Association of serum vitamin D3 deficiency with stage III periodontitis and periodontal evaluation of deficient individuals after high dose vitamin D3 supplementation

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Abstract

Aim: The aim of the present study was to find the association between serum vitamin D3 deficiency and stage III periodontitis. The adjunctive benefit of high dose vitamin D3 supplement in periodontal therapy was also evaluated.

Methods: Periodontally healthy and diseased individuals (n= 80, age 42.05± 6.7) were included in the study. Serum Vitamin D3 levels were assessed using electro chemiluminescence immunoassay. Vitamin D3 was correlated with full mouth bleeding score and pocket depth using pearson correlation coefficient. After scaling and root debridement vitamin D3 deficient periodontitis patients were provided 60,000 IU Vitamin D3 /week for 8 weeks. Re-evaluation was done after 2 months.

Results: Serum Vitamin D3 deficiency was significantly associated with periodontitis with an odds ratio of 16.24 and 95 % confidence interval of 5.39 – 48.91 ($p < 0.01$). Highly significant correlation was noticed with Vitamin D3 values and full mouth bleeding score and probing depth. Reduction in the periodontal parameters were higher in those who were supplemented with Vitamin D3 compared to non-supplemented group even-though it was not statistically significant.

Conclusion: The present study concludes that serum Vitamin D3 insufficiency is significantly associated with periodontitis. Vitamin D supplementation as an adjunct to mechanical therapy needs further evaluation.

Keywords: Periodontitis; bone remodelling; vitamin D deficiency; treatment outcome.

Introduction

Periodontitis is a globally prevalent disease and is a leading cause of tooth loss which can affect the quality of life and nutritional status (Tonetti *et al*., 2017). Nutritional imbalance in turn can play an important role in progression of periodontal disease. Vitamin D and calcium are fundamental for bone mineralization and its deficiency is found to affect bone homeostasis (Holick, 2008). It is likely that a chronically low intake of vitamin D and calcium may lead to a negative calcium balance, thus causing a secondary increase in calcium removal from bone, including the alveolar bone (Chapuy *et al*., 1997). Vitamin D also regulates innate and adaptive immune system (Hewison, 2012). The effect of vitamin D on oral structures is also influenced by its anti-inflammatory action through immunomodulatory properties and by the production of various antimicrobial peptides like cathelicidin (Wang *et al*., 2004).

Vitamin D deficiency is implicated in infectious diseases like pneumonia and chronic inflammatory diseases like rheumatoid arthritis which share many similarities with periodontal disease. The actions of the vitamin D hormone 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) are mediated by the vitamin D

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receptor (VDR). VDR genotype has been identified as a risk indicator for susceptibility to periodontitis (de Brito Júnior *et al*., 2004).

The evidence for the association between vitamin D deficiency and periodontitis are inconclusive according to a recent systematic review (Pinto *et al*., 2018). Similarly, the adjunctive role of vitamin D supplementation along with nonsurgical periodontal therapy is another grey area. So, the present study intends to find the association between serum vitamin D3 deficiency and periodontitis and evaluate the impact of high dose vitamin D3 supplement as an adjutant to scaling and root debridement in vitamin D3 deficient patients.

Materials and methods

The present case control study was conducted from April 2019 to December 2020 after obtaining clearance from institutional ethics committee [IEC NO: PMS/IEC/2018-19/15] and clinical trial registry of India (CTRI/2020/06/026236). The sample size was calculated using the formula n= 2 σ2 (Zα +Zβ) 2 ÷ δ. Type 1 error was kept as 0.05 and type 2 error as 0.20. Substituting the values and considering the chance of 10% dropouts during the study the minimum sample size required was calculated as 40 in each group.

We consecutively selected generalized stage III periodontitis patients and age and gender matched periodontally healthy individuals (40 in each group) (Papapanou *et al*., 2018). All participants were informed about study protocol and written consents were obtained. Patients who underwent periodontal therapy in the past 1 year, who consumed antibiotics or anti-inflammatory drugs during the past 6 months, pregnant and lactating females, patients with systemic diseases, those who were undergoing orthodontic treatment and immunocompromised patients were excluded from the study.

Serum Vitamin D Estimation

A 5-ml venous blood was collected to prepare serum which was transported to the laboratory at -20°C. Serum vitamin D3 levels were evaluated using electro-chemiluminescence immunoassay (Cobas 411 [Roche Diagnostics, Basel, Switzerland]). The functional sensitivity of the assay was 4.01 ng/mL. Patients were categorized based on Vitamin D levels as deficient (≤12 ng/ml), inadequate (12–19 ng/ml) and adequate (≥20 ng/ml). Adequate group was further divided into adequate (20–30 ng/ml) and optimal (>30 ng/ml) (Holick *et al*., 2011).

Procedure

Immediately after blood sample collection clinical parameters were recorded. It included full mouth plaque score (FMPS) (O'Leary *et al*., 1972), Full mouth bleeding score (FMBS) (Mühlemann and Son, 1971), probing depth (PD) and clinical attachment loss / level (CAL), using a manual periodontal probe (PW7, Hu-Friedy, IL, US) by a single calibrated examiner (RPG). Tooth staining index (Macpherson *et al*., 2000) was used to measure the intensity of staining. Supragingival scaling and polishing was performed for all the patients. Those who were found to have inadequate serum vitamin D3 levels were provided vitamin D3 supplements [Capsule Vitamin D3 60,000 IU once in a week for 8 weeks] after consulting with orthopaedician. The periodontitis patients underwent one-stage full-mouth root debridement by the same investigator (RPG), and proper oral hygiene instructions were given for maintenance. Re-evaluation was carried out after 2 months and clinical examination was repeated. Patients were instructed to bring the empty cover of vitamin D3 to ensure compliance. Study summary is provided in Figure 1.

Data analysis

Statistical analysis was performed using a statistical software package SPSS, version 20.0. Independent t test was used to compare quantitative parameters between categories. Paired t test was used to compare quantitative parameters before and after intervention. Chi-square test was used to find association between categorical variables. Odds ratio (OR) with 95 % confidence interval was used to explain association of periodontitis with vitamin D3 level. Mann-Whitney U Test was used to compare ordinal and non-parametric quantitative parameters between groups. Pearson correlation coefficient was used to correlate the clinical parameters with Vitamin D levels. For all statistical interpretations, $p<0.05$ was considered the threshold for statistical significance.

Results

The mean age was 41.5± 6.1 and 42.6 ± 7.3 in control and case group respectively. No significant difference was noticed among the groups for age and gender [$p = 0.47$, $p=0.82$ respectively]. The baseline characteristics of the study population such as FMBS, FMPS and PD were statistically higher in cases compared with control [$p<0.01$] and data are provided in Table 1.

Mean serum Vitamin D3 concentration in periodontally healthy individuals and periodontitis subjects were 25.8 ± 6.9 and 17.9 ± 5.7, respectively. Individuals in both groups were categorized into insufficient, adequate, and optimal based on their Vitamin D3 concentration. Among the 40 periodontitis patients, 31 were with insufficient, 6 had adequate level and 3 with optimal Vitamin D3 levels. In periodontally healthy individuals it was 7,22 and 11 respectively. For statistical analysis adequate and optimal categories were combined to make sufficient

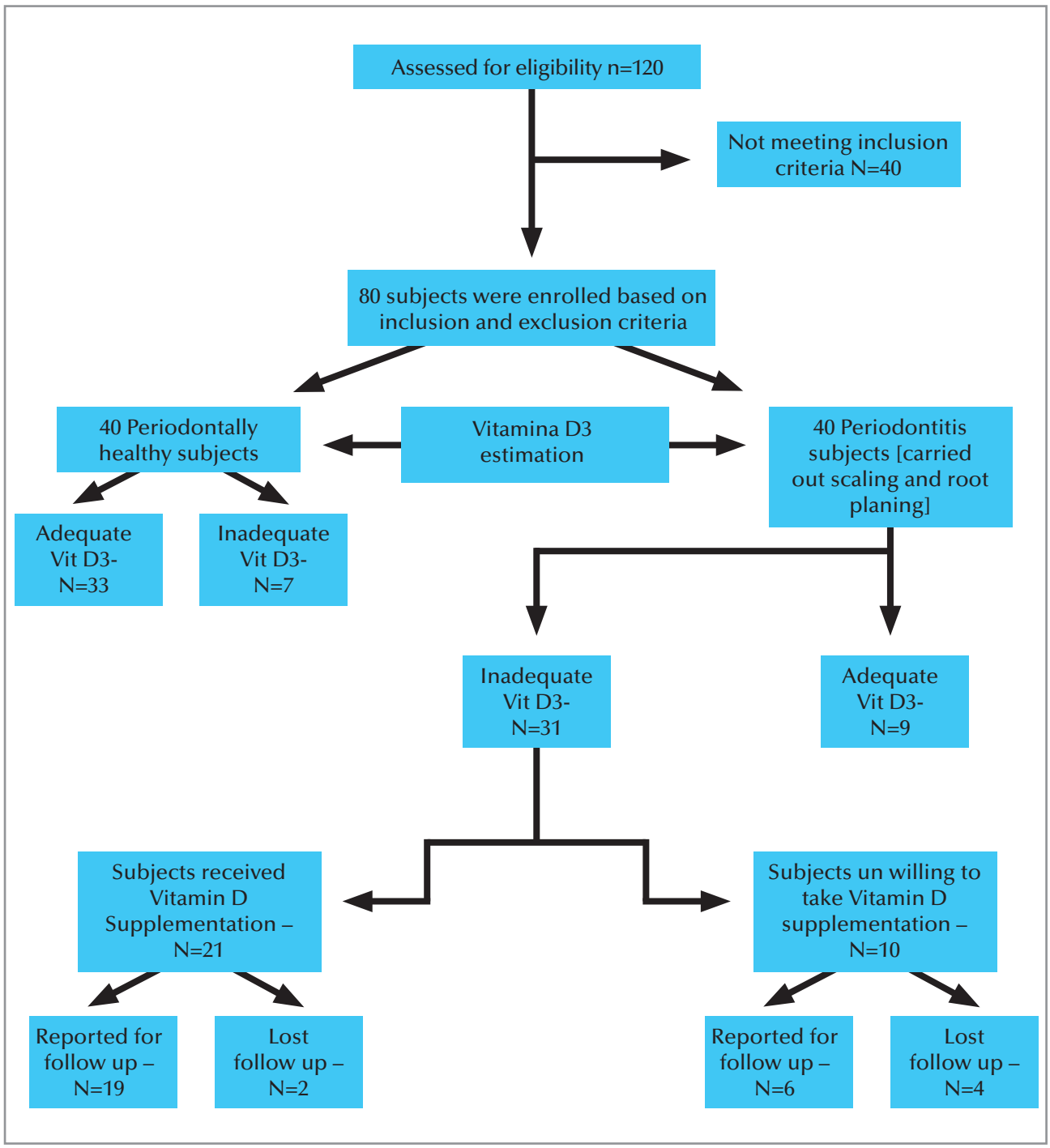

Figure 1. CONSORT flow diagram.

Table 1. Comparison of clinical parameters between Cases and Controls.

Clinical parameters	Control			Case			T	P
	Mean	SD	N	Mean	SD	N		
FMBS	54.7	10.4	40	80.4	8.3	40	12.24	p<0.01*
FMPS	54.3	7.6	40	81.0	6.7	40	16.71	p<0.01*
PD	2.5	0.2	40	3.6	0.4	40	14.18	p<0.01*

FMBS-Full mouth bleeding score, FMPS- Full mouth plaque score, PD-pocket depth, SD-standard deviation, * statistical significance

category. So, in periodontitis group 31 patients had insufficient vitamin D3 whereas it was 7 in periodontally healthy group. Statistical analysis was done using Chi square test and OR was found to be 16.24 with a 95 % Confidence Interval of 5.39 – 48.91. It was statistically significant with a p value of <0.01. Vitamin D3 value was correlated with FMBS, and PD. Correlation coefficient was -0.43 and -0.48 respectively and both results were statistically highly significant (p<0.0001). It is represented in Figure 2 and 3. No statistically significant difference in clinical parameters was noticed between patients with and without vitamin D3 deficiency in both periodontally healthy individuals as well as in periodontitis patients. Data is provided in Table 2.

Among the 31 periodontitis patients with vitamin D3 deficiency 21 patients received Vitamin D3 supplementation immediately after scaling and root debridement and the remaining were unwilling to take Vitamin D3 supplementation (n=10). Among the 21 patients who received supplementation 2 were lost to follow up (1 unable to contact & other shifted to a distant place). 100% compliance was observed in patients who returned for follow up. Among the 10 patients who have not taken Vitamin D3 supplementation 6 patients returned for follow up. Comparison of clinical parameters (FMBS, FMPS, PD and CAL) 2 months after scaling and root debridement was done in patients in both groups. Reduction in the periodontal parameters were higher in those who were supplemented with Vitamin D3 compared to non-supplemented group even-though it was not statistically significant. Data are provided in Table 3.

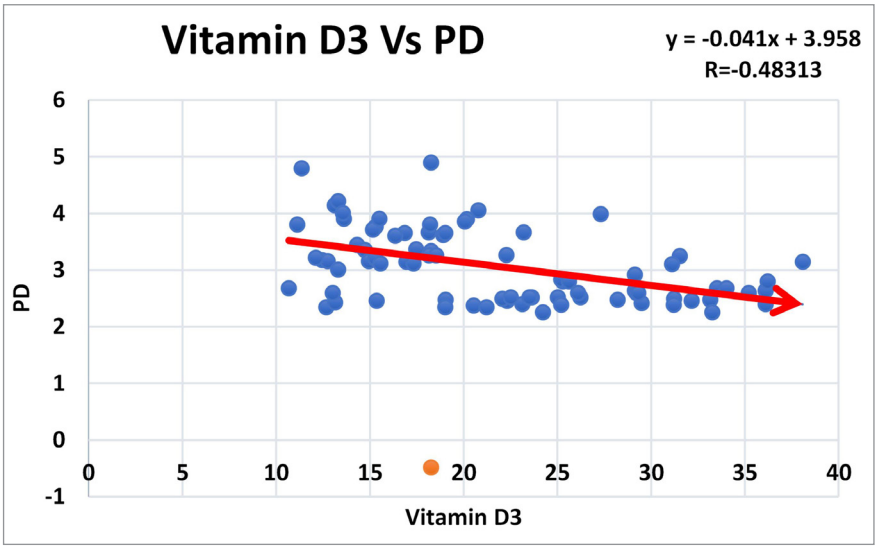

Figure 2. Correlation of Vitamin D3 with PD.

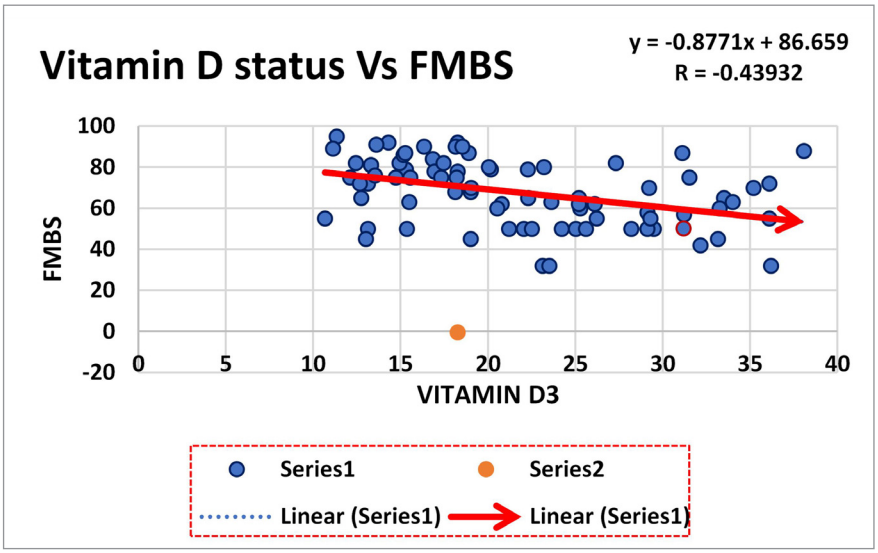

Figure 3. Correlation of Vitamin D3 with FMBS.

Table 2. Comparison of clinical parameters with vitamin D3 status in Cases and controls at baseline.

Clinical parameters	Insufficient			Adequate/Optimal			T	P
	Mean	SD	N	Mean	SD	N		
Periodontitis subjects								
FMBS	80.7	8.5	31	79.1	7.6	9	0.52	0.61#
FMPS	80.5	6.6	31	82.7	7.0	9	0.86	0.39#
PD	3.6	0.5	31	3.6	0.4	9	0.09	0.93#
CAL	3.7	0.5	31	3.6	0.4	9	0.18	0.86#
Periodontally healthy subjects								
FMBS	55.28	11.27	7	54.54	10.3	33	0.168	0.43#
FMPS	52.71	8.95	7	54.63	7.34	33	0.605	0.27#
PD	2.47	0.12	7	2.54	0.16	33	0.987	0.16#

FMBS-Full mouth bleeding score, FMPS- Full mouth plaque score, PD-pocket depth, CAL-Clinical attachment loss, SD-standard deviation, # statistically not significance

Table 3. % decrease in clinical parameters based on vitamin D3 supplementation.

	Vitamin Supplement	Median	Valid Value Count	Z#	p
FMPS	Yes	29.5	19	0.38	0.70#
	No	30.0	6		
FMBS	Yes	35.7	19	1.56	0.12#
	No	40.0	6		
PD	Yes	19.0	19	0.95	0.34#
	No	26.3	6		
CAL	Yes	24.2	19	0.7	0.48#
	No	26.7	6		

FMBS-Full mouth bleeding score, FMPS- Full mouth plaque score, PD-pocket depth, CAL-Clinical attachment loss, SD-standard deviation, # statistically not significance

Unusual black staining of teeth was noticed among vitamin D3 deficient individuals. Staining index in patients with Vitamin D3 deficiency in both cases and controls [N=38] and those who were having Adequate / Optimal Vitamin D3 levels [N=42] were compared using unpaired t test and it was statistically significant ($p < 0.01$). Staining index was also compared among cases and controls which was again statistically significant ($p < 0.01$). Percentage decrease in staining index based on vitamin D3 supplementation was also assessed among those who took supplementation [N=19] with those who were not supplemented [N=6] and no significant difference was noticed.

Discussion

Vitamin D insufficiency is a highly common condition affecting population worldwide and it varies among individuals based on race, sun exposure and presence of risk factors (James, 2008). Vitamin D plays a vital role in bone homeostasis and immunity. Oral epithelial cells convert inactive vitamin D to the active form of 25 (OH) D and induce expression of the antimicrobial peptide LL-37 and other host defense mediators (Ji *et al*., 2007). Biologically active form of vitamin D also function as an immunomodulatory agent because of its anti-inflammatory effect through inhibition of cytokine production (Zhang *et al*., 2012).

In the present case-control study, the association of periodontitis with serum Vitamin D3 deficiency was explored. OR was found to be 16.24 with a 95 % CI of 5.39 – 48.91with a statistical significance [p<0.01]. In a previous similar study conducted by Eshghi *et al*., 2016, there was a significant indirect relationship between the serum level of vitamin D and the periodontal indices (p<0.05). OR was 5.6 associating periodontitis with vitamin D levels less than 10 ng/ml (p=0.03), and it was 1.46 when vitamin D levels of 10-29 ng/ml was considered (p>0.05).

We observed mean Vitamin D3 concentration of 25.8 ± 6.9 and 17.9 ± 5.7 in periodontally healthy individuals and periodontitis subjects, respectively. Our results are comparable to a study done in the same ethnic population (22.32 ± 5.76 ηg/ml and 16.94 ± 5.58 ηg/ml). They have also evaluated serum vitamin D3 in periodontitis patients with diabetes mellitus, and it was found to be 14.06 ± 4.57 ηg/ml (Joseph *et al*., 2015). Statistically insignificant results were reported by Dasari *et al*., 2016 [23.34 ± 4.94 vs. 24.56 ± 4.82] which may be due to difference in disease definition and other confounding factors. Higher values of vitamin D3 were reported by Antonoglou *et al*., 2015 which might be because of the seasonal and racial variations.

Gingival bleeding was higher in Vitamin D3 insufficient patients included in cases as well as controls. But it was not statistically significant possibly because of inadequate samples in the comparison group. Dietrich *et al*., 2005 reported that participants with higher 25(OH)D levels were 20% less likely to have bleeding on probing. A recent cross-sectional study also reported that participants with deficiency of 25(OH)D have more gingival Inflammation (Bonnet *et al*., 2019). We have correlated FMBS & PD with Vitamin D levels using Pearson correlation coefficient, r value was found to be -0.43 for FMBS and -0.48 for PD. Both were statistically significant [p<0.0001]. Bhargava *et al*., 2018 also reported a similar correlation between Vitamin D3 deficiency and Gingival index [-0.45] as well as PD [-0.23].

We could observe a remarkable decrease [40 vs 34.7] in FMBS after vitamin D3 supplementation. Significant improvement in gingival index & bone density was reported by Perayil *et al*., 2015 after supplementing 500 mg calcium+250 IU vitamin D once daily for 3 months. Hiremath *et al*., 2013 also observed that vitamin D has a dose-dependent anti-inflammatory effect on gingivitis. For every unit (ng/ml) increase in serum 25 (OH) D levels, the odds of periodontal disease were significantly reduced by 12 % when vitamin D was supplemented in doses of 2000, 1000 and 500 international unit for 3months. Decrease in the systemic mediators of inflammation such as C-reactive protein (CRP) is also reported after Vitamin D supplementation (Chen *et al*., 2014).

Higher reduction in the periodontal parameters were noticed in patients who received Vitamin D3 supplementation. However, the data was not statistically significant which could be due to insufficient number of patients in the comparison group. We have included patients who did not comply with our instructions regarding vitamin D supplementation alone in the comparison group. Future studies recruiting larger samples can provide more useful information in this regard. Meghil *et al*., 2019 conducted a randomized controlled trial and concluded that Vitamin D supplementation has multiple benefits for reducing systemic inflammation and promoting induction of autophagy-related proteins related to anti-microbial functions.

Unlike most of the previous studies we have given Vitamin D3 supplementation for patients with Vitamin D3 deficiency alone which could be considered as the strength of our study. Additionally, we have prescribed a high dose of 60,000 IU Vitamin D3 once in a week for 8 weeks according to the recent evidence (Singh *et al*., 2019).

An interesting finding noticed in the present study was the presence of unusual black stains on teeth in patients with Vitamin D3 Deficiency irrespective of periodontal status (p <0.01). This could be considered as an alarming clinical sign indicating vitamin D deficiency for early detection before it makes irreversible changes in bone metabolism. Future studies are required to evaluate its prevalence and microscopic changes.

Vitamin D deficiency is recently suggested as a connecting link in many of these periodontal systemic interrelationships (Wang *et al*., 2020). Pregnant women with periodontitis presented with lower median Vitamin D3 levels (23.6 ng/ml) relative to periodontally healthy controls (40 ng/ml; p < 0.001). Patients with periodontitis with and without coronary heart disease (CHD) presented a significantly lower mean serum level of vitamin D (Isola *et al*., 2020). Future studies using biomarkers of inflammation and bone remodeling may be a useful tool for assessing the anti-inflammatory as well as bone sparing effects of vitamin D3. Similarly, the role of other confounding factors such as age, gender, ethnicity, seasonal variation, geographical latitude, and systemic diseases etc. also need clarification.

Patients with vitamin D deficiency were 4.6 times more likely to be positive for COVID-19 (indicated by the ICD-10 diagnostic code COVID19) than patients with no deficiency (p < 0.001). A recent study by Katz *et al*., 2021 reported that the association decreased significantly but remained robust (p < 0.001) after adjusting for race (OR = 3.76; p < 0.001), periodontal disease status (OR = 3.64; p < 0.001), diabetes (OR = 3.28; p < 0.001), and obesity (OR = 2.27; p < 0.001), and age groups (OR = 5.155; p < 0.001) respectively (32). Since periodontal disease is a highly prevalent

disease strongly associated with vitamin D deficiency management of periodontal disease and vitamin D supplementation may be useful for prevention of this pandemic and to reduce the post COVID 19 morbidities.

Conclusions

The present study concludes that serum Vitamin D3 deficiency is significantly associated with periodontitis. Assessment of serum 25(OH)D levels seems to be advisable in periodontitis patients. We could also observe that Vitamin D supplementation as an adjunct to mechanical therapy decrease gingival inflammation and periodontal pocket depth. Future well controlled multicentric trials can provide useful information in formulating universal guidelines and consensus regarding dose, duration, and frequency of vitamin D supplement in the management of periodontitis.

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