Author/s:
Lim, A;Shayan, R;Varigos, G

Title:
High serum vitamin D level correlates with better prognostic indicators in primary melanoma: A pilot study

Date:
2018-08-01

Citation:
Lim, A., Shayan, R. & Varigos, G. (2018). High serum vitamin D level correlates with better prognostic indicators in primary melanoma: A pilot study. Australasian Journal of Dermatology, 59 (3), pp.182-187. https://doi.org/10.1111/ajd.12648.

Persistent Link:
https://hdl.handle.net/11343/292691

# Higher serum vitamin D level correlates with better prognostic indicators in primary melanoma: a pilot study



Running title: Vitamin D improves melanoma prognosis

Alvin Lim, Ramin Shayan, George Varigos

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ajd.12648

Department of Dermatology, The Royal Melbourne Hospital,
Parkville, Victoria, Australia 3050



Dr Alvin Lim

2/55 Tenby Street, Mount Gravatt, QLD 4122

alvinlimdr@gmail.com

+61432 407 038

DR. ALVIN LIM (Orcid ID : 0000-0002-2436-8171)



Received Date : 03-Jan-2017

Revised Date : 06-Feb-2017

Accepted Date : 11-Feb-2017

Article type : Original Research

**Abstract**

**Background/Objectives:** Sunlight is a major risk factor for cutaneous melanoma. However, its interaction with melanoma is complex. In particular, vitamin D is a UVB-derived hormone which has been shown to have anti-cancer effects. In this pilot retrospective study, we sought to determine an association between clinicopathological features of melanoma and the corresponding patients' serum vitamin D level.

**Methods:** 109 primary melanomas diagnosed between 2001-2013 were identified from our institutional database with a corresponding 25-hydroxyvitamin D3 result within 6 months of diagnosis. Tumour clinical (age, sex, tumour location) and pathological (thickness, mitosis, ulceration, Clark level, subtype, metastatic status) parameters were correlated with vitamin D. For statistical analysis, unpaired Student t-test and ANOVA was used for categorical variables, and Spearman correlation for continuous variables.

**Results:** Vitamin D level was inversely associated with Breslow thickness as a dichotomous, categorical and continuous variable. The association remained significant when controlled for patient age and gender (P=.026). Vitamin D was higher in non-ulcerated tumours compared with ulcerated tumours (P=.006) and in tumours with mitotic rate $<1/mm^2$ compared with $\geq 1/mm^2$ (P=.036). A significant association was found between vitamin D level and tumour histological subtype (P=.019). On subgroup analysis, significant associations were found between SSM and NM (P=.026), and SSM and ALM (P=.007).

**Conclusion:** Higher vitamin D status may benefit prognosis in patients diagnosed with primary melanoma. A prospective cohort analysis with a larger sample and controlled for other vitamin D confounders would validate these findings.

**Keywords:** vitamin D, melanoma, cutaneous malignant melanoma, association, retrospective study



## Introduction

Cutaneous malignant melanoma comprises less than 2% of skin cancers but accounts for the vast majority of skin cancer deaths [1]. Important prognostic indicators in primary melanoma include Breslow thickness (vertical distance from granular layer of epidermis to tumour edge in millimetres), ulceration, and mitotic rate, with further contributions from age, gender and tumour anatomical site [2]. Increased public awareness has resulted in a majority of patients being diagnosed at an earlier stage, and complete excision remains the best form of treatment. However, patients have a long-term risk of developing a new melanoma or recurrence of the primary [3]. Furthermore, sentinel lymph node biopsy (SLNB) is not routinely indicated in

the most commonly diagnosed melanomas, thin tumours (≤1mm). Once established, tumours can locally invade and metastasise quickly. Understandably, novel interventions to modify the tumour presentation at the time of diagnosis will benefit prognosis.

Sunlight is a major risk factor for skin cancer; however, its interaction with melanoma is complex and not well understood. Although guidelines recommend sun protection following skin cancer diagnosis, recent interest has emerged regarding the protective role of vitamin D in cancer pathogenesis. Known as the 'sunshine vitamin', vitamin D is a hormone derived primarily by synthesis in the epidermis upon stimulation by UVB radiation [4]. Traditionally known to regulate calcium physiology and bone mineralisation [4], vitamin D supplementation is the mainstay of optimising bone health. Toxicity with overdosing is rare [5], precluding its safety as novel therapy.

More recently, vitamin D has been implicated in a wide range of diseases and human cancers [6]. Its association with cancer is supported by epidemiological observations of an inverse association of cancer incidence and mortality with latitude of residence, reflecting differences in regional sun exposure [7]. In vitro studies demonstrate vitamin D to have several anti-cancer effects: to promote cell differentiation and apoptosis, and to inhibit cell proliferation and tumour angiogenesis [8]. Observational studies also report that recently diagnosed melanoma patients with higher vitamin D status had thinner tumours [9], lower tumour stage [10], and reduced risk of relapse and death [9]. A limited number of prospective studies have shown lower vitamin D level at diagnosis was associated with more aggressive primary melanoma [11]. However, these benefits have not been validated in other studies [12-14], and data from randomized controlled trials is lacking on the effect of vitamin D intervention on melanoma outcomes. In one study, higher vitamin D level was associated with high naevus counts, considered to be a risk factor for melanoma [15]. Thus, the verdict still remains unclear.

In this single-centre pilot study, we correlated patient vitamin D status close to the time of primary melanoma diagnosis with clinicopathological features of their melanoma. Finding a positive association between vitamin D status and melanoma prognostic indicators would support the role of vitamin D supplementation in melanoma patients.

## Methods

This study was approved by the human research ethics committee at The Royal Melbourne Hospital, Melbourne, Australia (Approval no. QA2013147).

### Case recruitment

The study sample consisted of melanoma cases managed at The Royal Melbourne Hospital. Cases comprised patients with cutaneous melanoma who were diagnosed and treated, or referred for management at our institution. Thin melanoma was defined as tumour thickness ≤1mm.

Cases were retrospectively recruited by searching records of melanoma biopsy and excision specimens within the database of the pathology department between the years 2001-2013. Case selection was not restricted by age, gender, or ethnicity. The initial search yielded 468 cases, of which 226 were thin melanomas. Clinical details of the melanoma presentation (age, gender, tumour site, date of diagnosis) was obtained from the patient medical record.

Histopathological details of the melanoma (Breslow thickness, ulceration, mitotic rate, subtype, Clark level, metastasis if present) were obtained from the histology report. In patients with multiple documented melanomas, the earliest diagnosed or primary melanoma was selected for inclusion in the study. Tumour metastatic status was defined as local (recurrence in scar/skin as documented in serial reports), regional nodal (detection of tumour deposit in lymph node/s, through sentinel lymph node biopsy (SLNB) or fine needle aspiration) or distant (tumour detected in other sites by radiological imaging). To aid in determining metastatic status, both SLNB result (if performed) and patient medical record was searched. In cases which underwent a SLNB, the result was stated in the pathology report and these cases were classified as ‘metastatic’ or ‘non-metastatic’.

### Vitamin D level determination

Vitamin D was defined by serum 25-hydroxyvitamin D3 level, the active form which is conventionally measured. Melanomas of all thickness identified in the initial search were included in the analysis if there was a corresponding vitamin D result within 6 months of diagnosis. In our institution, vitamin D testing was not routinely performed in melanoma

patients in the study period. Hence, vitamin D results were obtained where available, which may have been indicated for other reasons, health-related or otherwise. Vitamin D results were retrieved from the electronic system of pathology results, medical record, or by contacting the patient's general practitioner clinic.

**Statistical analysis**

Statistical tests were applied with serum vitamin D level considered as a continuous variable throughout.

To analyse the association between vitamin D and Breslow thickness, thickness was treated as a dichotomous variable (thin vs thick) and unpaired Student's t-test was used to test the association. As a categorical variable, thickness was subdivided into 5 subcategories: <0.75, 0.75-1, 1-2, 2-3 and >3 (measured in millimetres), and subsequently correlated with serum vitamin D level using one way ANOVA. Finally, thickness was considered as a continuous variable and correlated with serum vitamin D level using Spearman correlation. Spearman correlation for non-parametric data was selected on the basis of the non-normal distribution of tumour thickness determined using Shapiro-Wilk test of normality. Outliers were calculated by Cook's distance, and subsequently excluded when identified outside of range. Multiregression analysis was then performed to predict a linear model between serum vitamin D level and Breslow thickness, controlling for patient age and gender.

Spearman correlation was used for statistical comparison between the continuous variables age and serum vitamin D level (both non-normally distributed).

Unpaired Student's t-test was used for comparisons involving serum vitamin D level and the dichotomous variables metastatic status (metastatic vs non-metastatic), gender (male vs female), ulceration (present vs absent) and mitosis ($\leq 1/mm^2$ vs $>1/mm^2$).

One way ANOVA was used for comparisons involving the categorical variables tumour site, Clark level, and histological subtype, each of which involved greater than two categories. Tumour site was subdivided as primary melanomas occurring on the 'head and neck', 'trunk', or 'limb'. Clark level was subdivided into 5 subcategories of invasion: I – melanoma in situ (confined to epidermis); II – invasion into papillary dermis but not reticular dermis; III – invasion into interface between papillary and reticular dermis; IV – invasion into reticular dermis; V – invasion into subcutaneous tissue. Tumour histological subtype was defined by 6 subtypes: superficial spreading melanoma (SSM), lentigo maligna/Hutchinson's melanotic

freckle (LM/HMF), lentigo maligna melanoma (LMM), acral lentiginous melanoma (ALM), nodular melanoma (NM) and desmoplastic melanoma (DM). Post-hoc analysis with Fisher's Least Significant Difference was used for subgroup comparisons when one way ANOVA yielded a significant result.

All P values were two sided with significance defined as less than 0.05. Statistical analyses were performed using SPSS version 22.

## Results

Selecting cases based on availability of a serum vitamin D result within 6 months of primary melanoma diagnosis resulted in many cases being excluded. In total, 109 confirmed primary melanomas met the inclusion criteria for having available relevant histopathology data and a recent serum vitamin D level.

Descriptive statistics of the study cohort are presented in Table 1. The mean age at diagnosis was 57.69 years (min 18, max 91; std deviation 18.05), among 51 males (47%) and 58 females (53%). Mean Breslow thickness was 1.72 mm (median 1.05, std deviation 2.17). Our sample included a roughly even proportion of tumours ≤1mm (49%) and >1mm (51%). Mean serum vitamin D level was 62.24 nmol/L (median 63.00, std deviation 24.39). 32% of patients met criteria for vitamin D deficiency (vitamin D level <50nmol/L).

### Vitamin D and Breslow thickness

Serum vitamin D level was significantly associated with Breslow thickness. When comparing thin (≤1mm) and thick (>1mm) tumours, mean serum vitamin D level in thin tumours (mean = 66.96 nmol/L) was higher than thick tumours (mean = 57.60 nmol/L; P=.045) (Table 2). Statistical significance remained in further subcategories of thickness, wherein a decreasing trend in vitamin D level corresponded with increasing tumour thickness up to 3mm (<0.75 mm, 67.19 nmol/L; 0.75-1 mm, 65.43 nmol/L; 1-2mm, 62.81 nmol/L; 2-3mm, 43.08 nmol/L; P=.044). Treating thickness as a continuous variable, Spearman correlation between Breslow thickness and serum vitamin D level was -.210 (P= .028) (See Supplemental Data, Table S2). Upon exclusion of 13 outliers (calculated by Cook's distance), higher serum vitamin D level was significantly associated with lower Breslow thickness, controlled for patient age and gender ($r^2$=0.055, test for linear trend P = 0.026, see Figure 1 and Supplemental Data Table S1).

**Vitamin D and ulceration**

A significant association was found between serum vitamin D level and the presence/absence of tumour ulceration. Mean serum vitamin D level was higher in non-ulcerated tumours (mean = 64.57 nmol/L) compared with ulcerated tumours (mean = 47.27 nmol/L; P=.006) (Table 2).

**Vitamin D and mitosis**

Furthermore, a significant association was found between serum vitamin D level and tumour mitotic rate. Mean serum vitamin D level was higher in tumours with <1 mitoses/$mm^2$ (mean = 68.81 nmol/L) compared to tumours with ≥1 mitoses/$mm^2$ (mean = 57.74 nmol/L; P=.036) (Table 2).

**Vitamin D and tumour subtype**

Histological subtype was also found to correlate with serum vitamin D level. Mean serum vitamin D level was highest in the DM subtype (mean = 79.29 nmol/L), followed by SSM (mean = 67.42 nmol/L), LM/HMF (mean = 63.50 nmol/L), LMM (mean = 61.33 nmol/L), NM (mean = 51.40 nmol/L) and ALM (mean = 43.56 nmol/L; P =.019) (Table 2). On subgroup analysis with Least Significant Difference testing, a significant association was found between SSM and ALM (67.42 and 43.56 nmol/L respectively, P =.007), and between SSM and NM (67.42 and 51.40 nmol/L respectively, P=.026).

**Vitamin D and other tumour parameters**

No statistically significant associations were found between serum vitamin D level and age at diagnosis, gender, metastatic status, site and Clark level respectively (Table 2).

## Discussion

In recent years there has been an increased impetus to promote vitamin D supplementation in the general population, out of emerging concern about the prevalence of vitamin D deficiency. Currently, controversy and practical difficulties surround testing and prescribing vitamin D. Many authorities report a serum level of 50-75 nmol/L is sufficient and >75 nmol/L is ideal, as it is the threshold for PTH suppression [16]. Although Melbourne has a more temperate climate compared to the northern parts of Australia (Latitude 37.8 ° South, Longitude 145°

East), in our study cohort we observed a mean vitamin D level of 62.24 nmol/L, although this was not corrected for other physiological variables. Approximately one-third (32%) of cases were vitamin D deficient (<50 nmol/L). However, it is uncertain how accurately this reflects the general population as we did not compare vitamin D status to disease-free controls. A similar study in Brisbane, Australia (Latitude 27.5° South, Longitude 153° East) with higher annual sun exposure reported a lower mean 25-hydroxyvitamin D level (58 nmol/L), with 36% prevalence of vitamin D deficiency in its melanoma cohort [11]. A higher prevalence of vitamin D deficiency is reported in studies conducted in the Northern hemisphere [11].

Although many factors influence vitamin D status, serum vitamin D level within 6 months of diagnosis was chosen to increase case availability, and to be consistent with previous similar study [11]. Until recent years, vitamin D level was not routinely measured in melanoma patients at our institution. Therefore, the majority of long-term patients did not have vitamin D level measured at initial diagnosis. Overall, in 56 of 109 cases (51%) vitamin D was measured within 3 months of diagnosis, which may be considered a suitable approximation (data not shown). Due to the retrospective nature of this study, vitamin D results obtained incidentally occurred near the time of melanoma diagnosis. It cannot be determined whether vitamin D measurement was performed as part of melanoma workup, or for another indication such as to evaluate a patient at risk of vitamin D deficiency. We acknowledge this may lead to bias in case selection. Moreover, the multiple sources of vitamin D result (hospital, GP practice) meant we were unable to account for variations in laboratory assays.

We have shown a significant association between vitamin D and Breslow thickness. Breslow thickness is the most significant prognostic indicator in primary melanoma and our data corroborate observational studies in other populations which also found an inverse relationship with vitamin D status [11, 17, 18]. We analysed vitamin D with respect to thickness as dichotomous, categorical and continuous variables and in each case found significant associations to suggest that higher vitamin D level correlates with lower thickness. Like our study, Newton-Bishop measured vitamin D within 6 months of diagnosis, but also followed up prospectively for survival, finding an inverse correlation with relapse-free survival [9]. However, Saiag et al found that change in vitamin D level post-diagnosis, rather than its level at diagnosis, was more significantly associated with survival [18]. Despite this, other prospective studies do not report a benefit of vitamin D on melanoma risk. Van der Pols et al. reported an increased risk of melanoma with higher vitamin D status [13], while others did not find an association [14, 19, 20]. Conflicting results in the literature may be due to

power of each sample size and lack of uniformity in adjusting for potential vitamin D confounders.

Our data also found significant associations with two traditional markers of tumour aggressiveness: ulceration and mitosis. Despite its prognostic importance, it is unknown how ulceration affects tumour invasion. Mitotic rate is another indicator of aggressiveness and the current AJCC staging protocol defines mitotic rate $<1/mm^2$ and $\geq 1/mm^2$ as prognostically important thresholds. We found higher vitamin D level in tumours which were non-ulcerated and had lower mitotic rate. The association with these favourable prognostic markers could imply a protective role. Interestingly, although the aforementioned results indicate lower vitamin D correlates with more aggressive tumour profile, there was no significant association with metastatic status. Possible explanations include limited power of our study, or selection bias of thicker tumours for metastatic workup with sentinel lymph node biopsy. More aggressive tumour features should logically pertain to increased metastatic risk.

We also report a statistically significant relationship between vitamin D and histological subtype. Surprisingly, desmoplastic melanoma, a rare form, had the highest mean vitamin D level. This could reflect a limitation with this analysis which is the uneven distribution, and low number of cases in most categories. An error on retrospect was the inclusion of several tumours with spindle cells in this category, despite not formally classified as desmoplastic melanoma. Therefore, lack of standardised terms in the pathology report may have contributed to erroneous classification of these particular tumours which were associated with higher vitamin D status. Nonetheless, these tumours were few and comprised only 8% of cases compared to superficial spreading (SSM), the most common subtype (55%), which had second highest mean vitamin D (67.42 nmol/L). There is extensive literature on melanoma subtypes bearing distinct evolutionary pathways [21]. SSM comprises the majority of clinically diagnosed melanoma and is considered to have better prognosis due to its slow growth and early detection. By contrast, NM is an aggressive form, characterised by its vertical growth phase and early invasion. Furthermore, these two subtypes bear several genetic distinctions. ALM, traditionally differentiated by its anatomical origin, is characterised by genomic instability, with lower BRAF mutation frequency compared to subtypes not related to chronic sun exposure [21]. Our results suggest that optimising vitamin D status may influence tumour type, although we have not controlled for factors such as genetics and anatomical site in presentation of these tumours. We also cannot rule out vitamin D as a proxy indicator of an unknown factor in the disease process. Currently,

knowledge is limited on how tumour subtype relates to prognosis, and consequently they are not incorporated into staging guidelines. Evidence from genetic and molecular studies could alter the existing classification system such that it is targeted toward prognostication and treatment.

Overall, our findings support the existing concept of vitamin D interaction with melanoma pathophysiology. The biological actions of vitamin D are mediated by its active form, 1,25-dihydroxyvitamin D, on the intranuclear vitamin D receptor (VDR). VDR is expressed in most tissues of the body, accounting for the pleiomorphic effects of the hormone and its links to an extensive array of diseases. Previous studies have found VDR polymorphisms with increased melanoma susceptibility [22], and association of VDR dysfunction with epidermal carcinogenesis [23]. Furthermore, VDR expression was higher in less aggressive melanoma and correlated with better prognosis [24]. Further studies are warranted to uncover the interaction of vitamin D with cancer cells.

Our results also support a future clinical trial examining the effect of vitamin D supplementation on melanoma prognosis. It is unclear how vitamin D supplementation influences melanoma risk, incidence and outcome. Studies to date were limited by the administration of subtherapeutic doses. Asgari et al did not find an association between vitamin D intake and melanoma risk [25]. A subgroup analysis of the Women's Health Initiative [26], in which postmenopausal women were randomised to take 400 IU/day vitamin D plus 1500 mg/day calcium or placebo, found women with previous NMSC taking supplements had reduced incidence of melanoma, though it is difficult to distinguish the effect of vitamin D from calcium. Doses of vitamin D in the higher range (1,100-4,000 IU/day) are believed to be necessary for its anticancer effects [27]. Clinical trials should be designed taking into account sources of vitamin D intake such as sun exposure and diet.

There were several limitations to this study. As a pilot study, we did not compare vitamin D levels with healthy control subjects. Due to the lack of available clinical data on patient vitamin D status, including sun exposure history, supplement use, skin phototype, BMI, and season of blood sampling, it was not possible to control for these possible confounders. For instance, in vitro studies on markers of obesity and insulin resistance (leptin, IGF-1) have described a link to melanoma progression [28]. Although controversial, obesity may also independently increase risk of developing skin cancer. A large cohort study also reported an association between higher BMI and lower vitamin D status [29]. As a marker of metabolic

status, future studies should account for vitamin D status in regard to other patient comorbidities, including obesity, liver and renal dysfunction. Furthermore, our time interval of vitamin D level within 6 months of diagnosis may not exclude reverse causation. That is, it is possible that vitamin D levels within this period reflect behavioural changes that have occurred after cancer diagnosis so that patients avoid sun exposure, resulting in lower vitamin D status. However, other studies have demonstrated that vitamin D level remains stable over time in cancer patients, and no significant variation in vitamin D level serially measured post-cancer diagnosis [30]. Thus, a single time-point measurement may reflect long-term status around the time of diagnosis. Nonetheless, more contemporary studies to this one have measured vitamin D prospectively at time of diagnosis and study recruitment, showing that higher vitamin D was associated with prognostically favourable tumour histology [11].

In conclusion, we found that higher vitamin D may benefit prognosis in patients presenting with melanoma. However, we have described an association with prognostic indicators and cannot confirm a causative relationship. Larger prospective cohort studies are needed in which vitamin D level is measured at time of diagnosis and controlled for confounding variables, in order to validate our findings. Future clinical trials should investigate the effect of vitamin D supplementation on melanoma outcomes. Optimising vitamin D status may be a safe, novel therapy to improve melanoma prognosis, in addition to other health benefits.

## List of Abbreviations

| | |
|---|---|
| AJCC | American Joint Cancer Committee |
| ALM | Acral lentiginous melanoma |
| ANOVA | Analysis of variance |
| BMI | Body mass index |
| DM | Desmoplastic melanoma |
| LM (HMF) | Lentigo maligna (Hutchinson's melanotic freckle) |
| LMM | Lentigo maligna melanoma |
| NM | Nodular melanoma |
| NMSC | Non-melanoma skin cancer |
| PTH | Parathyroid hormone |
| SLNB | Sentinel lymph node biopsy |
| SSM | Superficial spreading melanoma |
| UVB | Ultraviolet B |
| VDR | Vitamin D receptor |



## References


1. AIHW, *Cancer in Australia: an overview 2014*. 2014, Cancer series no. 90 Cat. no. CAN 88. Canberra: AIHW.
2. Abbas, O., D.D. Miller, and J. Bhawan, *Cutaneous malignant melanoma: update on diagnostic and prognostic biomarkers.* The American Journal Of Dermatopathology, 2014. **36**(5): p. 363-379.
3. Hohnheiser, A.M., et al., *Malignant melanoma of the skin: long-term follow-up and time to first recurrence.* World J Surg, 2011. **35**(3): p. 580-9.
4. Lips, P., *Vitamin D physiology.* Prog Biophys Mol Biol, 2006. **92**(1): p. 4-8.
5. Glade, M.J., *A 21st century evaluation of the safety of oral vitamin D.* Nutrition, 2012. **28**(4): p. 344-56.

6. Garland, C.F., et al., *The role of vitamin D in cancer prevention.* Am J Public Health, 2006. **96**(2): p. 252-61.
7. Grant, W.B., *Ecological studies of the UVB-vitamin D-cancer hypothesis.* Anticancer Res, 2012. **32**(1): p. 223-36.
8. Moukayed, M. and W.B. Grant, *Molecular link between vitamin D and cancer prevention.* Nutrients, 2013. **5**(10): p. 3993-4021.
9. Newton-Bishop, J.A., et al., *Serum 25-hydroxyvitamin D3 levels are associated with breslow thickness at presentation and survival from melanoma.* J Clin Oncol, 2009. **27**(32): p. 5439-44.
10. Nurnberg, B., et al., *Reduced serum 25-hydroxyvitamin D levels in stage IV melanoma patients.* Anticancer Res, 2009. **29**(9): p. 3669-74.
11. Wyatt, C., et al., *Vitamin D deficiency at melanoma diagnosis is associated with higher Breslow thickness.* PLoS One, 2015. **10**(5): p. e0126394.
12. Afzal, S., B.G. Nordestgaard, and S.E. Bojesen, *Plasma 25-hydroxyvitamin D and risk of non-melanoma and melanoma skin cancer: a prospective cohort study.* J Invest Dermatol, 2013. **133**(3): p. 629-36.
13. van der Pols, J.C., et al., *Vitamin D status and skin cancer risk independent of time outdoors: 11-year prospective study in an Australian community.* J Invest Dermatol, 2013. **133**(3): p. 637-41.
14. Major, J.M., et al., *Pre-diagnostic circulating vitamin D and risk of melanoma in men.* PLoS One, 2012. **7**(4): p. e35112.
15. Ribero, S., et al., *Positive Association Between Vitamin D Serum Levels and Naevus Counts.* Acta Derm Venereol, 2016.
16. Ross, A.C., et al., *The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know.* J Clin Endocrinol Metab, 2011. **96**(1): p. 53-8.
17. Bade, B., et al., *Low serum 25-hydroxyvitamin d concentrations are associated with increased risk for melanoma and unfavourable prognosis.* PLoS One, 2014. **9**(12): p. e112863.
18. Saiag, P., et al., *Prognostic Value of 25-hydroxyvitamin D3 Levels at Diagnosis and During Follow-up in Melanoma Patients.* J Natl Cancer Inst, 2015. **107**(12): p. djv264.
19. Skaaby, T., et al., *Prospective population-based study of the association between serum 25-hydroxyvitamin-D levels and the incidence of specific types of cancer.* Cancer Epidemiol Biomarkers Prev, 2014.

20. Ogbah, Z., et al., *Serum 25-hydroxyvitamin D3 levels and vitamin D receptor variants in melanoma patients from the Mediterranean area of Barcelona: 25-hydroxyvitamin D3 levels and VDR variants in melanoma patients from Barcelona.* BMC Med Genet, 2013. **14**(1): p. 26.
21. Whiteman, D.C., W.J. Pavan, and B.C. Bastian, *The melanomas: a synthesis of epidemiological, clinical, histopathological, genetic, and biological aspects, supporting distinct subtypes, causal pathways, and cells of origin.* Pigment Cell Melanoma Res, 2011. **24**(5): p. 879-97.
22. Zeljic, K., et al., *Melanoma risk is associated with vitamin D receptor gene polymorphisms.* Melanoma Res, 2014. **24**(3): p. 273-9.
23. Caini, S., et al., *Vitamin D and melanoma and non-melanoma skin cancer risk and prognosis: a comprehensive review and meta-analysis.* Eur J Cancer, 2014. **50**(15): p. 2649-58.
24. Brożyna, A.A., W. Jóźwicki, and A.T. Slominski, *Decreased VDR expression in cutaneous melanomas as marker of tumor progression: new data and analyses.* Anticancer Research, 2014. **34**(6): p. 2735-2743.
25. Asgari, M.M., et al., *A cohort study of vitamin D intake and melanoma risk.* J Invest Dermatol, 2009. **129**(7): p. 1675-80.
26. Tang, J.Y., et al., *Calcium plus vitamin D supplementation and the risk of nonmelanoma and melanoma skin cancer: post hoc analyses of the women's health initiative randomized controlled trial.* J Clin Oncol, 2011. **29**(22): p. 3078-84.
27. Garland, C.F., et al., *Vitamin D supplement doses and serum 25-hydroxyvitamin D in the range associated with cancer prevention.* Anticancer Res, 2011. **31**(2): p. 607-11.
28. Le Coz, V., et al., *IGF-1 contributes to the expansion of melanoma-initiating cells through an epithelial-mesenchymal transition process.* Oncotarget, 2016. **7**(50): p. 82511-82527.
29. Konradsen, S., et al., *Serum 1,25-dihydroxy vitamin D is inversely associated with body mass index.* European Journal Of Nutrition, 2008. **47**(2): p. 87-91.
30. Hofmann, J.N., et al., *Long-term variation in serum 25-hydroxyvitamin D concentration among participants in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial.* Cancer Epidemiology, Biomarkers & Prevention: A Publication Of The American Association For Cancer Research, Cosponsored By The American Society Of Preventive Oncology, 2010. **19**(4): p. 927-931.

**Figure 1.**

Linear regression analysis of serum vitamin D level and tumour thickness. After removal of 13 outliers (calculated by Cook's distance), adjusted for patient age at diagnosis and sex using linear regression model ($r^2$=0.055, P=.026). Serum vitamin D level in nmol/L and thickness in mm.

**Table 1.** Characteristics of the melanoma cases

| | |
|---|---|
| **Total cases (N)** | 109 |
| **Age at diagnosis (years)** | |
| Mean | 57.69 |
| Median | 59.00 |
| Range | 73 |
| Std deviation | 18.05 |
| **Gender (N)** | |
| Male | 51 |
| Female | 58 |
| **Primary tumour thickness (mm)** | |
| Mean | 1.72 |
| Median | 1.05 |
| Range | 12.20 |
| Std deviation | 2.17 |
| **Vitamin D (nmol/L)** | |

| | |
|---|---|
| Mean | 62.24 |
| Median | 63.00 |
| Range | 160 |
| Std deviation | 24.39 |
| **Vitamin D < 50nmol/L** | |
| N (%) | 35 (32) |
| Breslow ≤1mm, N (%) | 12 (22) |
| Breslow >1mm, N (%) | 23 (42) |

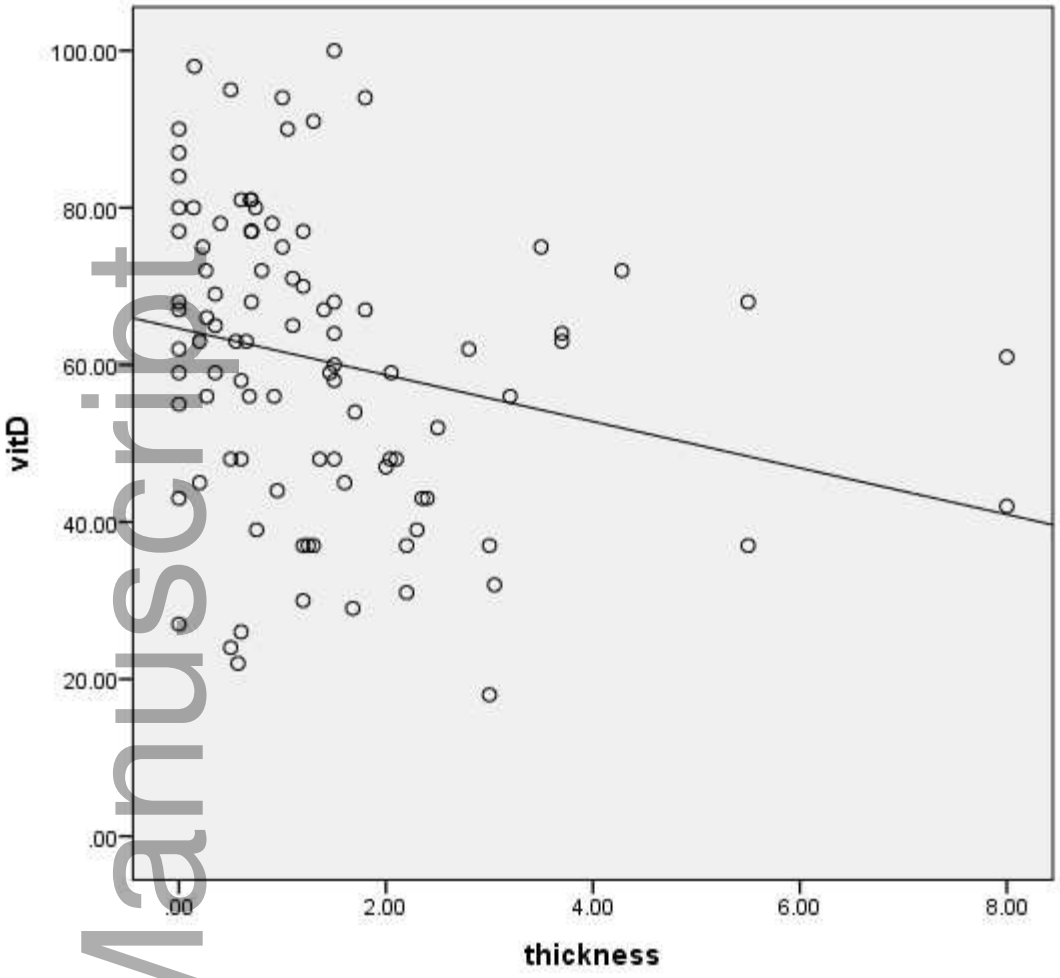


**Table 2.** Association between melanoma clinicopathological features and mean serum vitamin D level within 6 months of diagnosis[1]

| Feature | N (%) | Mean serum vitamin D level (nmol/L) | Standard deviation | P-value |
|---|---|---|---|---|
| **Metastatic status** | | | | |
| Metastatic | 22 (26) | 60.91 | 25.53 | .773 |
| Non-metastatic | 62 (74) | 62.69 | 24.56 | |
| **Tumour thickness** | | | | |

| | | | | |
|---|---|---|---|---|
| Thin (≤1.0 mm) | 54 (50) | 66.96 | 26.19 | .045 |
| Thick (>1.0 mm) | 55 (50) | 57.60 | 21.74 | |
| **Tumour thickness (mm)** | | | | |
| <0.75 | 47 (43) | 67.19 | 27.17 | .044 |
| 0.75-1 | 7 (6) | 65.43 | 19.81 | |
| 1-2 | 26 (24) | 62.81 | 22.71 | |
| 2-3 | 12 (11) | 43.08 | 12.08 | |
| >3 | 17 (16) | 59.88 | 21.93 | |
| **Gender** | | | | |
| Male | 51 (47) | 63.55 | 24.85 | .601 |
| Female | 58 (53) | 61.09 | 24.14 | |
| **Ulceration** | | | | |
| Present | 15 (19) | 47.27 | 22.35 | .006 |
| Absent | 65 (81) | 64.57 | 21.37 | |
| **Mitoses** | | | | |
| <1/mm$^2$ | 31 (44) | 68.81 | 22.38 | .036 |
| ≥1/mm$^2$ | 39 (56) | 57.74 | 20.83 | |
| **Tumour site** | | | | |
| Head/neck | 24 (22) | 64.88 | 20.07 | .524 |
| Trunk | 33 (30) | 64.73 | 19.96 | |
| Limbs | 52 (48) | 59.44 | 28.51 | |
| **Histological subtype** | | | | |
| Superficial spreading | 50 (55) | 67.42 | 26.70 | .019 |
| Lentigo maligna (HMF) | 6 (7) | 63.50 | 16.96 | |
| Lentigo maligna melanoma | 3 (3) | 61.33 | 17.62 | |
| Acral lentiginous | 9 (10) | 43.56 | 17.74 | |
| Nodular | 15 (17) | 51.40 | 21.02 | |
| Desmoplastic | 7 (8) | 79.29 | 20.56 | |
| **Clark level** | | | | |
| 1 | 19 (18) | 63.50 | 19.52 | .093 |
| 2 | 13 (12) | 78.25 | 31.03 | |
| 3 | 23 (21) | 59.45 | 24.05 | |
| 4 | 47 (44) | 59.38 | 22.90 | |
| 5 | 5 (5) | 69.80 | 24.30 | |

[1] Missing data not displayed were excluded from statistical analysis




ajd_12648_f1.jpg