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ORIGINAL ARTICLE

Evaluation and comparison of serum vitamin D and calcium levels in periodontally healthy, chronic gingivitis and chronic periodontitis in patients with and without diabetes mellitus – a cross-sectional study

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ABSTRACT

Objective: Limited data are available with respect to the relation of vitamin D and calcium with periodontal infections and type-2 diabetes mellitus (T2DM). The aim of this cross-sectional study was to evaluate the levels of vitamin D and calcium in serum of periodontally healthy, chronic gingivitis and chronic periodontitis patients with and without T2DM.

Material and methods: The study evaluated 100 patients equally divided into five groups (Group I to Group V) according to the inclusion criteria. Clinical parameters and serum 25-hydroxyvitamin D level were assessed. Other laboratory investigations comprised of random blood sugar, glycated haemoglobin and serum calcium.

Results: The probing pocket depth and clinical attachment loss were found to be greater in chronic periodontitis and chronic periodontitis with diabetes mellitus, while the vitamin D and calcium levels were found to be least in these groups. When vitamin D and calcium levels were compared between periodontal disease with diabetes to that of non-diabetics, statistically significant difference were found between the two with *p*-value of .001 indicating decrease in levels of vitamin D and calcium with increase in RBS and HbA1c values.

Conclusion: Vitamin D and calcium levels are inversely correlated with random blood sugar and glycated haemoglobin and also probing pocket depth and clinical attachment loss, thus contributing towards increase in periodontal disease severity.

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Calcium; gingivitis; periodontitis; vitamin D

Introduction

Periodontal disease is a chronic disease with underlying bacterial aetiology [1]. The bacterial aetiology of periodontal disease is complex, with a variety of organisms responsible for the initiation and progression of the disease [2]. The rate of disease progression is regulated by the impact of local, systemic or environmental factors that influence the normal host–bacterial interaction. Chronic periodontitis is also considered a potential risk factor for systemic diseases such as cardiovascular disease and type-2 diabetes mellitus (T2DM) [3].

Diabetes mellitus (DM) represents a group of metabolic diseases, characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action or both. Diabetes mellitus and its associated complications are some of the most crucial and alarming chronic health problems in the world, affecting around 415 million people, of which, approximately 90% of individuals present with T2DM. Diabetes increases the glucose concentration in the gingival crevicular fluid which further causes increase in plaque accumulation and change in its composition with increased number of gram-negative anaerobes. As a result, there is impaired cellular functions, impaired host defense, vascular alterations, prolonged inflammation, impaired bone formation or repair ultimately leading to increased severity of the disease resulting in tooth mobility and its loss. There is a two-way relationship between diabetes mellitus and periodontitis [4], where local periodontal infection if present, can exacerbate and cause progression of diabetes. The consequences of which are alterations in glucose metabolism and regulation resulting in difficulties in maintaining optimal glycemic control. Conversely, diabetes influences the periodontium mainly due to the classic microvascular and macrovascular complications, changes in subgingival microbiota and alterations in the host immuno-inflammatory response to potential periodontal pathogens [5].

There are several factors affecting the host immunity in inflammatory conditions, which also includes deficiency of vitamin D and calcium. Vitamin D influences the expression of inflammation related cytokines and plays an important role in many chronic inflammatory diseases. Also, association between vitamin D status and cardio-metabolic diseases, for example, metabolic syndrome, obesity, diabetes, and

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hypertension has been reported previously [4]. Thus, there is evidence relating vitamin D deficiency with both periodontal disease and T2DM [6]. The prevalence of vitamin D deficiency in the general population varies with ethnic background, sunlight exposure, and the presence of risk factors such as age, obesity, T2DM and other comorbidities. About one billion persons worldwide have been reported to have vitamin D deficiency or insufficiency [7].

Vitamin D plays a central role of the optimal functioning of cardiovascular, endocrine, and immune systems [8,9] along with its prominent role in calcium/phosphorus homeostasis and bone physiology [10]. It also helps in decreasing the risk of many chronic illnesses, like few cancers, autoimmune diseases, infectious diseases, hypertension, and cardiovascular diseases (CVDs) [8,9,11] CVD risk factors are also affected by serum calcium and parathyroid hormone. The active metabolite of vitamin D, 1,25 dihydroxy vitamin D inhibit cytokine production thus helping vitamin D exhibit potential anti-inflammatory effect via modulation of immune cells and stimulated secretion of peptides through monocyte–macrophage lineage [12–17]. Thus, vitamin D deficiency results in increased inflammation and bone loss by the virtue of its immuno-modulatory effects. Polymorphisms of the vitamin D receptor gene are found to be associated with periodontitis, alveolar bone loss, clinical attachment loss and/or tooth loss supporting the potential role of vitamin D in periodontal health [18]. Vitamin D may also improve insulin sensitivity and promote β-cell survival by directly modulating the generation and effects of cytokines. The direct effect of vitamin D may be mediated by binding of its circulating active form 1,25-OHD to the β-cell vitamin D receptor. The indirect effects of vitamin D may be mediated via its important and well-recognized role in regulating extracellular calcium and calcium flux through the β–cell [19].

There is preliminary evidence suggesting that calcium deficiency may also be one of the risk factors for periodontal disease [20], the association between the two was also studied later by Nishida et al. [21]. As the chronically low intake of calcium along with vitamin D may lead to a negative calcium balance, thus causing an increase in calcium removal from bone, including the alveolar bone as a secondary response. Calcium, along with vitamin D is also essential for insulin-mediated intracellular processes in insulin-responsive tissues with a very narrow range of calcium needed for optimal insulin-mediated functions. Changes in calcium in primary insulin target tissues may contribute to peripheral insulin resistance leading to decreased glucose transporter-4 activity. Insulin secretion is a calcium-dependent process and alterations in calcium flux can have adverse effects on β -cell secretory function [22]. Inadequate calcium intake or vitamin D insufficiency may interfere with normal insulin release, especially in response to a glucose load by altering the balance between the extracellular and intracellular β-cell calcium pools. Thus, altered vitamin D and calcium homeostasis may be contributing factors for the development of T2DM.

Dietrich et al. [23] in a study concluded that lower levels of vitamin D are related to inflammation in the gums, a precursor to gingivitis. Vitamin D can be supplemented orally for 2 to 3 months to achieve the desired results for patients with gingivitis, although 2000 IU of vitamin D per day can produce the anti-inflammatory effect sooner [24]. There is still need for more elaborate trials for better understanding the fluctuating vitamin D and calcium levels in inflammatory conditions like gingivitis, periodontitis and metabolic disease like diabetes mellitus which are co-related. Therefore, the present study was undertaken to evaluate and compare vitamin D and calcium levels in serum of periodontally healthy, chronic gingivitis and chronic periodontitis patients with and without T2DM with a hypothesis that vitamin D and calcium levels in serum of patients with gingivitis and chronic periodontitis with and without T2DM are reduced due to high inflammatory burden as compared to the healthy patients.

Material and methods

The present study included 100 patients attending the outpatient department, Department of Periodontics and Implantology, VSPM Dental College and Research Centre, Nagpur, India. Comparison of vitamin D levels of control and periodontitis group with T2DM resulted into higher effect size and thereby reduced sample size. Hence, in the proposed study, a sample of 20 patients per group was decided and accordingly the data were generated. The study design was reviewed and approved by the Institutional Ethics Committee and adhered to the provision of Helsinki declaration 1975, as revised in 2013 (IEC no. VSPMDCRC/IEC/PG/35 dated 18/07/17). Prior to the initiation of the study, an informed consent was obtained from all the patients who agreed to participate voluntarily. The study population which was 30–50 years of age were then categorized equally into 5 groups on the basis of periodontal (GI- gingival index, PI-plaque index, PPD- probing pocket depth, CAL- clinical attachment loss) and biochemical parameters (HbA1c-Glycated hemoglobin, RBS-random blood sugar), as mentioned in the Consort flow diagram (Figure 1) Chronic periodontitis was diagnosed as per the Armitage 1999 classification [25] periodontal disease classification. According to this classification, severity can be characterized on the basis of the amount of clinical attachment loss (CAL) as follows: Slight = 1 or 2 mm CAL, Moderate = 3 or 4 mm CAL, and Severe = ≥5 mm CAL. Orthopantomogram (OPG) was used to assess the bone loss in all participants [26]. Patients with duration of diabetes mellitus <5 years were selected for the study. All the diabetic participant were on oral diabetic agent.

1. Group I (PH) – Included patients who were systemically and periodontally healthy as assessed by
   GI < 1, PI < 1, PPD ≤ 3 mm,
   HbA1c levels < 6.5%, RBS levels ≤ 200 mg/dl.
2. Group II (CG) – Included patients with chronic gingivitis without T2DM i.e (CG) as assessed by
   GI ≥ 1, PI ≥ 1, PPD ≤ 3 mm,
   HbA1c levels < 6.5% and RBS levels ≤ 200 mg/dl.
3. Group III (CGDM) – Included patients with chronic gingivitis and with T2DM i.e (CGDM) as assessed by

GI $\geq$ 1, PI $\geq$ 1, PPD $\leq$ 3 mm.
HbA1c levels $\geq$ 6.5% and RBS levels $\geq$ 200 mg/dl.

4. Group IV (CP) – Included patients with chronic periodontitis and without T2DM i.e (CP) as assessed by
GI $\geq$ 1, PI $\geq$ 1, PPD $\geq$ 5mm and CAL $\geq$ 5mm,
HbA1c levels $<$ 6.5% and RBS levels $\leq$ 200 mg/dl.
5. Group V (CPDM) – Included patients with chronic periodontitis and with T2DM i.e (CPDM) as assessed by
GI $\geq$ 1, PI $\geq$ 1, PPD $\geq$ 5mm and CAL $\geq$ 5 mm,
HbA1c levels $\geq$ 6.5% and RBS levels $\geq$ 200 mg/dl.

Patients exhibiting any of the following conditions were excluded: (1) Medical disorders such as cardiovascular or renal disease, malignancies, multiple sclerosis, vitamin D deficiency disorders including bone diseases. (2) Tobacco chewers and smokers. (3) Pregnant, post-menopausal and lactating females. (4) History of antibiotic intake within 6 weeks. (5) History of any periodontal therapy within 6 months. (6) Patients on vitamin D supplementations.

Clinical procedures

All the clinical assessments, that is, GI [27], PI [28], PPD and CAL were performed on the participants before the start of the study by a single examiner (AK) using a manual periodontal probe (PCPUNC 15; Hu Friedy, Chicago, IL, USA). The measurements recorded on six sites around each tooth were rounded to nearest 0.5 mm. Readings were repeated by the same examiner (AK) 2 hours after the first measurement in order to perform intra-observer reproducibility analysis [29]. Intra-class correlation (ICC) coefficient with two-way mixed effects model were obtained for each measurement (between 0.92 and 0.99 in the groups, indicating excellent intra-examiner reproducibility. Plaque index and gingival index was scored at disto-facial, facial, mesio-facial and lingual surfaces by using mouth mirror and dental explorer. Full-mouth periodontal examination was performed with a Hu Friedy William's graduated periodontal probe, that is, PPD and CAL was measured in six sites per tooth (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, and disto-lingual) in all present teeth excluding third molars and the patients were categorized into healthy, gingivitis and chronic periodontitis groups accordingly.

Laboratory analysis

The blood glucose level of all the patients was tested by using Random blood sugar (RBS) and confirmed by glycated haemoglobin (HbA1c) tests on the basis of which the patients were grouped as diabetic and non-diabetic. Along with glucose levels, patients were also subjected to calcium tests.

A total of 4ml of blood was collected from antecubital fossa by venipuncture using a 20-gauge needle, serum was separated from blood by centrifuging at 30.24 g (g-force) for 5 minutes. The extracted serum was immediately transferred to a plastic vial and stored at $-80\,^{\circ}$C until assayed. Samples were assayed by another calibrated examiner (AA) for vitamin D levels using commercially available ELISA kit‡ and were analysed according to the instructions of the manufacturer protocol and at the Department of Biochemistry, NKP Salve Institute of Medical Sciences, Nagpur, India. Briefly serum samples were diluted with dilution buffer in the kit and the amount of vitamin D were determined. All samples were run in duplication.

Statistical analysis

The software used in the analysis was SPSS 17.0 and $p \leq .05$ was considered as the level of significance.

Analysis of the data was carried out by using descriptive and inferential statistics both. The sample size to ensure adequate power for this study was calculated based on the result of previous study [6], wherein, the authors reported mean vitamin D level of 22.3 ± 5.8 ng/dl in the control group, while 16.9 ± 5.6 ng/dl in the chronic periodontitis group. The data resulted into an effect size of 0.94. Accordingly, a sample of 19 patients will be required in each group that will provide this desired effect with 95% confidence level and 80% power. The data regarding the periodontal parameters like PPD, CAL, PI, GI for patients in all the five study groups was obtained. Frequency distribution and descriptive statistics like mean were obtained for periodontal parameters. Also, descriptive statistics obtained were compared across the five groups.

All intergroup comparisons were done by using Student's unpaired *t*-test. After intergroup comparisons, regression analysis was performed by keeping all the covariates constant and then evaluating the association of vitamin D and calcium with gingivitis and periodontitis across the groups using multiple regression tests.

Results

The mean levels of HbA1c in Group I were 4.65%. As shown in Table 1, all periodontal clinical parameters were significantly higher in case groups (Group II – Group V) as compared to control. Statistically significant difference ($p \leq .05$) in mean GI values between Group II (CG) and Group III (CGDM) were found (Table 2). As shown in Tables 2 and 3, the mean PPD and CAL values were also found to be statistically

Table 1. Mean of all the parameters for all groups.

	Mean				
Parameters	PH	CG	CGDM	CP	CPDM
Age (years)	44.65	41.45	46.23	39.29	46.22
GI	0.81	1.18	1.50	2.38	2.58
PI	0.36	1.38	1.39	2.57	2.62
PPD (mm)	2.12	2.28	2.57	5.79	6.38
CAL (mm)	0.0	0.0	0.0	6.50	6.70
HbAlc (%)	4.63	4.67	9.35	6.60	10.00
RBS (mg/dL)	97.65	180.35	241.87	170.76	263.5
Vitamin D (ng/ml)	66.87	49.05	37.22	26.94	18.05
Calcium (mg/dL)	10.99	10.41	8.38	7.24	5.93

PH: periodontally healthy (Group I); CG: chronic gingivitis without T2DM (Group II); CGDM: chronic gingivitis and with T2DM (Group III); CP: chronic periodontitis and without T2DM (Group IV); CPDM: chronic periodontitis and with T2DM (Group V).

Table 2. Clinical and biochemical parameters comparison of CG, CP and CGDM with PH group.

Parameters	PH	CG	CGDM	CP	CG vs. CP	PH vs. CG	CG vs CGDM	Parameters	PH
Mean ± SD									
GI	0.81 ± 0.42	1.18 ± 0.25	5.79 ± 1.22	1.50 ± 0.28	0.002‡	0.002‡	0.032§	0.001‡	0.001‡
PI	0.36 ± 0.30	1.38 ± 0.28	6.50 ± 1.35	1.39 ± 0.34	0.001‡	0.001‡	0.001‡	0.001‡	0.973
HbAlc (%)	4.63 ± 0.36	4.67 ± 0.37	6.60 ± 1.66	9.35 ± 3.75	0.001‡	0.965	0.002‡	0.001‡	0.001‡
RBS (mg/dL)	97.65 ± 47.47	180.35 ± 22.27	169.76 ± 41.59	241.87 ± 98.54	0.003‡	0.091	0.484	0.001‡	0.004‡
Vitamin D (ng/ml)	66.87 ± 6.92	49.05 ± 11.77	26.94 ± 1.84	37.22 ± 4.54	0.001‡	0.019§	0.001‡	0.001‡	0.001‡
Calcium (mg/dL)	10.99 ± 0.91	10.41 ± 0.94	7.24 ± 0.71	8.38 ± 1.30	0.091	0.055	0.088	0.001‡	0.001‡

§= significant; ‡ = highly significant.
PH: periodontally healthy (Group I); CG: chronic gingivitis without T2DM (Group II); CGDM: chronic gingivitis and with T2DM (Group III); CP: chronic periodontitis and without T2DM (Group IV); CPDM: chronic periodontitis and with T2DM (Group V).

Table 3. Clinical and biochemical parameters comparison of CP and CPDM with PH.

Parameter	PH	CP	CPDM	PH vs CP	PH vs CPDM	CP vs CPDM	CG vs CP
Mean ± SD							
PPD(mm)	2.12 ± 0.59	5.79 ± 1.22	6.38 ± 0.67	0.032§	0.041*	0.034§	0.046§
CAL(mm)	0.0	6.50 ± 1.35	6.70 ± 0.57	0.001‡	0.001‡	0.041§	0.001‡
HbAlc (%)	4.63 ± 0.36	6.60 ± 1.66	10.00 ± 2.42	0.002‡	0.001‡	0.003‡	0.029§
RBS (mg/dL)	97.65 ± 47.47	169.76 ± 41.59	263.50 ± 72.34	0.484	0.013*	0.003‡	0.127
Vitamin D (ng/ml)	66.87 ± 6.92	26.94 ± 1.84	18.05 ± 3.79	0.001‡	0.001‡	0.001‡	0.027§
Calcium (mg/dL)	10.99 ± 0.91	7.24 ± 0.71	5.93 ± 0.84	0.088	0.036§	0.001‡	0.014§

§= significant; ‡ = highly significant.
PH: periodontally healthy (Group I); CG: chronic gingivitis without T2DM (Group II); CGDM: chronic gingivitis and with T2DM (Group III); CP: chronic periodontitis and without T2DM (Group IV); CPDM: chronic periodontitis and with T2DM (Group V).

significant on intergroup comparisons except for that between Group I (PH) and Group III (CGDM).

Tables 2 and 3 show the mean RBS values of Group V (CPDM group) was 263.50 ± 72.34 mg/dl and Group III (CGDM group) was 241.87 ± 98.54 mg/dl which well-differentiated diabetics from non-diabetics (Groups I, II, IV). Similarly, the greater mean HbA1c values in Group III (CGDM) (9.35 ± 3.75%) and Group V (CPDM) (10 ± 2.42%) helped to differentiate these groups from Groups PH, CG and CP.

A gradual decrease in vitamin D level was found from Group PH to Group CPDM with statistically significant difference in vitamin D levels on intergroup comparisons as depicted in Tables 2 and 3. The mean vitamin D levels were found to be least i.e 18.05 ± 3.79 ng/ml in Group CPDM and highest in Group PH, that is, 66.87 ± 6.92 ng/ml. The inter-group comparisons between Groups CG and CGDM and in between Groups CP and CPDM was statistically significant with $p = .001$. Also, the intergroup comparisons Groups CG vs. CP and Groups CGDM vs. CPDM as shown in Table 3 was found to be statistically significant with $p = .001$ and $p = .027$ respectively. When each of these groups were compared with the control Group PH, statistically significant difference was found in between all the groups with Group PH ($p \leq .001$).

The mean calcium levels were found to be least, that is, 5.93 ± 0.84 mg/dL in Group CPDM and 7.24 ± 0.71 mg/dL in Group CP followed by 8.38 ± 1.3 mg/dL in Group CGDM, 10.41 ± 0.94 mg/dL in Group CG groups and highest in Group PH, that is, 10.99 ± 0.91 mg/dL. The inter-group comparisons between Group CG and Group CGDM, in between Group CP and Group CPDM were found to be statistically significant with p value of .001 as depicted in Table 2. Table 3 reveals statistically significant difference on intergroup comparison of calcium levels between groups CGDM and CPDM with p value of .014. When each of these groups were compared with the control Group PH, statistically significant difference was found with respect to Group CGDM (p value of .001) and Group CPDM (p value of .036).

Table 4. Regression analysis of vitamin D with Chronic Periodontitis parameters with DM.

	Unstandardized coefficients		Standardized coefficients		
Model	B	Std. error	Beta	*t* Value	*p* Value
PPD	−3.463	5.202	−0.541	−0.666	0.515
CAL	3.622	5.596	0.526	0.647	0.526

$R = 0.160$, $R^2 = 0.025$, Adjusted $R^2 = -0.089$.
$F = 0.222$, $p = .803 > .05$ (not significant), Standardized Error of the estimate = 3.62560.

When multiple regression analysis of vitamin D was done with chronic periodontitis parameters with T2DM, vitamin D was found to have a positive influence on CAL (Table 4, Figure 2(a)). Similarly, on keeping the other periodontal parameters constant in patients with T2DM, calcium had positive influence on CAL (Table 5, Figure 2(b)).

Discussion

Diabetes mellitus is known to cause an increased prevalence, severity and rapid progression of periodontal disease [30,31]. Type-2 diabetes mellitus had been recognized to be an independent risk factor for chronic periodontitis, with the three fold increased risk in diabetics as compared to non-diabetics [32]. It is said that cytokines which are secreted as a result of gingivitis may enhance insulin resistance which may further cause or exacerbate diabetes. Both periodontal infection and diabetes when concurrently present may reciprocate the ill effects of each other [33]. In the present study also, the increased and statistically significant mean values of PPD and CAL in Group CPDM as compared to Group CP, determines the increased periodontal destruction in periodontal disease patients with diabetes. These findings are similar to the ones reported by Choi et al. [34], where the authors observed that

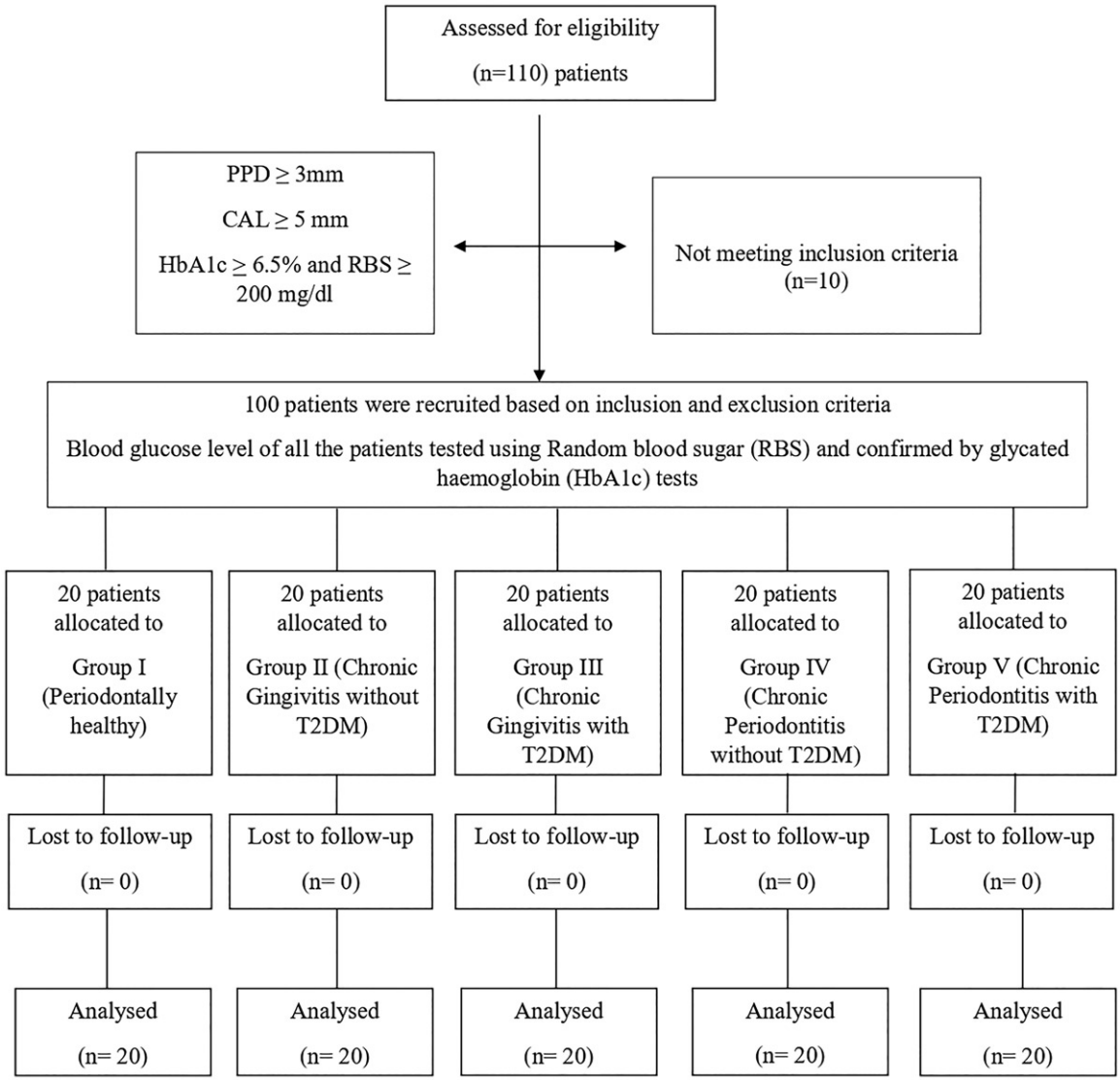

Figure 1. Consort flow diagram.

Table 5. Regression analysis of calcium with chronic periodontitis parameters with DM.

	Unstandardized coefficients		Standardized coefficients		
Model	B	Std. error	Beta	*t* Value	*p* Value
PPD	−2.273	0.768	−1.978	−2.959	0.009*
CAL	2.313	0.826	1.871	2.800	0.012*

*: significant.
R: 0.1583, R^2=0.340, Adjusted R^2: 0.263.
F: 4.383, $p = .029 < .05$(Significant), Standardized Error of the estimate: 0.53526.

the highest quintile of pocket depth and clinical attachment loss were possibly associated with impaired fasting glucose and diabetic status of the patients.

The increased probing depth in Group CGDM as compared to Group CG corresponds to raised RBS and HbA1c values in Group CGDM, similarly increased clinical attachment loss (6.70 ± 0.57mm) corresponds to the raised glycated haemoglobin level in Group CPDM as compared to AL (6.50 ± 1.35mm) in Group CP, suggestive of increased periodontal destruction in the presence of raised RBS and HbA1c levels in Groups CGDM and CPDM. Thus, the maximum destruction of periodontal tissues as indicated by increased pocket depth and clinical attachment loss was appreciated in Groups CGDM and CPDM. This can be attributed to several interacting factors such as altered polymorphonuclear cell function and derangements of inflammatory protein response coverage at the periodontium resulting in a higher prevalence and severity of periodontitis. In terms of mean HbA1c levels across these study groups, our results are in accordance with Apoorva S et al. [35] who studied the prevalence and severity of periodontal disease in T2DM patients and reported that as the glycated hemoglobin level increases, the severity of periodontal disease increases. However, in our study increased inflammatory burden in presence of raised glucose levels can be appreciated not only in Group CPDM but also in Group CGDM and was estimated with the help of RBS as well.

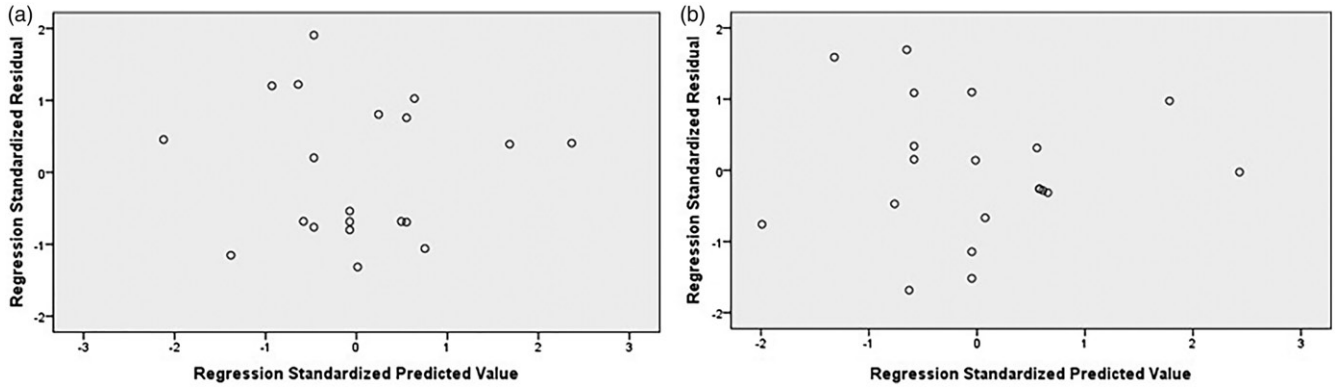

Figure 2. **a.** Regression analysis of vitamin D with chronic Periodontics parameters with T2DM. **b.** Regression analysis of calcium with chronic periodontics parameters with T2DM.

The vitamin D levels, an important finding of our study was found to be gradually decreasing from Groups PH to CPDM, with least in Group CPDM, that is, 18.05 ± 3.79 ng/ml. On the contrary, increase in probing pocket depth and clinical attachment loss can be appreciated from Group PH to Group CPDM indicating the reduction in vitamin D levels with increasing severity of inflammation. These findings are consistent with that of Dietrich et al. [36], who reported an inverse association between serum vitamin D and periodontal attachment loss and a strong negative association between serum vitamin D and gingival inflammation was also reported in one of his studies. As we estimated vitamin D and calcium levels in differing severities of inflammation (gingivitis and periodontitis) in the same study, a direct comparison of vitamin D levels within groups Group CG and CP revealed highly significant differences ($p = .001$) as depicted in Table 3. This is possibly because vitamin D has been found to inhibit cytokine production and cell proliferation in various tissues [12] and could thus affect the inflammatory resorption of alveolar bone [13]. Statistically significant low levels of vitamin D in Group CGDM and CPDM as compared to Group CG and CP, suggests that the vitamin D level decreases with increase in RBS and HbA1C levels thus resulting in an inverse correlation between vitamin D and HbA1c and RBS (Table 2).

Thus, vitamin D levels were found to be inversely related to RBS and HbA1c levels (Table 2). A meta-analysis for observational studies showed a relatively consistent association between low vitamin D status and T2DM [37], which might be due to the possibility that low vitamin D levels improved lipid metabolism disorder which lead to the disturbance in glucose metabolism sequentially. Thus, significant correlations were appreciated between vitamin D and RBS, HbA1c (Table 3). Lu et al. [38] reported that in their study population, the majority of patients with T2DM were vitamin D deficient.

Joseph et al. [6] observed low level of serum vitamin D levels in patients with chronic periodontitis and chronic periodontitis with T2DM and reported that this association could be due to the disease process rather than low vitamin D levels acting as a cause. Unlike this study we not only opted for glycated hemoglobin, a confirmatory blood glucose test but also estimated calcium levels along with vitamin D levels in Group CGDM along with Group CPDM.

As seen in Table 2, intergroup comparisons between Group II (CG) and Group III (CGDM) also Group IV (CP) and Group V (CPDM) revealed lesser vitamin D levels in T2DM groups as compared to Group CG and CP with highly significant differences ($p = .001$). Thus, an inverse correlation of vitamin D levels was found with both diabetes mellitus and inflammatory conditions like gingivitis and chronic periodontitis.

In an examination of data on 12,000 adults who took part in the Third National Health and Nutrition Examination Survey, it was found that low dietary intake of calcium increased attachment loss in a dose-dependent fashion [23]. Similar findings were observed in our study, wherein decreased calcium levels were found in Group CP as compared to Group CG and controls (PH) and thus was inversely correlated to both clinical attachment loss and probing pocket depth (Tables 3 and 5).

Vitamin D that is produced in the kidney in response to low serum calcium levels is transported to the small intestine and bone, where it interacts with vitamin D receptors to increase calcium absorption in the intestine and to release it from bone [39]. It also travels to various tissues, where it binds to membrane receptors and opens calcium channels [39]. This in turn can result in obesity leading to diabetes. Sarda T et al. [40] in their study confirmed a positive and significant association between obesity and chronic periodontitis. In support to these evidences, we estimated and compared serum calcium levels also across all the study groups. As depicted in Table 3, in our study, an inverse correlation was obtained between calcium levels and RBS and HbA1c levels on intergroup comparison between Groups CGDM and CPDM with a statistically significant difference ($p = .014$).

Our study groups were divided in such a way that the vitamin D and calcium levels could be estimated and compared in patients with different severities of inflammation and with/without diabetes. Thus, on the basis of the findings obtained, it can be stated that vitamin D and calcium levels found in chronic gingivitis and chronic periodontitis with T2DM were less as compared to other groups, that is,

Group CP and CPDM, thus suggesting the decline in vitamin D and calcium values with increase in severity of inflammation. Even though we took covariates into account, there could have been some other confounding variables such as sun exposure, dietary intake, regular exercise, all of which contributes to vitamin D synthesis but are not considered in the present study and thus is a limitation of this study.

It is emphasized that nutrition also has an impact on the inflammatory process. The present study also supports the previous evidence, where Miley D et al. [41] in their study found that in patients receiving periodontal maintenance therapy, there was a trend for better periodontal health with vitamin D and calcium supplementation.

Our study however, presents certain limitations such as smaller sample size for each group. Influence of gender on the vitamin D and calcium levels was not assessed, since vitamin D deficiency is predilected in females than males. Also, the duration of diabetes mellitus was not taken into consideration into the inclusion criteria for the study. However, taking into account the limitations of the study, it presents with an overview of influence of levels of vitamin D and calcium levels in different categories of periodontal disease and diabetic patients.

Conclusion

Within the limits of our study, it can be concluded that low vitamin D and calcium levels are associated with gingivitis and periodontitis. Not only inflammatory conditions, but it also affects the glycaemic status of individuals. Thus, the patients diagnosed with both periodontal disease and type 2 diabetes mellitus (T2DM) can be vitamin D and calcium deficient. Vitamin D and calcium levels are inversely correlated with random blood sugar and glycosylated haemoglobin and also probing pocket depth and clinical attachment loss, thus may contribute towards increase in the severity of periodontal disease activity. However, randomized interventional trials are needed to further substantiate these findings, before supplementation of vitamin D can be recommended for prevention of periodontitis with/without diabetes.

‡ – Krishgen Biosystems Vitamin D ELISA kit, India.

Disclosure statement

No potential conflict of interest was reported by the authors.

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