

## Reduced Serum 25-Hydroxyvitamin D Levels in Stage IV Melanoma Patients

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**Abstract.** *Background: Reduced serum 25-hydroxyvitamin D<sub>3</sub> (25(OH)D) levels are associated with an increased incidence and an unfavorable outcome of various types of cancer. However, the influence of serum 25(OH)D on the incidence and outcome of patients with malignant melanoma is unknown. Patients and Methods: The association between serum 25(OH)D levels and clinical and histopathological data among 205 patients with malignant melanoma was examined. Additionally, 141 healthy controls were investigated. All the blood samples were taken between October and April to minimize seasonal variations; basal serum 25(OH)D levels were analyzed using the LIAISON 25-OH Vitamin D-Assay (DiaSorin, Dietzenbach, Germany). The study started in 1997. The patients were observed until death or March 2007, whichever came first. Results: Serum 25(OH)D levels were significantly reduced in stage IV melanoma patients as compared to stage I melanoma patients ( $p=0.006$ ). A trend toward a greater tumor thickness of the primary cutaneous melanomas was seen in the patients with low ( $<10$  ng/ml) serum 25(OH)D levels (median: 2.55 mm) as compared to those with 25(OH)D serum levels  $>20$  ng/ml (median: 1.5 mm), although this difference was not statistically significant ( $p=0.078$ ). The patients with low 25(OH)D serum levels ( $<10$  ng/ml) had earlier distant metastatic disease (median: 24.37 months) as compared to those with 25(OH)D serum levels  $>20$  ng/ml (median: 29.47 months), although this difference was also not statistically significant ( $p=0.641$ ). Conclusion: Among the patients with*

*malignant melanoma, significantly reduced serum 25(OH)D levels were found in the stage IV patients as compared to stage I patients, and those with low 25(OH)D serum levels ( $<10$  ng/ml) may develop earlier distant metastatic disease compared to those with higher 25(OH)D serum levels ( $>20$  ng/ml). Further study of the vitamin D pathway and its influence on pathogenesis and progression of malignant melanoma is warranted.*

The vitamin D-cancer hypothesis has received strong epidemiological and experimental support over the past two decades. Experimental support is based on the almost ubiquitous expression of vitamin D receptors (VDR) (1, 2) and 1- $\alpha$ -hydroxylase (3) which convert serum 25-hydroxyvitamin D<sub>3</sub> (25(OH)D) into the biologically active vitamin D metabolite 1,25-dihydroxycholecalciferol (1,25(OH)<sub>2</sub>D) in the human body. The binding and transcriptional activation of VDR by 1,25(OH)<sub>2</sub>D exerts multiple cellular effects, including the induction of differentiation and apoptosis (4, 5) and inhibition of proliferation (6), angiogenesis (7, 8), and metastatic potential (9, 10). Prospective studies have convincingly shown that higher baseline serum levels of 25(OH)D are associated with a significant reduction in the risk of several types of cancer including colorectal cancer (11-16). Additionally, higher baseline serum levels of 25(OH)D are associated with a more favorable outcome in various malignancies (17-19). A meta-analysis of five epidemiological studies recently reported a 51% decrease in the risk of colorectal cancer associated with serum 25(OH)D levels in the highest *versus* lowest quintiles ( $p<0.0001$ ) (20). Furthermore, a prospective, randomized, placebo-controlled trial of vitamin D and calcium supplementation in 1,179 women found a 60% decrease in all-cancer risk in the intervention arm ( $p<0.03$ ) (21).

It is well known that lack of sun exposure leads to vitamin D deficiency, because 90% of all requisite vitamin D has to be formed in the skin through the action of the sun (22, 23). The role of solar UV-exposure on the incidence and

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prognosis of malignant melanoma is not well understood, and the influence of vitamin D on the incidence and outcome of patients with malignant melanoma is unknown (24, 25). On the one hand, intensive short duration and recreational sun exposure increases the risk of melanoma, especially in fair-skinned individuals (26, 27). On the other hand, according to new scientific findings, moderate sun exposure is associated with a relatively favorable prognosis and increased survival rates in melanoma patients (24, 25, 28). Therefore, the possible association of a direct measure of vitamin D status, serum levels of 25(OH)D, and of an indirect measure of vitamin D status, namely UV-exposure in recent years as evaluated by a questionnaire, on the incidence and clinical outcome of melanoma patients was examined.

## Materials and Methods

The study population consisted of 205 patients with histologically proven cutaneous melanomas of different stages. The patients were treated from December 1997 to March 2007 at the Department of Dermatology of the Saarland University Hospital (n=140) or at the Department of Dermatology of the University of Mannheim (n=65). The histopathological and clinical data of the melanoma patients were obtained from these institutions. The control group consisted of 141 healthy individuals, in part volunteers (n=71) who visited the Department of Dermatology, while others (n=70) were patients at the Department of Orthopaedic Surgery of the Saarland University Medical Center Homburg. Their blood samples were stored at the Institute of Virology, The Saarland University Hospital Homburg, after the completion of routine preoperative serological diagnostics.

The venous blood samples taken for the biochemical analyses were immediately processed and separated. The serum samples were aliquoted and stored at  $-40^{\circ}\text{C}$ . All the blood samples were taken between October and April to minimize seasonal variations. Basal 25(OH)D serum levels were analyzed at the Department of Clinical Chemistry and Laboratory Medicine of the Saarland University Hospital in Homburg using the LIAISON 25-OH Vitamin D-Assay (DiaSorin, Dietzenbach, Germany). The lower detection limit of this assay is 7 ng/ml.

Using a self-administered questionnaire, the study participants were asked to provide additional information including history of sun exposure, skin type, number of painful sunburns and use of sunscreens (melanoma patients: n=58; control group: n=42). Those data were correlated with the 25(OH)D serum levels to ensure that the single measurement of circulating 25(OH)D was representative of the long-term vitamin D status.

The study population was divided into certain groups of interest (such as 25(OH)D level, tumor stage, gender, age) and the medians, means and standard deviations were calculated. Because the 25(OH)D serum levels could not be assumed to be normally distributed (demonstrated by Kolmogorov-Smirnov-Test), differences in the quantitative variables between individual groups were analysed with the Mann-Whitney *U*-test. A *p*-value of  $<0.05$  was considered statistically significant. All the statistical analyses were performed using the statistical package SPSS Version 13 (SPSS Inc., Chicago, IL, USA).

Table I. Patient characteristics.

Variable	Melanoma patients (n=205)	Control group (n=141)	Total (n=346)
Gender			
Men	55.1% (113)	42.6% (60)	100% (173)
Women	44.9% (92)	57.4% (81)	100% (173)
Age group			
14-34 years	4.9% (10)	28.4% (40)	14.5% (50)
35-64 years	52.7% (108)	46.8% (66)	50.3% (174)
$\geq 65$ years	42.4% (87)	24.8% (35)	35.3% (122)
Body mass index			
$<20\text{ kg/m}^2$ (underweight)	2.6% (2)	11.9% (5)	5.9% (7)
$20-24\text{ kg/m}^2$ (normalweight)	33.8% (26)	47.6% (20)	38.7% (46)
$25-29\text{ kg/m}^2$ (overweight)	42.9% (33)	28.6% (12)	37.8% (45)
$\geq 30\text{ kg/m}^2$ (obese)	20.8% (16)	11.9% (5)	17.7% (21)
Serum 25(OH)D (ng/ml)			
N	205	141	346
Mean	16.93	18.77	17.68
Standard deviation	9.34	11.12	10.13
Median	14.3	15.6	14.75
25(OH)D group			
$\leq 20\text{ ng/ml}$	78.1% (160)	63.1% (89)	72.0% (249)
$<10\text{ ng/ml}$	20.0% (41)	27.7% (39)	23.1% (80)
10-20 ng/ml	58.1% (119)	35.5% (50)	48.8% (169)
$>20\text{ ng/ml}$	22.0% (45)	36.9% (52)	28.0% (97)

## Results

*Vitamin D and 25(OH)D serum levels in melanoma patients and controls.* The characteristics of the study population are summarized in Table I. A high prevalence of vitamin D deficiency, defined as serum 25(OH)D levels  $<20\text{ ng/ml}$ , was found both in the melanoma patients (78.1%) and the controls (63.1%).

The median 25(OH)D serum levels were slightly lower in the melanoma patients (14.3 ng/ml, n=205) as compared to the controls (15.6 ng/ml, n=141), although this difference was not statistically significant ( $p=0.44$ ) (Figure 1). No statistically significant associations were found when the 25(OH)D levels were compared with respect to age, gender or body mass index (BMI). Among the melanoma patients, an age-related trend ( $p=0.053$ ) was observed concerning 25(OH)D levels: the 14-34 years group had a median 25(OH)D serum level of 16.95 ng/ml compared to 14.3 ng/ml in the older population ( $>65$  years).

*25(OH)D serum levels and solar UV-exposure.* When the 25(OH)D serum levels were compared with sun exposure within the previous 2 years, a statistically significant ( $p=0.001$ ) difference was demonstrated between those patients with infrequent sun exposure ( $<50$  days within the previous 2

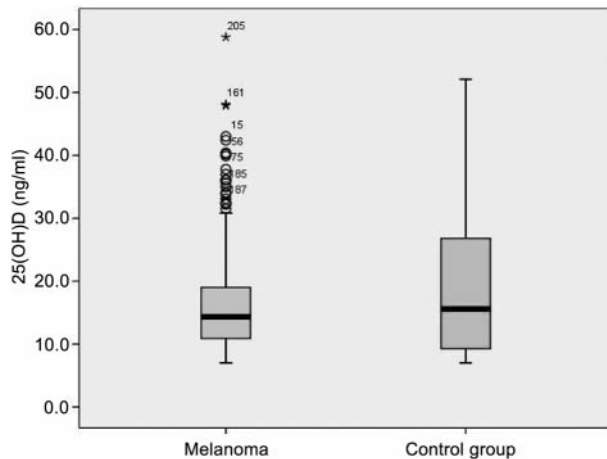


Figure 1. 25(OH)D serum levels in melanoma patients and controls. Median 25(OH)D serum levels (black horizontal line) in melanoma patients (14.3 ng/ml, n=205) as compared to controls (15.6 ng/ml, n=141) ( $p=0.44$ ). Boxes represent the values within the 25th and 75th percentiles.

years, median 8.16 ng/ml) and those with more frequent sun exposure (>150 days within the previous 2 years, median 25.90 ng/ml) (Table II).

**25(OH)D serum levels in stage IV as compared to stage I melanoma patients.** The median 25(OH)D serum levels were significantly ( $p=0.006$ ) lower in the stage IV melanoma patients (13.10 ng/ml, n=115) as compared to the stage Ia/b melanoma patients (16.40 ng/ml, n=50) (Figure 2).

**Tumor thickness in primary cutaneous melanoma.** The patients with low 25(OH)D serum levels (<10 ng/ml) had primary cutaneous melanomas with greater tumor thickness (2.55 mm, n=28) as compared to the patients with serum levels >20 ng/ml (1.5 mm, n=35), although this difference was not statistically significant ( $p=0.078$ ) (Table III).

**Distant metastatic disease.** The patients with low serum 25(OH)D levels (<10 ng/ml) had earlier distant metastatic disease (24.37 months, n=31) as compared to the patients with 25(OH)D serum levels >20 ng/ml (29.47 months, n=21), although this difference was not statistically significant ( $p=0.641$ ) (Table IV).

**Season of diagnosis and clinical outcome.** In the patients diagnosed in summer, the median time between primary excision and lymphogenous metastasis was 13.17 months as compared to 1.20 months in the patients diagnosed in autumn ( $p=0.486$ ) (Table V). There was also a trend for an earlier appearance of distant metastasis in the patients

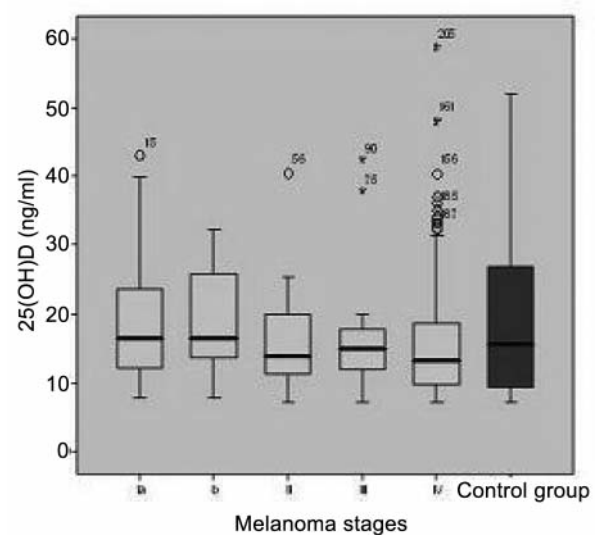


Figure 2. 25(OH)D serum level and stage. The median 25(OH)D serum levels (black horizontal line) in stage IV melanoma patients (13.10 ng/ml, n=115) as compared to stage Ia/b melanoma patients (16.40 ng/ml, n=50) ( $p=0.006$ ). Boxes represent the values within the 25th and 75th percentiles.

Table II. Comparison of solar UV-exposure within the previous 2 years and 25-hydroxyvitamin D serum levels.

Sun exposure within previous 2 years (days)	25(OH)D serum level (ng/ml)			
	n	Median	Mean	Standard deviation
0-50	20	8.16	10.12	3.75
51-100	40	12.65	13.01	5.54
101-150	29	17.6	19.98	8.37
> 150	11	25.90	25.53	12.96

25-Hydroxyvitamin D values with solar UV-exposure <50 days within the past 2 years compared with solar UV-exposure >150 days within the past 2 years,  $p=0.001$ .

Table III. Comparison of 25(OH)D serum levels and primary cutaneous tumor thickness in melanoma patients.

25(OH)D serum level (ng/ml)	Tumor thickness (mm)			
	n	Median	Mean	Standard deviation
<10	28	2.55	3.00	2.32
10-20	97	1.63	2.76	3.30
>20	35	1.50	2.33	2.28

Median 25(OH)D serum levels <10 ng/ml as compared to >20 ng/ml,  $p=0.078$ . (n=melanoma patients with documented tumor thickness).

Table IV. Distant metastatic disease in melanoma patients.

25(OH)D serum level (ng/ml)	Period of time between primary excision and distant metastatic disease (months)			
	n	Median	Mean	Standard deviation
<10	31	24.37	34.81	35.50
10-20	66	25.25	41.58	45.26
>20	21	29.47	42.14	43.36

Median time to distant metastatic disease with serum 25(OH)D levels <10 ng/ml as compared to >20 ng/ml,  $p=0.64$ . (n=melanoma patients whose clinical outcome could be analyzed; some cases were lost to follow-up).

Table V. Season of diagnosis time between primary excision and development of lymphogenous metastasis (months).

Season of diagnosis	Period of time between primary excision and lymphogenous metastasis (months)			
	n	Median	Mean	Standard deviation
Winter	40	16.40	35.43	48.24
Spring	34	3.70	24.00	36.61
Summer	33	13.17	22.48	30.66
Autumn	29	1.20	22.12	31.70

Patients whose melanomas were diagnosed in spring or autumn developed earlier lymphogeneous metastatic disease as compared to patients diagnosed in summer or winter, although this difference was statistically not significant ( $p=0.486$  for patients with malignant melanomas diagnosed in autumn as compared to those diagnosed in summer). For details concerning generation of the data and statistical analysis, see Materials and Methods (n=melanoma patients whose clinical outcome could be analyzed; some cases were lost to follow-up).

diagnosed in autumn (14.20 months) compared with those patients diagnosed in summer (31.70 months) ( $p=0.057$ ) (Table VI).

## Discussion

A high prevalence of vitamin D deficiency was observed in the study population. Although there is still no consensus on optimal levels of 25(OH)D (29-32) at present, most experts define vitamin D deficiency as serum 25(OH)D of  $\leq 20$  ng/ml (23, 32-35). In the melanoma patients and controls, 78.1% and 63.1%, respectively, showed 25(OH)D serum levels  $\leq 20$  ng/ml, which was comparable with results of other recently published investigations among the German population (36). Interestingly, a statistically significant ( $p=0.001$ ) difference

Table VI. Season of diagnosis and time between primary excision and development of distant metastatic disease (months).

Season of diagnosis	Period of time between primary excision and distant metastatic disease (months)			
	n	Median	Mean	Standard deviation
Winter	36	25.93	49.43	51.94
Spring	26	21.57	40.60	46.13
Summer	30	31.70	38.45	30.78
Autumn	26	14.20	27.68	33.04

Patients whose melanomas were diagnosed in spring or autumn developed earlier distant metastatic disease as compared to patients diagnosed in summer or winter, although this difference was statistically not significant ( $p=0.057$  for patients with malignant melanomas diagnosed in autumn as compared to those diagnosed in summer). For details concerning generation of the data and statistical analysis, see Materials and Methods (n=melanoma patients whose clinical outcome could be analyzed; some cases were lost to follow-up).

was found when the serum 25(OH)D levels were compared according to individual sun exposures (<50 days of solar UV exposure within the previous 2 years compared with than 150 days). This finding supported the hypothesis that, at least in the present study population, serum 25(OH)D levels mainly depended on solar UV exposure and indicated that the measured serum 25(OH)D levels not only reflected the recent sun exposure, but could also be considered to be representative for a period of at least several years. Previously, some studies reported that serum 25(OH)D levels were associated with BMI (36-41), while others did not support this finding. In the present study, no statistically significant associations were found when the serum 25(OH)D levels were compared with age, gender, BMI or additional factors that included skin type, number of painful sunburns and the use of sunscreens.

The median 25(OH)D serum level (16.9 ng/ml) of all the analysed melanoma patients was slightly lower compared to the controls (18.8 ng/ml, n=141), although this difference was not statistically significant ( $p=0.44$ ), in accordance with our previous pilot study of 25(OH)D serum levels in melanoma patients (42).

This was the first study identifying patients with metastasized melanoma to be at high risk of having vitamin D deficiency. In the present study, the serum 25(OH)D levels were significantly lower in the stage IV melanoma patients as compared with the stage Ia/b patients ( $p=0.006$ ). It remains to be investigated whether reduced serum 25(OH)D levels in patients with metastasized melanoma are caused by reduced outdoor activities (resulting in less solar UV-exposure) or are due to other unknown factors. Although it can only be speculated whether reduced serum 25(OH)D



levels affect melanoma progression directly, we concluded that it is of high importance to detect and to treat vitamin D deficiency in metastasized melanoma, at least to avoid other vitamin D deficiency-associated diseases including bone diseases, fractures and falls (43). Vitamin D deficiency can be treated by oral vitamin D as recommended previously (22, 23). A single dose of 50,000 IU vitamin D once a week for 8 weeks has also been shown to be an efficient and safe treatment (22, 23). Another means of guaranteeing vitamin D sufficiency is to give 50,000 IU of vitamin D once a month.

Tumor thickness at the time of diagnosis is strongly associated with melanoma prognosis and considered to be the best predictor of progression. Interestingly, in the present study, the patients with low serum 25(OH)D levels (<10 ng/ml) had primary cutaneous melanomas with greater tumor thickness (2.55 mm) as compared to those with serum 25(OH)D levels >20 ng/ml (1.5 mm). Furthermore, the patients with low 25(OH)D serum levels (<10 ng/ml) had earlier distant metastatic disease (24.37 months) as compared to those with higher levels (>20 ng/ml, 29.47 months). Due to limitations that included a limited number of cases, the present study did not allow a definite conclusion as to whether low serum 25(OH)D levels were associated with greater tumor thickness at the time of diagnosis and/or with a more unfavorable course of the disease.

Additionally, seasonal variations were noticed, with more rapid tumor progression of melanomas diagnosed in the spring and autumn compared to those diagnosed in winter or summer. The literature provides controversial data in respect of seasonal variations regarding fatality. In Australia, melanomas diagnosed in summer showed lower fatality compared with those diagnosed in winter (44), while in Spain, mortality from melanoma was highest when diagnosed during July and August (45). In fact, 25(OH)D serum levels, as well as melanoma fatality are influenced by various factors (*e.g.* skin type, regional and climatic variations). The lower fatality, of melanomas diagnosed in summer might be due to earlier diagnosis or to increased sun exposure. Due to limitations including a limited number of cases, the present study did not allow a definite conclusion as to whether the season of diagnosis affected the prognosis of malignant melanoma.

## Conclusion

This is the first study identifying patients with metastasized melanoma to be at high risk of developing vitamin D deficiency. Although a possible role of serum 25(OH)D in the pathogenesis and progression of malignant melanoma is indicated, due to the study limitations (relatively small retrospective study), definite conclusions cannot be drawn. Further study of the vitamin D pathway and its influence on pathogenesis and progression of malignant melanoma is warranted.

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