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Measurement of 25-Hydroxy Vitamin D3 and Osteocalcin in Chronic Periodontitis Patients: A Clinical and Laboratory Study

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ABSTRACT

This study was conducted to evaluate the level of 25-Hydroxy vitamin D3 and osteocalcin in GCF and serum befor and after scaling and root planning in chronic periodontitis patients. A total of forty patients (20 with 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. The respective local osteocalcin level are 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). 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.

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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]. Soft and hard tissues destruction in periodontitis caused by a large number of cytokines as well as due to the presence of other effector molecules released by resident and migrating cells [5,6].

Bone homeostasis maintains by a coupled process of resorption followed by formation which reflect a change in bone turnover [7]. Markers of bone formation are proteins revealing osteoblast activity and are byproducts of collagen synthesis, matrix proteins or osteoblastic enzymes [8,9].

Vitamin D is important in mediating calcium absorption and regulating musculoskeletal health [10]. It has also been demonstrated to function in the regulation of cardiovascular health, immune responses, wound healing and cancer prevention [11]. Also, 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, fortify dairy products with vitamin D due to its scarcity in natural foods [11]. Finally, various forms of vitamin D are available in over-the-counter dietary supplements [12]. It is 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 [13].

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 [14]. Higher doses (800-1,000 IU) of vitamin D are recommended for osteoporosis prevention [15]. It is estimated that 1 billion people worldwide have vitamin D deficiency [16,17] 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. Binding of

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vitamin D activates the VDR, which acts as a transcription factor for target genes [18].

PATIENTS AND METHODS

Study design and patients

A cross-sectional 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 Periodontology, Faculty of Dentistry. 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. 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).

- Gingival index.
- Plaque index.
- Bleeding on probing index.
- Clinical attachment level.
- Probing pocket depth.

Data collection

A baseline visit was conducted by a periodontist for two groups, and then study group underwent an initial periodontal therapy consisting of scaling and root planing (SRP) and oral hygiene instructions. The treatments were completed in 6 weeks.

GCF samples and serum were obtained at the two visits at baseline and after 6weeks for 20 study group. And for 20 control group GCF, serum samples collected at baseline only.

GCF collection and processin

Subjects were recalled for GCF sampling. In the CP groups, GCF samples were collected from one sites of tooth with PD≥4 (pocket). In the control group, GCF samples were collected from one site of tooth with PD≤3 mm (sulcus). Prior to GCF sampling, the supragingival plaque was removed from the interproximal surfaces with a sterile curette these surfaces were dried gently by an air syringe and were isolated by cotton rolls. GCF was sampled with filter paper

Paper strips were carefully inserted into the pocket until mild resistance was felt and left there for 30 seconds. Care was taken to avoid mechanical injury. Strips contaminated with blood were discarded. pooled and placed into a sterile eppendorff placing containing 250µl of phosphate buffer saline (PBS).

Statistical analysis

Numerical data were presented as mean and standard deviation (SD) values. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Age data showed parametric distribution while all clinical measurements data showed non-parametric distribution. For parametric data, Student's t-test was used to compare between age values of the two groups.

For non-parametric data, Mann-Whitney U test was used to compare between the two groups. Wilcoxon signed-rank test was used to study the changes after treatment within each group.

Gender data (Qualitative data) were presented as frequencies (n) and percentages (%). Chi-square (*x2*) test was used to compare between the two groups. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

RESULTS

All patients completed the entire study. No adverse effects, such as discomfort, dentin hypersensitivity, or pain related to the scaling and root plaining 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 35-56years. 35.3±2.1 were in control group, their age ranged from 30-43 years.

Table 1. Baseline demographic data

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

Tables 2 showed the variation of mean values and standard deviation of GI, PI, BOP, PD and CAL of cases 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.

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

Par	Before	After	At baseline	p-value
GI	1.88 ± 0.29	**0.72±0.22	0.16±0.16	< 0.001
PI	2.02±0.44	**0.72± 0.28	0.27±0.24	< 0.001
BOP	0.91 ± 0.08	*0.18±0.09	0 ±0	< 0.001
PD	5.27 ± 0.77	**4.35 ± 0.73	0.45 ±0.36	< 0.001
CAL	5.84 ± 0.79	**5.02 ± 0.71	0 ±0	< 0.001

*BOP = Bleeding on probing; P = Probability of significance;** Highly significant at P < 0.001; PD = Pocket depth in millimeters; CAL = Clinical attachment level in millimeters; M = Mean; SD = Standard deviation; GI= Gingival index; PI= Plaque index; Par= Parameter*

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 plaining, 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 value > 0.05).

Table 3. Osteocalcin levels in GCF and serum (ng/ml) in study group at baseline and after SRP compared to control group

Osteocalcin	Study group		Control group	p-value
	Before	After		
GCF	9.56 ± 2.18**	7.38 ± 1.96*	2.65 ± 0.65	< 0.001
Serum	10.85 ± 3.29*	14.00 ± 1.65**	17.74 ± 4.09	< 0.001

***,*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

25-(OH) vitamin D3	Study group		Control group	p-value
	Before	After		
GCF	3.41± 2.25	4.57± 2.33*	4.86± 1.12*	< 0.001
Serum	39.88 ±13.64	41.48 ± 13.58	38.40 ± 16.53*	>0.005

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

DISCUSSION

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 [19]. 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 20.

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 [21]. 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. 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. 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.

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.

CONCLUSION

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. Futureintervention 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.

Conflict of interest. Nil

REFERENCES

1. Madianos P.N. Generation of inflammatory stimuli: how bacteria set up inflammatory responses in the gingiva. J Clin Periodontol 2005; 8: 57–71.
2. Amano A. Host–parasite interactions in periodontitis: micro- bial pathogenicity and innate immunity. . Periodontol 2000, 2010; 53: 9-14.
3. Lian J.B, Hauschka P.V, Cole D.E, Gundberg C.M. Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. Physiol Rev 1989; 69: 990–1047.
4. Page R.C, Tonetti M.S, Kornman K.S. The host response to the microbial challenge in periodontitis: assembling the player. Periodontol 2000 1997; 14: 33–53.
5. Kinugawa S, Koide M, Takahashi N, Udagawa N. Osteo-clastic bone resorption induced by innate immune responses. Periodontol 2000, 2010; 54: 235–246.
6. Genco RJ. Host responses in periodontal diseases. J Periodontol; 1992.; 63: 338-355.
7. Slatopolsky E Brown AJ Dusso AS,Vitamin D. Am J Physiol 2005; 289: 8-28.
8. Liu Y, Giovannucci E, Hollis BW, Rimm EB. 25-Hydroxyvitamin D and Risk of Myocardial Infarction in Men. Arch Intern Med 2008; 168: 1174-1180.
9. Knekt P, Kilkkinen A, Aro A. Vitamin D Status and the Risk of Cardiovascular Disease death. Am J Epidemiol 2009; 170: 1032-1039.
10. Atia A, Arhoma S. Epidemiological study of Vitamin D deficiency among Libyan patients. MRIMS Journal of Health Sciences. 2022 Jan 1;10(1):14-7.
11. Michaud DS Skinner HG, Giovannucci E, Willett WC, Colditz GA. Skinner HG, Michaud DS, Giovannucci E, Willett WC, Colditz GA. Cancer Epidemiol Biomarkers Prev 2006; 15: 1688-1695.
12. AbuRedwan MA, Blhaj AA, Aboushkeewah AM. Prevalence of Vitamin D Deficiency among Libyan Pregnant Women. Attahadi Medical Journal. 2024 Jun 27:22-4.
13. Ahmed A, Amr M, Almoner B, Salim M, Abudaber S. Evaluation of vitamin D status among adult population in Tripoli Region, Libya. AlQalam Journal of Medical and Applied Sciences. 2023 Oct 15:626-34.
14. Abushhewa M, Salem A, Mahmoud A, Mohsen R, Aqila E. Preliminary Study of Vitamin D Deficiency and Its Associated Risk Factors in Libya. AlQalam Journal of Medical and Applied Sciences. 2024 Jan 3:7-10.
15. Mikkelsen K, Glerup H, Poulsen L. Commonly Recommended Daily
16. Intake of Vitamin D is Not Sufficient if Sunlight Exposure is Limited. . J Intern Med 2000; 247: 260-268.
17. Alasbily H, Abdalrahman S, Netfa M, Elemmami A, Aldebani A. The Association Between Vitamin D Status and Glycemic Control in Children and Adolescents with Type 1 Diabetes. AlQalam Journal of Medical and Applied Sciences. 2024 Jul 3:470-6.
18. LÖe H, Acta. Silness P. periodontal disease in pregnancy. . Odontol. Scanad 1964; 22: 121.
19. Son S, Mühleman H. Gingival sulcus index. A leading symptom in initial gingivitis. HelvOdontolActa 1971; 15: 107-113.
20. Lucy Chandler1 Pedro Bullon1, Juan J. Segura Egea1, Ramón Perez Cano2, Angel Martinez Sahuquillo1. Osteocalcin in serum, saliva and gingival crevicular fluid: their relation with periodontal treatment outcome in postmenopausal women. Med. oral patol. oral cir.bucal 2007; 12: 3: 46-98.
21. Reid I. R, Bolland M. J. Skeletal and nonskeletal effects of vitamin D: is vitamin D a tonic for bone and other tissues Osteoporos Int 2014;
22. Wilson A. Bone turnover markers inserumandperiodontalmicroenvironments. J Periodontal Res 2003; 38: 4: 355-61.