

Association between Serum Vitamin D Status and Gingival Bleeding among School-Aged Children: A Cross-Sectional Study

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Abstract: Background: Vitamin D (VD) has immunomodulatory and anti-inflammatory effects that may influence the health of gingiva. Current pediatric evidence, however, remains limited, particularly in South Asia where deficiency is common.

Objective: To examine the association between serum vitamin D status and gingival inflammation, measured by bleeding on probing percentage (BOP%), among school-aged children in Lahore, Pakistan.

Materials and Methods: This comparative cross-sectional study was conducted from 1st Feb 2023 to April 30th 2024, after approval from the ERB of the University of Lahore (REC-UOL-/577/11/23), dated: 27th January 2023. Two hundred children aged 8 to 16 years were recruited using consecutive sampling and grouped by serum 25-hydroxyvitamin D [25(OH)D] as sufficient (30–100 ng/mL; n=100) or deficient (<30 ng/mL; n=100). BOP was recorded using a WHO probe at six sites per tooth for erupted permanent teeth; the primary outcome was BOP%. Group differences were evaluated using an independent samples t-test. A univariate general linear model assessed whether the association persisted after adjusting for covariates.

Results: BOP% was significantly lower in the VD sufficient group (22.25±1.16) than the deficient group (24.96±1.16), with a mean difference of -2.71 percentage points (95% CI: -3.03 to -2.38; p<0.001). In the adjusted model, VD status showed a strong association with BOP% (F=251.12, p<0.001; partial η^2 =0.567; adjusted R²=0.569).

Conclusion: VD deficiency was independently associated with increased gingival bleeding in school-aged children, suggesting VD status contributes to early gingival inflammatory burden in this population.

Keywords: Gingiva, Pediatrics, Micronutrients, Deficiency, Inflammation.

INTRODUCTION

Vitamin D (VD) is a fat-soluble vitamin that, through metabolic activation, forms a biologically active hormone involved in calcium and phosphate homeostasis along with skeletal development [1]. Beyond its role in bone metabolism, VD also influences immune regulation and inflammatory responses through the VD receptor, a nuclear transcription factor in multiple tissues [2]. The majority of VD is synthesized endogenously following exposure to ultraviolet radiation, whereas dietary intake contributes a smaller proportion. Previous literature suggests that an increase of 100 IU of daily VD intake is

associated with a rise in serum 25-hydroxyVD concentrations of about 1 ng/mL [3].

VD deficiency is a global public health issue, especially in children and adolescents [4]. Population-based studies from high-income countries reported varied prevalence based on age, season, and diagnostic thresholds; however, significantly higher deficiencies have been documented in South Asian countries [5-7]. Previous South Asian studies reported that VD deficiency is widespread across pediatric and adolescent populations, ranging from moderate to severe deficiency levels, based on region, age, and season of assessment, indicating VD deficiency as a common nutritional concern [8, 9].

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Although VD's role in periodontal health is still being investigated, the existing studies report that VD deficiency may lead to periodontal issues, especially with compromised bone health [10]. VD has an important role in bone metabolism, calcium homeostasis, and inflammatory responses as it suppresses pro-inflammatory cytokines and synthesis of antimicrobial peptides in monocytes and macrophages leading to host defense [11, 12]. Therefore, lower VD levels may lead to infections including periodontal infections [10]. Data from the NHANES-III reported that intake of low dietary calcium, reflective of suboptimal VD levels, was linked to advanced periodontitis [13].

A previous study has reported that there is an inverse association between serum VD levels and attachment loss of periodontal ligament fibers among adults 50 years and above, and reported that lower VD levels were linked to higher BOP, suggesting that VD's anti-inflammatory properties may play a role in reducing gingivitis [14]. Similarly, individuals receiving more than 400 IU/day of VD along with over 1000 mg/day of calcium for at least 18 months have reported significant improvements in periodontal health [15].

Periodontal health is shaped both by oral biofilm and the host inflammatory response and can be assessed objectively using BOP. Low VD levels can increase susceptibility to gingivitis [10].

The study aimed to examine the association between serum VD and Gingival Inflammation, using BOP scores of children aged 8-16 years old in Lahore, Pakistan. The objectives were to compare gingival inflammation between children with sufficient versus deficient serum VD levels, and to understand the association between BOP% and serum VD.

MATERIALS AND METHODS

This comparative cross-sectional study was done to compare BOP% between children with VD sufficiency and deficiency. The study was conducted from 1st Feb 2023 to April 30th 2024 at The University of Lahore, Pakistan. Ethical approval was obtained from the Institutional Review Board, (Ref. No. REC-UOL-/577/11/23), dated: 27th January 2023. Written informed consent was obtained from all parents/guardians.

The sample size of 200 participants was calculated using an effect size of 0.42, a significance level (α) of 0.05, and a statistical power of 0.84, 100 participants were taken from each group [7].

Children aged 8 to 16 years old were recruited using consecutive sampling technique from University Dental Hospital, The University of Lahore. Focus on this age range was done due to the fact that the presentation of gingivitis in this age group is common, reversible and responds to early intervention. Participants were screened for and excluded based on history of any chronic systemic diseases affecting immunity or bone metabolism, acute infection at the time of examination, VD supplementation, recent antibiotic use and periodontal treatment, and those who refused to be the part of the study.

Venous blood was collected by trained staff using standard aseptic technique. Serum VD was assessed by measuring concentration of 25-hydroxyVD (25[OH]D). VD deficiency was defined as <30 ng/mL, and sufficiency as 30–100 ng/mL.

Participants were categorised into Group A (sufficient), i.e., 30–100 ng/mL, and Group B (deficient), i.e., <30 ng/mL. Oral examinations were performed by calibrated examiners. BOP was recorded using a WHO probe with gentle probing at six sites per tooth for all erupted permanent teeth. Bleeding was scored as present if bleeding occurred within 10 to 15 seconds of probing. The primary outcome, percentage BOP sites (BOP%), was calculated as:

$$BOP\% = \frac{\text{number of bleeding sites}}{\text{total sites examined}} \times 100$$

Gingivitis is plaque-mediated; however, plaque index was not recorded in this study. Adjustments were made for available oral health behaviors, including brushing frequency, dental visits and sociodemographic factors, but residual confounding by plaque accumulation and related behaviors may remain.

STATISTICAL ANALYSIS

Data was analyzed using IBM Statistical Package for Social Sciences (SPSS), Version 24 (IBM Corp), and was summarized using descriptive statistics. Independent-samples t-test was used to compare BOP between VD sufficient vs deficient groups, whereas homogeneity of variance was assessed using Levene's test. To determine whether the link between VD and BOP, after accounting for potential confounders, a univariate general linear model (GLM) was fitted with BOP as the dependent variable and VD level as the main factor, adjusting for parental education, residence, family type, dental visit frequency, and daily brushing habits. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 200 children were included in the study, with 100 participants in each group. Group A comprised children with sufficient serum VD levels, while Group B included children with deficient VD levels. The baseline/demographic characteristics were comparable between children with sufficient and deficient VD levels (Table 1). The only significant difference was observed in maternal education ($p=0.038$), indicating children in the lower VD level group were more likely to have mothers with a lower education level. This was thus included in the adjusted model, due to its role as a potential confounder.

Mean BOP% significantly differed between the two groups (Table 2). Children in the VD sufficient group had a significantly lower mean BOP% compared to children in the VD deficient group ($t=-16.49$, $df=198$, $p<0.001$).

To evaluate whether the difference in BOP% persisted after accounting for confounders, a univariate GLM was applied with BOP% as the dependent variable and VD level as the primary factor, adjusting for parental education, residence, family type, dental visit frequency, and brushing habits (Table 3).

The overall model was statistically significant ($F=38.53$, $p<0.001$) with around 56.9% of the variance in BOP% (adjusted $R^2=0.569$). After adjustment, VD level was an independent predictor of BOP%, with children in the VD-deficient group showing higher BOP% ($F=251.12$, $p<0.001$). The magnitude of this association was large (partial $\eta^2=0.567$), indicating that VD levels were significantly related to variation in BOP. Because exposure and outcome were measured simultaneously, these adjusted findings represent independent associations rather than causal effects.

Table 1. Baseline Characteristics of Participants by Vitamin D Status.

Characteristic	Vitamin D sufficient (Group A, n = 100) n	Vitamin D deficient (Group B, n = 100) n	p
Gender (n)			
Male	61	53	0.25
Female	39	47	
Daily Brushing (n)			
None	1	2	0.67
Once daily	58	62	
Twice daily	41	36	
Father's Education (n)			
Illiterate	8	16	0.177
Intermediate	41	31	
Graduation	42	47	
Post-graduation	9	6	
Mother's Education (n)			
Illiterate	5	9	0.038
Intermediate	36	46	
Graduation	43	24	
Post-graduation	16	21	
Residence			
Urban	73	82	0.128
Rural	27	18	
Type of Family			
Nuclear	34	44	0.147
Extended	66	56	
Yearly Dental Visit Frequency			
Only in case of pain	76	74	0.939
1-2 times	19	21	
More than 2 times	5	5	

p-values were calculated using Pearson's chi-square test; Fisher's exact test was used when expected cell counts were <5 .

Table 2. Comparison of Bleeding on Probing (%) by Vitamin D Status.

Vitamin D Sufficient (Group A, n = 100)	Vitamin D Deficient (Group B, n = 100)	Mean Difference (95% CI)	p
22.25±1.16	24.96±1.16	-2.71 (-3.03 to -2.38)	<0.001

Table 3. Multivariable Analysis of Factors Associated with Bleeding on Probing Percentage.

Variable	F	p	Partial η^2
Vitamin D status	251.12	<0.001	0.567
Father's education	0.03	0.860	<0.001
Mother's education	1.96	0.164	0.010
Residence (urban/rural)	0.18	0.672	0.001
Type of family	0.14	0.713	0.001
Dental visit frequency	0.28	0.597	0.001
Daily brushing habit	0.20	0.655	0.001

Multivariable univariate general linear model (GLM) with BOP% as the dependent variable. Model was adjusted for parental education, residence, family type, dental visit frequency, and daily brushing habits. Partial eta squared (η^2) represents effect size.

DISCUSSION

This study reported a significant link between VD deficiency and gingivitis, as indicated by higher BOP among school-aged children. Recent studies have found a link between VD and oral inflammatory conditions. A review reported that lower serum VD is linked to poor periodontal and gingival health across diverse populations [16]. The study also indicates that VD influences early gingivitis, aligning with the objectives and outcomes of this study.

Evidence using a large population dataset (NHANES) has shown a direct negative relation of serum VD concentrations with poor periodontal outcomes, including bleeding and inflammation indicators, after adjustment for key covariates and behaviors [17]. Maternal education differed significantly at baseline in the present study, and it stands to reason that it influences both nutritional status and oral health behaviors. Therefore, it was considered a potential confounder; however, the association between vitamin D status and BOP% remained strong after adjustment. This association is particularly relevant in South Asian populations as VD deficiency is prevalent across South Asia, presenting it as a common contributor to the population burden of gingival inflammation [18].

In the pediatric and adolescent populations, studies linking VD to periodontal outcomes remain limited as most available data is derived from adult studies [19]. This study contributes novel evidence of the pediatric population by demonstrating a clinically meaningful difference in bleeding on probing between sufficient and deficient children.

The association in this study is biologically plausible given VD's established immunomodulatory effects and its ability to suppress the excessive production of pro-inflammatory cytokines, thus enhancing innate immune defenses. This includes antimicrobial peptide expression in oral epithelium tissues [20]. In the gingiva, this balance allows for control of microbial biofilm-induced inflammation and lowering the incidence of tissue damage. VD has an influence on vascular permeability and endothelial stability, which may directly affect gingival bleeding responses during probing. Deficiency may therefore amplify clinical signs of inflammation even in the absence of advanced periodontal breakdown, commonly observed in pediatric gingivitis [21, 22].

These findings have important implications in low- and middle-income settings such as Pakistan, where VD deficiency is high among children [23]. Gingival bleeding is often considered a minor or transient finding; however, if this inflammation persists through childhood and adolescence it may increase susceptibility to periodontal disease later in life [24, 25]. Addressing VD deficiency through nutritional intervention programs and public health strategies may allow a complementary approach to conventional oral hygiene-based prevention programs to tackle Gingival and Periodontal Disease.

LIMITATIONS

There are certain limitations to this study as participants were recruited using consecutive sampling from a single hospital-based setting, which may introduce selection bias and limit generalizability to all school-aged children in Lahore or Pakistan.

The cross-sectional design prevents establishing causality; therefore, the observed association between vitamin D status and BOP% should not be interpreted as causal.

Gingivitis is plaque-mediated, yet a plaque index was not recorded, and residual confounding related to plaque accumulation and oral hygiene technique may remain.

Serum vitamin D was measured at one time point, which may not reflect longer-term vitamin D status and may vary with season, sun exposure, and recent diet.

Several potential confounders were not captured, including sunlight exposure, dietary vitamin D and calcium intake, body mass index, socioeconomic indicators beyond those recorded, Pubertal status and use of supplements outside the exclusion window.

BOP% is a standardized clinical indicator but can be influenced by examiner technique and probing force; thus measurement variability cannot be fully excluded.

Exact age in years was not recorded or used in analysis. Thus, age-based differences between groups or analysis adjusting for age as a potential confounder were not done.

FUTURE RECOMMENDATIONS

Future studies should use prospective cohort designs to clarify the overtime relationship between vitamin D status and gingival inflammation in children and adolescents. Randomized controlled trials evaluating vitamin D supplementation with standardized dosing, adherence monitoring and follow-up are recommended to determine whether improving vitamin D status leads to meaningful reductions in BOP%. Future work should also include a standardized plaque measure such as plaque index and record additional determinants such as sun exposure, dietary vitamin D and calcium intake. Seasonal variation should also be accounted for by recording the season of blood sampling or sampling across seasons. Multi-center studies across different socioeconomically diverse areas of Pakistan are recommended to improve generalizability.

CONCLUSION

The children with VD deficiency showed significantly higher gingival bleeding, reflected by increased BOP, as compared with VD-sufficient children, and this association was significant after adjustment for sociodemographic factors and oral health behaviors. These findings

do not establish causality and should be confirmed in longitudinal or interventional studies. Future studies should also include a standardized plaque measure and test whether VD remains associated with BOP after plaque adjustment. Optimising VD levels may be a relevant strategy alongside routine oral hygiene measures in reducing early gingival inflammation in children.

AUTHORS' CONTRIBUTIONS

Arooj ul Hassan and Asif Hanif: Conceptualization, study design, methodology, data analysis and interpretation, and drafting the manuscript.

Muhammad Hassan: Study design, methodology, data analysis and interpretation, drafting the manuscript, and critical review and revision of the manuscript.

Obaid Bajwa: Conceptualization, methodology, data analysis and interpretation, critical review and revision of the manuscript, and final approval of the version to be published.

Muhammad Zaeem Ihsan: Study design, methodology, data analysis and interpretation, and drafting the manuscript.

All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work, including ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Declared none.

ETHICAL DECLARATIONS

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request, subject to applicable ethical, privacy, and institutional requirements.

Ethical Approval

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki, as revised in 2024, and was approved by the Institutional Review Board of The University of Lahore (Ref. No. REC-UOL-/577/11/23; dated 27th January 2023).

Consent to Participate

Written informed consent was obtained from the parents or legally authorized guardians of all participating children before enrollment in the study. Age-appropriate assent was also obtained from the children, where applicable.

Consent for Publication

Written informed consent for publication was obtained from the parents or legally authorized guardians of participants whose individual information or other potentially identifiable material is included in this article, where applicable.

Declaration of Competing Interest

The authors declare that they have no known financial or non-financial competing interests that could have influenced the work reported in this study.

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Use of AI-Assisted Technology

The authors used ChatGPT (OpenAI) to assist with rephrasing selected sections of the manuscript to improve language and readability. The AI tool was not used for data collection, data analysis, interpretation of results, or generation of scientific conclusions. All AI-assisted output was critically reviewed, edited, and verified by the authors, who take full responsibility for the accuracy, integrity, originality, and final content of the manuscript.

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