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# EVALUATION OF THE EFFECT OF VITAMIN D ON THE SALIVARY AND SERUM LEVELS OF INTERLEUKIN-8 IN PATIENTS WITH PERIODONTITIS STAGE I AND II GRADE A

Ahmed Elmorsy Saleh Elmorsy [1*], Mohamed.M. Fekry Khedr [2], Mostafa Mohamed Hosny [3]

## *ABSTRACT*

**Objectives:** Evaluate the effect of vitamin D in conjugation with phase 1 periodontal therapy on the GCF, salivary and serum level of IL-8 in periodontitis stage I and II grade A patients. **Subjects and Methods:** A total of 40 medically healthy adult subjects with periodontitis stage I and II grade A. Twenty patients received a full mouth supra –and sub-gingival scaling and root planning, in addition to vitamin D (one alpha 0.5mcg) one capsules daily for 6 months. Control group: twenty patients received a full mouth supra – and sub-gingival scaling and root planing only. Samples Collection of Gingival Crevice Fluid, blood samples and saliva before treatment and 3 and 6 months after: Clinical evaluation and Serum level of vitamin D, Serum, salivary and GCF levels of IL-8. **Results:** Study group after treatment for 6 months showed a higher mean vitamin D % increase (↑117.41 ± 46.82) from baseline than control group (↑2.77±20.34) The results showed, also,that study group reported a reduction in GCF IL-8 % while control group increased, at both observation period of 3and 6 month. At 3 months, study group showed a reduction in Serum IL-8% from baseline than control group. At 6 months, Study group showed a reduction in Serum IL-8% from baseline while control group increased. There was a negative correlation between vitamin D and IL-8. **Conclusion:** Vitamin D supplementation may reduce the severity of periodontal disease. Vitamin D may have a potential role in the inhibition of periodontal inflammation by inhibiting the IL-8 expression.

**KEYWORDS**: Periodontitis stage I and II grade A, vitamin D, interleukin-8.

## INTRODUCTION

Periodontitis is an inflammatory disease of the periodontium, characterized by loss of connective tissue and alveolar bone, resulting in tooth loss eventually. Dental plaque is the primary cause for periodontitis, however the nutritional and endocrinological conditions can influence the host's susceptibility to periodontal disease [(1)].

Vitamin D is a fat-soluble vitamin that occurs naturally in very few foods, is added to other foods, and is available as a dietary supplement. It is also produced endogenously when ultraviolet rays from sunlight hit the skin and stimulate vitamin D synthesis. It is biologically inert and must undergo two hydroxylations in the body to activate. The first occurs in the liver and converts vitamin D to

1. Masters Candidate, Dentist at Ministry of Health
2. Professor, Department of Oral Medicine, Periodontology, Oral Diagnosis and Radiology, Faculty of Dental Medicine. Vice president of Al-Azhar University for the girls branch
3. Ass. Professor, Department of Oral Medicine, Periodontology, Oral Diagnosis and Radiology, Faculty of Dental Medicine. Cairo, Boys, Al-Azhar University

• ***Corresponding author*:** dr.dentist.201216@gmail.com

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25-hydroxyvitamin D [25 (OH) D], also known as calcidiol. The second occurs primarily in the kidneys and forms the physiologically active 1,25-dihydroxyvitamin D [1,25 (OH) 2D], also known as calcitriol [2].

Studies have shown a significant association between periodontal health and vitamin D and calcium intake. And dietary supplements containing calcium and vitamin D can improve periodontal health, increase bone mineral density in the lower jaw, and prevent alveolar bone resorption [3–4]. It has been discovered that vitamin D can be actively involved in regulating the immune system and inflammation, and vitamin D exerts its biological effects by binding to the vitamin D receptor (VDR). Previous research has shown the link between VDR gene polymorphisms and periodontal disease, suggesting that vitamin D / VDR may play an important role in the development and course of periodontal disease, but it is uncertain whether vitamin D is used to improve gum health or to help it Damage from periodontal disease can contribute [5].

Vitamin D might affect periodontal disease through the effect on bone mineral density (BMD) and through immunomodulatory effects as it is well established as being essential for bone growth and preservation. In the elderly, supplementation with vitamin D and calcium is effective in preventing non vertebral fractures. A potential anti-inflammatory effect of vitamin D may be attributed to active metabolite of 25-hydroxyvitamin D, 1,25 dihydroxyvitamin D, ability to inhibit cytokine production and cell proliferation [6].

Interleukin-8 (IL-8) is an important proinflammatory cytokine and chemokine, which can potently induce the chemotaxis in its target cells, e.g, polymorphnuclear leukocytes, leading to periodontal inflammation and tissue destruction[7]. IL-8 can also stimulate the granule exocytosis and release of myeloperoxidase, elastase, and b-glucuronidase. Excessive IL-8-mediated processes within the periodontal tissues may contribute to local periodontal tissue destruction [8,9]. In view of thesis ,it was felt that measuring the level of IL-8 in saliva and serum of patients with periodontitis stage I and II grade A may aid in clarification of role of Vit D and IL-8 in natural history of periodontitis stage I and II grade A.

## SUBJECTS AND METHODS

**Study design:** Analytical descriptive study

A total of 40 medically healthy adult subjects with periodontitis stage I and II grade A attending at the Department of Oral Medicine and Periodontology, Faculty of Dental Medicine (Boys, Cairo) Al-Azhar University, were included in this study.

**Inclusion criteria:**

Patients diagnosed as having mild to moderate periodontitis stage I and II grade A; with probing pocket depth ≥4mm and clinical attachment loss >1mm in 30% of sites with radiographic evidence of bone loss. Patients had a free medical history according to criteria of dental modification of Coronall Medical Index [10].

**Exclusion criteria:**

1. Patients with renal disorders and hyperparathyroidism and hypercalcemia.
2. Patients with sensitivity to the medication used in the study i.e. (one alpha 0.5mcg)
3. Patients with previous periodontal treatment including scaling and root planing and periodontal surgery in the last 3 and 6 months, respectively.
4. Smokers, pregnant, and lactating women.

**Groups:** Study group: twenty patients received a full mouth supra –and sub-gingival scaling and root planning, in addition to vitamin D (one alpha 0.5mcg) one capsules daily for 6 months. Control group: twenty patients received a full mouth supra – and sub-gingival scaling and root planning only.

**Samples Collection of Gingival Crevice Fluid[11]:** Subjects selected for the study were instructed to sit comfortably in an upright position on the dental chair with proper illumination. Crevicular fluid was obtained by placing calibrated volumetric micro-capillary pipettes at the gingival margin. A standardized volume of (100 microlitre) crevicular fluid was collected by placing the tip of the pipettes extra-crevicularly. Samples of GCF contaminated by blood or saliva were discarded.

**Collection of blood samples:** Venous blood samples were collected from each subject, from the antecubital vein, in 4 ml vacutainer glass blood collection tubes. The samples will be immediately transported to the laboratory and samples will be centrifuged and the supernatant will be stored at -70 degree.

**Collection of saliva:** The patients were instructed to rinse their mouths several times with distilled water and then relax for 5 minutes. The samples were collected at rest with leaning the patient's head forward over the funnel test tube by keeping the mouth slightly opened to allow saliva to drain into the tube. At the end of collection period, the patients were asked to collect any remaining saliva in the mouth and spit it into the test tube [12].

**Postoperative evaluation: The following parameters were recorded for each patient before treatment and 3 and 6 months after:**

1. Serum level of vitamin D.
2. Serum, salivary and GCF levels of IL-8.
3. Clinical periodontal parameters including:
4. Plaque Index (Pl) [13].
5. Gingival Index (GI) [14].
6. Clinical Attachment loss (CAL)[15] .
7. Probing pocket depth (PPD)[15].
8. Bleeding index (BI) [16].

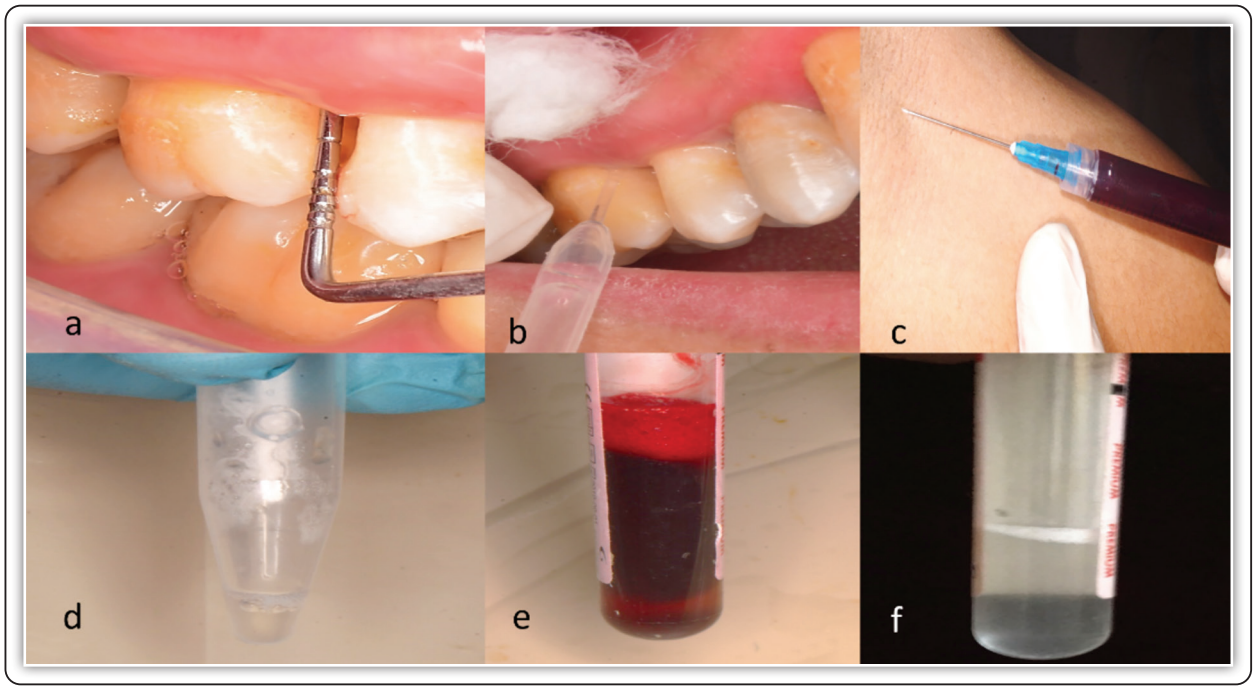


FIG (1) a, Intraoral photograph probing depth showing pocket depth=4 mm, b, withdrawal of GCF sample, c, Withdrawal of blood sample, d, GCF sample Collection e, Blood sample collection, and f, The collected saliva sample.

## RESULTS

Twenty patients ranged in age between 33.0 – 60.0 years with a mean age of 44.65 ± 7.18 years for control group and twenty patients ranged in age between 33.0 – 62.0 years with a mean age 45.40±8.41 years for study group. Control group had 8 males and 12 females, while study group had 8 males and 12 females. Study group after treatment for 6 months showed a higher mean vitamin D % increase (↑117.41±46.82) from baseline than control group (↑2.77±20.34) with improved periodontal health after Vit D supplements. At 3 and 6 months, study group showed a higher mean pocket depth reduction than control group. At 3 and 6 months, study group showed a higher mean attachment gain than control group. The results showed, also,that study group reported a reduction in GCF IL-8% while control group increased, at both observation period of 3 and 6 month. At 3 months, study group showed a reduction in Serum IL-8% from baseline than control group. At 6 months, Study group showed a reduction in Serum IL-8% from baseline while control group increased. There was a negative correlation between vitamin D and IL-8.

**TABLE (1)** Comparison between the two studied groups according to Plaque Index, Gingival Index, Bleeding Index, Pocket Depth, and Attachment loss.

| | Control (n = 20) | Study (n = 20) | p |
|---|---|---|---|
| Plaque Index | | | |
| Baseline | 3.0 ± 0.0 | 3.0 ± 0.0 | 1.000 |
| 3 months | 1.90 ± 0.31 | 1.60 ± 0.50 | 0.108 |
| 6 months | 0.80 ± 0.41 | 0.40 ± 0.50 | 0.030* |
| Gingival Index | | | |
| Baseline | 3.0 ± 0.0 | 3.0 ± 0.0 | 1.000 |
| 3 months | 1.80 ± 0.41 | 1.60 ± 0.50 | 0.289 |
| 6 months | 0.60 ± 0.68 | 0.40 ± 0.50 | 0.461 |
| Bleeding Index | | | |
| Baseline | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.000 |
| 3 months | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.000 |
| 6 months | 0.50 ± 0.51 | 0.50 ± 0.51 | 1.000 |
| Pocket Depth | | | |
| Baseline | 5.51 ± 0.21 | 5.45 ± 0.45 | 0.597 |
| 3 months | 4.70 ± 0.27 | 4.01 ± 0.41 | <0.001* |
| 6 months | 4.0 ± 0.28 | 2.09 ± 0.21 | <0.001* |
| % reduction from baseline to | | | |
| 3 months | 14.75 ± 2.83 | 26.40 ± 5.58 | <0.001* |
| 6 months | 27.41 ± 4.24 | 61.62 ± 3.18 | <0.001* |
| Attachment loss | | | |
| Baseline | 3.02 ± 0.27 | 3.58 ± 0.67 | 0.002* |
| 3 months | 2.48 ± 0.36 | 2.08 ± 0.38 | 0.001* |
| 6 months | 1.97 ± 0.37 | 1.16 ± 0.11 | <0.001* |
| % increase from baseline to | | | |
| 3 months | 18.0 ± 5.51 | 41.58 ± 5.18 | <0.001* |
| 6 months | 35.08 ± 7.20 | 66.92 ± 4.18 | <0.001* |

*t: Student t-test*
*p: p value for comparing between the studied groups*
**: Statistically significant at p ≤ 0.05*

**TABLE (2)** Comparison between the two studied groups according to Vitamin D (ng/ml), Salivary Il-8 (pg/ml), and Serum IL-8

| | Control (n = 20) | Study (n = 20) | p |
|---|---|---|---|
| Vitamin D (ng/ml) | | | |
| Baseline | 27.45 ± 5.35 | 25.85 ± 3.59 | 0.273 |
| 3 months | 25.85 ± 4.36 | 24.70 ± 2.89 | 0.332 |
| 6 months | 27.60 ± 5.43 | 55.10 ± 7.81 | <0.001* |
| % from baseline to | | | |
| 3 months | ↓2.25 ± 23.71 | ↓3.74 ± 9.24 | 0.862 |
| 6 months | ↑2.77 ± 20.34 | ↑117.41 ± 46.82 | <0.001* |
| Salivary Il-8 (pg/ml) | | | |
| Baseline | 1094.1 ± 73.99 | 1101.5 ± 73.20 | 0.751 |
| 3 months | 978.7 ± 61.07 | 1156.1 ± 92.81 | <0.001* |
| 6 months | 907.5 ± 40.58 | 1010.7 ± 70.69 | <0.001* |
| % redaction from baseline to | | | |
| 3 months | ↓10.32 ± 6.09 | ↑5.44 ± 11.46 | <0.001* |
| 6 months | ↓16.77 ± 5.73 | ↓7.85 ± 9.08 | 0.001* |
| GCF il-8 (pg/ml) | | | |
| Baseline | 1333.1 ± 133.4 | 1386.8 ± 149.5 | 0.239 |
| 3 months | 1371.5 ± 99.95 | 1067.2 ± 164.9 | <0.001* |
| 6 months | 1410.2 ± 93.80 | 506.1 ± 84.33 | <0.001* |
| % reduction from baseline to | | | |
| 3 months | ↑3.64 ± 10.64 | ↓23.02 ± 8.53 | <0.001* |
| 6 months | ↑6.53 ± 9.99 | ↓63.11 ± 7.35 | <0.001* |
| Serum IL-8 (pg/ml) | | | |
| Baseline | 15.12 ± 1.69 | 15.65 ± 1.96 | 0.365 |
| 3 months | 14.22 ± 1.32 | 11.73 ± 1.19 | <0.001* |
| 6 months | 14.58 ± 1.49 | 7.87 ± 0.87 | <0.001* |
| % reduction from baseline to | | | |
| 3 months | ↓5.43 ± 8.37 | ↓24.17 ± 10.56 | <0.001* |
| 6 months | ↑2.77 ± 12.38 | ↓49.13 ± 7.06 | <0.001* |

*t: Student t-test*
*p: p value for comparing between the studied groups*
**: Statistically significant at p ≤ 0.05*

**TABLE (3)** Correlation between Vitamin D (ng/ml) with il-8 in study group (n = 20)

| | Vitamin D (ng/ml) | |
|---|---|---|
| | R | p |
| Salivary il-8 (pg/ml) | | |
| Baseline | -0.366 | 0.113 |
| 3 months | -0.256 | 0.275 |
| 6 months | 0.053 | 0.823 |
| GCF il-8 (pg/ml) | | |
| Baseline | 0.426 | 0.061 |
| 3 months | 0.247 | 0.294 |
| 6 months | -0.250 | 0.288 |
| Serum il-8 (pg/ml) | | |
| Baseline | 0.113 | 0.636 |
| 3 months | 0.481 | 0.032* |
| 6 months | 0.069 | 0.773 |

*r: Pearson coefficient*

**: Statistically significant at p ≤ 0.05*

## DISCUSSION

Periodontitis is a chronic inflammatory condition induced by periodontopathic bacteria that leads to destruction of the supporting tissues of teeth. It is the leading cause of tooth loss in adults; Therefore, the focus should be on prevention rather than treatment of this disease [17, 18]. Studies have shown a significant association between periodontal health and vitamin D and calcium intake [19] and that vitamin D supplementation can improve periodontal health, increase mineral density in the mandible, and prevent alveolar bone loss [20, 21].

The immunomodulatory and anti-inflammatory roles of vitamin D have been reported. Studies have shown a significant association between periodontal health and vitamin D and calcium intake, and that vitamin D supplementation can improve periodontal health, increase lower jaw bone mineral density, and prevent alveolar bone resorption [20, 21].

Calcium and vitamin D supplementation has been reported to reduce the severity of periodontal disease when used in doses greater than 800-1000 IU daily, and supports the rationale for examining the potential positive role of vitamin D. in periodontal disease in randomized clinical trials. They also found that vitamin D, in addition to its role in bone homeostasis and calcium, acted as an anti-inflammatory agent by inhibiting cellular expression of immune cells and causing monocytes / macrophages to secrete molecules with potent antibiotic effects. In fact, vitamin D deficiency can be linked to an increased risk of infectious diseases. This suggests that vitamin D may be useful in treating periodontal disease, not only because of its direct effects on bone metabolism, but also because it can have an antibiotic effect on periodontal pathogens and inhibit inflammatory mediators that contribute to periodontal destruction. [22].

The present study was to evaluate the effect of vitamin D in conjugation with phase 1 periodontal therapy on the GCF, salivary, and serum level of IL-8 in periodontitis stage I and II grade A patients. A correlation between Vit D insufficiency and several inflammatory and infectious conditions, including periodontal disease, has been observed[23]. Therefore, the risk of gingivitis and periodontitis stage I and II grade A can be decreased by adequate consumption of Vit D [24, 25]. Dietrich et al. reported a negative correlation between Vit D level and periodontal disease. In the present study, At 6 months, Study group after treatment showed a higher mean vitamin D % increase (↑117.41 ± 46.82) from baseline than control group (↑2.77 ± 20.34) with improved periodontal health after Vit D supplements[26].

In accordance, Miley et al. observed improved periodontal health after Vit D supplements [24]. Anbarcioglu et al. observed an increased prevalence of Vit D deficiency in patients with aggressive periodontitis, suggesting that decreased Vit D level could be a risk factor, and screening is recommended where deficiency is suspected[27].

The obtained findings are in favor of available evidence from other studies on VD and periodontitis support the perio-protective role of Vit D. There was a tendency towards a higher degree of periodontal healing in the study group, expressed through a greater reduction in the number of residual PPD ≥4mm. Additionally, the study group showed a statistically highly significant intra-group diminution in the number of residual PPD ≥ 4 mm at 6 months. Therefore, supplementation of vit D could have a beneficial impact on PD in promoting the healing after the non-surgical treatment of periodontitis. Study group showed a higher mean pocket depth reduction than control group and higher mean attachment gain than control group[28,29].

The association between vitamin D serum level and the incidence and severity of periodontitis stage I and II grade A indicate that, a sufficient serum level of vitamin D is necessary for the maintenance of periodontal health[30]. One possible mechanism for this association is that vitamin D reduces the risk of gingivitis through the induction of cathelicidin. The vitamin D pathway has been shown to be present in human periodontal fibroblasts and tooth-supporting ligament cells and to play an important role in the immune defense in periodontal soft tissues by activating the human antimicrobial protein cathelicidin [30-34]. More recently, serum 25 (OH)D deficiency has been associated with decreased levels of hBD 2 and cathelicidin in periodontal tissue in stage I and grade II gingivitis and periodontitis [34].

In this study, at 3 and 6 months, Study group showed a reduction in GCF IL-8 % while control group increased. At 3 months, Study group showed a reduction in Serum IL-8 % from baseline than control group. At 6 months, Study group showed a reduction in Serum IL-8 % from baseline while control group increased. In the present study, there was a negative correlation between vitamin D and IL-8. Astudy found that the endogenous and P. gingivalis-induced expression of IL-8 was significantly down-regulated by Vitamin D, which suggests that Vitamin D may decrease the periodontal inflammation partly by inhibiting the IL-8 expression [35]. Vitamin D has been reported to inhibit IL-8 expression by various mechanisms. Another study found that Vitamin D suppressed the expression of IL-8, but they also showed that Vitamin D only moderately suppressed IL-8 promoter and NF-kB activities and supposed that Vitamin D may affect the stability of IL-8 mRNA[36]. Additionally, it was reported that vitamin D reduces IL-8 secretion from NK cells through the blocking effect on the P38 MAP-Kinase pathway [37]. Vitamin D may potentially inhibit the periodontal inflammation induced by P. gingivalis partly by decreasing the IL-8 expression in hPDLCs[2].

## CONCLUSION

In conclusion, the results suggest that vitamin D could play a potential role in periodontal inflammation by inhibiting interleukin-8 expression. As far as we know, this is the first evidence of the anti-inflammatory effects of vitamin D. Further studies, the different ways in which vitamin D regulates IL-8 and the precise ways in which it is regulated must, however, clarify this aspect.

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