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Original Article

Correlation of Vitamin D3 Level and Periodontal Health

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ABSTRACT

Objective: To determine whether periodontitis severity, complexity, and progression risk are associated with variations in vitamin D3 levels, providing insights into potential links between this essential nutrient and periodontal health.
Study Design: Comparative cross sectional study
Place and Duration of Study: Armed forces Institute of Dentistry (AFID), Rawalpindi Pakistan, from Mar to Oct 2024.
Methodology: Two hundred and eighty individuals were included. Participants were categorized into two groups (Group-A included 140 individuals with a healthy periodontium and Group-B had 140 diagnosed with periodontitis). Clinical attachment loss (CAL), probing pocket depth (PPD), and bleeding on probing (BOP) were taken as a measure of periodontal clinical parameters. Vitamin D3 concentration in serum was tested by 25-hydroxy vitamin D (25(OH)D) test after attained radiographs.
Results: Between healthy and patients with periodontitis, the differences were found to be significant for PPD, CAL, BOP, and serum vitamin D_3 levels. Median vitamin D_3 levels progressively reduced as the stage and grade of periodontitis increased (41.5 ng/mL in Stage 1 to 20.5 ng/mL in Stage 4) and (41.6 ng/mL in Grade A to 24.1 ng/mL in Grade C). Vitamin D_3 was inversely related to CAL (r = –0.658), PPD (r = –0.487), BOP (r = –0.658), and BMI (r = –0.140). Regression indicated that CAL was the most predictive of vitamin D deficiency.
Conclusion: Serum D3 levels of vitamin have a significant association with periodontitis disease.

Keywords: Bleeding, Clinical Attachment Loss, Periodontitis, Probing Pocket Depth, Vitamin D3 Deficiency.

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INTRODUCTION

Vitamin D3 is a vitamin with hydrophobic structure, contributing to the absorption of calcium ions, bone metabolism, and overall health of the musculoskeletal system.[1] One of the biggest public health problems, vitamin D deficiency, develops due to limited sunlight exposure, poor dietary intake and certain systemic diseases.[2,3] In addition to its importance in maintaining skeletal health, vitamin D3 has immunomodulatory and antimicrobial effects, suggesting its broader relevance for systemic and oral health.[4]

Multiple studies have examined the role of vitamin D3 in relation with periodontal health.[5,6] Machado *et al.*, reported a significant independent association between a low vitamin D3 levels in the serum and an increased periodontitis prevalence, with patients of periodontitis showing 22% lower mean values of vitamin D in comparison with individuals without periodontitis.[7] Similarly, El Jilani *et al.*, demonstrated that mean concentration of vitamin D3 was significantly lower (35.4±7.6 nmol/L) among patients having chronic periodontitis as opposed to healthy subjects (49.2±9.1 nmol/L), which collectively revealed the biological association between vitamin D3 and periodontal inflammation.[8] Adding to this, a large Brazilian population study conducted by Nascimento *et al.*, indicated that low vitamin D dietary intake raised the chances of periodontitis by 27% and supported the nutritional role in periodontal health.[9] Lindholm *et al.*, identified that adults who were in adequate vitamin D3 status had remarkable lower prevalence of periodontitis (15.7%) compared to individuals with lower levels (28.4%).[10]

In spite of this increasing body of evidence, there are inconsistencies in the existence and extent of the association between the severity of periodontitis and vitamin D3 levels. The aim of this study was to provide a comparative analysis of the serum concentrations of serum vitamin D3 between both healthy subjects and patients having periodontitis, and correlate these levels with periodontitis severity, extent, and risk of progression.

METHODOLOGY

This comparative cross-sectional study was conducted at the Armed Forces Institute of Dentistry Rawalpindi, Pakistan, from March to October 2024,

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after approval from the Institutional Review Board (vide letter no. 918/Trg dated 13 May, 2020).

Inclusion Criteria: Participants of either gender older than 18 years, nonsmokers, not exhibiting signs of caries, inflammatory lesions and oral epithelial dysplasia were included.

Exclusion Criteria: Individuals with antibiotic use within the past six months, or the use of corticosteroids, nonsteroidal anti-inflammatory drugs, or supplementary multi-vitamins within three months, pregnant individuals and those who had undergone periodontal treatment or chemo/radio therapy in the last six months were excluded.

WHO calculator was used to determine sample size, a medium effect size of 0.5 for the difference in serum Vitamin D3 levels between periodontitis and healthy participants was assumed, and the final sample size was calculated to be around 280 participants.[10] We initially assessed 300 individuals. After a drop out of 20 individuals, a total of 280 individuals were further allocated into two groups. These included 140 people with healthy periodontal conditions (Group-A), and 140 participants diagnosed with periodontitis (Group-B). Patient flow can be seen in Figure. Non-probability consecutive sampling was used to collect data. Informed consent was taken from all participants.

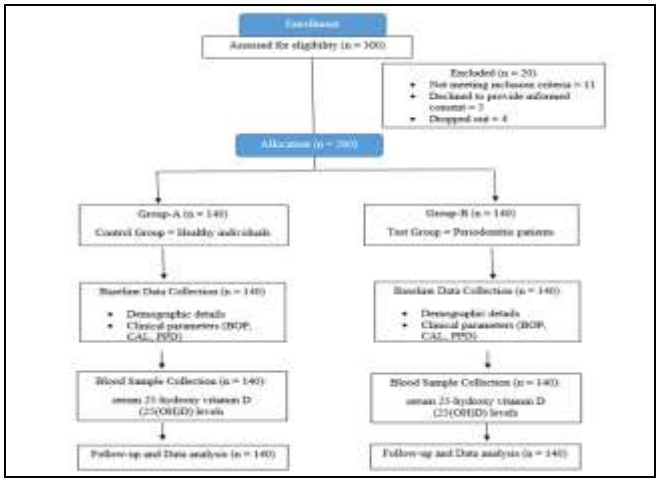

Figure: Patient Flow Diagram (n=280)

Periodontitis diagnosis was dependent upon evaluations, both clinical and radiological, as per the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. The staging system (I–IV) was used to evaluate extent and severity of the disease on the basis of tissue destruction and complexity, while grading assessed the progression rate of periodontitis, guided by clinical attachment loss (CAL) and other relevant factors. Healthy subjects were those who had a clinically healthy periodontium and did not exhibit any signs of loss of attachment, probing depth >3mm, or bleeding on probing. Subjects were recruited through dental check-up visits, routine prophylaxis visits, and accompanying relative who agreed to take part in the study. They were meticulously checked for the absence of periodontal disease in order to have an adequate control group with which to compare periodontitis patients.

Demographic details (age, gender), clinical data including probing pocket depth (PPD), bleeding on probing (BoP), clinical attachment level (CAL)), biochemical data (Serum 25-hydroxy vitamin D (25(OH)D) levels) were obtained of each participant. Baseline data were gathered and compiled during scheduled periodontal appointments, including demographic details of participants, medical and dental history, past administration of medicines or drugs, smoking habits, and oral hygiene practices. A single examiner assessed all periodontal clinical parameters such as the bleeding on probing (BoP), pocket probing depth (PPD), and clinical attachment level (CAL) by using a periodontal probe (PCP-UNC 15, Hu-Friedy, USA). Disease progression grading was carried out using indirect methods, including the bone loss (%) relative to the patient's age and the case phenotype.

Qualified nursing assistants collected venous blood (4mL) from each individual at baseline in pathology laboratory after clinical data collection was completed. The blood samples were then sent for determining the concentration of serum 25-hydroxy vitamin D (25(OH)D) using the 25(OH)D test. Participants received thorough pre- and post-procedure instructions to ensure compliance and safety. Thorough documentation of all clinical parameters was done in standard periodontal charts and further studied based on the established clinical stages and grades of periodontitis.

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 20. Normality of data was assessed using Shapiro-Wilk test. Quantitative variables including age, PPD, CAL, and vitamin D3 levels were presented with median and interquartile range (IQR). Categorical variables including gender, BOP and periodontal status were represented as frequency and percentage. Chi-square test was used to assess differences in categorical variables, such as

gender and BOP distribution, across periodontal status groups. Comparison of age, PPD, CAL and vitamin D3 levels in healthy controls and periodontitis patients was assessed using Mann-Whitney U test. Relationship between serum Vitamin D_3 levels and clinical variables (periodontal stages and grades of periodontitis) was evaluated using the Kruskal–Wallis test, whereas between-disease-localization comparisons were carried out utilizing the Mann–Whitney U test. Correlations between serum Vitamin D_3 and both clinical and demographic variables were evaluated using Spearman's rank correlation for continuous variables and point-biserial correlation for binary variables. Multiple linear regression was subsequently conducted to determine independent predictors of Vitamin D_3, such as age, gender, BMI, PPD, CAL, and BOP. A p-value ≤0.05 was considered statistically significant.

RESULTS

The final analysis included 280 participants, including 140 who were periodontally healthy (Group-A) and 140 who were diagnosed with periodontitis (Group-B). Age and gender distribution across both groups were the same since no significant difference was observed (p=0.387 and p=0.304 respectively). Significant differences in PPD, CAL, BOP and vitamin D levels were observed between the groups (p<0.001) as indicated in Table-I.

Table-I: Demographic and Clinical Characteristics of Study Participants (n=280)

Variables	Group-A (n=140) n(%)	Group-B (n=140) n(%)	*p*-value
Age			
Median (IQR)	36.00(26.00)	42.50(22.75)	0.300
Range	18.00-64.00	18.00-72.00	
Gender			
Male	144.00(51.43%)	136(48.57%)	0.304
Female	68.00(48.57%)	52(37.14%)	
PPD Median (IQR)	2.40(1.50)	5.75(1.60)	<0.001
Range	0.40-4.10	4.10-9.50	
CAL Median (IQR)	0.00(0.00)	3.65(3.95)	<0.001
Range	0.00	2.10-9.60	
BOP n(%)			<0.001
positive	0.00(0.00%)	118.00(84.29%)	
negative	140.00(100.00%)	22.00(15.71%)	
Vitamin D3 Serum Concentration Median (IQR)	40.25(3.50)	30.60(12.00)	<0.001
Range	36.60-46.80	18.70-46.10	

**PPD = probing pocket depth, CAL = clinical attachment loss, BOP: Bleeding on Probing*

Serum vitamin D_3 level decreased substantially with the severity of periodontitis (Table-II). Median (IQR) levels of vitamin D_3 reduced progressively with the stages: Stage 1 = 41.50 (4.90), Stage 2 = 31.20 (2.01), Stage 3 = 25.70 (1.50), and Stage 4 = 20.50 (1.45), p<0.001. Likewise, grading showed the highest levels in Grade A [41.55 (5.05)], followed by Grade B [31.00 (2.80)] and Grade C [24.10 (1.45)] (p<0.001). Patients with localized periodontitis also had significantly higher vitamin D_3 levels [34.85 (10.70)] than those with generalized disease [24.65 (5.13)] (p<0.001).

Table-II: Categorization of Vitamin D3 Levels by Periodontitis Stage, Periodontitis Grade, and Extent of Periodontitis Distribution

Variables	Categories	Vitamin D Serum Concentration Median (IQR)	*p*-value
Stages	1	41.50 (4.90)	<0.001
	2	31.20 (2.01)	
	3	25.70 (1.50)	
	4	20.50 (1.45)	
Grade	A	41.55 (5.05)	<0.001
	B	31.00 (2.80)	
	C	24.10 (1.45)	
Localization	Localized	34.85 (10.70)	<0.001
	Generalized	24.65 (5.13)	

Table-III: Correlation of Clinical and Demographic Variables with Serum Vitamin D Levels

Variable	Correlation with Vitamin D (r / ρ)	*p*-value
PPD	-0.487	<0.001
CAL	-0.658	<0.001
BOP (point-biserial)	-0.658	<0.001
Age	0.008	0.898
BMI	-0.140	0.019
Gender (point-biserial)	-0.010	0.870

**PPD = probing pocket depth, CAL = clinical attachment loss, BoP: Bleeding on Probing*

Vitamin D was highly significantly inversely correlated with probing pocket depth (PPD; r=–0.487, p<0.001), clinical attachment loss (CAL; r=–0.658, p<0.001), bleeding on probing (BOP; r=–0.658, p<0.001), and body mass index (BMI; r=–0.140, p=0.019). No considerable correlation was found for age (r=0.008, p=0.898) or gender (r=–0.010, p=0.870) as shown in Table-III. The regression model picked CAL (β=–0.716, p<0.001), BOP (β=–0.388, p<0.001), PPD (β=0.284, p<0.001), BMI (β=–0.132, p<0.001), and age (β=0.090, p=0.006) as independent predictors of Vitamin D. Gender was not found to have a significant association (β=–0.012, p=0.724). The highest negative predictor was CAL (B=–1.878), whereas PPD was positively correlated (B=0.916) as evident from Table-IV.

Table-IV: Multiple Linear Regression analysis of Predictors of Vitamin D Levels

	Unstandardized Coefficients		Standardized Coefficients	t	*p*–value
	B	Std. Error	Beta		
Constant	44.838	2.044		21.931	<0.001
Age	0.045	0.016	0.090	2.776	0.006
BMI	-0.319	0.079	-0.132	-4.033	<0.001
PPD	0.916	0.171	0.284	5.366	<0.001
CAL	-1.878	0.148	-0.716	-12.670	<0.001
Gender	-0.171	0.483	-0.012	-0.354	0.724
BOP	-5.711	0.790	-0.388	-7.233	<0.001

**PPD = probing pocket depth, CAL = clinical attachment loss, BoP: Bleeding on Probing*

DISCUSSION

This study revealed a progressive reduction of serum vitamin D_3 levels with worsening periodontitis, with median values ranging from 41.50 ng/mL in Stage 1 to 20.50 ng/mL in Stage 4. Highly significant negative correlations were found between Vitamin D_3 and CAL (r = –0.658), BOP (r = –0.658), PPD (r=–0.487), and BMI (r = –0.140). Regression analysis yielded CAL as the strongest of the negative predictors (β = –0.716), seconded by BOP (β = –0.388) and BMI (β = –0.132), and PPD was positively related (β = 0.284). Age also had a weak but significant influence (β = 0.090) with gender being unrelated (β = –0.012).

These results are in accordance with other studies that have examined the correlation of low serum vitamin D3 and periodontal disease, with similar statistical results. Liang *et al.,* indicated that patients having periodontitis had much lower mean levels of serum 25(OH)D with an estimated mean difference of 8.52 nmol/L, which almost replicates the 9.48 nmol/L found in the present study.[11] Alzahrani *et al.,* also documented mean vitamin D3 levels of 32.5±7.4 nmol/L among periodontitis patients and 42.1±5.8 nmol/L in healthy controls (p<0.001), a trend very similar to the observations in the current study.[12] Along with this, Zhou *et al.,* also noted that participants with severe periodontitis exhibited lower serum vitamin D3 concentrations (26.3±5.2 nmol/L) compared with healthy patients, which is comparable to the levels in stage III and stage IV periodontitis groups observed in the present study.[13]

With regard to the severity of the disease, Saleh *et al.,* reported a gradual reduction with advancing stages of periodontitis, recording 39.2±4.3 nmol/L in stage I, 30.8±3.7 nmol/L in stage II, and 22.7±3.2 nmol/L in stage IV, the trend being statistically significant (p<0.001) and replicated closely by the results for stages I to IV, respectively in the current study.[14] Likewise, in the case of periodontitis grading, the current study's results are supported by those of Aydin *et al.,* who observed considerably lower total and bioavailable vitamin D3 levels in stage III patients with periodontitis compared to controls (p<0.001).[15]

Generalized periodontitis in the present research revealed significantly lower serum vitamin D3 concentrations (23.95±3.4 nmol/L) than localized periodontitis (35.86±5.3 nmol/L), as described by Madi *et al.,* who highlighted a more severe deficiency in generalized disease (p<0.001), supporting the association between increased disease severity and systemic vitamin D3 deficiency.[16] Also in agreement with the current study's findings, Wang *et al.,* showed that type 2 diabetes patients with advanced chronic periodontitis exhibited significantly lower vitamin D3 concentrations (21.7±4.8 nmol/L) than those presenting with mild disease (35.6±5.1 nmol/L, p<0.001), confirming the negative correlation between disease severity and vitamin D3 status.[17] Likewise, one study indicated much lower mean vitamin D3 concentrations among patients with periodontitis (29.6±7.2nmol/L) than among healthy subjects (41.3±5.7nmol/L), which is reflective of the statistically significant difference observed in the present study.[18] Ali *et al.,* further established low vitamin D3 concentrations in periodontitis patients (30.2±6.1 nmol/L) versus a healthy group (39.8±4.9 nmol/L), thus reinforcing the outcome of the current study in a similar regional setting.[19]

The inverse strong correlation found between Vitamin D_3 and CAL and BOP is reflected in national findings from NHANES, which found a linear negative relationship between vitamin D_3 and progression of attachment loss (β ≈ –0.0183 per nmol/L).[20] Mechanistically, vitamin D's bone-metabolism and anti-inflammatory functions add biological credence to the reverse association with CAL and BOP: decreased vitamin D inhibits the expression of antimicrobial peptides and regulates host inflammation, which may enhance attachment loss and bleeding on probing observed both in our findings and in interventional studies where vitamin D–sufficient patients achieved superior pocket-depth changes following therapy.[21] The negative correlation between vitamin D and BMI (r = –0.140; regression β = –0.132) found in the current study has also been extensively reported; large cohort studies have demonstrated increasingly lower 25(OH)D across higher BMI categories and impaired response to

supplementation in obesity, which may be explained by volumetric dilution and sequestration of this fat-soluble vitamin in adipose tissue.[22] In consideration of the regression result that CAL was the best negative predictor (β = –0.716) while PPD had a positive adjusted coefficient (β = 0.284) even though it was negatively correlated bivariately, this must be due to the distinct clinical information that CAL (cumulative, irreversible attachment loss) and PPD (which can be conditioned upon inflammation, gingival swelling, and probing force) measure. When both measures are entered into the same multivariable model together, their common variance and opposing pathophysiological connotations can create suppression or co-efficient sign-changes; other researchers have characterized such modeling subtleties and thus urge that the more clinically stable measure (CAL) be used in emphasis when interpreting vitamin D associations.[23] Local literature on assessment of vitamin D3 levels among periodontitis patients also found similar results such as that conducted by Tariq *et al*.[24]

LIMITATION OF STUDY

In addition to these strengths, the current study has a few limitations. As a single-center, cross-sectional study, causality or control of regional or seasonal differences in vitamin D3 cannot be determined. Dietary intake, genetic factors, sun exposure, and socioeconomic status, which are potential confounders, were not controlled for. Vitamin D-binding protein and bioavailable vitamin D3, which can possibly more accurately represent the functional state of this vitamin, were not measured.

CONCLUSION

In conclusion, the serum concentrations of vitamin D3 was found to be lower compared to healthy individuals. Furthermore, the increase in stage and grade of periodontitis was positively correlated with decreasing levels of serum vitamin D3. Future studies should be directed to examine additional factors that could influence this association to provide greater insight into the association between vitamin D3 and periodontal health.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

AA & MSA: Data acquisition, data analysis, critical review, approval of the final version to be published.

AWA & YI: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

NF & SAT: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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