Multivariate analysis of association of serum vitamin D levels of 25(OH)D with marginal gingivitis

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ABSTRACT

Purpose: Gingivitis is the inflammation of gingiva and a mildest form of periodontal disease. Earlier studies have revealed that vitamin D has an anti inflammatory effect on gingiva and low levels of serum vitamin D are associated with many chronic diseases.. The objective of this study was to assess the association of serum level of 25-OHD to marginal gingivitis. **Materials and Methods:** This was a cross sectional study conducted in Maratha Mandal dental college, Belgaum, India. Out of the 451 people screened, 96 subjects who had Gingivitis score more than 1 and who satisfied the inclusion criteria and signed the informed consent were considered for the study. The gingival status was assessed by Loe and Silness Index and the serum vitamin D levels of 25-OHD were analysed by The Diasorin vitamin D Direct Elisa kit Germany. Also the information on the Demographic profile which included the age, sex, weight and calculus status was collected in the form. **Results:** On statistical analysis the relationship of serum vitamin D with gingival status was found to be negative and statistically significant at $P < 0.05$. Further with multivariate analysis the total contribution of age, sex, vitamin D, weight and calculus on gingival status was 38.92% in which the calculus status contributed maximum of 20.11% compared to minimum of 0.83% by gender, followed by serum vitamin D 11.77%, age by 3.96% and weight by 2.25% respectively. **Conclusion:** Based on this analysis we can conclude that serum vitamin D and calculus are the most important contributors for the gingival status.

Key words: 25 hydroxy vitamin D levels, gingival status, multivariate analysis.

INTRODUCTION

Gingivitis is the inflammation of gingiva and a mildest form of periodontal disease. It affects 50% to 90% of adults worldwide. Clinically gingivitis is recognized by the signs of inflammation such as redness, swelling, bleeding, and exudation but less frequently by pain. Gingivitis is an inflammatory process limited to the mucosal epithelial tissue surrounding the cervical portion of the teeth and the alveolar processes.[1,2] Gingivitis has been classified by clinical appearance (eg, ulcerative, hemorrhagic, necrotizing, purulent), etiology (eg, drug-induced, hormonal, nutritional, infectious, plaque-induced), and duration (acute, chronic).[3] The most common type of gingivitis is a chronic form induced by plaque. It involves the marginal gingiva and is brought on by the accumulation of microbial plaques in persons with inadequate oral hygiene. Gingivitis proceeds through an initial stage to produce early lesions, which then progress to advanced disease. The cause of gingivitis is primarily bacterial plaque at the gingival margin however possible nutritional influences on the development and progression of this disease cannot be ignored. [4]

Researchers analyzed government data on calcium consumption and periodontal disease indicators in nearly 13,000 U.S. adults. They found that men and women who had calcium intakes of fewer than 500 milligrams, or about half the recommended dietary allowance, were almost twice as likely to have gum disease, as measured by

the loss of attachment of the gums from the teeth. The association was particularly evident for people in their 20s and 3o.[6]

Vitamin D is a vital nutrient in bone strength and prevention of osteoporosis, but what many people don't know is that vitamin D has many other functions. Vitamin D sufficiency is required for optimal health. The consensus of scientific understanding appears to be that vitamin D Deficiency is reached for serum 25-hydroxyvitamin D (25(OH)D) levels less than 20 ng/mL (50 nmol/L), insufficiency in the range from 20–32 ng/mL, and sufficiency in the range from 33–80 ng/mL, with normal in sunny countries 54–90 ng/mL, and excess greater than 100 ng/mL.[5,6,7] The conditions with strong evidence for a protective effect of vitamin D include several bone diseases, muscle weakness, more than a dozen types of internal cancers, multiple sclerosis, and type 1 diabetes mellitus. There is also weaker evidence for several other diseases and conditions.[8,9,10]

Some studies have suggested that vitamin D may have beneficial effect because of its anti inflammatory effects.[11,12,13] However a study on association between serum vitamin D levels and periodontal disease as well as tooth loss in U S population proved to be positive with high prevalence associated with deficiency in Vitamin D[14], A study by Boston University is worth mentioning where in the evaluation of the association between the serum concentration of 25-hydroxyvitamin D and gingival inflammation was assessed They conclude that Vitamin D may reduce susceptibility to gingival inflammation through its anti inflammatory effect by inhibiting antigen induced T cell proliferation and cytokine production.[15]

However, in India studies on whether there is an association between 25(OH)D serum concentrations on marginal gingivitis has not been investigated. The objective of this study was to assess the relationship between 25(OH)D serum concentrations and marginal gingivitis. Also the Investigator was interested to evaluate the influence of variables like age, sex, weight and calculus on the gingival status with a multivariate analysis. To restrict the analysis to marginal gingivitis, the participants with clinical attachment loss >2 mm were excluded from the study.

MATERIALS AND METHODS

This was a cross sectional study which was done to assess the association of serum vitamin D levels with the gingival status in people residing in Belgaum, India. The study was conducted in Maratha Mandal dental College Belgaum India, from February 2010 to may 2011. The protocol prepared at the start of the study was reviewed and approved by K L E University Belgavi, institutional review board (IRB) and local ethical committee prior to the start of the study.

The Inclusion Criteria included male and females in good general health aged 18–64 years diagnosed with different clinical stages of gingivitis and willing to sign the Informed Consent Form and who demonstrated a willingness to comply with all study procedures and clinical examination schedules were enrolled for the study. Also a minimum of 20 natural teeth with facial and lingual scorable surfaces with adequate oral hygiene and no signs of oral neglect with calculus + score were preferred. The selected subjects had to have periodontal pockets less than 2 mm. and gingivitis score of <1 measured by Loe and Sillness Gingivitis Index – 1963. Also the subject need to have Serum 25-hydroxyvitamin D concentration of 20 ng/mL to 65 ng/mL The exclusion Criteria included Subjects who had mobile teeth and having history of active severe periodontal disease with bleeding gums, Gross dental caries, severe generalized cervical abrasion and/or enamel abrasion, large fractured or temporary restorations (based on visual examinations) were not be included in the tooth count. Also subjects with fixed or removable orthodontic appliance or removable partial dentures, with history of dental prophylaxis or treatments in the past month were excluded from the study. Women who are postmenopausal, pregnancy or planned pregnancy within the period of the trial were not considered for the study. Subjects who were currently smoking or former smokers with cessation <5 years ago and people who regularly used medication for prevention or treatment of disease (including Aspirin, NSAIDs, corticosteroids, but NOT including contraceptives) were excluded.

Examination was done by a single examiner. Prospective subjects were scheduled during initial screening and the results of this examination recorded in the Initial Screening Form. Total of 452 people were screened and 110 participants who satisfied the inclusion and exclusion criteria were selected for the study. A subject demographics form was completed for all enrolled subjects which also included their age, sex, weight and calculus status also.

Clinical examinations and Blood sample collection after enrollment: Enrolled subjects were scheduled for a clinical examination. A whole mouth assessment of gingivitis by the Löe and Silness gingival Index (1963) was

completed and recorded in the appropriate forms during the examination.

BLOOD COLLECTION FOR BIOCHEMICAL ANALYSIS FOR SERUM VITAMIN D[24]

Tourniquet was applied and area disinfected Later 3ml of blood was drawn from the participants with an sterile 5ml Syringe and were transferred to test tubes and were allowed to clot for a period of one hour. The blood samples were centrifuged to separate the serum from plasma. The serum was then stored at –2 degree centigrade In the lab situated at Maratha Mandal Dental College to analyse for serum vitamin D level. The Diasorin vitamin D Direct Elisa kit was provided by Konark Enterprises Hubli India imported from Immunotek Germany, was used for serum Vitamin D analysis. Results from the biochemical assessments were reported and entered in the computer excel sheet5E.

STATISTICAL ANALYSIS:

The statistical analysis was performed by STATA 10.0 version.

Karl Pearson's Correlation test was conducted to know the relationship between gingival scores with vitamin D and other variables like age, sex, weight and calculus status.

Simple multiple regression test was done for analysis of Gingival scores by Serum vitamin D

Also test was done for multiple regression analysis of gingival scores with other variables. Relative contribution of different variables on gingival scores.

RESULTS

There were a total of 451 non- smokers aged 18 to 60 years with 25(OH)D serum concentrations and periodontal data available with complete data on covariates. Of them, 120 subjects had no teeth with attachment loss >2 mm. To restrict the analysis to participants with marginal gingivitis, we excluded those with clinical attachment loss > 2 mm (ie, bone loss resulting from periodontal disease). Furthermore, because smoking suppresses the bleeding response of the gingival tissues (18, 19), the analysis was restricted to subjects who reported to be never smokers.

After signing the informed consent a total of 96 samples were included in the study in which males were 55 and females were 41. The mean age of male respondents was 32.65 years ± 10.26 and females 31.90±10.95 respectively. (Table: 1)

Table 2: It can be seen that the relationship of vitamin D with gingival status is found to be negative and statistically significant at P<0.05, but age, weight, calculus were showing significant and positive relationship with gingival status, and above mentioned significant variables were dependent on each other.

Table 3: The influence of serum vitamin D was found to be negative and significant on gingival status of study subjects. Therefore we can say that serum vitamin D would be the best predictor of gingival status.

Table 4: Adjusting for sex, age, weight, and calculus on gingival status, the results presented in this table. Even after adjusting the other factors, the influence of serum

Table 1: Distribution of study subjects by gender with mean and SD age

Sex	Number of respondents	Mean age	SD age
Male	55 (57.29%)	32.65	10.26
Female	41 (42.71%)	31.90	10.95
Total	96 (100.00%)	32.33	10.51

Table 2: karl pearson's Correlation analysis between gingival scores and other variables

Independent variables	r-value	t-value	P-value
Sex	0.0900	0.8789	0.3817
Age	0.2300	2.1032	**0.0381***
Serum vit D	−0.4800	−3.5308	**0.0006***
Weight	0.2600	2.8622	**0.0052***
Calculus status	0.5300	5.7296	**0.0000***

*p<0.o5.

Table 3: Simple Linear multiple regression analysis of Gingival scores by serum D

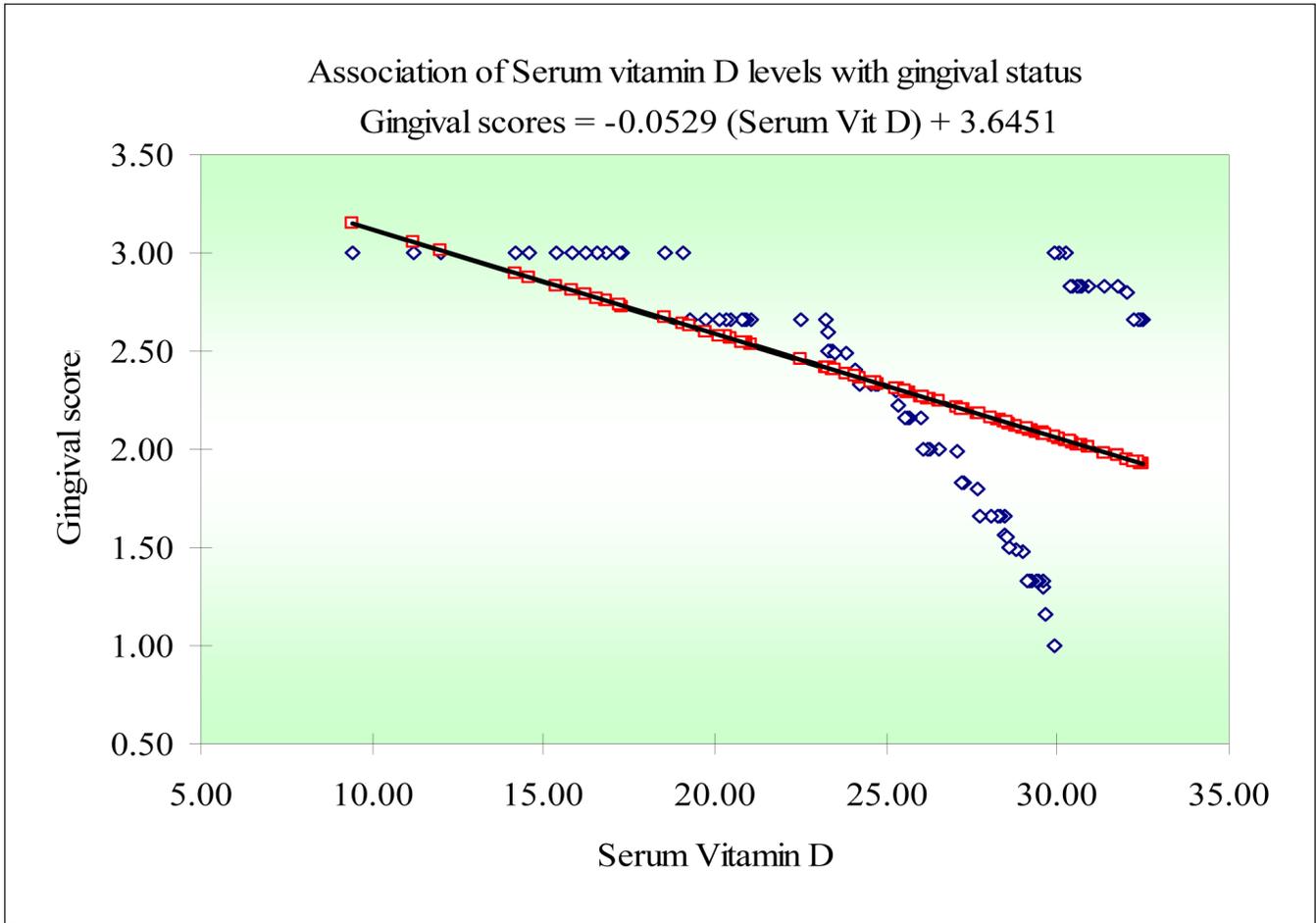| Inpt var	Regression coefficient	SE of reg coeffi.	t-value	p-value
Intercpt	3.6451	0.2578	14.1412	**0.0000***
Serum Vit D	−0.0529	0.0100	−5.3079	**0.0000***

*p<0.o5.

Figure 1:

Table 4: Linear Multiple regression analysis of gingival scores with other variables

Indpt vars	Regression coefficient	SE of reg coeffi.	t-value	p-value
Intercpt	2.0027	0.5536	3.6175	**0.0005***
Sex	0.1091	0.0973	1.1214	0.2651
Age	0.0096	0.0047	2.0590	**0.0424***
Serum vit D	−0.0270	0.0112	−2.4077	**0.0181***
Weight	0.0045	0.0048	0.9418	0.3488
Calculus status	0.4447	0.1107	4.0173	**0.0001***

R=0.6239, R^2=0.3892, Adjusted R^2=0.3574,
F(5,90)=11.570 p<0.05, S, Std.Error of estimate: .46963

*p<0.05.

Table 5: Relative contribution of different variables on gingival scores

Indpt vars	Beta	r-value	Beta x r	% of contribution
Sex	0.0926	0.0900	0.0083	0.8333
Age	0.1720	0.2300	0.0396	3.9559
Serum vit d	−0.2453	−0.4800	0.1177	11.7723
Weight	0.0866	0.2600	0.0225	2.2506
Calculus status	0.3794	0.5300	0.2011	20.1085
				38.9207

vitamin D is significant and negative on gingival status. Also after adjustment the influence of age and calculus status was found to be positive and significant on gingival status at P<0.05.

Table 5:The total contribution of age, sex, vitamin D, weight and calculus on gingival status was 38.92% in which

the calculus status contributed maximum of 20.11% compared to minimum of 0.83% by gender, followed by serum vitamin D 11.77%, age by 3.96% and weight by 2.25% respectively

So based on this analysis we can conclude that serum vitamin D and calculus are the most important contributors' for the gingival scores.

DISCUSSION

In this study, we found an inverse association between serum concentrations of 25(OH)D and chronic gingivitis as measured by Loe and silness gingival Index among participants who never smoked aged 18 to 65 y. To restrict the analysis to participants with marginal gingivitis, we excluded subjects with clinical attachment loss >2 mm (ie, bone loss resulting from periodontal disease). Furthermore, because smoking suppresses the bleeding response of the gingival tissues only non smokers were included in the study.[20,21] This approach allowed us to adjust for important covariates that are strong predictors of gingival bleeding age, sex, weight and calculus.

In the present study It can be seen that the relationship of vitamin D with gingival status is found to be negative and statistically significant at $P<0.05$, but age, weight, calculus were showing significant and positive relationship with gingival status, and above mentioned significant variables were dependent on each other. (Table2). This is in agreement with the earlier study done by Dietrics (2005) Who found a strong negative association between serum concentrations of 25(OH)D and prevalence of bleeding on probing. Compared with the lowest 25(OH)D quintile, sites in subjects in the highest 25(OH)D quintile had 20% (95% CI: 8%, 31%) lower odds of bleeding on probing. An increase in serum concentration of 25(OH)D of 30 ng/ml was associated with sites having 10% (95% CI: 5%, 14%) lower odds for bleeding. Therefore we could say that serum vitamin D would be the best predictor of gingival status.[15]

In a cross-sectional study of 116 subjects, serum concentrations of 25(OH)D were negatively correlated with serum concentrations of C-reactive protein. In a sub-sample of 24 patients from that study, vitamin D supplementation significantly reduced serum concentrations of C-reactive protein by 23%.[6] This further demonstrates the inverse association of inflammation and serum 25(OH)D levels.

A study by Catherine A Peterson, and Mary E Heffernan (2008)[19] showed that Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum vitamin D concentrations in healthy women. This supports the findings of the present study. Further adjusting for sex, age, weight, and calculus on gingival status, the influence of serum vitamin D was significant and negative on gingival status. Where as, the influence of age was found to be positive and significant on gingival status at $P<0.05$. This is not in agreement with the study

done by Dietrics (2005) where no significant interactions were observed with race-ethnicity, age, or sex, and effect estimates were similar across strata. The reason could be that the finding are in consistent with usual trend in this part of the world where gingivitis increases with age.

The multivariate analysis shows that the total contribution of age, sex, vitamin D, weight and calculus on gingival status was 38.92% in which the calculus status contributed maximum of 20.11% compared to minimum of 0.83% by gender, followed by serum vitamin D 11.77%, age by 3.96% and weight by 2.25% respectively

So based on this analysis we can conclude that though calculus is the most important contributor for the gingival scores, the serum vitamin D also contributes to a larger extent for the same.

The study has several limitations. The bacteria and bacterial products of dental plaque are the main cause of gingival inflammation. Plaque is an important confounder variable in the present study. Unfortunately, plaque levels were not measured. Therefore, the role of plaque cannot be explained. Also in this study subjects with chronic systemic diseases were excluded so that the analysis could be restricted to subjects with normal health. However, further studies may be needed to know the contribution of the above mentioned confounding factors in patients with common systemic disease like diabetes and hypertension and the vitamin D levels in the serum that could influence the gingival status.

CONCLUSION

The inverse association of serum hydroxy vitamin D concentration and gingivitis is an important finding as it indicates the individuals with low levels of vitamin D would be susceptible to gingivitis. Though other factors like calculus, age, gender, weight also contribute to the gingival status, the serum hydroxy vitamin D contributes substantially for the same.

ACKNOWLEDGEMENT

REFERENCES

1. John W. *Stamm. Epidemiology of Gingivitis Journal of Clinical Periodontology* 1986; **13**(5): 360–366.

2. Proskin HM (1992)62 Some thoughts on the measurent of gingivitis. *J Periodont Res* 1992; **27**: 369–372.

3. Caton J: Biological and measurement issues critical to design of gingivitis trials. *J Periodontal Res* 1999; **27**: 364–368.

4. Walls AW, Steele JG. Oral Health and Nutrition in Elderly people. *Journal of Public Health Dentistry* 2000; **60**: 304–7.

5. Dawson, Hughes B *et al.* Effect of calcium and vitamin D supplementation on bone density in men and women aged 65 yrs and above. *N. Eng. J Med* 1997; **33**(7):610–16 .

6. TimmsPM, Mannan N, Hitman GA *et al.* Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders. *Q J Med* 2002; **95**: 787–96.

7. Van den Berghe G, Van Roosbroeck D, Vanhove P, Wouters PJ, De Pourcq L, Bouillon R. Bone turnover in prolonged critical illness: effect of vitamin D. *J Clin Endocrinol Metab* 2003; **88**: 462332.

8. Krall EA, Wehler C, Garcia RI, Harris SS, Dawson-Hughes B. Calcium and vitamin D supplements reduce tooth loss in the elderly. *Am J Med* 2001; (11):452–466.

9. Chill KC, Chu A. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J of Cli Nut* 2004; **70**(3):820–25.

10. Holick MP. Vitamin D importance in prevention of cancers, Type 2 Diabetes, heart diseases and osteoporosis. *Am Journal Clinical Nutrition* 2004; **79**(3): 362–71.

11. Bhalla AK, Amento EP, Serog B, Glimcher LH. 5-Dihydroxyvitamin D3 inhibits antigen-induced T cell activation. *J Immunol* 1984; **133**: 174854.

12. Rigby WF, Denome S, Fanger MW. Regulation of lymphokine production and human T lymphocyte activation by 5-dihydroxyvitamin D3. Specific inhibition at the level of messenger RNA. *J Clin Invest* 1987; **79**: 165964.

13. Relationship between vitamin D receptor gene polymorphism and periodontitis. *J Periodontal Res* 2002; **37**: 2637.

14. Dietrich T, Joshipura KJ, Dawson-Hughes B. *et al*. Association between serum concentrations of 25-hydroxyvitamin D3 and periodontal disease in the US population. *Am J Clin Nutr* 2004; **80**: 108–113.

15. Dietrich Thomas, Nunn Martha, ans Heik Bess Dawson. Association between serum concentration of 25-hydroxyvitamin D and gingival inflammation. *Am J Clin Nutr* 2005; **82**: 575–580.

16. Heany RP. Long latency deficiency disease insights from calcium and vitamin D. *Am J Cl Nut* 2003; **78**(5): 91–8.

17. Grant William B, MichelF, Holock. Benefits and Requirements of Vitamin D for Optimal Health. *Alternative Medicine Review* 2005; **10**: 23–33.

18. Nagaki K, Krall EA, Fleet JC, Garcia RI. Vitamin D receptor alleles, periodontal disease progression, and tooth loss in the VA dental longitudinal study. *J Dent Res* 2003; **74**: 1617.

19. Peterson Catherine A, Heffernan Mary E. Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum 25 (OH)D concentrations in healthy women. *Journal of inflammation* 2008; (5):10–15.

20. Bergstrom J, Preber H. The influence of cigarette smoking on the development of experimental gingivitis. *J Periodontal Res* 1986; **21**: 66876.

21. Dietrich T, Bernimoulin J-P, Glynn RJ. The effect of smoking on gingival bleeding. *J Periodontol* 2004; **75**: 10.

22. Dixon D, Hildebolt CF, DMiley D: Calcium and vitamin D use among adults in periodontal disease maintenance programmes. *British Dental Journal* 2009; **206**,627–631.

23. Sema Puri, Raman K. Marwaha, Neha Agarwal, Nikhil Tandon *et al*. Vitamin D status of apparently healthy schoolgirls from two different socio-economic strata in Delhi: relation to nutrition and lifestyles. *British Journal of Nutrition* 2008; **99**: 876–882.

24. Carter G, Carter R, Gunter E *et al*. Measurement of vitamin D metabolites: an international perspective on methodology and clinical interpretation. *J Steroid Biochem Mol Biol* 2004; 89–90: 467–71.