

CORRELATION OF INVASION PARAMETERS IN BASAL CELL CARCINOMA WITH PRE-DIAGNOSTIC VITAMIN D INSUFFICIENCY LEVELS

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ABSTRACT

Purpose: The relationship between vitamin D and non-melanoma skin cancers remains controversial. Elevated serum vitamin D levels above 60 nmol/L (24 ng/mL) have been associated with an increased risk of basal cell carcinoma. This study aims to examine the association between tumor invasion parameters in basal cell carcinoma and pre-diagnostic vitamin D insufficiency levels.

Material and Methods: A retrospective analysis was conducted on basal cell carcinoma cases from 2014 to 2024, where serum vitamin D levels were below 25 ng/mL. Histopathological evaluations included measurements of Breslow thickness and assessments of the Clark level.

Results: The study population consisted of 45.5% males and 54.5% females, with a mean age of 67.95 ± 8.6 years. The mean serum vitamin D level was 15.69 ± 6.56 ng/mL. Non-aggressive histological subtypes exhibited lower Clark levels ($p < 0.05$). The high Clark level group had a greater Breslow thickness (2409.2 ± 253.05 μ m) compared to the low Clark level group (2120.13 ± 832.19 μ m) ($p < 0.05$). However, no significant differences were observed in mean vitamin D levels across Clark level or tumor localization groups. Furthermore, serum vitamin D levels did not show a significant correlation with Breslow thickness or Clark level ($p > 0.05$).

Conclusion: In basal cell carcinoma cases with vitamin D insufficiency, serum vitamin D level does not correlate with Clark level or Breslow thickness. Therefore, prediagnostic vitamin D insufficiency levels in basal cell carcinoma are not significantly associated with invasion parameters.

Keywords: calcifediol, neoplasm invasiveness, skin neoplasms

INTRODUCTION

Over the past decade, there has been a notable increase in the global incidence of skin cancers, posing significant challenges to public health systems worldwide. Although keratinocytic tumours, including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), have relatively low mortality rates, they impose substantial burdens on both quality of life and healthcare expenditure (1). Recent

epidemiological studies suggest that fair-skinned individuals have a 30% lifetime risk of developing at least one BCC (2).

Various factors contribute to the development and aggressiveness of BCC, including exposure to sunlight, individual pigment characteristics, and family history (1). Tumor localization and histological subtypes play crucial roles in determining the aggressiveness and recurrence rates of BCC.

Typically, low-risk areas include the trunk and limbs, while high-risk areas, such as the nose and periorificial regions, are associated with more aggressive subtypes, including micronodular, morpheaform, and infiltrative types (3).

The relationship between vitamin D and non-melanoma skin cancers remains a topic of debate in the scientific literature (1, 4). Recent insights from a meta-analysis conducted in 2020 suggest a significant increase in the risk of developing BCC with serum vitamin D levels surpassing approximately 60 nmol/L (24 ng/mL) (1). Vitamin D levels are commonly classified as follows: sufficient (≥ 30 ng/mL), insufficient (20-29 ng/mL), deficient (< 20 ng/mL), and severely deficient (< 7 ng/mL) (5). In this study, we focused on individuals with prediagnostic vitamin D levels below 25 ng/mL.

The primary objective of our study is to explore the potential correlation between prediagnostic vitamin D insufficiency levels and the invasive behavior of BCC tumors. We employed established methodologies such as the Clark classification and Breslow thickness assessment, commonly utilized in the evaluation of melanoma, to achieve this aim. Through this research, we aim to enhance the understanding of vitamin D insufficiency's role in BCC invasiveness, providing valuable insights into skin cancer prevention and management.

MATERIAL AND METHODS

Analysis of Patient Data and Exclusion Criteria in a Retrospective Manner

Basal cell carcinoma (BCC) cases archived in the Pathology Department of Research Hospital from 2014 to 2024 were retrospectively examined. Cases with measured serum vitamin D levels during the one-year pre-diagnostic period were included in the study. Cases with positive surgical margins were excluded as they could affect Breslow thickness.

Ethical Consideration

Ethical approval for the study was obtained from the Ethics Committee of Bilecik University (Date: 11.12.2023, Decision number: 2023/8-11), ensuring full compliance with the ethical guidelines outlined in the Declaration of Helsinki.

Histopathology

Following skin tissue removal, samples were fixed in 10% neutral buffered formaldehyde and processed through routine tissue processing steps including

dehydration, clearing, and paraffin embedding. Five-micrometer sections were obtained from paraffin blocks and mounted on standard slides. Following the process of deparaffinization and rehydration, tissue sections were stained with hematoxylin and eosin (H&E). Histopathological evaluations included Clark level and Breslow thickness measurements. The Clark classification categorizes tumor invasion into five levels based on the invasion depth into the layers of the skin: Clark I to Clark V. Breslow thickness was measured perpendicular to the epidermis, from the surface of the granular layer to the deepest point of tumor invasion (6).

Histological Classification

The histological classification of BCC includes nodular and superficial subtypes, categorized as non-aggressive groups (3). Nodular BCCs are characterized by solid tumor nodules that may extend into subcutaneous tissues, while superficial BCCs exhibit multifocal nests of atypical basaloid epithelium originating as buds from the basal layer of the epidermis (7). Additionally, nodulocystic and adenoid variants were included in the non-aggressive category. In contrast, the aggressive group comprises micronodular, morpheaform, and infiltrative subtypes (3). Micronodular BCCs are characterized by multiple small tumor nests, and morpheaform BCCs exhibit strands of atypical basaloid epithelium within a densely fibrotic stroma. Infiltrating BCC consists of tumor nests containing more than 5-8 cells, displaying an irregular, infiltrative pattern (7).

Statistical Analysis

The normal distribution of data was assessed using the Shapiro-Wilk test. Non-parametric data were compared using the Mann-Whitney U test, while parametric data were analyzed using the Student's t-test. Spearman's correlation test was applied to non-parametric data, whereas Pearson's correlation test was used for parametric data. The chi-square test was conducted for the analysis of categorical data. Statistical significance was set at $p < 0.05$.

RESULTS

Demographic Characteristics of Patients and Histopathologic Parameters

Among the cases, 54.5% were female, and 45.5% were male. The mean age of patients with basal cell carcinoma (BCC) was 67.95 ± 8.6 years. The most common localization of BCC cases was in the nasal

region (59.1%), followed by other areas such as the trunk, scalp, and face (40.9%). In terms of histological classification, 81.8% of cases were classified as non-aggressive BCC, while 18.2% were categorized as aggressive BCC (Table 1).

Histopathology

In the low Clark level group, basaloid tumor areas exhibit hyperchromatic nuclei with narrow cytoplasm, peripheral nuclear palisading, and cleft formation extending from the epidermis into the papillary dermis (Figure 1A). Conversely, in the high Clark level group, tumoral nodules originate from the epidermis and extend into the reticular dermis. These nodules were composed of hyperchromatic nuclei with narrow cytoplasm, displaying nuclear palisading (Figure 1B).

Comparison of Clark Level, Localization, Histological Type, and Gender Groups

Clark levels were categorized into two groups: Clark I-II-III and Clark IV-V. The difference in mean Breslow thickness between the low ($2120.13 \pm 832.19 \mu\text{m}$)

and high Clark level ($2409.2 \pm 253.05 \mu\text{m}$) groups was statistically significant ($p < 0.05$), indicating a correlation between Clark level and tumor thickness. No significant difference was observed in the mean age (70 ± 6.1 vs. 66.54 ± 9.97 years) or vitamin D levels (16.98 ± 6.16 vs. 13.83 ± 7.04 ng/mL) between the high and low Clark level groups ($p > 0.05$).

Additionally, while no significant differences were observed in age, vitamin D levels, or Breslow thickness among different localization, histological type, and gender groups ($p > 0.05$). However, it's noteworthy that non-aggressive BCCs (16.91 ± 6.59 ng/mL) exhibited relatively higher vitamin D levels compared to the aggressive subtype (10.2 ± 2.52 ng/mL) (Table 1).

No significant relationship was observed between Clark level and gender or localization ($p > 0.05$). However, a notable association was detected between non-aggressive BCCs and low Clark levels ($p < 0.05$) (Table 2). This finding underscores the importance of Clark level as a potential indicator for tumor behavior in basal cell carcinoma cases.

Table 1. Demographic, histopathologic and clinic parameters of BCC patients. SD: Standard deviation. Student t-test, Mann-Whitney U test * $p < 0.05$

Gender	Male	Female	Total	P
Total (%)	45.5%	54.5%	100.0 %	
Age (Mean $\pm$ SD)	68.4 $\pm$ 8.3	67.58 $\pm$ 9.2	67.95 $\pm$ 8.6	0.831
Vitamin D, ng/mL, (Mean $\pm$ SD)	18.17 $\pm$ 6.18	13.65 $\pm$ 6.4	15.69 $\pm$ 6.56	0.112
Breslow thickness, μm , (Mean $\pm$ SEM)	2896.44 $\pm$ 1026.82	1690 $\pm$ 278.78	2238.38 $\pm$ 494.76	0.497
Clark level	Clark I-II-III	Clark IV-V		P
Total (%)	59.1%	40.9%		
Age, (Mean $\pm$ SD)	66.54 $\pm$ 9.97	70 $\pm$ 6,1		0.366
Vitamin D, ng/mL, (Mean $\pm$ SD)	16.98 $\pm$ 6.16	13.83 $\pm$ 7.04		0.280
Breslow thickness, μm , (Mean $\pm$ SEM)	2120.13 $\pm$ 832.19	2409.2 $\pm$ 253.05		0.03*
Localization	Nose	Others		P
Total (%)	59.1%	40.9%		
Age, (Mean $\pm$ SD)	67.77 $\pm$ 10.2	68.22 $\pm$ 6.18		0.907
Vitamin D, ng/mL, (Mean $\pm$ SD)	15.77 $\pm$ 7.42	15.58 $\pm$ 5.51		0.948
Breslow thickness, μm , (Mean $\pm$ SEM)	2714.36 $\pm$ 805,93	1550.86 $\pm$ 251.86		0.587
Histologic type	Non-aggressive	Aggressive		P
Total (%)	81.8%	18.2%		
Age, (Mean $\pm$ SD)	67 $\pm$ 9.09	72.25 $\pm$ 4.43		0.280
Vitamin D, ng/mL, (Mean $\pm$ SD)	16.91 $\pm$ 6.59	10.2 $\pm$ 2.52		0.062
Breslow thickness, μm , (Mean $\pm$ SEM)	2208.61 $\pm$ 604	2372.37 $\pm$ 337.84		0.902

Table 2. Association of Clark levels with gender, histologic type and localization. Chi-square test, * $p < 0.05$

		Clark I-II-III	Clark IV-V	P
Total (%)		45.5%	54.5%	
Gender	Male	70%	30%	0.415
	Female	50%	50%	
Histologic type	Non- aggressive	72.2%	27.8%	0.017*
	Aggressive	0%	100%	
Localization	Nose	53.8%	46.2%	0.674
	Others	66.7%	33.3%	

Table 3. Correlation analysis of age, vitamin D levels, Breslow thickness and Clark levels in BCC patients. r: Correlation coefficient, Spearman test, Pearson test, * $p < 0.05$

		Age	Vitamin D	Breslow thickness	Clark level
Age	r		-0.372	0.027	0.251
	P		0.088	0.904	0.259
Vitamin D	r	-0.372		-0.188	-0.220
	P	0.088		0.403	0.326
Breslow thickness	r	0.027	-0.188		0.634
	P	0.904	0.403		0.002*
Clark level	r	0.251	-0.220	0.634	
	P	0.259	0.326	0.002*	

Correlation Between Vitamin D Levels, Age, and Invasion Parameters

A correlation coefficient of 0.634 was found between Clark level and Breslow thickness as indicators of invasion ($p < 0.05$). No significant relationship was found between vitamin D levels and age, Clark level, or Breslow thickness ($p > 0.05$) (Table 3).

DISCUSSION

The relationship between vitamin D and non-melanoma skin cancers remains a contentious topic in the literature (1, 4). Eide et al. found no significant association between vitamin D levels ≥ 15 ng/mL and BCC (8). Liang et al. reported a twofold increase in BCC risk at vitamin D levels of approximately > 30 ng/mL compared to levels < 20 ng/mL (9).

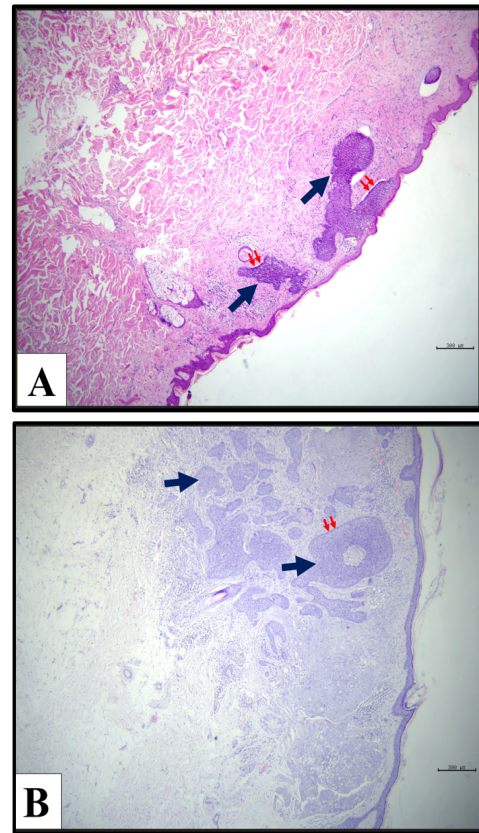


Figure 1. Hematoxylin and eosin staining of low and high Clark level groups. A) Low Clark level group; showing nuclear palisading (red arrow), cleft formation extending into the papillary dermis (black arrow). B) High Clark level group; displaying nuclear palisading (red arrow), tumoral nodules extending into the reticular dermis (black arrow). Scale bars: 300 μ m

A meta-analysis in 2020 evaluated data from three studies and found a significant increase in BCC risk with serum vitamin D levels rising above approximately 60 nmol/L (24 ng/mL). A nonlinear graph was also demonstrated between 30 nmol/L and 60 nmol/L (1). In contrast to these studies, Tang et al. reported that vitamin D levels > 32 ng/mL reduced the risk of non-melanoma skin cancer by 40% (10). Studies on BCC regarding vitamin D deficiency have also reported that supplementation reduces recurrence (11). In our study, we investigated the relationship between serum vitamin D levels and tumor invasion in BCC cases with levels below 25 ng/mL during the prediagnostic period. Unlike other studies, we examined the average serum vitamin D levels during a one-year prediagnostic period. The results of the research showed no difference in vitamin D levels between high and low Clark level groups. Additionally, vitamin D levels did not correlate

with Clark level or Breslow thickness ($p>0.05$). Therefore, it was concluded that serum vitamin D levels in BCC cases with vitamin D insufficiency were not significantly associated with tumor invasion.

Hakverdi et al. (2011) reported a higher incidence of BCC in males (55.8%) and predominantly nodular BCCs, with a preference for of nasal localization (12). Similarly, Scrivener et al. (2002) found a gender ratio (M/F) of 0.92 and a mean age of 65, with predominantly head-localized nodular BCCs (13). More recent data from 2018 indicated a female ratio of 57%, with an average age of 66.15 and increasing frequency of nodular BCCs with age, predominantly localized on the head (14). A study conducted in 2022 also found a higher incidence of BCC in older female patients (15). In our study, among BCC cases with vitamin D insufficiency, the female proportion was observed to be 54.5%. The findings of our study, with an average age of 68.37 ± 9 , 81.8% non-aggressive types, and 59.1% nasal localization, are consistent with previous studies.

Ince et al. (2019) demonstrated an increased recurrence of BCC with vitamin D levels below 25 ng/mL (16). In our study, prediagnostic vitamin D levels did not show a significant difference between localization and histological type groups ($p>0.05$). However, vitamin D levels were relatively higher in low-risk non-aggressive BCC cases with lower Clark levels (16.91 ± 6.59 ng/mL) compared to aggressive cases (10.2 ± 2.52 ng/mL) ($p=0.062$). This difference was attributed to the relationship between vitamin D levels and recurrence.

CONCLUSION

Previous studies have primarily focused on preoperative samples, often overlooking the potential influence of vitamin D supplementation during the prediagnostic period. Given the controversial relationship between serum vitamin D levels and non-melanoma skin cancer, future research should carefully consider these aspects. Our study, limited by its single-center retrospective design, did not reveal a significant difference in average vitamin D levels below 25 ng/mL during a one-year prediagnostic period among Clark level and localization groups. However, relatively lower vitamin D levels were observed in aggressive subtypes thought to be associated with recurrence. In conclusion, our findings indicate that vitamin D insufficiency below 25 ng/mL is not directly

associated with invasion parameters in BCC, but its role in recurrence warrants further investigation.

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