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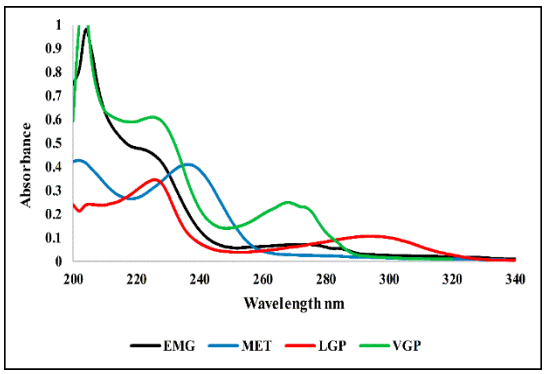

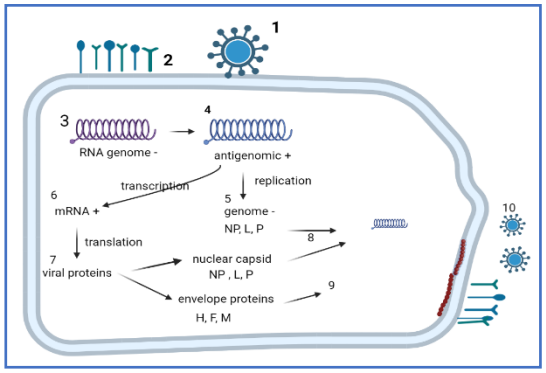

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Vitamin D and C Supplementation as an Adjunct to Non-surgical Therapy in the Treatment of Chronic Periodontitis: A Clinical Study

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Abstract

Background: Periodontitis is a common disorder affecting the bone and soft tissues of the periodontal complex. When untreated, it may lead to severe mobility or even loss of teeth. Recent studies highlight the alleged role of vitamin C (Vit C) and vitamin D (Vit D) in developing the disease. **Objectives:** To summarize recent knowledge on the role of Vit C and Vit D in the pathogenesis and treatment of periodontitis. **Patients and methods:** Forty-five patients with moderate to severe chronic periodontitis were selected and randomly divided into three equal groups. The first group was the control group, which underwent scaling, polishing and root planning (SRP; conventional periodontal treatment) without medication. The second group underwent SRP and received Vit D3 (1000 IU) with Vit C (500 mg) orally and daily. The third group received Vit D3 (1000 IU) with Vit C (500 mg) orally and daily without SRP. All treatments were continued for two months. Then, plaque index (PI), gingival index (GI), and clinical attachment loss (CAL) were evaluated after two months. **Results:** Significant PI, GI, and CAL reductions were detected after two months of periodontal treatment with Vit D3 and C supplementation in the second group compared to other groups. **Conclusions:** Vit C and Vit D reduced gum bleeding and can be used to improve patient's quality of life.

Introduction

Gingivitis is inflammation of the gingiva, defined as inflammation of the gingiva in which the junctional epithelium remains attached to the tooth at the original level [1]. Periodontitis is a ubiquitous chronic inflammatory disease affecting the supporting tissues of the teeth. The disease is caused by infection with specific microorganisms, leading to progressive destruction of the periodontal ligament (PDL) and alveolar bone. The progression appears to depend on abnormal host response to the biofilm organisms [2].

Well-known and preventable biological risk factors related to periodontitis are diabetes, high blood pressure, genetic factors, obesity and high blood cholesterol. In contrast, behavioral risk factors are an unhealthy diet, physical inactivity, and tobacco use [3]. When periodontitis develops, reactive oxygen species (ROS) are mostly overproduced by hyperactive neutrophils, cannot be balanced by the body's antioxidant defense system, and lead to tissue damage. This is characterized by protein damage, increased metabolites of lipid peroxidation, and DNA damage [4]. Chronic periodontitis/gingivitis is stimulated and sustained by the pathogens of the dental plaque. The microbial biofilm comprises up to 150 species in a single person, and up to 800 species have been identified in dental plaque [5].

Furthermore, it requires a combination of periodontal therapeutic modalities to prevent disease progression and symptoms and possibly restore lost tissues according to the patient's periodontal status. The treatment can include oral hygiene, sub-gingival instrumentation to remove plaque and calculus, local and systemic pharmacotherapy and periodontal surgery [6].

Evidence addressing nutritionally adequate approaches must be generated to prevent periodontal disease. Several epidemiological studies have evaluated the association between vitamin C intake and periodontal disease. In addition, the ability of vitamin C to avoid periodontal disease has been analyzed in clinical studies [7]. Vit C belongs to the scavenging (chain-breaking) group of antioxidants [8]. It plays a vital role in maintaining the integrity of connective tissue, osteoid tissues, and dentine, apart from its activity as an enzyme cofactor in its ionic form as ascorbate [9]. It is also considered an important dietary antioxidant in the maintenance of periodontal health and decelerates the rate of progression of periodontitis by stimulating the differentiation of progenitor cells of the periodontal ligament [10].

Vitamin D is a fat-soluble vitamin obtained from sunlight and diet [11]. 25(OH)D is used to assess vitamin D levels since it is present in the circulation for 20 days, unlike 1,25(OH)2D, which is present only for four hours. No consensus exists on the optimal Vit D level, but Vit D <20 ng/mL is considered insufficient [12]. 1,25(OH)2D works through the Vit D nuclear receptor (VDR), which functions as a transcription factor to modulate multiple biological processes, including inflammation, cell-mediated immunity, calcium/bone homeostasis, and apoptosis [13]. The VDR/1,25 (OH)2D pathway could facilitate antibacterial, antiviral, and anti-inflammatory action. This pathway influences bone remodeling mechanisms by triggering the differentiation of osteoclasts and osteoblasts. Consequently, Vit D deficiency affects bone density, leading to pathological fracture, osteomalacia, and osteoporosis [14,15].

Recently, the role of vitamin D in periodontal disease pathogenesis has been established as affecting bone mineral density and resorption [16]. Vit D deficiency has been predicted to influence the initiation and progression of periodontal disease [17]. So, this study aimed to evaluate the clinical efficacy of systemic use of Vit D and C as an adjunct to SRP in treating chronic periodontitis patients.

Materials and methods

Study setting and design

A randomized clinical controlled trial enrolled 45 patients at the Dental Clinic of the College of Dentistry, University of Duhok, Duhok, Iraq, from January 2023 to December 2023.

Study protocol

Patients (no=45) were randomly allocated into three equal groups. Group 1 (control group) patients did not receive any medication, just scaling and root surface debridement (SRP), and they had normal serum Vit D. Group 2 received Vit D3 and Vit C with SRP. They had insufficient Vit D. Group 3 received Vit D3 and Vit C alone, and they had inadequate Vit D. All treatments continued for two months. Vit D was given orally/daily at 1000 IU, while Vit C was given orally/daily at 500 mg.

Inclusion criteria

Patients aged 20–55 years old suffering from moderate to severe chronic periodontitis, with the presence of a periodontal pocket of ≥5 mm, indicated for SRP and radiographic bone defects of ≥3 mm.

Exclusion criteria

Pregnant/lactating women, those with exposed bifurcation areas in molars, those with systemic illnesses, those on medications (antibiotic treatment within the past six months and received periodontal therapy within the past three months) and smokers were excluded.

Laboratory investigation

Venous blood was taken to measure serum 25(OH)D3 concentrations before and after one month and two months of periodontal treatments. Vit D levels were categorized into sufficient (>20 ng/ml), insufficient (<20 ng/ml), and deficient (<12 ng/ml) [18].

Clinical examination

Clinical examinations were done before treatments and one month and two months after treatments using UNC15 Probe that has circumferential markings at 5, 10, and 15 mm with a dental mirror, including plaque index (PI), gingival index (GI), probing pocket depth (PPD), and clinical attachment level (CAL). PI was estimated by measuring the thickness of plaque on all teeth for four surfaces, mid labial (buccal), disto-labial (buccal), mesio-labial (buccal) and mid lingual(palatal) surfaces and given a score from 0-3 (Table 1) [19].

Table 1. Criteria used for measuring plaque index (PI) [19].

Score	Criteria
0	No plaque
1	A thin layer of plaque on the gingival margin was only detected by scraping the probe
2	There is a moderate layer of plaque on the gingival margin; interdental space is free, but plaque is visible to the naked eye
3	There is abundant plaque along the gingival margin; interdental space is filled with plaque.

GI was estimated by measuring the extent and severity of gingival inflammation for mid-labial (buccal), disto-labial (buccal), media-labial (buccal) and mid-lingual (palatal) surfaces and giving a score from 0-3 (Table 2) [19].

Table 2. Criteria used for measuring gingival index (GI) [19].

Score	Criteria
0	Normal gingival
1	Mild inflammation: a slight change in colour and slight oedema. No bleeding on probing
2	Moderate inflation: redness, oedema and glazing. Bleeding on probing
3	Severe inflation: Marked redness, oedema, ulceration and tendency toward the spontaneous bleeding

PPD was assessed by measuring the distance from the free gingival margin to the base of the pocket, and clinically normal gingival sulcus in humans is 2-3 mm. The examination was done by three readings for the labial/buccal site (mesio-buccal, mid-buccal, and disto-buccal) and one reading for the lingual/palatal site(mid-palatal). CAL was estimated by measuring the distance from the free gingival margin to the cementoenamel junction and from the free gingival margin to the bottom of the gingival sulcus in millimetres. If the free gingival margin is on the cementum, its distance from the cementoenamel junction is recorded as unfavourable. Then, oral hygiene instruction was given to the patient about brushing techniques, mouthwash, and dental floss.

Ethical considerations

Permission from the College of Dentistry, University of Duhok, Duhok, Iraq, was taken before starting the study. Written informed consent was also obtained from volunteers.

Data analysis

Obtained data were analyzed using Statistical Package for Social Science (SPSS, Chicago, USA, version 25). The paired t-test test was used for categorical variables, while the analysis of variance (ANOVA) test was

used to determine the difference in numerical variables. P-value considered as high significant ($p<0.001$), significant ($p<0.05$), non-significant ($p>0.05$), and very highly significant ($p<0.000$).

Results

In the first group, the mean difference between the variables (CAL, PPD, GI, and PI) at baseline and after two months of treatments is highly significant ($p=0.000$). The mean difference between Vit D at baseline and after 2-month treatments is insignificant ($p=0.333$) (Table 1).

Table 1. Intra-comparison of parameters among patients of the control group.

Variable		Mean	SD	p-value
CAL	Baseline	4.20	0.414	0.000**
	After two months	3.27	0.536	
PPD	Baseline	5.20	0.414	0.009**
	After two months	4.80	0.414	
GI	Baseline	3.00	0.000	0.000**
	After two months	0.749	0.036	
PI	Baseline	2.75	0.332	0.000**
	After two months	1.32	0.607	
Vit D	Baseline	9.34	1.510	0.333
	After two months	82.44	282.884	

PI: Plaque index, GI: Gingival index, PPD: Probing pocket depth (PPD), CAL: Clinical attachment level, Vit D: Vitamin D. **: Highly significant difference using paired t-test

In the second group, there are highly significant differences between the variables (CAL, PPD, GI, and PI) at baseline and after two months of treatments ($p=0.000$) (Table 2).

Table 2. Intra-comparison of parameters among patients of the second group.

Variable		Mean	SD	p-value
CAL	Baseline	4.266	0.961	0.000**
	After two months	2.513	1.555	
PPD	Baseline	2.500	0.582	0.000**
	After two months	0.913	0.264	
GI	Baseline	2.759	0.639	0.000**
	After two months	1.046	0.489	
PI	Baseline	5.333	0.617	0.000**
	After two months	3.666	0.899	
Vit D	Baseline	15.610	2.072	0.000**
	After two months	18.900	1.365	

PI: Plaque index, GI: Gingival index, PPD: Probing pocket depth (PPD), CAL: Clinical attachment level, Vit D: Vitamin D. **: Highly significant difference using paired t-test

In the third group, there are no significant differences between the variables (CAL, PPD, and PI) at baseline and after two months of treatments ($p>0.05$). At the same time, a significant difference between the variables (GI and Vit D) at baseline and after two months of treatments was seen ($p<0.05$) (Table 3).

The ANOVA test results indicate significant differences ($p<0.05$) in the means of GI2 and PI2, with highly significant differences ($p<0.000$) in the means of PPD2 and D across groups. At the same time, no significant differences ($p>0.05$) were observed for each CAL, CAL2, GI, PI, PPD2, and Vit D2 among groups (Table 4).

Table 3. Intra-comparison of parameters among patients of the third group.

Variable		Mean	Std. Deviation	P-value
CAL	Baseline	3.586	1.322	0.25
	After two months	2.933	1.416	
PPD	Baseline	4.286	6.585	0.06
	After two months	2.453	3.281	
GI	Baseline	2.566	0.717	0.006*
	After two months	1.549	0.700	
PI	Baseline	6.553	10.733	0.26
	After two months	3.293	1.085	
Vit D	Baseline	8.886	1.742	0.008*
	After two months	12.353	3.406	

PI: Plaque index, GI: Gingival index, PPD: Probing pocket depth (PPD), CAL: Clinical attachment level, Vit D: Vitamin D. *: Significant difference using paired t-test

Table 4. Comparison between three groups at baseline and after two months.

Variable		Sum of Squares	df	Mean Square	F	p-value
CAL	Between Groups	4.215	2	2.108	2.222	0.121
CAL2	Between Groups	4.706	2	2.353	1.750	0.186
GI	Between Groups	25.488	2	12.744	0.875	0.424
GI2	Between Groups	31.248	2	15.624	4.470	0.017*
PI	Between Groups	0.360	2	0.180	0.522	0.597
PI2	Between Groups	2.777	2	1.389	4.145	0.023*
PPD	Between Groups	16.688	2	8.344	0.216	0.806
PPD2	Between Groups	22.478	2	11.239	16.838	0.000**
Vit D	Between Groups	423.695	2	211.847	66.120	0.000**
Vit D2	Between Groups	45600.519	2	22800.260	0.855	0.433

PI: Plaque index, GI: Gingival index, PPD: Probing pocket depth (PPD), CAL: Clinical attachment level, Vit D: Vitamin D. *: Significant difference; **: Highly significant difference using ANOVA test

Discussion

The periodontitis prevalence ranges from 10 to 90% in adults [20]. Factors underlying oral bacterial infection can lead to periodontal disease and bone destruction [21]. The role of vitamins begins to attract the attention of researchers and clinicians. Non-surgical treatments such as taking vitamins can reduce periodontal inflammation [22]. Several studies hypothesized that reduced serum levels of vitamin D may be intimately correlated with persistent periodontal diseases [23]. The long-term study of the relationship between vitamin D levels and periodontal diseases remains diverse. Thus, the present study aimed to determine whether vitamin D deficiency is significantly associated with deteriorated periodontal status.

In this study, we found that daily supplementation with Vit D (1000 IU) and Vit C (500 mg) for two months resulted in a significant elevation in Vit D levels and significant improvement of GI, PI, CAL and PPD for moderate and deep pockets in patients with moderate to severe periodontitis. In addition, Vit D supplementation was well tolerated, and no adverse events were reported. There is conflicting evidence regarding the association between vitamin D and periodontal disease. Most cross-sectional studies reported

that higher serum concentrations of vitamin D were associated with low disease prevalence [17,24] and mean CAL and PPD [25].

Also, Vit C has an inhibitory effect on oxidative stress and the synthesis of proinflammatory mediators, while Vit D has an inhibitory effect on the secretion of proinflammatory cytokines [26]. This influence on the inflammatory process could have a beneficial effect on periodontitis. In this study, the intervention group received conventional periodontal care with pharmaceutical intervention (Vit D3 1000 IU + Vit C 500 mg) for the treatment of Vit D deficiency, and the results showed a significant decrease in the clinical periodontal among patients to control and non-interventional group (First and third groups). Similarly, in a clinical trial study, the test group was supplemented with a combined dose of calcium (500 mg) and Vit D (250 IU) once daily for three months in addition to scaling and root planning. Intergroup comparison of clinical parameters (GI, PI, PPD, and CAL) at the end of the study showed a significant difference ($p<0.05$) with no significant difference for Vit D. The non-significance in the latter measurements might be due to the low dose of Vit D the patients received [27]. Current guidelines propose a dose of 50,000 IU of Vit D taken orally once weekly for up to 3 months to treat Vit D deficiency, after which a maintenance dose of 800 to 2000 IU is needed to avoid recurrent deficiency [28].

Furthermore, several studies demonstrate improvements in periodontal health with Vit D supplementation [14,29]. However, adequate/optimal Vit D status levels and optimal doses of Vit D supplementation remain uncertain. The biological effects of vitamin D have been demonstrated to be related to its serum concentrations [30]. Thus, vitamin D serum levels should be maintained at ≥75 nmol/L, and optimal levels of vitamin D supplementation used for treating most chronic diseases should range from 90-100 nmol/L [31].

In the present study, the GI index showed a significant difference between study groups ($p<0.0001$), with a higher median value in the third group compared to other groups. The second group had a lower median GI compared to the third group. The significant decrease in the GI values in the test group at 2-month intervals may be attributed to the anti-inflammatory effects of vitamin D supplements, which is another study [14]. Botelho et al. investigated the relationship between serum Vit D levels and periodontal disease. They indicated that serum Vit D levels were significantly lower in chronic periodontitis patients, which aligns with the present study [32]. Also, Millen et al. reported correlations between serum Vit D levels and periodontal disease in postmenopausal women. They found that Vit D status was inversely associated with periodontal disease, which was confirmed by the present study [33].

Moreover, in another study, the effect of periodontitis and systemic sclerosis on vitamin D levels was investigated in a randomized experimental study. Vit D level was negatively correlated with probing depth, clinical attachment level, and bleeding on probing. Vit D levels in patients with periodontitis were significantly lower than those without [34]. Kim et al. investigated whether serum Vit D levels are associated with periodontal disease status and tooth loss. The PD was ≥4 mm, and CAL was ≥4 mm. Low serum vitamin D levels were highly related to tooth loss and severe periodontitis [35]. Also, GI and CAL determined a significant association between CAL and vitamin D levels in Canadian people [32].

Conclusions

Both vitamin D and C may decrease gum bleeding, which can be used to improve a patient's quality of life. A growing number of studies suggest that adequate vitamin C and vitamin D intake could benefit the prevention and treatment of periodontal disease. However, further research regarding vitamins' role is crucial to understanding their effect on periodontal tissues.

Conflict of Interest

The authors declare no conflicts of interest regarding this manuscript's publication and/or funding.

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