 46 patients (46.46%). Family history of CMM was reported by 8 patients (8.08%), and personal history by 1 (1.01%). Atypical nevi were present in 32 patients (32.32%). Risk factors for CMM among our melanoma patients are depicted in Table 2, while clinical and histological characteristics of melanoma are presented in Table 3.

Serum total 25-hydroxyvitamin D levels were measured in 99 melanoma patients at the time of diagnosis and in 97 age- and sex-matched healthy controls at the time of recruitment. Of the melanoma patients, 49 (49.49%) had deficient serum total 25-hydroxyvitamin D (<20 ng/mL), 23 (23.23%) had insufficient levels (21-29 ng/mL), and 27 (27.27%) had adequate 25-hydroxyvitamin D levels (>30 ng/mL). Two melanoma patients with serum total 25-hydroxyvitamin D levels between 20 and 20.9 ng/mL and 3 patients with values between 29 and 29.9 ng/mL were included in the insufficient class of the melanoma patients group. Of the healthy controls, 42 (43.30%) had deficient serum levels of total 25-hydroxyvitamin D (<20 ng/mL), 18 (18.56%) had insufficient levels (21-29 ng/mL), and 37 (38.14%) had adequate 25-hydroxyvitamin D levels (>30 ng/mL). The median serum total 25 hydroxyvitamin D levels were lower in melanoma patients (20.62 ng/mL vs 24.71 ng/mL) compared with those in healthy controls (P = 0.051). Statistical analysis is presented in Table 1.

We tested the correlation of serum total 25-hydroxyvitamin D levels at melanoma diagnosis with demographic

characteristics and risk factors for CMM. No statistically significant association between serum total 25-hydroxyvitamin D levels and the tested parameters was found (Table 2). We also tested the correlation of serum total 25-hydroxyvitamin D levels at melanoma diagnosis with prognostic factors, such as Breslow thickness and ulceration, as well as with clinical characteristics, such as melanoma stage, clinical type, and location, after classifying patients into 2 groups: those with BMI >25 (n = 57) and those with BMI ≤25 (n = 42) (see Table e1 of supplementary content). Although no statistical difference was documented, we observed that a higher percentage (33.33%) of melanoma patients with ulceration and BMI >25 had insufficient serum total 25-hydroxyvitamin D levels compared with those (23.81%) without ulceration. Moreover, Breslow thickness was not associated with serum concentrations of 25-hydroxyvitamin D (P = 0.370), and the correlation remained nonsignificant even when the patients were classified into 2 subgroups of early-onset (<40 years, P = 0.295) and late-onset melanoma (>40 years, P = 0.284) (see Table e2 of supplementary content).

Table 3. Descriptive Statistics of Clinical and Histological Characteristics and Association With Serum 25-Hydroxyvitamin D Levels

	N (%)	P Value[a]
Melanoma location		0.139[b]
Trunk	45 (45.45)	
Extremities	43 (43.43)	
Face and neck	11 (11.11)	
Melanoma type		0.128[b]
LMM	3 (3.03)	
SSM	76 (76.77)	
NM	16 (16.16)	
ALM	2 (2.02)	
Other	2 (2.02)	
Stage		0.602[b]
In situ	5 (5.05)	
I	63 (63.64)	
II	19 (19.19)	
III	11 (11.11)	
IV	1 (1.01)	
Deaths		0.566
Yes	4 (4.04)	
No	95 (95.96)	
Recurrence		0.359[b]
Yes	6 (6.06)	
No	93 (93.94)	
Breslow level		0.370
<1 mm	52 (52.53)	
1-4 mm	33 (33.33)	
>4 mm	14 (14.14)	
Ulceration		0.494
Yes	31 (31.31)	
No	68 (68.69)	

[a]Chi-square test (unless specified otherwise).
[b]Fisher exact test.
N (%) = frequency (percentage); LMM = lentigo malignant melanoma; SSM = superficial spreading melanoma; NM = nodular melanoma; ALM = acral lentiginous melanoma.

Discussion

In the present study, we found lower median serum total 25-hydroxyvitamin D levels among melanoma patients compared with healthy controls, but the difference did not reach statistical significance (P = 0.051). Possible explanations for this finding may be the relatively small number of patients, though representative of the low incidence of melanoma in Greece, and the narrow variations of the median (interquartile range) serum 25-hydroxyvitamin D levels between patients and healthy controls (Table 1), as both groups had median values of serum total 25-hydroxyvitamin D in the range of vitamin D insufficiency (20.62 ng/mL for the melanoma patients and 24.71 ng/mL for the healthy controls.

In contrast, no statistically significant associations were documented between serum total 25-hydroxyvitamin D levels at melanoma diagnosis and demographic characteristics, risk factors for CMM, clinical characteristics, and prognostic factors, such as Breslow thickness, ulceration, and melanoma stage. To our knowledge this is the first study to assess serum total 25-hydroxyvitamin D at the time of melanoma diagnosis and examine its correlation with clinical and histological variables in a low-incidence Southern European country with high year-round ambient UVR levels.

In recent years, various studies have focused on the role of vitamin D status in CMM risk and progression. Overall lower serum vitamin D levels at melanoma diagnosis have been associated with an increased incidence of CMM and worse prognosis. In a prospective study of 271 melanoma patients in UK, Newton-Bishop et al found that higher 25-hydroxyvitamin D_3 levels were associated with lower Breslow thickness at diagnosis and were independently protective of relapse and death [14]. Among 2,182 participants in the Leeds Melanoma Cohort, lower 25-hydroxyvitamin D levels and smoking were associated with ulceration of primary melanomas and poorer melanoma-specific survival [15]. In accordance with these findings, other authors have found significantly lower median serum 25-hydroxyvitamin D concentrations in melanoma patients compared with healthy controls and combined these lower serum 25-hydroxyvitamin D concentrations found in melanoma patients with greater Breslow thickness

and worse survival [16]; association between lower serum 25-hydroxyvitamin D levels, higher Breslow thickness, and higher AJCC melanoma stage [17]; a nearly 4-fold increase in risk of having a thicker tumor that was associated with low serum vitamin D levels (<50 nmol/L) [18]; inversely associated Breslow thickness with vitamin D levels [19]; and higher vitamin D in nonulcerated tumors and in tumors within mitotic rate <1/mm^2 [23].

Other studies, however, have argued against the prognostic value of serum 25-hydroxyvitamin D in CMM. In a cohort of 1,171 patients with invasive melanoma, Saiag et al tested the prognostic value of serum 25-hydroxyvitamin D_3 concentrations and concluded that only variation during follow-up is an independent melanoma prognostic marker, but not levels at diagnosis [24]. Previously reported associations between low 25-hydroxyvitamin D_3 at diagnosis and poor prognosis they believe to be due to insufficient adjustment for prognostic factors [24]. Other authors found significantly higher levels of vitamin D_3 in melanoma patients than in healthy controls, but both inferior of normal values [25]; significantly reduced serum 25-hydroxyvitamin D levels only in the stage IV melanoma patients compared with stage I patients, suggesting that patients with low serum vitamin D levels may develop earlier distant metastatic disease [26]; and significantly lower serum vitamin D binding protein levels in melanoma patients compared with healthy controls, emphasizing the prognostic significance of vitamin D binding protein rather than of 25-hydroxyvitamin D levels [27].

In contrast to what one might expect, ie, the typical fair-skinned patient with melanoma with a history of excessive sun exposure would have normal vitamin D levels at diagnosis, a significant number of melanoma patients in the cohorts studied were serologically vitamin D-deficient [21]. This observation is difficult to interpret. Although serum 25-hydroxyvitamin D concentration is the best determinant of vitamin D status because of its long half-life (more than 250 hours), its value is influenced by several factors, such as variation in sun exposure due to latitude, season, or time of day, clothing, sunscreen use, skin pigmentation, age, and obesity [28]. In addition, suboptimal serum 25-hydroxyvitamin D levels are common in adults in many countries and are highly prevalent in the Middle East and Asia [29]. Serum 25-hydroxyvitamin D levels are higher in Northern than in Southern Europe, and in Western than in Eastern Europe, presumably owing to differences in skin phototypes among European populations [30]. In Mediterranean countries such as Spain, Italy, and Greece, low serum 25-hydroxyvitamin D may be attributed to darker skin pigmentation and reduced sun exposure resulting from urbanization [29]. Also in Greece dairy product fortification and vitamin D supplementation is not a usual practice, especially for younger age groups (median age of our melanoma patients was 50 years). We found deficient total 25-hydroxyvitamin D levels among our healthy controls, in agreement with previous Greek experience in various settings. This raises the discussion on what is sufficient and necessary for different population types [31,32]. Our findings are more consistent with the US Institute of Medicine cutoff levels for serum 25-hydroxyvitamin D sufficiency in almost the entire (97.5%) Caucasian population (>20 ng/mL) and less consistent with the Endocrine Society standards (>30 ng/mL) [33,34].

The prognostic significance of serum 25-hydroxyvitamin D levels at melanoma diagnosis remains controversial. In the present study, no significant association with Breslow thickness and melanoma stage was documented. A possible explanation for that could be that our sample consisted mostly of thin melanomas (52.38% of them had Breslow thickness <1 mm) and there was only 1 stage IV case. It is of note that most studies showing significant association between serum 25-hydroxyvitamin D and Breslow thickness and/or melanoma stage originate from countries with high melanoma incidence rates such as Australia, United Kingdom, Germany, and Italy [14,16-19]. In contrast, the incidence in Greece is low and it is estimated to be about 4 (4.01 females and 4.05 males) cases/100,000/year [35]. It can be speculated that in countries with high melanoma rates, the prognostic significance of vitamin D status becomes more evident, possibly because of a complex and yet unknown interplay between genetic and environmental factors that affect simultaneously both vitamin D status and melanoma development and progression.

The question remains: Should serum vitamin D levels be determined in any newly diagnosed patient with melanoma? Literature review provides contradicting evidence. Moreover, a meta-analysis of studies that looked at serum vitamin D levels and the incidence of skin cancer—cutaneous melanoma and nonmelanoma skin cancer—found no association between vitamin D level and risk of melanoma development, although there was a clear association with nonmelanoma skin cancer risk [36]. As mentioned before, 25-hydroxyvitamin D concentration at melanoma diagnosis is influenced by many factors, and it is not by any means representative of the vitamin D status during the whole process of melanoma genesis. The use of "snapshot" single measurements of 25-hydroxyvitamin D when attempting to demonstrate health risks associated with vitamin D deficiency is in dispute. In addition, serum 25-hydroxyvitamin D level is not the sole determinant of vitamin D activity. Vitamin D binding protein, polymorphisms in the vitamin D receptor, calcium ingestion, renal activation of vitamin D, among others, may also play an important part [37,38]. On the other hand, the large number of melanoma patients who have low or deficient vitamin D levels at diagnosis, combined with the routine recommendation for avoidance of unprotected sun exposure thereafter,

along with the real possibility that patients with lower vitamin D levels may fare worse than those with higher levels, seems to provide strong rationale for routine determination of the 25-hydroxyvitamin D level at initial diagnosis, followed by oral supplementation and monitoring for patients with low or deficient levels [21].

In view of the well-established pathogenetic role of UVR in skin carcinogenesis and the controversy over vitamin D, changes in current recommendations for strict photo-protection as a major target of skin cancer prevention are not warranted [21]. Most importantly, sun exposure alone does not guarantee vitamin D sufficiency [15,22,39]. Nevertheless, an optimum balance between sun protection and exposure is advocated [1].

The present study has some limitations. The sample size of melanoma patients was relatively small and monocentric compared to other studies, although it originated from the largest referral center of melanoma patients in central Greece. In addition, most cases were early-stage melanomas. It is known that serum total 25-hydroxyvitamin D levels are usually not affected until the BMI is >30 ng/mL. In our melanoma group no patient had BMI >30 so we divided our patients into 2 groups, one with BMI ≤25 and the other with overweight patients and BMI >25. Another limitation of studies including ours, assessing serum 25-hydroxyvitamin D levels, is that the ideal levels are a matter of controversy and concentrations that would be beneficial for patients with melanoma are not adequately documented.

Conclusions

We found lower serum total 25-hydroxyvitamin D levels among our melanoma patients in a case-control study performed in a low-risk Southern European population. However, no statistically significant difference was documented, and these levels were not associated with established risk factors and prognostic variables for CMM. More studies are needed to elucidate the complex effect of vitamin D in the development and progression of CMM. Better understanding of the role of vitamin D may provide new insights applicable to melanoma prevention and treatment.

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Table e1. Association of Serum 25(OH)D Levels With Clinical and Histopathological Characteristics in Different Levels of BMI

	Serum 25(OH)D, N (%)			Total	P Value
	<20 ng/mL	21-29 ng/mL	>30 ng/mL		
Patients with BMI ≤25					
Breslow					0.473[a]
<1 mm	8 (40)	8 (40)	4 (20)	20 (100)	
1-4 mm	9 (60)	2 (13.33)	4 (26.67)	15 (100)	
>4 mm	3 (42.86)	3 (42.86)	1 (14.29)	7 (100)	
Total	20(47.62)	13(30.95)	9 (21.43)	42 (100)	
Patients with BMI >25					
Breslow					0.338[a]
<1 mm	14 (43.75)	9 (28.13)	9(28.13)	32 (100)	
1-4 mm	7 (38.89)	5 (27.78)	6 (33.33)	18 (100)	
>4 mm	6 (85.71)	1 (14.29)	0	7 (100)	
Total	27 (47.37)	15 (26.32)	15 (26.32)	57 (100)	
Patients with BMI ≤25					
Ulceration					0.423
Yes	6 (37.50)	5 (31.25)	5 (31.25)	16 (100)	
No	14 (53.85)	8 (30.77)	4 (15.38)	26 (100)	
Total	20(47.62)	13 (30.95)	9 (21.43)	42 (100)	
Patients with BMI >25					
Ulceration					0.735
Yes	6 (40)	5 (33.33)	4 (26.67)	15 (100)	
No	21 (50)	10 (23.81)	11 (26.19)	42 (100)	
Total	27(43.37)	15 (26.32)	15 (26.32)	57 (100)	
Patients with BMI ≤25					
Melanoma located in sun-exposed body parts					0.943
Yes	12 (46.15)	8 (30.77)	6 (23.08)	26 (100)	
No	8 (50)	5 (31.25)	3 (18.75)	16 (100)	
Total	20 (47.62)	13 (30.95)	9 (21.43)	42 (100)	
Patients with BMI>25					
Melanoma located in sun-exposed body parts					0.926
Yes	13 (46.43)	8 (28.57)	7 (25)	28 (100)	
No	14 (48.28)	7 (24.14)	8 (27.59)	29 (100)	
Total	27 (47.37)	15 (26.32)	15 (26.32)	57 (100)	

[a]Result derived from Fisher exact test.

Sun-exposed body parts = extremities and head and neck; 25(OH)D = 25-hydroxyvitamin D; BMI = body mass index.

Table e2. Association of Serum 25(OH)D Levels With Breslow in Different Age Groups

	Serum 25(OH)D, N (%)			Total	P Value
	<20 ng/mL	21-29 ng/mL	>30 ng/mL		
Age ≤40 years					
Breslow					0.295[a]
<1 mm	8 (66.67)	0	4 (33.33)	12 (100)	
1-4 mm	9 (64.29)	3 (21.43)	2 (14.29)	14 (100)	
>4 mm	4 (80)	1 (20)	0	5 (100)	
Total	21 (67.74)	4 (12.90)	6 (19.35)	31 (100)	
Age >40 years					
Breslow					0.284[a]
<1 mm	14 (35)	17 (42.50)	9 (22.50)	40 (100)	
1-4 mm	7 (36.84)	4 (21.05)	8 (42.11)	19 (100)	
>4 mm	5 (55.56)	3 (33.33)	1 (11.11)	9 (100)	
Total	26 (38.24)	24 (35.29)	18 (26.47)	68 (100)	

[a]Result derived from Fisher exact test.

25(OH)D = 25-hydroxyvitamin D.