

Vitamin D levels in patients with periodontal disease and dark skin

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Abstract. Periodontal diseases are prevalent chronic inflammatory conditions. Vitamin D deficiency may influence immune and bone pathways relevant to periodontal pathology, and cutaneous synthesis can be reduced in individuals with darker skin phototypes. The present study investigated serum vitamin D levels in patients with the dark skin phototype diagnosed with periodontitis. The present cross-sectional study included 79 adults. A survey was applied regarding sources of vitamin D intake and skin phototype according to the Fitzpatrick scale. Periodontal indicators were clinically assessed, and serum vitamin D levels were measured using ELISA. Statistical analyses included two-sided Fisher's exact tests for categorical associations and one-way ANOVA and Kruskal-Wallis tests for vitamin D comparisons, with Tukey's HSD and Dunn-Bonferroni post hoc tests, respectively ($\alpha=0.05$). The results revealed that 89.9% of the patients did not report risk habits, such as smoking. As regards the oral diagnosis, 10.1% of the patients were healthy, 41.8% had gingivitis, 44.3% had stage I-II periodontitis 3.8% had stage III-IV periodontitis. The mean vitamin D level was 22.6 ± 7.1 ng/ml; 83.5% of patients had insufficient vitamin D levels. The vitamin D levels differed across the diagnostic groups ($P=0.005$ and $P=0.00051$), with lower levels observed in patients with periodontitis and gingivitis. No significant associations were found between vitamin D sources and periodontal diagnosis (dairy borderline, $P=0.054$). Phototype distributions differed across the diagnostic categories; however, phototype was not associated with vitamin D categories. On the whole, insufficient vitamin D levels were common, and levels were lower in those with advanced-stage periodontitis. These findings support considering the vitamin D status in periodontal care for patients with darker skin phototypes and motivate longitudinal, multivariable studies with

validated dietary assessment to clarify causal pathways and test targeted interventions.

Introduction

Periodontal diseases are among the most prevalent oral conditions worldwide (1). In Colombia, the Fourth National Oral Health Survey (ENSAB IV) revealed that 61.8% of the population presented with periodontitis of varying severity, although with a higher frequency of moderate periodontitis (43.46%), followed by advanced periodontitis (10.62%). Only 38.2% of the population was found to be free of periodontitis, demonstrating that it is a highly prevalent oral pathology (2).

Periodontitis is a chronic infectious and inflammatory disease that is multifactorial and complex, affecting the supporting and protective tissues of the teeth. In general terms, it results from an imbalance in the immunological interaction between the host and the subgingival biofilm (3,4). The disruption in the uptake of vitamin D sources, due to various endogenous and/or exogenous factors, diminishes its levels, which may represent an indicator at the time of the diagnosis of these types of periodontitis (5), particularly considering that the critical physiological action of this vitamin is to increase intestinal calcium reabsorption, facilitate its renal absorption and promote bone mineralization. Therefore, vitamin D deficiency may be a risk factor for the development of gingivitis and periodontitis, highlighting the importance of this vitamin for bone and periodontal health (6).

The main clinical interest in vitamin D lies in the discovery of its receptors and the expression of the enzyme 1α -hydroxylase in various tissues. This has led to the identification of a wide range of effects of vitamin D in different tissues and physiological processes, such as antitumor activity, DNA repair, apoptosis control, oxidative stress, immunomodulation, cell adhesion, metabolism and another functions. Although research to date remains inconclusive, an association between low levels of vitamin D and various infectious, autoimmune, chronic and oral diseases has been suggested, linking it to periodontal health, given that insufficient vitamin D levels have been identified in patients with clinical attachment loss in the periodontium (6).

Vitamin D is acquired through dietary intake and cutaneous synthesis triggered by exposure to sunlight. Specifically, solar radiation induces the photolytic conversion of 7-dehydrocholesterol into previtamin D3 (precholecalciferol), which is

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Table I. Factors related to a systemic condition.

Condition	Yes		No		Total
	n	(%)	n	(%)	n (%)
Smoker	8	(10.1)	71	(89.9)	79 (100)
Presence of stress	21	(26.6)	58	(73.4)	79 (100)
Presence of allergies	11	(13.9)	68	(86.1)	79 (100)

then rapidly transformed into vitamin D3 (cholecalciferol). Notably, excessive sun exposure does not result in vitamin D toxicity (7,8).

Furthermore, the amount of melanin present in the skin affects the amount of vitamin D that can be produced. Individuals with greater amounts of melanin in the skin (those with darker skin types) have greater protection against the sun; however, they require a greater amount of sun exposure to synthesize vitamin D, rendering them more likely to experience vitamin D deficiency. Therefore, it is necessary to conduct examinations to assess their vitamin D levels and, if required, initiate treatment to improve vitamin D acquisition (9,10).

Several clinical trials have investigated the association between periodontal disease and vitamin D levels. These studies have included diverse patient populations receiving vitamin D supplementation, either alone or in combination with calcium or other medications. The findings suggest that vitamin D supplementation may reduce the risk of tooth loss (11), exert a dose-dependent anti-inflammatory effect on gingivitis (12) and inhibit pro-inflammatory cytokines, such as IL-1, IL-6, TNF- α and IL-10 in individuals with periodontitis (13).

Likewise, cross-sectional studies have demonstrated an association between low concentrations of vitamin D and periodontal disease (14,15); however, no significant association has been found between vitamin D deficiency and age (16). The association between vitamin D and skin phototype has also been evaluated, with findings indicating that in the majority of subjects with dark to very dark skin phototypes, there is a high prevalence of vitamin D deficiency, despite high sun exposure during the summer months (17). This suggests that the dark skin phototype is an endogenous factor that, even in a state of general health, may produce levels below the normal range of vitamin D and a possible tendency for the development of periodontitis.

Therefore, it is crucial to elucidate the association between periodontal disease and the consequences of vitamin D deficiency, particularly in patients with dark skin phototypes, due to their potential susceptibility to developing periodontal disease. Currently, to the best of our knowledge, there are no studies available on the association between vitamin D levels and periodontitis in patients with dark skin phototypes. Thus, the aim of the present study was to evaluate vitamin D levels in patients with dark skin phototypes who have been diagnosed with periodontitis.

Subjects and methods

Study population. A descriptive cross-sectional study was conducted. The study population consisted of a sample of

79 patients who attended dental evaluations at a university clinic and at the Oral Health Center-Naval Hospital of Cartagena (Cartagena, Colombia) between September and December, 2023. Participants were included upon signing the written informed consent agreeing to the use of their samples in this scientific investigation and meeting the following inclusion criteria: Individuals diagnosed with periodontitis or gingivitis according to the 2018 periodontal diagnosis classification (18), with a dark skin phototype in accordance with the Fitzpatrick scale (types III, IV and V) (19) and of legal age.

The exclusion criteria were the following: Pregnant or lactating women, individuals taking medications that could affect bone and mineral metabolism; those taking multivitamins or dietary supplements containing vitamin D; subjects with systemic diseases or diagnosed syndromes, specifically malabsorption syndrome, or patients with chronic diarrhea. Participants were recruited over a 4-month period.

Assessment of vitamin D sources and skin phototype. To evaluate vitamin D sources and skin phototypes, a structured questionnaire was administered. This tool gathered information on sun exposure and the consumption of various food groups, including dairy products, cereals, natural juices and animal-derived protein. Additionally, the questionnaire incorporated parameters from the Fitzpatrick scale to determine the skin phototype of each participant. The Fitzpatrick scale is a dermatological classification system that categorizes skin according to color and response to ultraviolet radiation, into six phototypes, as follows: Type I, pale white skin (extremely sensitive skin, always burns, never tans); type II, white skin (very sensitive skin, burns easily, tans minimally); type III, light brown skin (sensitive skin, sometimes burns, slowly tans to light brown); type IV, moderate brown skin (mildly sensitive, burns minimally, always tans to moderate brown); type V, dark brown skin (resistant skin, rarely burns, tans well); type VI, deeply pigmented dark brown to black skin (very resistant skin, never burns, deeply pigmented) (19). Initial classification was based on self-assessment and subsequently confirmed by a dermatologist at the Naval Hospital of Cartagena.

Measurement of periodontal indicators. Calibrated dentists evaluated the probing depth (PD), clinical attachment level (CAL), bleeding on probing (BoP), bone level and tooth loss due to periodontitis in the patients. Following this examination, the periodontal status of the included subjects was confirmed based on the following criteria: No periodontitis: No clinical attachment loss (CAL) and no radiographic bone loss (RBL). PD \leq 3 mm, assuming there were no pseudopockets. Subjects

Table II. Sources of vitamin D uptake in the study participants.

Sources of vitamin D uptake	Periodontitis						P-value ^a			
	Healthy		Gingivitis		Stage I-II			Stage III-IV		
	Yes	No	Yes	No	Yes	No		Yes	No	
	n (%)		n (%)		n (%)		n (%)			
Sun exposure	5 (62.5)	3 (37.5)	22 (66.7)	11 (33.3)	21 (60)	14 (40)	1 (33.3)	2 (66.7)	79 (100)	0.768
Dairy consumption	5 (62.5)	3 (37.5)	32 (97)	1 (3)	30 (85.7)	5 (14.3)	3 (100)	0 (0)	79 (100)	0.054
Cereal consumption	1 (12.5)	7 (87.5)	19 (57.6)	14 (42.4)	16 (45.7)	19 (54.3)	1 (33.3)	2 (66.7)	79 (100)	0.126
Natural juice consumption	7 (87.5)	1 (12.5)	27 (81.8)	6 (18.2)	30 (85.7)	5 (14.3)	3 (100)	0 (0)	79 (100)	0.940
Animal protein consumption	7 (87.5)	1 (12.5)	29 (87.9)	4 (12.1)	32 (91.4)	3 (8.6)	2 (66.7)	1 (33.3)	79 (100)	0.690

^aData were analyzed using Fisher's exact test (Freeman-Halton extension for RxC tables).

were further categorized according to BoP into: Healthy periodontium (<10% BoP) and gingivitis (≥10% BoP). Periodontitis was categorized as: Periodontitis stages I and II, PD of 3-5 mm, mostly horizontal RBL, and no tooth loss associated with a periodontal cause. CAL of 1-2 mm (stage I) or 3-4 mm (stage II), with bone loss limited to the coronal third (<15% for stage I and 15-33% for stage II). Periodontitis stages III and IV: At least two non-adjacent sites with CAL ≥5 mm, RBL extending to the mid-third of the root, and PD ≥6 mm. Evidence of tooth loss due periodontal disease (4).

Measurement of vitamin D levels. Vitamin D levels in serum were measured in duplicate using the 25OH Vitamin D Total ELISA kit (Cat. no. KAP1971, DiaSource®; DIAsource ImmunoAssays SA). The reaction readings on the microplates were taken at 450 nm using the Multiskan™ GO reader (Thermo Fisher Scientific, Inc.). The suggested values on the kit were considered for the interpretation of the vitamin D levels of each patient, as follows: <10 ng/ml (deficient), 10-30 ng/ml (insufficient), 30-100 ng/ml (sufficient) and >100 ng/ml (intoxication).

Statistical analysis. A descriptive analysis was carried out, applying measures of central tendency and dispersion (frequency, mean ± standard deviation). Categorical associations (given small expected cell counts) were assessed using two-sided Fisher's exact tests; for RxC tables the Freeman-Halton extension was used (conditioning on row/column margins). For the continuous outcome [serum 25(OH)D], normality within each diagnosis group was evaluated with the Shapiro-Wilk test and homogeneity of variances with Levene's test. As the sample sizes were unequal and one group was very small (Stage III-IV, n=3), distributional assumptions could be fragile; therefore, a dual approach was prespecified: One-way ANOVA when assumptions were approximately met, complemented by the Kruskal-Wallis test as a distribution-free sensitivity analysis. Post-hoc multiple comparisons were applied only for 25(OH)D: Tukey's HSD following ANOVA and Dunn's test with Bonferroni correction following Kruskal-Wallis. A two-sided α=0.05 was adopted. A value of P<0.05 was considered to indicate a statistically significant difference. All analyses were conducted using IBM SPSS Statistics, v21 (IBM Corp.).

Results

A total of 79 patients who met all the inclusion criteria were enrolled. Of these, 72 (91.1%) were male and 7 (8.9%) were female. The average age of the patients was 33.4 years (SD ± 14.6), ranging from 18 to 75 years. As regards oral health, 8 patients (10.1%) had a healthy periodontium, 33 (41.8%) were diagnosed with gingivitis, 35 (44.3%) with stage I-II periodontitis and 3 (3.8%) with stage III-IV periodontitis. Some participants reported having risk factors related to their systemic condition, such as smoking, stress or allergies (Table I).

When assessing various sources of vitamin D uptake, such as sun exposure and the consumption of dairy products, cereals, natural juices and animal-derived protein, no statistically significant associations with periodontal diagnosis were observed; the association for dairy consumption exhibited a borderline trend (P=0.054; Table II).

Table III. Serum vitamin D levels in the study participants.

A, Serum 25(OH)D descriptive statistics and vitamin D categories by periodontal diagnosis				
Parameter	Healthy	Gingivitis	Periodontitis stage I-II	Periodontitis stage III-IV
No. of participants	8	33	35	3
Min (ng/ml)	18.8	4.5	2.0	13.6
Max (ng/ml)	33.8	43.3	42.0	14.8
Mean (\pm SD)	26.7 \pm 4.9	20.3 \pm 6.8	24.4 \pm 7.1	14.4 \pm 0.7
Deficient, n (%)	0 (0)	1 (3)	1 (2.9)	0 (0)
Insufficient, n (%)	5 (62.5)	30 (90.9)	28 (80)	3 (100)
Sufficient, n (%)	3 (37.5)	2 (6.1)	6 (17.1)	0 (0)

B, Global tests for serum vitamin D across the periodontal diagnostic groups		
Test	Statistic (df)	P-value
ANOVA (one-way)	F (3.75)=4.636	0.0050
Kruskal-Wallis	H (3)=17.684	0.00051

C, Pairwise comparisons of serum vitamin D across the periodontal diagnostic groups.		
Comparison	Tukey's HSD	Dunn (Bonferroni correction)
Healthy vs. gingivitis	0.086	0.034
Healthy vs. stage I-II periodontitis	0.834	0.999
Healthy vs. stage III-IV periodontitis	0.041	0.014
Gingivitis vs. stage I-II periodontitis	0.062	0.026
Gingivitis vs. stage III-IV periodontitis	0.462	0.645
Stage I-II vs. stage III-IV periodontitis	0.069	0.034

Data are presented as the minimum, maximum, and mean \pm standard deviation (ng/ml). Vitamin D status categories: Deficient (<20 ng/ml), insufficient (20-29 ng/ml) and sufficient (\geq 30 ng/ml). Two-sided tests were performed ($\alpha=0.05$). ANOVA reports F (3.75); Kruskal-Wallis reports H (3). Group sizes: Healthy (n=8), gingivitis (n=33), stage I-II periodontitis (n=35), stage III-IV periodontitis (n=3). Post-hoc tests were applied only to the continuous outcome [serum 25(OH)D]: Tukey's HSD following one-way ANOVA and Dunn's test with Bonferroni correction following the Kruskal-Wallis test (two-sided $\alpha=0.05$).

The serum levels of vitamin D ranged between 2.0 and 43.3 ng/ml, with a mean of 22.6 \pm 7.1 ng/ml. Furthermore, all participants were categorized based on their serum vitamin D levels into sufficient, insufficient, or deficient levels. The majority of the patients were classified as having insufficient levels of vitamin D (Table IIIA). Across the periodontal diagnostic categories, serum 25(OH)D levels differed significantly overall (ANOVA $P=0.005$; Kruskal-Wallis test, $P=0.00051$) (Table IIIB). Post-hoc tests [applied only to 25(OH)D] indicated lower levels in those with stage III-IV periodontitis vs. healthy subjects (Tukey's test, $P=0.041$; Dunn-Bonferroni test, $P=0.014$), vs. those with stage I-II periodontitis (Dunn-Bonferroni test, $P=0.034$); a difference was also observed between those with gingivitis and stage I-II periodontitis (Dunn-Bonferroni test, $P=0.026$) (Table IIIC).

Additionally, when applying the Fitzpatrick criteria to determine skin phototype, 8 participants (10.1%) were classified as phototype III, 54 (68.4%) as phototype IV and 17 (21.5%) as phototype V. When these were associated with the patient

groups, phototype IV was found to be the most prevalent among those with gingivitis and stage I-II periodontitis. Pairwise exact comparisons of phototype distributions across the diagnostic categories revealed differences for stage III vs. IV periodontitis ($P=0.017$), stage III vs. V periodontitis ($P=0.018$), and stage IV vs. V periodontitis ($P<0.0001$) (Table IVA).

Similarly, phototypes III, IV and V were compared against the vitamin D categories, no significant pairwise differences were detected (stage III vs. IV periodontitis, $P=0.999$; stage III vs. V periodontitis, $P=0.999$; stage IV vs. V periodontitis, $P=0.673$). Notably, both individuals with deficient vitamin D were phototype IV, and the majority of participants with phototypes IV and V were classified as insufficient (Table IVB).

Discussion

The present study involved 79 patients, with a notable predominance of male participants (91.1%), which may reflect a gender disparity in the pursuit of dental care or a higher prevalence of

Table IV. Comparisons of phototypes vs. diagnoses and vitamin D status.

A, Comparison of phototypes vs. diagnoses

Phototype	Healthy	Gingivitis	Periodontitis I and II	Periodontitis III and IV	Total	P-value
Phototype III, n (%)	0 (0)	1 (3)	6 (17.1)	1 (33.3)	8 (10.1)	0.017 ^a
Phototype IV, n (%)	4 (50)	25 (75.8)	24 (68.6)	1 (33.3)	54 (68.4)	0.018 ^b
Phototype V, n (%)	4 (50)	7 (21.2)	5 (14.3)	1 (33.3)	17 (21.5)	<0.001 ^c
Total	8 (100)	33 (100)	35 (100)	3 (100)	79 (100)	

B, Comparison of phototypes vs. categories of vitamin D

Phototype	Deficient	Insufficient	Sufficient	Total	P-value
Phototype III, n (%)	0 (0.0)	7 (87.5)	1 (12.5)	8 (100.0)	0.999 ^a
Phototype IV, n (%)	2 (3.7)	45 (83.3)	7 (13.0)	54 (100.0)	0.999 ^b
Phototype V, n (%)	0 (0.0)	14 (82.4)	3 (17.6)	17 (100.0)	0.673 ^c
Total	2 (2.5)	66 (83.5)	11 (13.9)	79 (100.0)	

The data in part A were analyzed using pairwise Fisher's test (2x4): ^aPhototype III vs. IV, ^bphototype III vs. V, ^cphototype IV vs. V. The data in part B were analyzed using Fisher's exact test (2x3): ^aPhototype III vs. IV, ^bphototype III vs. V, ^cphototype IV vs. V.

periodontal conditions among males. This observation aligns with existing evidence identifying male sex as a non-primary risk factor for the development of chronic periodontitis (10). Moreover, the sex distribution within the Colombian armed forces, where males outnumber females at a ratio of ~60:1 may also explain this imbalance (20).

The oral health status varied considerably within the study sample, with a marked number of participants diagnosed with gingivitis or periodontitis at various stages, indicating a high burden of periodontal disease in this clinical setting. This pattern is consistent with reports of a higher periodontal disease burden among individuals with darker skin phototypes and in populations of African origin (21), although causal inferences cannot be drawn from our cross-sectional design. Consistent with the pairwise analyses, phototype distributions differed across periodontal diagnosis categories (Table IIIB). These observations underscore the importance of tailored periodontal care and preventive strategies.

The presence of systemic risk factors, such as smoking, stress and allergies further illustrates the multifactorial nature of periodontal disease and highlights the importance of a holistic, patient-centered approach to oral health and management.

As regards sources of vitamin D uptake, no statistically significant associations with periodontal diagnosis were observed; dairy intake exhibited only a borderline trend (P=0.054; Table II). Diet remains a plausible contributor to the vitamin D status and promoting vitamin D-rich or fortified foods may be considered (22), particularly given the predominance of insufficiency in our sample. Previous research has reported inverse associations between vitamin D and periodontal disease (23); however, the cross-sectional design of the present study and the absence of a direct analysis of dietary intake vs. vitamin D preclude causal inferences. The widespread insufficiency also raises concerns about bone

integrity and immune function, both relevant to periodontal health, and supports the need for longitudinal and interventional studies to clarify the impact of improving vitamin D status on periodontal outcomes (21).

Concerning skin phototype and vitamin D categories, no statistically significant pairwise differences were detected (Fisher's test 2x3: stage III vs. IV periodontitis, P=0.999; stage III vs. V periodontitis, P=0.999; stage IV vs. V periodontitis, P=0.673; Table IVB). While both participants with deficient vitamin D levels were phototype IV, this subgroup was very small (n=2), and the pattern should be interpreted with caution. Given the influence of skin pigmentation on cutaneous vitamin D synthesis, phototype remains a relevant clinical consideration when evaluating vitamin D status and its potential implications for oral health (22).

However, certain limitations of the present study should be acknowledged. The relatively small sample size and the potential for selection bias may restrict the generalizability of the findings. Moreover, the lack of a large control group limits the ability to draw definitive conclusions regarding the association between vitamin D levels and periodontal disease within this population. Future research is thus required to prioritize longitudinal analyses for a more accurate investigation of the association between vitamin D status, oral health outcomes and systemic risk factors over time. Additionally, implementing targeted interventions to improve vitamin D levels and evaluating their effects on periodontal health could provide critical evidence to guide clinical practice and public health strategies.

In conclusion, the majority of the participants in the present study exhibited insufficient vitamin D levels, and lower levels were observed in those with advanced periodontitis. While phototype distributions varied across periodontal diagnosis categories, the phototype was not associated with vitamin D categories. No statistically significant associations

were identified between sources of vitamin D intake and periodontal diagnosis (dairy intake exhibited only a borderline trend). These findings support considering vitamin D status within periodontal care for patients with darker phototypes and motivate longitudinal studies, ideally with validated dietary assessment and multivariable adjustment, to delineate causal pathways and evaluate targeted interventions.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

DOC performed the vitamin D quantification and data analysis, and wrote the manuscript. JOP participated in data analysis and in the reviewing of the manuscript. WGVS managed the patients and performed the periodontal analysis. RP managed the patients, performed the periodontal analysis and data management, and was involved in the reviewing of the manuscript. RP and WGVS confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Rafael Nuñez University Corporation, Cartagena, Colombia (code CURN0605-CE052020). Participants were included upon signing the written informed consent agreeing to the use of their samples in this scientific investigation.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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