Regular use of vitamin D supplement is associated with fewer melanoma cases compared to non-use: a cross-sectional study in 498 adult subjects at risk of skin cancers

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There are conflicting results on the role of vitamin D system in cutaneous carcinogenesis. Therefore, it was investigated whether the use of oral vitamin D supplements associates with photoaging, actinic keratoses, pigment cell nevi, and skin cancers. In this cross-sectional study, 498 adults (aged 21–79 years, 253 males, 245 females, 96 with immunosuppression) subjects at risk of any type of skin cancer were examined, and possible confounding factors were evaluated. The subjects were divided into three groups based on their self-reported use of oral vitamin D supplements: non-use, occasional use, or regular use. The serum level of 25-hydroxyvitamin-D3 was analyzed in 260 subjects. In 402 immunocompetent subjects, vitamin D use did not associate with photoaging, actinic keratoses, nevi, basal, and squamous cell carcinoma. In contrast, there were lower percentages of subjects with a history of past or present melanoma (32/177, 18.1% versus 32/99, 32.3%, *P*=0.021) or any type of skin cancer (110/177, 62.1% versus 74/99, 74.7%, *P*=0.027) among regular users compared to non-users. In the logistic regression analysis, the odds ratio for melanoma was 0.447 (*P*=0.016, 95% confidence interval, 0.231–0.862) among regular users. Furthermore, the investigator-estimated risk class of skin cancers was significantly lower among regular users. Serum 25-hydroxyvitamin-D3 did not show marked associations with skin-related parameters. The results on 96 immunosuppressed subjects were somewhat similar, although the number of subjects was low. In conclusion, regular use of vitamin D associates with fewer melanoma cases, when compared to non-use, but the causality between them is obscure. *Melanoma Res* XXX: XXXX–XXXX

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Introduction

The incidences of cutaneous malignant melanoma and nonmelanoma skin cancers (NMSC, keratinocyte skin cancers) have steadily been increasing in Western populations during the last decades owing to increased exposure to ultraviolet (UV) radiation from the sun with resultant photocarcinogenesis through DNA damage and immunosuppression [1–3]. The role of immunosuppression in these events is highlighted by reports that in organ transplant recipients (OTRs) the risk of actinic keratosis and squamous cell carcinoma (SCC), but also basal cell carcinoma (BCC) and melanoma, is increased [4,5]. In addition, numerous studies have shown the co-existence or co-risk of different types of NMSC, melanoma, and precursor lesions in the same subjects [6–8], and therefore they are closely interrelated skin malignancies.

Even though solar UV radiation is a well-known risk factor for skin cancers, the other side of the coin is that public sun protection campaigns have led to alerts that insufficient sun exposure is a significant public health problem resulting in insufficient vitamin D status [9]. Serum level of 25-hydroxyvitamin-D3 (25(OH)-D3, calcidiol) has generally been used to assess the vitamin D status of an individual in clinical practice. However, human skin itself can express the CYP enzymes that generate biologically active vitamin D metabolites (CYP27A1 for calcidiol and CYP27B1 for calcitriol) or inactivate them (CYP24A1), and the balance between their expression may have a marked impact on the final concentration of these active metabolites in the microenvironment within epidermis, dermis and skin tumors [10–12]. Consequently, these vitamin D metabolites can regulate the cutaneous immune system, photoaging, and carcinogenesis [13–15].

In numerous previous studies, the serum level of 25(OH)-D3 has been the golden standard to investigate the role of vitamin D status in skin cancers, although its concentration in the tissue microenvironment is actually unclear. Vitamin D status is dependent, for example, on the exposure level to sunlight, age, BMI, skin phototype, pregnancy, breastfeeding, air pollutants, dietary intake, smoking, and some genes of skin pigmentation (reviewed in [14]). In a recent review and meta-analysis,

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Mahamat-Saleh *et al*. [16] reported that a circulating level of 25(OH)-D3 is associated with higher risks of melanoma, BCC, and SCC. Similarly, in a registry-based study with 247 574 individuals from primary healthcare in Denmark, Vojdeman *et al*. [17] found that higher levels of vitamin D were associated with a higher incidence of NMSC [hazard ratio (HR) 1.09] and melanoma skin cancer (HR 1.1). Another large Danish study concluded that genetically determined high 25(OH)-D levels, by analyzing four genetic variants near *DHCR7* and *CYP2R1*, did not appear to protect against NMSC, whereas high plasma 25(OH)D concentrations were associated with a risk of NMSC, although the association likely reflects confounding by sun exposure rather than causality [18]. A recent large Canadian registry-based study on 71 171 subjects reported that serum 25(OH)-D3 is associated with elevated NMSC risk, but not significantly with melanoma risk, and this association is likely due to sun exposure [19]. Also, Stenehjem *et al*. [20] found in a large case-control study in Norway that there is no persuasive evidence for an association between prediagnostic serum 25(OH)-D3 and melanoma risk overall, but serum levels within the medium range (between 60 and 85 nmol/L) might be associated with reduced risk. An Australian study used the Mendelian randomization approach on five single-nucleotide polymorphisms associated with 25(OH)-D in 12 874 cases and 23 203 controls and found that 25(OH)-D levels may not be causally associated with melanoma risk [21]. However, another Australian pilot study with 109 primary melanomas found that high serum 25(OH)-D3 level correlates with better prognostic indicators, such as low Breslow thickness, non-ulcerated tumor, and low mitotic rate, in primary melanoma [22]. Similar results have been obtained in a Spanish study with 204 melanoma patients [23], and in a UK study with 2183 melanoma patients [24].

In a recent meta-analysis, it was reported that the intake of vitamin D from diet or supplements does not have associations with melanoma and SCC risk, except for a weak positive association with elevated BCC risk [16]. More recently, a randomized placebo-controlled trial of adjuvant vitamin D supplementation in stage II melanoma in 109 patients revealed that regardless of the increase in 25(OH)-D levels in the active arm, subjects who had a Breslow thickness ≥3 mm at diagnosis experienced a lower increase in 25(OH)-D levels and were more prone to relapse in the future, as compared to subjects with a Breslow <3 mm at diagnosis [25]. With regard to vitamin D and NMSC risk, a recent Italian meta-analysis led to the conclusion that there appears not to be any strong relationship between vitamin D metabolism and NMSC risk [26]. Also, Ali *et al*. [27] reported recently in a randomized placebo-controlled trial that vitamin D supplementation did not reduce the incidence of keratinocyte carcinoma (both SCC and BCC combined) and other actinic lesions, although it increased the incidence of keratinocyte carcinomas in adults aged over 70 years. In a multicenter, double-blind, placebo-controlled, randomized clinical trial of vitamin D, calcium, or both, Passarelli *et al*. [28] concluded that calcium alone (HR 0.60) or in combination with vitamin D (HR 0.42), but not vitamin D alone (HR 0.79), may reduce the risk of SCC, but not BCC.

There are controversies with regard to the role of vitamin D or its metabolites in NMSC and melanoma. Theoretically, vitamin D metabolites may protect the skin from damaging oxidative stress, photoaging, premalignant skin lesions, and carcinogenesis induced by UV radiation, in addition to the modulation of the cutaneous immune system [29,30], although clinical studies on human subjects dealing with the role of vitamin D in these carcinogenetic events are sparse. However, there are recent reports showing that serum 25(OH)-D3 level or vitamin D supplementation is positively associated with a better outcome of head actinic keratosis treatment with photodynamic therapy [31,32]. In this present cross-sectional study, the purpose was to investigate whether an oral vitamin D supplementation can have associations with cutaneous photoaging level, actinic keratoses, pigment cell nevi, and skin cancers. To obtain the study cohort with a sufficient number of cases with skin-related parameters, 498 adult subjects at risk of any type of skin cancer were recruited, interviewed, and examined. Thereafter, the subjects were divided into 3 groups based on their self-reported use of oral vitamin D supplements. Because gender differences may exist with respect to skin cancers, male and female subjects were analyzed separately, too. In addition, serum 25(OH)-D3 was analyzed in about half of the subjects in each group and correlated to skin-related parameters.

Methods

Study subjects

For the study cohort, 498 subjects (aged 21–79 years, 253 males, and 245 females) were recruited at the dermatological outpatient clinic of Kuopio University Hospital (Kuopio, Finland). The inclusion criteria were that a subject is 18–80 years old and may be at increased risk of any type of skin cancer. Subjects with a neurological or psychiatric disorder affecting significantly the mental health, memory, and capability to understand decision-making, or were convicted prisoners, were excluded. Pregnant and breastfeeding females were excluded, too. The risk assessment was based, for example, on past or present skin cancers or their precursor lesions, cutaneous photoaging severity, nevus count, atypical nevi, immunosuppression, skin phototype, and family history of melanoma as evaluated by an experienced dermatologist [33]. Consequently, the subjects were placed into a low, moderate, or high-risk group, using a risk classification system modified from the previous report [4]. A typical subject in the low-risk group showed mild photodamage and an

occasional premalignant lesion or dysplastic nevus without marked immunosuppression. Before entering the study, the subjects read an informative material related to the study protocol and then filled out a questionnaire with questions on demographic details, Fitzpatrick skin type, different aspects of the skin exposure to UV radiation, previous or current diseases in internal organs or skin, family history of melanoma, and tobacco smoking, as described in Results and Table 1 in more detail. The Fitzpatrick skin type was also assessed by skin reactions to sun exposure producing a maximum score of 32 points. During the recruitment, the medical staff of the hospital clinic, unrelated to the study, was instructed to provide all candidate subjects referred to the clinic with the possibility to participate in the study by sending in advance an invitation letter, an informed consent form, and a four-page informative material. After arrival, the subject declared whether he/she is (or is not) willing to voluntarily participate in the study. All study subjects signed informed consent before participation. The study was approved (71/2017) by the Ethics Committee of Kuopio University Hospital, Kuopio, Finland, and it followed the principles of the declaration of Helsinki.

Grouping of study subjects

All 498 recruited subjects were divided into three groups based on their answer to the following question: Do you use orally ingested vitamin D preparations? The answer options were (1) 'no' (group 1), (2) 'occasionally' (group 2), and (3) 'yes, regularly' (group 3). These three groups were compared with each other. The exact dose of vitamin D could not be defined reliably, because there was heterogeneity in answers and many aged subjects were not aware of the dose upon examination by dermatologists. The same applies to dietary issues. In addition, the use of vitamin D preparations was coincidental or seasonal in several cases and therefore these subjects belong to group 2. The subjects were recruited to the study throughout the year, from May 2017 to October 2020, except for the mid-summer months, June and July. In this study, a special interest was to compare the subjects in group 3 to those in group 1.

The immunosuppression state of subjects was evaluated as described recently [33]. There were 96 subjects with immunosuppression: 12 subjects in group 1 (5 OTRs), 20 subjects in group 2 (5 OTRs), and 64 subjects in group 3 (28 OTRs). The OTRs and subjects with another immunosuppression state due to immunosuppression medication for a variety of immune-mediated diseases in different tissues and organs were studied separately from the 402 immunocompetent subjects, because of their significantly uneven distribution in groups 1–3 ($P < 0.001$, χ^2 test).

The exposure of skin to UV radiation was clarified with different questions. The self-estimated lifetime exposure was studied with the following question 'How often have you exposed yourself to sunlight during your lifetime?' The answer options were (1) 'seldom', (2) 'occasionally', (3) 'often', or (4) 'very often'. The sunburn history was studied with the following question: how often has your skin been burned due to sunlight during your lifetime? The answer options were (1) 'seldom', (2) 'occasionally', or (3) 'often'. The answer options for the question of 'Main environment in working history' were (1) 'outdoor', (2) 'indoor', or (2) 'variably both'. The answer options in the case of exposure of skin to UV-light treatment prescribed by a physician or exposure to the solarium (an artificial indoor tanning device with UVA radiation) were (1) 'never', (2) '0–30 times', or (3) '31–100 times'. None of the subjects reported to had been experienced more than 100 exposure times.

Malignancies in the skin and extracutaneous site

There were 295 subjects with a history of past or present cutaneous malignancy: 100 with melanoma, 213 with BCC, and 41 with SCC. Some of the subjects revealed a history of more than one malignancy type. Subjects with in-situ melanoma ($n = 15$) were included in the melanoma group (all melanomas). Also, subjects with carcinoma *in situ* (Bowen's disease) ($n = 5$) were included in the SCC group. In 70 subjects of 498, a previous malignancy in another organ or tissue than skin (termed as extracutaneous site, ECS) was documented, including breast (22), prostate (12), kidney (2), bladder (3), intestine (6), hematological malignancy ($n = 12$: three subjects with past follicular lymphoma, four with past large B cell lymphoma, one with past Hodgkin's disease, two with past acute lymphatic leukemia, and two with past chronic lymphatic leukemia), thyroid (2), ovary (2), uterus (3), eye (1), eyelid (1), salivary gland (1), tongue (2), lip (2), and cervix (1). Two subjects had a history of two different cancer types, the first one had breast and intestinal cancer, and the second one had bladder and salivary gland cancer.

Examination of skin photoaging, actinic keratoses, and pigment cell nevi

All skin sites were examined by experienced dermatologists, and a PhotoAging Area and Severity Index (PAASI) score were calculated as described [33]. The skin sites were evaluated using the following scoring: 0 = no marked photoaging (intrinsic skin aging), 1 = mild, 2 = moderate, 3 = severe photoaging with actinic keratosis, and 4 = very severe photoaging with several actinic keratoses. The PAASI score ranges from 0 to 400.

Actinic keratoses were counted and the subjects were divided into six subgroups: (1) 0; (2) 1; (3) 2; (4) 3; (5) 4–10; and (6) >10 actinic keratoses [33]. If necessary, a diagnostic biopsy was taken. Pigment cell nevi (≥2 mm in diameter) were counted and the subjects were divided accordingly into subgroups with (1) 0–20; (2) 21–50; (3) 51–100; or (4) >100 nevi.

Table 1 The characteristics of 402 immunocompetentsubjects divided into three groups according to the self-reported use of oral vitamin D supplements

	Group 1, 'No' (n=99)	Group 2, 'Occasionally' (n=126)	Group 3, 'Yes, regularly' (n=177)	P value, statistical test
Age, mean±SD	65.0±11.1 (range 22–79)	60.2±15.0 (range 21–79)	62.9 ± 13.6 (range 24–79)	0.030
				ANOVA
Gender (male/female)	73/26	61/65	68/109	<0.001
	73.7%/26.3%	48.4%/51.6%	38.4%/61.6%	χ^2
BMI	27.1±4.7	27.2±5.0	26.6±5.0	0.544
				ANOVA
Serum 25(OH)-D3 (nmol/L)	n=47 (47.5%)	n=69 (54.8%)	n=91 (51.4%)	<0.001
	61.3±19.3	69.5±19.2	83.1±23.5	ANOVA
Education:				0.004
1. Comprehensive school	1. 33.3%	1. 13.5%	1. 20.9%	χ^2
2. Upper secondary	2. 39.4%	2. 57.1%	2. 45.2%	
3. Higher education of applied sciences	3. 14.1%	3. 11.1%	3. 10.2%	
4. Academic degree	4. 13.1%	4. 18.3%	4. 23.7%	
Main environment in working history:	n=98	n=125	n=175	<0.001
Outdoor	18.4%	1.6%	5.7%	χ^2
Indoor	55.1%	75.2%	69.7%	
Variably both	26.5%	23.2%	24.6%	
Skin type:	n=94	n=119	n=168	0.318
I	6.4%	5.9%	4.2%	χ^2
II	42.6%	48.7%	45.8%	
III	43.6%	42.0%	47.6%	
IV	5.3%	3.4%	2.4%	
Fitzpatrick score	n=97	n=125	n=177	0.888
	14.1±4.6	14.0±4.7	13.9±4.4	ANOVA
Lifetime exposure to sunlight:	n=99	n=125	n=175	0.220
Seldom	27.3%	13.6%	18.3%	χ^2
Occasionally	36.4%	38.4%	36.0%	
Often	26.3%	30.4%	30.3%	
Very often	10.1%	17.6%	15.4%	
Lifetime sunburns:	n=99	n=126	n=176	0.669
Seldom	33.3%	29.4%	27.3%	χ^2
Occasionally	43.4%	42.9%	49.4%	
Often	23.2%	27.8%	23.3%	
Solarium exposure:	n=99	n=126	n=175	0.004
Never	84/99=84.8%	80/126=63.5%	116/175=66.3%	χ^2
≤30 times	14/99=14.1%	37/126=29.4%	46/175=26.3%	
31–100 times	1/99=1.0%	9/126=7.1%	13/175=7.4%	
UV-light treatment:	n=94	n=122	n=165	0.845
Never	87/94=92.6%	108/122=88.5%	149/165=90.3%	χ^2
≤30 times	5/94=5.3%	11/122=9.0%	11/165=6.7%	
31–100 times	2/94=2.1%	3/122=2.5%	5/165=3.9%	
Tobacco pack years (mean±SD)	n=98	n=126	n=176	<0.001
	9.7±16.8	2.9±6.6	3.9±10.6	ANOVA
Any smoking history	n=99	n=126	n=176	0.238
	Yes 52	Yes 56	Yes 74	χ^2
	No 47	No 70	No 102	
Hemoglobin (g/L)	n=97	n=125	n=176	<0.001
	145.6±10.7	142.0±12.5	139.9±11.4	ANOVA
Blood leukocytes ($\times10^9$/L)	n=97	n=125	n=176	0.169
	6.5±1.8	6.1±1.5	6.2±1.7	ANOVA
Neutrophils ($\times10^9$/L)	n=95	n=123	n=172	0.563
	4.0±1.5	3.6±1.1	4.0±5.3	ANOVA
Monocytes ($\times10^9$/L)	n=95	n=122	n=171	0.002
	0.43±0.16	0.37±0.13	0.38±0.12	ANOVA
Lymphocytes ($\times10^9$/L)	n=95	n=122	n=171	0.979
	1.9±0.8	1.9±0.6	1.9±0.7	ANOVA
NLR	n=95	n=122	n=171	0.310
	2.4±1.4	2.0±0.8	2.3±2.7	ANOVA
Thrombocytes ($\times10^9$/L)	n=97	n=125	n=176	0.775
	241.3±59.4	246.7±63.4	243.3±53.5	ANOVA
PAASI score	69.9±43.0	66.3±42.8	65.3±43.3	0.691
				ANOVA
Facial photoaging score	n=99	n=126	n=177	0.017
0	2/99=2.0%	7/126=5.6%	4/177=2.3%	χ^2
1	18/99=18.2%	33/126=26.2%	41/177=23.2%	
2	51/99=51.5%	46/126=36.5%	78/177=44.1%	
3	28/99=28.3%	35/126=27.8%	54/177=30.5%	
4	0/99=0%	5/126=4.0%	0/177=0%	
Actinic keratosis count	n=99	n=126	n=177	0.904
0	50.5%	53.2%	55.9%	χ^2
1	14.1%	15.1%	10.2%	
2	4.0%	5.6%	7.3%	
3	7.1%	5.6%	6.8%	
4–10	12.1%	12.7%	11.3%	
>10	12.1%	7.9%	8.5%	

(Continued)

Table 1 (Continued)

	Group 1, 'No' (n=99)	Group 2, 'Occasionally' (n=126)	Group 3, 'Yes, regularly' (n=177)	P value, statistical test
Nevus count:	n=98	n=126	n=177	0.394
0–20	46.9%	39.7%	50.3%	χ^2
21–50	24.5%	30.2%	18.6%	
51–100	15.3%	17.5%	18.1%	
>100	13.3%	12.7%	13.0%	
Subjects with past or present:				χ^2
Melanoma (all)	32/99=32.3%	26/126=20.6%	2/177=18.1%	0.021
Melanoma malign	26/99=26.3%	23/126=18.3%	29/177=16.4%	0.128
Melanoma *in situ*	6/99=6.1%	3/126=2.4%	3/177=1.7%	0.110
BCC	46/99=46.5%	54/126=42.9%	86/177=48.6%	0.614
SCC	11/99=11.1%	7/126=5.6%	11/177=6.2%	0.220
Any type	74/99=74.7%	73/126=57.9%	110/177=62.1%	0.027
Risk class	N=99	N=126	N=177	0.003
Low	21.2%	26.2%	38.4%	χ^2
Moderate	49.5%	51.6%	48.6%	
High	29.3%	22.2%	13.0%	
Family history of melanoma	n=79*	n=110*	n=155*	0.038
	Yes 9 (11.4%)	Yes 28 (25.5%)	Yes 26 (16.8%)	χ^2
	No 70	No 82	No 129	
Subjects with past or present malignancy in ECS	12/99 (12.1%)	18/126 (14.3%)	28/177 (15.8%)	0.702 χ^2
Subjects with past lymphatic malignancy	2/99=2.0 %	3/126=2.4 %	5/177=2.8 %	0.915 χ^2

The P values shown refer to the comparison of all three groups.
ANOVA, analysis of variance; BCC, basal cell carcinoma; ECS, extracutaneous site; NLR, neutrophil-to-lymphocyte ratio; PAASI, PhotoAging Area and Severity Index; SCC, squamous cell carcinoma; 25(OH)-D3, 25-hydroxyvitamin-D3 '*', number of subjects with reliable information on the family history of melanoma.

Blood tests

All blood samples were analyzed in the hospital laboratory (ISLAB) of Kuopio University Hospital. The level of hemoglobin, white blood cell count and differential, and thrombocytes were analyzed. The neutrophil-to-lymphocyte ratio (NLR) [34] was calculated and compared between subgroups. A serum sample taken from 260 subjects (53 with immunosuppression) after the recruitment was analyzed for 25(OH)-D3 levels using an immunochemiluminometric assay. The recommended reference value is >50 nmol/L.

Statistics

The differences between continuous variables were tested using the unpaired, two-tailed t-test or one-way ANOVAtest after checking the normal distribution of variables. The χ^2 test was used in categorical variables. The correlation between continuous variables was tested using the Spearman correlation test. The logistic regression analysis was used to identify the factors related to the significant odds ratio (OR) with 95% confidence interval (CI) for melanoma, SCC, or any type of skin cancer, and all these three endpoint variables were individual endpoints in the analysis. In addition, a multivariate approach was used in each endpoint variable, and a stepwise approach in the case of melanoma, too. A P value less than 0.05 was considered to be statistically significant.

Results

The characteristics of 402 immunocompetent subjects

The 402 non-immunosuppression subjects were first analyzed separately because the percentage of subjects with immunosuppression differed significantly between the three groups. The serum concentration of 25(OH)-D3 was measured in 207 of 402 subjects, that is, in about half of the subjects in each group (Table 1), and it increased significantly in a dose-dependent manner from group 1 to 3. Owing to the recruitment system, the subjects with 25(OH)-D3 measurements were evenly distributed in these three groups by chance. This suggests that the division of patients into three groups based on their self-reported oral use of vitamin D was reasonable.

The results of 402 non-immunosuppression subjects are summarized in Table 1. Even though the age between the three groups was significantly different, it was not significantly different between groups 1 and 3 (P=0.181). The subjects with regular use of vitamin D in group 3 were more educated (P=0.032), showed less frequently an outdoor working history (P=0.003), lower tobacco pack years (P=0.001), and more frequent solarium exposures (P=0.002) than the subjects in group 1. However, there were no significant differences between the groups in the self-estimated lifetime exposure to sunlight or lifetime sunburns as well as in the Fitzpatrick skin type or score, UV-light treatments, BMI, PAASI score, actinic keratosis count, nevus count, or cancers in ECS. The slightly, although significantly lower level of hemoglobin (P=0.001) and monocytes (P=0.005) in group 3 than 1 is considered to be clinically non-relevant. Of note is the result that there were no differences in the previously proposed prognostic cancer marker, NLR [34]. Even though there was a statistically significant difference in the family history of melanoma between the groups

(Table 1), there was no difference between groups 3 and 1 ($P=0.275$). The case is similar with respect to the facial photoaging score, that is, there was no significant difference between groups 3 and 1 ($P=0.656$).

The comparison of groups with regard to a history of past or present skin cancer revealed significant differences, that is, there were fewer subjects with past or present melanoma ($P=0.007$ when all types included), or any type of skin cancer ($P=0.033$), in the group 3 than 1. Furthermore, the investigator-estimated risk class of skin cancers was significantly lower in group 3 than in 1 ($P=0.001$). The difference in malignant melanoma turned out to be significant ($P=0.049$), when comparing the subjects in group 3 to those in group 1 and excluding the subjects in group 2. In addition, a tendency towards fewer subjects with past or present SCC was apparent (Table 1), but significant differences were not seen when comparing the subjects in group 3 ($P=0.150$) or group 2 ($P=0.127$) to those in group 1. In contrast to melanoma and SCC, the subjects with past or present BCC were very evenly distributed in these 3 groups.

Next, the non-immunosuppression subjects were divided according to gender (data not shown). The significant differences observed were that (1) males revealed less frequently an outdoor working history in group 3 than 1 ($P=0.036$), (2) serum 25(OH)-D3 increased in both genders in parallel with the vitamin D use ($P<0.001$), (3) there were fewer male subjects with melanoma *in situ* in the group 3 (0%) than 1 (6.8%) ($P=0.044$), and (4) there were fewer female subjects with high-risk class in the group 3 (8.3%) than 1 (26.9%) ($P=0.01$).

The logistic regression analysis in 402 immunocompetent subjects

In the logistic regression analysis of the subjects with or without a history of melanoma, relevant variables shown in Table 1 were chosen for the analysis and then were stepwise omitted from it. The results of univariate and multivariate ORs are shown in Table 2. The regular use of vitamin D in group 3 produced a statistically significant multivariate OR of 0.447 ($P=0.016$), but the occasional use of vitamin D in group 2 did not ($P=0.08$, OR 0.540) when compared to controls in group 1. In the case of other variables tested, that is, the age in years, gender, education, the main environment in working history, malignancy in ECS, smoking history, tobacco pack years, hemoglobin, monocytes, and indoor tanning (solarium), no significant univariate, or multivariate ORs were observed (Table 2). During the stepwise omission of variables, only the regular use of vitamin D in group 3 remained steadily significant ($P\leq0.025$) in every test. In the last test with only the variables of vitamin D use, age, and sex, also the occasional use of vitamin D in group 2 turned to produce a significant multivariate OR of 0.523 ($P=0.041$; 95% CI, 0.281–0.974), whereas the OR in regular users in this test was 0.445 ($P=0.008$; 95% CI, 0.245–0.809), but the ORs by age ($P=0.670$; OR 0.996; 95% CI, 0.976–1.014) and gender ($P=0.751$; OR 0.922; 95% CI, 0.558–1.524) were non-significant.

Table 2 The logistic regression analysis and consequent odds ratios for subjects with a history of melanoma compared to control subjects without it

Variable	Univariate odds ratio	95% Confidence interval	*P* value	Multivariate odds ratio	95% Confidence interval	*P* value
Vitamin D use:						
No use	Ref., 1	0.298–0.995	0.048	Ref., 1	0.271–1.076	0.080
Occasional use	0.544	0.262–0.816	0.008	0.540	0.231–0.862	0.016
Regular use	0.462			0.447		
Age in years	0.998	0.981–1.015	0.821	1.003	0.982–1.024	0.795
Gender:						0.305
Female	Ref., 1			Ref., 1		
Male	1.075	0.674–1.715	0.761	0.712	0.372–1.363	
Education:						
Comprehensive school	Ref., 1			Ref., 1		
Upper secondary	1.132	0.610–2.101	0.694	1.292	0.626–2.663	0.488
Higher education of applied sciences	1.353	0.585–3.128	0.480	1.574	0.597–4.147	0.359
Academic degree	1.068	0.506–2.255	0.862	1.623	0.652–3.988	0.301
Working environment:						
Outdoor	Ref., 1			Ref., 1		
Mixed	0.513	0.208–1.267	0.148	0.623	0.233–1.668	0.346
Indoor	0.554	0.246–1.248	0.154	0.536	0.204–1.408	0.206
Indoor tanning (solarium):						
Never	Ref., 1			Ref., 1		
<30 times	1.167	0.680–2.003	0.576	1.418	0.779–2.582	0.253
31–100 times	0.986	0.352–2.761	0.978	1.184	0.399–3.516	0.761
Malignancy in extracutaneous site:						
No	Ref., 1			Ref., 1		
Yes	0.778	0.385–1.571	0.484	0.639	0.278–1.471	0.293
Any smoking history						
No	Ref., 1	0.579–1.483	0.752	Ref., 1	0.489–1.554	0.641
Yes	0.927			0.871		
Tobacco pack years	1.002	0.983–1.022	0.822	1.003	0.979–1.029	0.791
Hemoglobin level	1.009	0.989–1.029	0.383	1.010	0.984–1.036	0.466
Monocyte count	0.988	0.170–5.738	0.989	0.683	0.092–5.081	0.710

The logistic regression analysis was also used to study risk factors in subjects with or without a history of any past or present skin cancer. In this analysis, the OR was 0.478 in group 3 ($P = 0.032$; 95% CI, 0.243–0.939) and 0.543 in group 2 ($P = 0.061$; 95% CI, 0.287–1.028) suggesting a lower risk for any skin cancer in the group 3 than 1. However, the OR was 1.049 in relation to the age of subjects ($P < 0.001$; 95% CI, 1.029–1.070). All other variables tested (see above) were non-significant.

In the case of subjects with or without a history of SCC, only the age produced a significant OR of 1.134 ($P = 0.001$; 95% CI, 1.050–1.224), but neither vitamin D use (group 3: OR 0.562; $P = 0.243$; 95% CI, 0.214–1.477), PAASI (OR 1.009; $P = 0.065$; 95% CI, 0.999–1.018), gender, solarium, nor tobacco pack years produced significance.

The characteristics of 96 immunosuppressed subjects

The 96 subjects with immunosuppression were analyzed separately and similarly as shown in Table 1. The only difference observed in variables was in the history of the malignant type of melanoma; 1 of 12 cases in group 1, 4 of 20 cases in group 2, and 2 of 64 cases in group 3 ($P = 0.04$).

Serum 25-hydroxyvitamin-D3, photoaging, nevi, and skin cancers in 207 immunocompetent subjects

A slightly positive correlation between the age and the serum level of 25(OH)-D3 was observed in the Spearman correlation test in group 3 ($r = 0.226$, $P = 0.031$), but not in groups 1 and 2. A slight positive correlation between the age and 25(OH)-D3 was also found ($r = 0.178$, $P = 0.010$) when all 207 subjects were combined for the test.

The correlation test between the serum level of 25(OH)-D3 and PAASI showed that there was no significant correlation between these variables in group 1 ($n = 47$, $r = 0.174$, $P = 0.242$), group 2 ($n = 69$, $r = 0.161$, $P = 0.187$) and group 3 ($n = 91$, $r = 0.057$, $P = 0.592$). In addition, a significant correlation was not reached ($r = 0.133$, $P = 0.056$), when testing all 207 subjects combined.

The serum level of 25(OH)-D3 was also compared in each group in relation to actinic keratosis count, nevus count, or facial photoaging score. However, no significant differences were noted in this concentration as tested with ANOVA, that is, 25(OH)-D3 did not associate with actinic keratoses, nevi, and facial photoaging in any group. No relevant association was seen either when testing the whole group.

In addition, the serum level of 25(OH)-D3 was compared between subjects with or without a history of past or present melanoma, SCC, BCC, or any type of skin cancer in these three groups, but no significant differences were noted, as tested with an unpaired *t*-test. No relevant association was seen either when testing the whole group combined.

After the division of these 207 non-immunosuppression subjects into a group with <50 (deficient level), 50–70, or >70 nmol/l 25(OH)-D3, the percentage of subjects with past or present melanoma was 34.6% (9/26), 21.7% (15/69), or 25.0% (28/112) ($P = 0.435$), respectively. Thus, there was a tendency towards a higher percentage of melanoma cases in the <50 nmol/L groups, but the difference was not significant.

Serum 25-hydroxyvitamin-D3, photoaging, nevi, and skin cancers in 53 immunosuppressed subjects

The age of immunosuppression subjects in group 3 correlated slightly to the serum level of 25(OH)-D3 ($n = 41$, $r = 0.364$, $P = 0.019$), but not so in groups 1 ($n = 6$) and 2 ($n = 6$) probably due to small number of subjects. When testing all 53 subjects combined, a significant positive correlation was seen, too ($r = 0.357$, $P = 0.009$).

The serum level of 25(OH)-D3 did not correlate significantly to PAASI in any of the three groups or in the whole group of 53 subjects ($r = 0.078$, $P = 0.578$).

When studying the serum level of 25(OH)-D3 in relation to actinic keratosis count, mole count, or facial photoaging score, no significant or relevant associations were seen in the whole group. The serum level of 25(OH)-D3 did not differ significantly between the subjects with a past or present melanoma, BCC, or any skin cancer and those without a corresponding skin cancer. Unexpectedly, the serum level of 25(OH)-D3 was higher in subjects with a history of past or present SCC ($n = 6$, 115.5 ± 46.8 nmol/L) than in those without an SCC history ($n = 47$, 77.0 ± 22.7 nmol/L) ($P = 0.029$), although the number of cases was small and variation high, and consequently, a subgroup analysis could not be done.

Discussion

In the present cross-sectional cohort of adult subjects at risk of any type of skin cancer, the essential finding was that the regular use of vitamin D supplements associated with fewer melanoma cases, especially, when compared to non-users of vitamin D. Furthermore, the logistic regression analysis suggests that the regular use of vitamin D may be an independent protective factor of melanoma. The strength of this study is that all subjects were carefully examined and interviewed by experienced dermatologists. Also, the self-reported use of vitamin D was associated in a dose-dependent manner with serum 25(OH)-D3 concentration. Therefore, a simple and practical division based on vitamin D use can be considered to be reasonable for characterizing the vitamin D status in this study. A single measurement of serum 25(OH)-D3 may not necessarily represent a long-term status of vitamin D, as it can vary according to the season and many other factors [14]. Thus, serum 25(OH)-D3 can reflect the level of sun exposure, rather than being a causal factor of skin cancer [18,19]. Nevertheless, the temporal and causal connection between the self-reported use of oral vitamin D and the history of past or

present skin cancer should be interpreted with caution. Theoretically, it is possible that a subject has changed the behavior toward vitamin D supplementation after a skin cancer diagnosis. The Finnish Food Authority (https://www.ruokavirasto.fi/en/) recommends vitamin D supplementation for adults over 75 years of age, but it is not specifically recommended for people after skin cancer. However, skin cancer patients are instructed to be careful about sun protection measures and consequently, it may lead to vitamin D supplementation in response to decreased sun exposure. It is also of note that the age of study subjects upon recruitment does not refer to the age at skin cancer diagnosis. The other weakness is that the study cohort does not represent the general population because the subjects were recruited at the university hospital policlinic based on their risk for skin cancers. Also, it is noteworthy that the cohort size is low for epidemiological studies, vitamin D dose could not be defined reliably, and the self-reported vitamin D supplementation may be biased.

In this study, the indicators of cutaneous photoaging and carcinogenesis, PAASI, and actinic keratosis, did not show any significant association with vitamin D use or serum 25(OH)-D3. One possibility for this is that the photoaging develops within tens of years, but neither the self-reported vitamin D use nor serum 25(OH)-D3 upon recruitment hardly has a similar extension to the patient's background history. It is also possible that the indicators, PAASI, and actinic keratosis, do not sufficiently take into account the fact that the photoaging can be very local, for example, in the face or dorsal aspects of hands. With regard to the association of serum 25(OH)-D with facial photoaging, both positive associations in middle-aged white women in the USA [35] and no association in male and female adult subjects in Egypt [36] have been reported. Therefore, in this study, the facial photoaging score was analyzed separately and compared to vitamin D use or serum 25(OH)-D3, but no relevant association was seen in any of the tests supporting the findings by Dawoud *et al* [36]. Even though there were no statistical differences between groups 3 and 1, there were some tendencies towards more favorable variables of the carcinogenetic line of SCC in group 3 than 1, that is, the PAASI score tended to slightly decrease ($P = 0.398$) and there were fewer subjects with high actinic keratosis count ($P = 0.663$) or those with a history of past or present SCC ($P = 0.150$). Possibly this picture can be turned to be clear by increasing the number of study subjects. Nevertheless, prospective follow-up studies and intervention trials with oral or topical vitamin D supplements are needed to demonstrate a possible causal connection of vitamin D to photoaging, actinic keratoses, and SCC, like the studies published recently on the positive effect of vitamin D in the photodynamic therapy of actinic keratoses [31,32], or the study suggesting a reduced risk of SCC by the combination of calcium and vitamin D [28].

The intake of vitamin D from supplements or diet can associate with a slightly increased BCC risk [16], although some other studies have reported no risk [26,28]. In this study, the subjects with a history of past or present BCC were very evenly distributed into groups 1 through 3. Further, there were no significant differences in serum 25(OH)-D3 levels between the subjects with a history of past or present BCC and those without BCC history. One possibility for the weak or no effect of vitamin D supplementation on BCC may be that unhydroxylated vitamin D3 generated in the skin by UV radiation, or topically applied vitamin D, may suppress BCC growth through effective hedgehog inhibition, but oral vitamin D may not do so because of its hydroxylation in the liver and kidney leaving nonefficient hedgehog inhibitor [37]. In addition, the supplementation with high-dose vitamin D capsules biweekly for 8–9 weeks has been found to induce the expression of CYP24 mRNA, but not vitamin D receptor, in photodamaged and photoprotected human skin [38], and therefore orally administered vitamin D may, in fact, regulate the levels of active vitamin D metabolites within the cutaneous microenvironment. In future studies, topical unhydroxylated vitamin D preparations may be recommended in interventional trials to study their role in preventing BCC. However, when putting a strict focus on studying one NMSC type only, like BCC, it should be remembered that skin cancer increases the risk of other types of skin cancers, too [6–8].

Pigment cell nevi are known to associate with melanoma risk, although only 29.1% of melanomas have been reported to originate from a preexisting nevus, as has been concluded in a meta-analysis [39], and there are differences with respect to tumor characteristics and phenotype of subjects between subjects with nevus-associated melanoma and those with *de novo* melanoma [40]. Previously, a positive association has been found between serum 25(OH)-D and nevus count in 3501 adults (aged 18–79, mean 46.5) female subjects in the UK [41]. In this study; however, there were no significant differences with respect to nevus count between the three groups, neither in females nor in males with a clearly higher mean age than that in the aforementioned UK report. To further elucidate this possibility of an association, the serum level of 25(OH)-D3 was studied and correlated to nevus count in each of the three groups as well as in the whole group of 207 subjects, but no marked differences in 25(OH)-D3 were observed. Therefore, the present study cannot show any association between vitamin D and nevi. The reason may be that the mean age of subjects was relatively high and the selection criteria of subjects were different.

In this study, there were significantly lower percentages of subjects with a history of past or present melanoma (all types) and malignant melanoma in group 3 than 1

suggesting a beneficial role for vitamin D status in melanoma, like it has been associated with better prognostic indicators or survival [22–24,42,43]. The same was noted in the case of any type of skin cancer, which probably reflects the sum effect of melanoma and SCC. In parallel with this result, the investigator-estimated risk class of skin cancers was significantly lower in group 3 than 1. Several possible confounding factors were evaluated and taken into consideration, such as age, gender, BMI, blood cells, NLR, nevi, photoaging level, skin phototype, different aspects of UV exposure, immunosuppression, cancers in ECS, family history of melanoma, and smoking, but only the regular vitamin D use was significantly associated with fewer melanoma cases. According to the logistic regression analysis, the result suggests that even the occasional use of vitamin D may have a beneficial effect on melanoma risk. In the case of immunosuppression subjects, a similar tendency was noted in melanoma, although the number of immunosuppression subjects was low, especially in the control group 1. However, it is still possible that some other, yet unidentified or untested, factors can still confound the present result. The serum level of 25(OH)-D3, albeit associated with vitamin D use, revealed no marked associations with skin-related parameters. It appears, in light of the previous numerous studies and the present one, that serum 25(OH)-D3 alone may not be a strong marker to study the role of the vitamin D system in skin carcinogenesis. It is possible that the cutaneous CYP enzymes involved in vitamin D metabolism/catabolism and vitamin D receptor, or mutations in their genes, are needed to complement the repertoire of markers [10–12,44]. Nevertheless, the causal link between vitamin D and melanoma cannot be confirmed by the present results.

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Conflicts of interest

There are no conflicts of interest.

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