Assessment of 25-Hydroxy Vitamin D3 and Osteocalcin in Chronic Periodontitis Patient (Clinical and Laboratory Study)

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Abstract:

Objectives: Evaluate the level of 25-Hydroxy vitamin D3 and osteocalcin in GCF and serum before and after scaling and root planning in chronic periodontitis patients.

Methods: A total of forty patients (20 with a moderate to severe chronic periodontitis (study group) and 20 chronic gingivitis patients were chosen as a control group) were selectively collected for contribution in the present study. The diseased patients (study group) received initial periodontal therapy (SRP). Gingival crevicular fluid (GCF) and serum sample were collected at baseline and six weeks after therapy for study group and at baseline for control group. Systemic and local levels of 25-hydroxy vitamin D3, osteocalcin were measured using radioimmunoassay or enzyme-linked immunosorbent assay kits and compared.

Results: The respective local osteocalcin level is significantly dropped from baseline to six weeks after (SRP) (9.56ng/ml versus 7.38ng/ml, P=0.001). The respective systemic osteocalcin level significantly increased after six weeks from SRP (10.85ng/ml versus 17.74 ng/ml, P=0.001). The respective local 25-Hydroxy vitamin D3 level are significantly increased from baseline to six weeks after (SRP) (3.41ng/ml versus 4.57ng/ml, P=0.001). The respective systemic 25-Hydroxy vitamin D3 level significantly increased after six weeks from SRP (39.88ng/ml versus 41.48 ng/ml, P=0.001).

Conclusions: Scaling and root planing (SRP) is the mainstay of treatment of periodontal diseases as SRP was effective in improving clinical parameters in patients with chronic periodontitis. 25-hydroxy vitamin D3 might have an important role in the pathogenesis of periodontal disease and could be used as adjunctive therapeutic modality for the prevention and treatment of different types of periodontitis. Osteocalcin could be used as a potential diagnostic marker for periodontal disease activity in both serum and gingival crevicular fluid.

Keywords: 25-Hydroxy Vitamin D3, Osteocalcin, Chronic Periodontitis.

Introduction

Current knowledge about the pathogenesis of periodontal disease suggests that the central cause of periodontal disease is the loss of a healthy balance between microbial virulence agents and host inflammatory response [1,2]. The immune system while protecting the host against microbial dental plaque, also participates in attacking the host. Inflammation and tissue destruction are early and [3] mediated process in response to the bacterial infection [4]. Periodontal diseases may differ in their etiological factors and pattern of progression. This variability can be attributed to differences in the presence of factors that might modify the host response to microbial pathogens. Chronic periodontitis (CP) and aggressive periodontitis [5], forms of inflammatory periodontal disease, differ from each other in terms of the magnitude, sequel and control of the response [5].

The destruction of soft and hard tissues seen in periodontitis is caused by a large number of cytokines as well as due to the presence of other effector molecules released by resident and migrating cells [6,7].

Among many inflammatory and immune mediators identified in gingival crevicular fluid (GCF), cytokines have attracted particular attention and are suspected of involvement in both inflammation-related alteration and repair of the periodontal tissues [8]. Certain cytokines have been proposed as potentially useful diagnostic or prognostic markers of periodontal destruction [9,10]. For example, site-specific increases of IL-1 were observed in untreated periodontitis [11] and in experimental gingivitis models [12].

It has also been reported that old and young subjects with initially normal gingiva present similar levels of IL-1a and IL-1. However, during a 3 week period without oral hygiene, both groups developed increased levels of IL-1a, whereas IL-1 levels increased only in the old adult group indicating that there are differences between the inflammatory responses in young and old adult individuals [13]. Treatment of periodontitis resulted in a dramatic local decrease of IL-1, suggesting that this molecule is crucial in periodontal tissue destruction [14,15].

Bone homeostasis maintains by a coupled process of resorption followed by formation which reflect a change in bone turnover [16]. Markers of bone formation are proteins revealing osteoblast activity and are byproducts of collagen synthesis, matrix proteins or osteoblastic enzymes [17,18]. Osteocalcin is a small (5.4 kDa), calcium-binding protein of bone accounting for 10–20% of the non-collagenous protein in bone matrix. It has three residues of a calcium-binding amino acid, gamma-carboxyglutamic acid (Gla), that allow specific conformational changes enabling its binding to hydroxyapatite and later accumulation in bone matrix [18,19]. This vitamin K- and D-dependent protein produced by mature osteoblasts, osteocytes and odontoblasts, is found...
Vitamin D plays a critical role in mediating calcium absorption and regulating musculoskeletal health [21]. It has also been demonstrated to function in the regulation of cardiovascular health, immune responses, wound healing and cancer prevention [22]. Vitamin D is a fat soluble vitamin obtained from three sources. Endogenous synthesis of vitamin D occurs in the skin and is induced via ultraviolet radiation. It may also be obtained exogenously through dietary sources that include oily salt fish (mackerel, salmon, sardines and tuna), cod liver oil and egg yolk. Many countries, including the United States of America, fortify dairy products with vitamin D due to its scarcity in natural foods [22].

Finally, various forms of vitamin D are available in over-the-counter dietary supplements [23]. Vitamin D obtained through supplements is converted to 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D through the same pathway that keratinocytes utilize when ultraviolet radiation stimulates its synthesis from a cholesterol precursor in the epidermis [23].

The current recommended daily intake for vitamin -D and calcium are 400-600 IU and 1,000 to 1,200 mg, respectively, for people over 50 years of age [24]. Higher doses (800-1,000 IU) of vitamin D are recommended for osteoporosis prevention [25]. It is estimated that 1 billion people worldwide have vitamin D deficiency [26,27] to target organs and tissues. Vitamin D then mediates its effects via binding to the vitamin D receptor (VDR), a member of the steroid superfamily receptors. inding of vitamin D activates the VDR, which acts as a transcription factor for target genes [28].

Patients and methods
The present study was carried out on forty patients there age ranged from 35-60 years (both males and females) which who were selected from the Department of Oral Medicine and Periodontology, Faculty of Dentistry, Mansoura University. Those only 20 patients were diagnosed as having chronic periodontitis after obtaining proper case history, thorough clinical examination and according to the clinical and radiographic criteria and 20 suffering from chronic gingivitis.

The selected patients were free from any systemic disease, and receiving no medication for the present condition three months prior to the study. Furthering, none of them had previous periodontal treatment including scaling, root planning, and periodontal surgery in the last six months. On other hand, smokers and pregnant females were excluded from the present study.

Clinical measurements
The following clinical parameters were be measured before and after treatment (at baseline, after 6weeks)
- Probing pocket depth [32].
- Plaque index [30].
- Gingival index [31].
- Clinical attachment level [32].

Results
All patients completed the entire study. No adverse effects, such as discomfort, dentin hypersensitivity, or pain related to the scaling and root planning were reported by any of the patients.

Table 1 showed the demographic characteristics and baseline data of the patients enrolled in the study. The mean value and standard deviation of the age of individuals was 40.8±7.7 years in study group. Their age ranged from 35-56 years. And 35.3±2.1 were in control group, their age ranged from 30-43 years.

Assad Amara Matoug et al.
Tables 2 showed the variation of mean values and standard deviation of GI, PI, BOP, PD and CAL of individuals participated in the study. For the study group, the mean value and standard deviation of gingival index (GI) at baseline was 1.88 ± 0.29 and after SRP was 0.72±0.22. So, there was a statistically significant decrease in GI values post-operatively (P < 0.001). Moreover, the mean value and standard deviation of plaque index (PI) before treatment was 2.02 ± 0.44. After treatment, the mean value was 0.72±0.28. So, there was a highly statistically significant difference between values of PI before and after treatment in the study group (at P < 0.001).

It was obviously observed that there was a statistically significant reduction in bleeding on probing (BOP) scores at baseline in study group with mean values 0.91 ± 0.08 compared to scores after treatment which was 0.18±0.09 (at P < 0.001).

The mean value and standard deviation of probing pocket depth (PD) at baseline in the study group was 5.27 ± 0.77 mm and after therapy, it was 4.35 ± 0.73 mm. As a result, there was a statistically significant decrease in mean PD post-operatively (at P < 0.001). Moreover, the mean value and standard deviation of clinical attachment level (CAL) at baseline in the study group was 5.84 ± 0.79 mm. After treatment, the mean value and standard deviation of CAL in the same group was 5.02 ± 0.71 mm. Therefore, in periodontitis patients; there was a statistically significant decrease in mean CAL post-operatively (at P < 0.001).

Table 3.4 showed the comparison of GCF and serum level of osteocalcin and 25-Hydroxy vitamin D3 with ng/ml in group of patients with CP before and after SRP with control group. The mean value and standard deviation of osteocalcin in SRP group in GCF at baseline was 9.56±2.18 and after therapy (SRP), it was 7.38± 1.96. As a result, there is a statistically significant decrease in mean osteocalcin post-operatively (with P < 0.001). The mean value and standard deviation in control group in GCF was 2.65± 0.65. Pre-operatively as well as post-operatively, Study group showed statistically significantly higher mean Osteocalcin than Control group (at P < 0.001). The mean value and standard deviation of osteocalcin in (SRP) group in serum at baseline was 10.58±3.29 and after scaling and root planning, it was 14.00±1.65. As a result, there is a statistically significant increase in mean osteocalcin post-operatively (at P < 0.001). The mean value and standard deviation in control subject of GCF was 17.74±4.09. As a result Pre-operatively as well as post-operatively, control group showed statistically significantly higher mean osteocalcin than study group with (P < 0.001).

The mean value and standard deviation of 25(OH) vitamin D3 in study group in GCF at baseline was 3.41±2.25 and after therapy (SRP), it was 4.57±2.33. As a result, there was a statistically significant increase in mean post-operatively at p value 0.022. The mean value and standard deviation in control subject in GCF was 4.86±1.12. SO, Pre-operatively, control group showed statistically significantly higher mean 25 (OH) vitamin D3 than study group (with P < 0.001). The mean value and standard deviation of SRP group in serum at baseline was 39.88±13.64 and after therapy (SRP), it was 41.48±13.58. As a result, there no statistically significant change in mean 25 (OH) vitamin D3 post-operatively. The mean value and standard deviation in control group in serum was 38.40±16.53. Pre-operatively as well as post-operatively, there was no statistically significantly difference between the two groups with (p > 0.05).

Discussion

Chronic periodontitis is a chronic condition where bacterial biofilms leads to host responses within periodontal tissues, and inducing inflammatory damage resulting in breakdown of the connective tissue that anchors teeth to alveolar bone [20]. In periodontitis, there is an increased turnover of alveolar bone although there may be a dominance of bone resorption over bone formation leading to alveolar bone loss and loss of attachment [30].

Previous reports revealed evidence that periodontal disease is linked to low serum 25-hydroxyvitamin D concentrations in addition to recognized risk factors like diabetes and smoking. Evidence for plausibility includes that vitamin D increases calcium absorption and protects bone strength [31]. It induces formation of cathelicidin (LL-37) and other defensins that combat bacterial infection; hence reduces tissue production of destructive matrix metalloproteinases actively associated with periodontal disease [32]. It was also found that the prevalence of periodontal disease varies with common [33,34]. Experimental evidence from limited supplementation studies [using calcium and vitamin D] shows that supplementation reduces tooth loss [35]. Thus, existing evidence for hypovitaminosis D as a risk factor for PD to date meets scientific criteria for causality in a biological system [36].

Gingival crevicular fluid (GCF), which is an exudate that can be harvested from the sulcus or periodontal pocket, has been regarded as a promising medium for the detection of periodontal disease activity. So, the majority of new diagnostic tests for periodontal disease utilize GCF [37]. Several investigations on osteocalcin levels in GCF from patients with periodontitis have been reported, suggesting that osteocalcin levels in GCF may reflect inflammation at diseased sites and there has been recent interest in osteocalcin as a potential marker of bone turnover in periodontal disease [38]. However, the role of osteocalcin in periodontal disease progression and periodontal treatment outcome is still unclear [39].

Thus, the present clinical study was designed to assess the levels of 25-Hydroxy vitamin D3 and osteocalcin in GCF and serum before and after scaling and root planing (SRP) in chronic periodontitis patients.

The results of our study showed improvement of all clinical periodontal parameters (GI, PI, BOP, PD and CAL) after SRP in chronic periodontitis patients (study group) and exhibited significant improvements of all values after therapy compared to baseline records. These findings are in agreement of several studies and many consensus reports as SRP is considered the basic therapeutic modality of chronic periodontitis [40-43].

Our results showed a significant reduction in GCF osteocalcin level in the study group after SRP. However, there was a significant increase in serum osteocalcin after SRP. Regardless of the decrease or increase either in GCF or serum of the test group, the values of the control group showed statistically significant variation. This comes in agreement with the results obtained by other investigators.

Assad Amara Matoug et al.
They showed that osteocalcin in gingival crevicular fluid collected from periodontitis sites were significantly higher than those found in healthy and gingivitis sites [44].

Moreover, the results of the present study were in accordance with those of the study done by Ignoble and his colleagues to correlate the levels of osteocalcin to the progression of experimental alveolar bone loss in the beagle dogs. The results revealed significant elevations in gingival crevicular fluid osteocalcin in experimental periodontitis sites as compared to baseline and controls throughout disease progression. The osteocalcin in gingival fluid appeared to correlate well with periodontal disease and bone turnover as evidenced by significant elevations in gingival crevicular fluid osteocalcin during the more active periods of bone loss. A fall in the levels of gingival crevicular fluid osteocalcin accompanied removal of the ligatures showing that osteocalcin increased with bone resorption and decreased with periodontal healing [45].

The results of the present study are consistent with the results achieved by Button and his colleagues to assess serum and gingival crevicular fluid levels of osteocalcin and correlate them with periodontitis. They revealed that gingival crevicular fluid level of osteocalcin in periodontitis group is significantly higher than healthy group. However, they found that there is no significant difference in the serum osteocalcin levels between healthy and diseased group [46].

Some investigators showed that the levels of osteocalcin in peri-implant crevicular fluid from peri-implantitis patients were significantly higher than healthy implants and thus concluded that, osteocalcin in peri-implant crevicular fluid may reflect increased local bone turnover around implants [47].

The results of the present study were in disagreement with those reported by Golub and colaborators who failed to detect any changes in osteocalcin levels after periodontal therapy in patients with chronic periodontitis. The possible explanation may be related to the adjunctive use of antibiotic therapy in his study which eliminated the microbial insult and achieved appropriate environment that favors periodontal healing [48].

The same was shown by some investigators who demonstrated that although osteocalcin is specifically produced by osteoblasts and is found exclusively in mineralized tissues, it is still plausible that the measurable levels in gingivitis may represent osteocalcin released during normal bone homeostasis [49]. On the contrary, Kinney and his colleagues reported that osteocalcin could not be detected in patients with gingivitis and this is because they did not use high sensitive detection ELISA kit in their analysis [50].

In a cross-sectional study aimed to detect the levels of osteocalcin in gingival crevicular fluid from healthy and diseased subjects with chronic periodontitis in order to further investigate its potential role as a possible marker of the disease process, they reported no differences in gingival crevicular fluid osteocalcin levels between diseased and healthy sites in the same patients [51].

Vitamin D is necessary for bone formation and proper immune function, which are also important to the success of periodontal therapy [52]. The role of vitamin D in reducing the risk of periodontitis and gingivitis is induction of LL-37 and defensins by 1, 25-dihydroxyvitamin D. A recent study found that saliva from orally healthy individuals is capable of protecting LL-37 from proteolysis by Porphyromonas gingivalis, a bacterium associated with chronic periodontitis. This protective activity was found to be necessary to enable the direct bactericidal activity of LL-37 (as tested on E. coli) in the presence of virulence associated proteases of Porphyromonas gingivalis [53].

Previously, only a few studies had assessed the role of vitamin D on periodontal disease status [54].

In the present study, there was a statistically significant increase in the mean GCF 25-Hydroxy vitamin D3 after performing SRP in the study group. However, there was no statistically significant variation of mean values of serum 25-OH vitamin D3 level after SRP of the same group. These findings come in agreement with other investigators. However, they evaluated 25-OH vitamin D3 in aggressive periodontitis individuals [55].

In previous studies, which found that the low vitamin D levels in GCF have been associated with increased tooth loss, clinical attachment loss, and maternal periodontal disease during pregnancy [56,57] and this finding is consistent with our results [58,59].

Investigators found an evidence for the association of periodontal disease with low serum 25(OH) D concentrations [55]. This result comes in disagreement with our findings that showed no difference of serum 25(OH) D concentrations between periodontitis patients at baseline and after therapy and also compared to chronic gingivitis group. This can be explained as Hills and his coworker's evaluated serum 25(OH) vitamin D concentrations in postmenopausal women, however, subjects included in our study were healthy and had no systemic diseases.

In our current study, 25-Hydroxy vitamin D serum level showed no significant difference in study group following treatment. These results were in disagreement with those reported by other investigators who detected a transient increase in serum levels of 25(OH) D that coincided with the six-week healing phase. This may have an important anti-inflammatory effect of vitamin D and could explain the early improvements in CAL gain and PD decreases in the vitamin-D deficient teriparatide patients (drug therapy of osteoporosis) [59,60]. The association found in this study is unrelated to any effects of vitamin D on bone.

However, chronic marginal gingivitis may eventually lead to loss of alveolar bone and, thus, evolve into periodontal disease in susceptible patients. Because bone loss is pathognomonic for periodontal disease, studies have linked vitamin D status and polymorphisms of the vitamin D receptor genes to periodontal disease and tooth loss [60,61]. Much of the action of vitamin D is achieved through the VDRs. There are many VDR polymorphisms, BsmI, Apal, TaqI and FokI being the best known. VDR polymorphisms appear to be associated with variation in expression of various other genes even though polymorphisms such as Apal, BsmI, FokI and TaqI are in non-expressed portions of the VDR gene, possibly due to variation in the induction of tertiary structure of the VDR [33,62,63].

It was previously shown that there was an inverse association between serum concentrations of 25(OH) D and prevalence of periodontal disease as measured by

Assad Amara Matoug et al.
periodontal attachment loss [64]. However, this association was independent of bone mineral density. The previous data are not consistent with our results. The patients selected for participation in the previous study were suffering from aggressive periodontitis. We can only speculate why the association between serum 25(OH) D3 concentrations and clinical attachment loss (CAL) was limited to the older subjects of both sexes. One reason may be the higher prevalence and extent of AL in older subjects than in younger subjects. In this way, older subjects may be more susceptible to a potential benefit of vitamin D.

In another study, it was found that there was a significant inverse association between serum 25(OH) D3 concentrations and periodontal disease in both the men and the women aged ≥50 y. This association was independent of race or ethnicity, socioeconomic status, estrogen use among the women, smoking, and gingival bleeding [65]. However, in a longitudinal study of 550 predominantly white, middle-aged, elderly men found no association between vitamin D intake from foods and supplements and the number of teeth with progression of periodontal bone loss over a 4-years period [66].

The anti-inflammatory effect of vitamin D on gingival inflammation may be an alternative pathway by which vitamin D may be beneficial for the prevention of periodontal disease [67]. Furthermore, cross-sectional study and a causal effect of 25(OH) D concentrations on gingival inflammation cannot be established on the basis of these data. However, the concomitant assessment of 25(OH) D concentrations and gingival inflammation is not likely to be an important problem because gingival inflammation develops and resolves rapidly. Intervention studies will be necessary to establish whether increased intake of vitamin D can reduce gingivitis susceptibility. Marginal gingivitis may be a useful model to study the anti-inflammatory effects of vitamin D in humans [68].

It was concluded from the current study that 25-hydroxy vitamin D3 might have an important role in the pathogenesis of periodontal disease and could be used as adjunctive therapeutic modality for the prevention and treatment of different types of periodontitis. It was also concluded that osteocalcin could be used as a potential diagnostic marker for periodontal disease activity in both serum and gingival crevicular fluid.

One of the limitations of the present study is the small number of patients enrolled to participate in this investigation. Thus, large scale projects are recommended to generalize and confirm our results. Future intervention therapeutic studies with vitamin D supplementation are also recommended in both chronic periodontitis and aggressive periodontitis patients to completely evaluate the role of vitamin D that might play in the treatment of periodontal disease.

**Conclusion**

- Scaling and root planing (SRP) is the mainstay of treatment of periodontal diseases as SRP was effective in improving clinical parameters in patients with chronic periodontitis.
- 25-hydroxy vitamin D3 might have an important role in the pathogenesis of periodontal disease and could be used as adjunctive therapeutic modality for the prevention and treatment of different types of periodontitis. Laser therapy exhibit bactericidal effect in the periodontal pocket of patients with chronic periodontitis.
- Osteocalcin could be used as a potential diagnostic marker for periodontal disease activity in both serum and gingival crevicular fluid.

**Table 1: Baseline demographic data.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study group (SRP) (M±SD)</th>
<th>Control group (M±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (n)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Average</td>
<td>40.8±7.7</td>
<td>35.3±2.1</td>
</tr>
<tr>
<td>Age (year)</td>
<td>35-56</td>
<td>30-43</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>(8/12)</td>
<td>(9/11)</td>
</tr>
</tbody>
</table>

**Table 2: The mean values (±SD) of GI, PI, BOP, PD and CAL of study group before and after (SRP) and control group.**

<table>
<thead>
<tr>
<th>par</th>
<th>Before</th>
<th>After</th>
<th>At baseline</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>1.88±0.29</td>
<td>0.72±0.22**</td>
<td>0.16±0.16</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PI</td>
<td>2.02±0.44</td>
<td>0.72±0.28**</td>
<td>0.27±0.24</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BOP</td>
<td>0.91±0.08</td>
<td>0.18±0.09*</td>
<td>0±0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PD</td>
<td>5.27±0.77</td>
<td>4.35±0.73**</td>
<td>0.45±0.36</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CAL</td>
<td>5.84±0.79</td>
<td>5.02±0.71**</td>
<td>0±0</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

BOP = Bleeding on probing
Table 3: Osteocalcin (OC) levels in GCF and serum (ng/ml) in study group at baseline and after SRP compared to control group.

<table>
<thead>
<tr>
<th></th>
<th>Study group</th>
<th>Control group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td>GCF</td>
<td>9.56 ± 2.18**</td>
<td>7.38 ± 1.96*</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Serum</td>
<td>10.85 ± 3.29*</td>
<td>14.00 ± 1.65**</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

**, *p < 0.001 i.e., There is significant difference.

GCF = gingival crevicular fluid, OC = osteocalcin, ng/ml = nanogram (10^-9)/milliliter

Table 4: 25-Hydroxy vitamin D3 levels in GCF and serum (ng/ml) in study group at baseline and after SRP in comparison to control group.

<table>
<thead>
<tr>
<th>25-(OH) vitamin D3</th>
<th>Study group</th>
<th>Control group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td>GCF</td>
<td>3.41 ± 2.25</td>
<td>4.57 ± 2.33*</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Serum</td>
<td>39.88 ± 13.64</td>
<td>41.48 ± 13.58</td>
<td>&gt;0.005</td>
</tr>
</tbody>
</table>

*p < 0.001 i.e., There is significant difference

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ingival crevicular fluid calprotectin, in beagle -


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Assad Amara Matoug et al.


