Determinants of 25-hydroxyvitamin D Status in a Cutaneous Melanoma Population

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Vitamin D status is influenced by well-known determinants, but factors associated with low 25-hydroxyvitamin D levels in the cutaneous melanoma population are not well defined. The aim of this study was to confirm the well-known determinants and to assess new determinants for 25-hydroxyvitamin D levels in a cutaneous melanoma population. In a prospectively included cohort of 387 patients with cutaneous melanoma the association of 25-hydroxyvitamin D levels with sex, age, body mass index, time of blood withdrawal, Fitzpatrick phototype, vitamin D supplementation, score for intensity of lifetime sun exposure, smoking, education level, hair and skin colour, eye colour, total number of benign naevi, freckles and parameters of chronic sun damage was investigated. In addition, 25-hydroxyvitamin D levels were correlated with pathological parameters of the primary tumour and melanoma stage (8th edition of the American Joint Committee on Cancer (AJCC)). Univariate and multivariate logistic regressions were performed using R software. The following factors had a significant effect on vitamin D status: body mass index, seasonal time of blood sampling, vitamin D supplementation, and a subtype of skin, and hair colour.

Key words: melanoma; vitamin D; body mass index.

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Cutaneous melanoma (CM) is a form of skin cancer with increasing incidence rates worldwide. In 2020, an estimated 9,272 deaths in central and eastern Europe were caused by CM (1). According to the National Cancer Institute in the USA, CM was responsible for 5.6% of all new cases of cancer and 1.2% of all cancer deaths in 2021 (2).

Vitamin D (VD) is a secosteroid produced mainly in the human skin upon ultraviolet B (UVB) radiation. The other source of VD is exogenous via dietary intake or supplements. Metabolization of VD is required to form biologically active products. The classical metabolic pathway is a 2-step process with a 25-hydroxylation in the liver to form 25-hydroxyvitamin D (25OHD), followed by 1-alpha hydroxylation in the kidney to form 1-alpha,25-dihydroxyvitamin D (3). An alternative metabolic pathway of VD is via cytochrome P450 side-chain cleavage enzyme (CYP11A1), whereby multiple hydroxymetabolites of VD are produced (4–8). Measurement of the level of 25OHD in blood serum is the best index of VD stores (9,10). However, this may have some limitations, since not all metabolites are measured. 25OHD levels in the general population are influenced by well-known determinants, such as body mass index (BMI), age, gender, season of blood sampling, sun protection (clothing and use of sunscreen), incidence of several chronic illnesses, and skin type (11). Levels of 25OHD are also influenced by genetic variants of VD pathway genes (12). Levels of 25OHD below 20 ng/ml are associated with the greatest risk of cancer, infections, cardiovascular and metabolic diseases (13).

Besides its well-known function in calcium/phosphate homeostasis, VD has also pleiotropic anti-cancer effects (3). In vitro and in vivo studies have demonstrated an anti-melanoma activity of VD, with effects on cellular
growth, differentiation, apoptosis, malignant cell invasion, and metastasis (14, 15).

Various observational studies have shown a beneficial effect of higher 25OHD levels on overall survival, relapse-free survival, and prognostic pathology parameters, such as Breslow thickness and ulceration of the primary tumour (16–22). In molecular and clinicopathological studies, defects in VD signalling pathways are correlated with CM progression and reduced disease outcome (23).

Given the association of VD status and clinical outcome, the question arises as to whether VD supplementation is useful in the (adjuvant) treatment of patients with CM. If VD supplementation proves useful, as currently assessed in ongoing trials, one question will be which patients with CM are prone to have low 25OHD levels, and hence would potentially benefit more from supplemental therapy with VD. The aim of this study is to define which factors are associated with 25OHD levels < 20 ng/ml. Thus, in a well-defined prospective population of patients with CM, levels of 25OHD lower than 20 ng/ml were assessed, and 25OHD levels were correlated with basic patient demographics. A further aim was to investigate the relationship between 25OHD levels, tumour-node-metastasis (TNM) stage (8th AJCC) (24) and histopathological parameters in the study population.

**MATERIALS AND METHODS**

**Study population, recruitment and study procedure**

This study is part of a multicentre randomized double-blind placebo-controlled phase III trial investigating the effect of VD supplementation on melanoma outcome (ViDMe trial). The full protocol of the ViDMe trial has been published (25). Briefly, patients with stage IB to III CM (according to the 7th AJCC staging), age 18–80 years, were randomized in the ViDMe trial at the University Hospitals Leuven, the University Hospitals of Antwerp, the Centre Hospitalier Universitaire de Liège in Belgium and at the Clinical Center at the University of Debrecen in Hungary if the only treatment was surgery. Most patients were recruited in the study centres in Belgium, only 4 patients were recruited in Hungary. For inclusion and exclusion criteria we refer to the published study protocol (25). The ViDMe trial, which is still ongoing, was approved by the local ethics committees, and written informed consent was obtained from all participants. A total of 387 patients prospectively recruited from Q4 2012 to Q1 2020 with a baseline serum sample available were included in this study.

**Determination of clinical parameters**

Basic demographic data on age, sex, tumour site and clinical stage were collected. At randomization, patients were questioned on ethnicity, current smoking status, highest education level, personal medical history, previous and current sun exposure. Current (concomitant) drug intake, VD supplementation (via over-the-counter supplements before randomization) and calcium supplementation were registered. A full skin examination was performed by a physician to assess skin phototype, naevoid phenotype, presence of freckles, and signs of chronic sun damage. The latter was determined by the presence of actinic keratoses, solar lentigines and guttate hypomelanosis. The patient’s height and weight were measured in order to estimate BMI.

**Statistical analyses**

Clinical and pathological parameters were represented as means and standard deviations (SD) for continuous variables, and frequencies or proportions (%) for categorical variables between group A (25OHD < 20 ng/ml) and group B (25OHD ≥ 20 ng/ml). Initial melanoma stage (AJCC 7th edition) was recalculated to AJCC 8th edition for statistical analysis of the pathological parameters. Univariate logistic regression analysis and multivariate logistic regressions were performed in order to assess the associations between VD status and the clinical parameters.

The variable selection for the final multivariate model was carried out using a forward stepwise variable selection based on Akaike Information Criterion (AIC) using the data omitting missing values (n = 387). The model with the lowest AIC was chosen as a final model and re-fitted with the whole dataset (n = 387). Multicollinearity was checked by variance inflation factors for each variable with a threshold < 5. The results of the logistic regression models were represented as odds ratios (ORs) on 25OHD levels < 20 ng/ml and 95% confidence intervals (95% CI). All analyses were performed with R software (version 4.0.3).

**RESULTS**

In total, 387 serum samples were analysed. Of these, 155 (40%) had 25OHD levels < 20 ng/ml and 232 (60%) had 25OHD levels ≥ 20 ng/ml. Mean 25OHD plasma concentration for the whole study population was 23.33 (SD 8.77) ng/ml.

**Clinical characteristics of group A (25OHD < 20 ng/ml) vs group B (25OHD ≥ 20 ng/ml)**

Median age was 56 years in group A and 54 years in group B. In general, inclusion in the ViDMe study occurred more in the winter period (December to February). In group A, most patients (46%) had a Fitzpatrick phototype III and, in group B, most patients (42%) had
Pathological characteristics of group A (25OHD < 20 ng/ml) vs group B (25OHD ≥ 20 ng/ml)

In both groups the most dominant histological subtype was superficial spreading melanoma, with 58% of patients in group A and 39% of patients in group B having this subtype. Regarding primary tumour thickness, the mean Breslow thickness for group A was 1.48 mm and for group B 1.30 mm, and most tumour tissue in both groups was Clark level 4. For other pathological parameters, both groups showed the same trend, with, mostly, no presence of ulceration, mitosis, vascular invasion, or microsatellites, but presence of TILs. For perineural invasion, information on primary tumour tissue was, in the majority, unknown or missing. In the group with 25OHD levels < 20 ng/ml, most patients were stage IB followed by stage III. In comparison in the group with 25OHD levels ≥ 20 ng/ml, most patients were stage IB, followed by stage IA (Table SI).

Univariate analysis between 25OHD status and clinical parameters

Having a high BMI ≥25–30 kg/m² or ≥30 kg/m² was a significant risk factor for 25OHD levels <20 ng/ml compared with patients with CM with a normal BMI (≥18.5–<25 kg/m²) (OR 2.21, 95% CI 1.36–3.63, p=0.002 and OR 5.03, 95% CI 2.73–9.48, p<0.001, respectively). Patients included during winter and spring had a significantly higher risk for 25OHD levels <20 ng/ml than patients included during summertime (OR 2.14, 95% CI 1.20–3.91, p=0.011; OR 2.09, 95% CI 1.11–4.02, p=0.024, respectively) (Table II).

Patients with CM with light skin and blond/light-brown hair had a higher risk of 25OHD levels ≥20 ng/ml compared with patients with CM with light skin and dark-brown/black hair (OR 0.47, 95% CI 0.29–0.77, p=0.003). Compared with patients with idiopathic guttate hypomelanosis as a sign of chronic sun damage, patients

Table I. Clinical characteristics of the study population (N=387)

| Characteristics | Group A | Group B |
|-----------------|---------|---------|
| 25(OH)D3 <20 ng/ml | n=155 (39.7%) | n=232 (59.4%) |
| Sex, n (%) | | |
| Male | 78 (50) | 95 (41) |
| Female | 77 (50) | 137 (59) |
| Age, mean (SD) | 56 (47, 66) | 54 (45, 64) |
| <40 years | 16 (10) | 37 (16) |
| 40–60 years | 79 (51) | 118 (51) |
| >60 years | 60 (39) | 77 (33) |
| Body mass index, n (%) | | |
| ≤18.5 kg/m² | 1 (0.6) | 1 (1.7) |
| ≥18.5–<25 kg/m² | 34 (22) | 101 (44) |
| ≥25–<30 kg/m² | 75 (49) | 101 (44) |
| ≥30 kg/m² | 44 (29) | 26 (11) |
| Missing | 1 (0) | 0 |
| Season of blood draw, n (%) | | |
| Winter (Dec–Feb) | 62 (40) | 73 (31) |
| Spring (Mar–May) | 39 (25) | 47 (20) |
| Summer (Jun–Aug) | 23 (15) | 58 (25) |
| Autumn (Sept–Nov) | 31 (20) | 54 (23) |
| Fitzpatrick phototype, n (%) | | |
| Ia | 17 (11) | 26 (11) |
| Ib | 51 (33) | 96 (42) |
| Ic | 72 (46) | 77 (33) |
| IV + V + VIa | 15 (9.7) | 32 (14) |
| Missing | 0 | 1 |
| Vitamin D supplementation, n (%) | | |
| No | 150 (97) | 184 (79) |
| Yes | 5 (3.2) | 48 (21) |
| Score for intensity of lifetime sun exposure, n (%) | | |
| Normal sun exposure | 87 (56) | 120 (52) |
| Low sun exposure | 51 (33) | 89 (39) |
| High sun exposure | 17 (11) | 22 (9.5) |
| Missing | 0 | 1 |
| Smoking, n (%) | | |
| Never smoked | 64 (41) | 100 (43) |
| Current smoking | 28 (18) | 35 (15) |
| Ex-smoker | 63 (41) | 97 (42) |
| Education level, n (%) | | |
| Primary school | 7 (4.5) | 9 (3.9) |
| Secondary school | 55 (35) | 67 (29) |
| Vocational training | 35 (23) | 43 (19) |
| Vocational university | 34 (22) | 74 (32) |
| University graduated | 23 (15) | 38 (16) |
| Other | 1 (0.6) | 1 (0.4) |
| Hair colour + skin colour, n (%) | | |
| Light skin, red or red-blond hair | 15 (9.7) | 14 (6.1) |
| Light skin, blond or light-brown hair | 69 (45) | 137 (59) |
| Light skin, brown or black hair | 52 (34) | 49 (21) |
| Medium tone skin, brown or black hair or | 19 (12) | 31 (13) |
| dark-brown or black hair | | |
| dark-brown or black hair or | | |
| missing | 0 | 1 |
| Eye colour, n (%) | | |
| Brown | 29 (19) | 39 (17) |
| Blue/green/grey | 109 (70) | 163 (71) |
| Hazel (brownish green) | 16 (10) | 25 (11) |
| Missing | 0 | 1 |
| Total number of benign naevi, n (%) | | |
| <25 | 71 (46) | 97 (42) |
| 25–49 | 32 (21) | 58 (25) |
| 50–100 | 29 (19) | 47 (20) |
| >100 | 22 (14) | 30 (13) |
| Missing | 1 | 0 |
| Solar lentigines, n (%) | | |
| Presence | 117 (75) | 173 (75) |
| No presence | 117 (75) | 173 (75) |
| Back of the hands, n (%) | | |
| Presence | 42 (27) | 68 (29) |
| No presence | 113 (73) | 164 (71) |
| Shoulders, n (%) | | |
| Presence | 27 (17) | 47 (20) |
| No presence | 128 (83) | 185 (80) |
| Freckles, n (%) | | |
| Presence | 128 (83) | 186 (80) |
| No presence | 27 (17) | 46 (20) |
| Guttate hypomelanosis, n (%) | | |
| Presence | 143 (92) | 198 (85) |
| No presence | 12 (7.7) | 34 (15) |
| Actinic keratosis, n (%) | | |
| Presence | 132 (85) | 204 (88) |
| No presence | 23 (15) | 52 (12) |
**Table II. Associations between 25-hydroxyvitamin D (25OHD) status and clinical parameters, univariate analysis**

| Characteristics | 25OHD <20 ng/ml vs ≥20 ng/ml |
|-----------------|-----------------------------|
| **Sex (n = 387)** |                            |
| Male (ref)       |                            |
| Female           |                            |
| **Age, years (n = 387)** |                |
| 100              |                            |
| **Body mass index (n = 386)** |            |
| <18.5 kg/m²      |                            |
| ≥18.5–<25 kg/m² |                            |
| ≥25–<30 kg/m²   |                            |
| ≥30 kg/m²       |                            |
| **Season of blood draw (n = 387)** |          |
| Winter           |                            |
| Spring           |                            |
| Summer           |                            |
| **Fitzpatrick phototype (n = 386)** |        |
| I                |                            |
| II               |                            |
| III              |                            |
| IV + V + VI (ref) |                        |
| **Vitamin D supplementation (n = 387)** |        |
| Yes (ref)        |                            |
| No               |                            |
| **Score for intensity of lifetime sun exposure (n = 386)** |          |
| 0=no normal sun exposure |            |
| 1=low sun exposure |                       |
| 2=high sun exposure (ref) |                  |
| **Smoking (n = 387)** |                          |
| No smoking       |                            |
| Current smoker   |                            |
| Never smoked     |                            |
| **Education level (n = 387)** |                      |
| Primary (ref)    |                            |
| Secondary school |                            |
| **Hair colour + skin colour (n = 386)** |          |
| Light skin, red or red-blonde hair |         |
| Light skin, blond or light-brown hair |        |
| Light skin, brown or black hair (ref) |      |
| Medium tone skin, brown or black hair or brown skin, dark-brown or black hair or black skin, dark-brown or black hair | |
| **Eye colour (n = 386)** |                         |
| Brown (ref)      |                            |
| Blue, green, grey |                      |
| Hazel (brownish green) |                |
| Unknown          |                            |
| **Total number of benign naevi (n = 386)** |         |
| <25 (ref)        |                            |
| 25–49            |                            |
| ≥50              |                            |
| **Solar lentigines (n = 387)** |               |
| Head–neck region |                            |
| No presence      |                            |
| Presence (ref)   |                            |
| **Back of the hands** |                        |
| No presence      |                            |
| Presence (ref)   |                            |
| **Shoulders**    |                            |
| No presence      |                            |
| Presence (ref)   |                            |
| **Freckles (n = 387)** |                      |
| No presence      |                            |
| Presence (ref)   |                            |
| **Guttate hypomelanosis (n = 387)** |            |
| No presence      |                            |
| Presence (ref)   |                            |
| **Actinic keratosis (n = 387)** |            |
| No presence      |                            |
| Presence (ref)   |                            |

Overall p-values are represented for each variable, p-values for comparisons of each category levels are represented only for those with more than 2 levels. OR: odds ratio on 25OHD levels <20 ng/ml; CI: confidence interval; ref: reference.

DISCUSSION

This study investigated the correlation of socio-demographic variables (sex, age and educational level), clinical variables (BMI, Fitzpatrick phototype, hair/skin/eye colour, naevus phenotype, and signs of chronic sun damage), behavioural variables (smoking and sun exposure habits), season, and VD supplementation with 25OHD status in a well-defined carefully phenotyped and assessed CM population.
To our knowledge, this is the first study to investigate a wide range of potential determinants of VD status (measured as 25OHD levels in serum) simultaneously in a prospectively recruited CM population.

Low 25OHD levels (< 20 ng/ml) were found in 40% of patients with CM, and a mean 25OHD level of 23.33 ng/mL in the CM population in this study. Low 25OHD levels (with the strongest association for levels < 20 ng/ml) are associated with cancer in general (13), but also with CM, as established in previous studies that showed higher 25OHD levels in the serum of healthy controls than in patients at the time of CM diagnosis (27). Higher 25OHD levels at the time of diagnosis of the primary melanoma have been shown to predict a lower risk of relapse and increased survival, independent of Breslow thickness (16, 22). In contrast, in another study, a change in 25OHD levels during follow-up after CM diagnosis, and not the 25OHD level at diagnosis, was ascribed as contributing to the effect on CM survival (28). Further research is necessary in patients with CM to explore the utility of determination of 25OHD status and VD dynamics.

In the current study population with CM, a clear and significant correlation of overweight (BMI ≥ 25–< 30 and obesity BMI ≥ 30 kg/m²; p < 0.001) with 25OHD levels < 20 ng/ml was observed. This is in line with the literature demonstrating an association between low VD status and obesity in the general population (29). An inverse correlation between serum 25OHD levels, BMI, body weight and fat mass is a consistent finding in both adults and children of different ethnicities in a range of geographical locations (30).

It is not known if this association is causative, or is just a bystander effect (i.e. a not yet defined underlying condition may lead to low VD status), or if it simply originates from an association with common lifestyle factors (13, 29).

Multiple possible mechanisms are proposed as the cause of low 25OHD levels in obesity (30): lower sun exposure, low dietary intake, impaired synthesis in the skin, variance in VD metabolism (due to alterations in the vitamin D binding protein (VDBP) or more rapid metabolic clearance), or increased volumetric dilution into a larger tissue volume.

Sunlight habits differ geographically and between cultural groups, but previous studies did not show a difference in sunlight exposure and dietary intake between normal weight and obese adults (32, 33). A similar cutaneous production of VD was observed between normal weight and obese people (33).

Circulating VD is protein bound to VDBP and albumin. Alterations in the concentrations of these proteins and genetic variations in the binding affinity of VDBP affect 25OHD measurement. No differences were found between obese and normal weight people concerning those proteins, and no differences in VDBP genotypes were observed (31, 34).

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VD is fat soluble and distributed in the liver, muscle, fat and, in smaller amounts, in other tissues. The volume of these compartments is increased in obese patients. A logical consequence of more widely distributed VD in a larger tissue volume is a lower level of distributed VD in the serum. The volumetric dilution has clinical implications if oral VD supplementation is given to obese patients. A smaller increase in 25OHD levels is seen in obese patients compared with normal weight patients, therefore loading doses of VD will need to be larger to achieve the same 25OHD levels (31).

In the current study population, intake of VD supplements was a significant determinant of 25OHD level (OR no VD supplementation = 8.69, p-value < 0.001). Further research is required into the effect of VD supplementation on 25OHD levels and, subsequently, the effect on outcome in patients with CM, especially those in the obese and overweight group.

Univariate and multivariate analyses showed a significant correlation between seasonal collection of blood and 25OHD levels. Blood sampling in winter had a lower chance of normal 25OHD levels compared with the summer period (OR 2.74; p = 0.022). This is in line with the fact that VD is produced in the skin upon UVB-exposure, which is more pronounced during the summer period.

In both univariate and multivariate analysis, a protective effect of having a light skin in combination with blond or light-brown hair was seen in terms of normal 25OHD levels, compared with patients with light skin and brown or black hair. We cannot explain this finding, nor have we found any similar documentation in the literature.

We further investigated sociodemographic, and other clinical and behavioural parameters as possible deter-
minants for 25OHD levels, but there were no statistically significant correlations with regard to sex, age, Fitzpatrick phototype, score for intensity of lifetime sun exposure, smoking, education level, eye colour, total number of benign naevi, solar lentigines, freckles, idiopathic guttate hypomelanosis, and actinic keratosis. In the univariate analysis, there was a significant correlation of idiopathic guttate hypomelanosis with 25OHD levels. Idiopathic guttate hypomelanosis is a marker of chronic sun damage and thus can be linked with higher 25OHD levels. However, the correlation with idiopathic guttate hypomelanosis was no longer significant in the multivariate analysis. Other parameters of chronic sun damage, such as solar lentigines and actinic keratosis, were not statistically significantly associated with 25OHD levels.

Univariate analysis demonstrated a statistically significant association between higher tumour stage and lower 25OHD levels, in line with previous studies (16, 17, 35, 36). We found no significant correlation between 25OHD level and histological subtype of primary tumour tissue, Clark level, mitosis, vascular invasion, perineural invasion, regression, or TILs.

In conclusion, this study found a clear significant association between 25OHD levels and the following factors: BMI, takingVD supplements, season of blood drawing, hair and skin colour. Low levels of 25OHD were associated with a higher tumour stage. Further research is ongoing to investigate whether VD supplementation after removal of the primary melanoma has a protective effect on melanoma outcome and could be used as adjuvant therapy in patients with CM. It will be of interest to investigate the benefit of VD supplementation in the overweight and obese CM population.

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