ORIGINAL RESEARCH

Correlation of Serum Calcium and Vitamin D Levels in Patients with and without Periodontitis before and after Nonsurgical Periodontal Therapy

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Abstract

Aim: To evaluate and correlate levels of serum calcium and 25-hydroxycholecalciferol (vitamin D) in subjects with periodontitis and in subjects with healthy periodontium before and following nonsurgical periodontal therapy (NSPT).

Materials and methods: A total of 52 patients were enrolled in the study with 26 patients in each group. The control group included patients without periodontitis, whereas the study group included patients with periodontitis. On the first visit, ~ 3–5 mL of venous blood was collected from all the participants for the assessment of calcium and Vitamin D levels in the blood. The various parameters evaluated included probing depth, clinical attachment level (CAL), gingival index (Loe and Silness 1963). Plaque index (Silness and Loe 1964), and IOPA (site having greatest pocket depth). Participants of both the groups received NSPT which involved extensive scaling and root planing. On the second visit (after 3 months), the same clinical parameters except IOPA were recorded in all the participants. On the third visit (after 6 months from the first visit), the blood sample was again collected for the evaluation of serum calcium and Vitamin D, and all the parameters of baseline were recorded.

Result: The result of the study showed that Vitamin D levels at baseline were less in test subjects (12.73 ± 2.25) as compared to controls (17.81 ± 4.03), which improved at 6 months for the test group (24.53 ± 1.98) but not the control group (17.8 ± 4.14) following NSPT.

All the clinical parameters improved for both groups. There was no statistically significant difference in the calcium levels of both groups at baseline or 6 months.

Conclusion: Vitamin D levels were found to be lower in periodontitis patients than healthy controls, vitamin D levels improved in the test subjects along with the clinical parameters following NSPT highlighting the beneficial role of Vitamin D in maintaining periodontal health. No changes were seen in calcium levels in either of the groups at any point in time.

Clinical significance: Vitamin D can reduce the incidence and severity of periodontal disease by its various anti-inflammatory and immunomodulatory effects in addition to its traditional role in bone metabolism. Thus, maintaining adequate levels of systemic vitamin D could be critical to periodontal health. This study justifies this notion and further suggests the adjunctive role of vitamin D supplementation to improve therapeutic outcomes.

Keywords: Periodontal disease, Periodontal inflammation, Vitamin D.

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Introduction

Periodontitis is an inflammatory disease of the tissues that provide support to the teeth and is caused by a group of specific microorganisms, resulting in progressive destruction of periodontal ligament and destruction of alveolar bone with increased pocket depth, recession, or both.[1] Vitamin D is well known as a fat-soluble vitamin that can be acquired from being exposed to sunlight, dietary sources, and nutritional supplements. Vitamin D is metabolized in the liver to 25-hydroxycholecalciferol [25(OH)D] and then metabolized in the kidneys to its active form 1,25-dihydroxycholecalciferol [$1,25(OH)_2D$]. As the principal metabolite in serum, 25(OH)D is evaluated to determine a patient's vitamin D status.[2]

Vitamin D mainly acts as a hormone, and its endocrine activity promotes blood calcium and phosphate balance through the modulation of intestinal absorption.[3–5] In addition, Vitamin D also acts as an autocrine and paracrine agent performing varied functions such as regulating cell differentiation, cell maturation, and innate immune system.[6]

Vitamin D has recently proven its role as a potent immunomodulator due to its anti-inflammatory effect through inhibition of cytokine synthesis by cells of the immune system and stimulation of monocytes or macrophages to secrete peptides with potent antibiotic activity such as beta-defensin-4 and cathelicidin.[7] As vitamin D plays a crucial role in bone equilibrium and immunity,

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there is biological rationale to suspect that vitamin D deficiency could negatively affect the periodontium.[8]

Given climate variations and recent lifestyle changes that limit outdoor sun exposure, vitamin D deficiency has become a widely prevalent global health problem.[2] Therefore, it is highly likely that many patients suffering from periodontitis, also suffer from vitamin D deficiency and could benefit from restoring the vitamin D levels to normal values.

Plethora of research exists exploring the function of vitamin D in the prevalence and progression of periodontitis and investigating the effect of Vitamin D supplements on periodontal treatment results but very few studies have investigated the role of nonsurgical periodontal therapy (NSPT) on Vitamin D levels. Vitamin D also plays an important role in the reabsorption of calcium.[9]

The effect of NSPT on serum Vitamin D and calcium levels is worth exploring as NSPT is known to reduce many inflammatory markers including Vitamin D levels, which in turn could improve calcium levels.[10]

This study aimed to evaluate and correlate levels of serum calcium and 25-hydroxycholecalciferol (vitamin D) in patients suffering from periodontitis and healthy controls without periodontitis before and following NSPT.

Materials and Methods

After getting approval from the institutional ethical committee (reference number IEC/Perio/4/21), a total of 100 patients willing to participate in the study and reporting to the outpatient department of Periodontics were enrolled, with 50 patients each in the test and control groups, respectively. The study was done over a period of 1 year from 2022 to 2023 in Greater Noida. Sampling was done by a method of convenience sampling. The control group included patients without periodontitis, whereas the study group included patients with periodontitis.

A total of 52 participants could complete the study with 26 patients each in the test and control groups, respectively.

The included patients were aged 18–65 years of both genders, subjects suffering from periodontitis (according to AAP classification 2017), and subjects without periodontitis (according to AAP classification 2017). Pregnant and lactating females, patients on hormone replacement therapy, receiving treatment with bisphosphonates, receiving multivitamins or food supplements containing calcium or vitamin D, receiving immunosuppressive drug therapy, and patients with syndromes and genetic disorders affecting bone metabolism were excluded from the study.

The participants were evaluated on three visits, at baseline, 3 months from the baseline visit, and 6 months from the baseline visit. On the first visit, ~ 3–5 mL of venous blood was collected from all the participants for the evaluation of serum calcium and Vitamin D levels. The various parameters evaluated included probing depth, clinical attachment level (CAL), gingival index (Loe and Silness 1963), plaque index (Silness and Loe 1964), and IOPA (at the site having greatest pocket depth).

Participants of both the groups received NSPT which included extensive scaling and root planing using ultrasonic scalers and curettes.

On the second visit (after 3 months), only the clinical parameters recorded at the first visit were again recorded.

On the third visit (after 6 months from the first visit), the blood sample was again collected for the assessment of serum calcium and Vitamin D levels (colorimetric analysis) and all the parameters that were recorded at the first visit were recorded again.

Statistical Product and Service Solution (SPSS) version 21 for Windows (Armonk, NY: IBMcorp) software was used to analyze the data. Statistical analysis was done by using tools of descriptive statistics such as mean, and SD for representing quantitative data.

Probability $p < 0.05$, considered as significant as alpha error set at 5% with confidence interval of 95% set in the study. The power of the study was set at 80% with beta-error set at 20%. The normality of data was checked using Shapiro–Wilk test. Unpaired *t*-test was used to find significant differences between both groups for parametric distribution data. Paired *t*-test was used to find significant differences within each group for parametric distribution data. Chi-square test was used to find the statistically significant difference for intergroup and intragroup comparison for percentage/proportion data.

Results

A total of 52 subjects completed the study with 26 participants in each group. Few of the participants dropped out of the study because of time constraints that did not allow them to report for follow-up visits.

Demographic details of the participants: Test group had 14 (53.8%) males and 12 (46.2%) females. The control group included 10 (38.5%) males and 16 (61.5%) females.

Evaluation and comparison of clinical parameters at baseline, 3 months, and 6 months are summarized in Table 1.

At the first visit (baseline), the mean plaque index score (2.05 ± 0.57 and 1.63 ± 0.67) showed a statistically significant difference in the values between the test and control groups ($p = 0.02$). At 3 months, the mean plaque index score (1.15 ± 0.63 and 0.82 ± 0.580.46) also showed a statistically significant difference in the values between the test and control groups ($p = 0.048$). At 6 months, the mean plaque index score (0.46 ± 0.4 and 0.26 ± 0.35) was not statistically significant between the test and control groups ($p = 0.07$).

At the first visit (baseline), the mean gingival index score (2.1 ± 0.51 and 1.39 ± 0.45) showed a significant statistical difference between the test and control groups ($p < 0.001$). At 3 months, the mean gingival index score (1.18 ± 0.56 and 0.86 ± 0.39) showed a statistically significant difference between the two groups ($p = 0.022$). At 6 months, the mean gingival index scores (0.42 ± 0.28 and 0.48 ± 0.3) were not statistically significant between the two groups ($p = 0.487$).

For the measurement of pocket probing depth (PPD) and CAL, at baseline, the mean values showed a significant statistical difference between the two groups ($p < 0.001$). At 3 months, the values again showed a significant statistical difference between the two groups ($p < 0.001$). At 6 months, the mean values for the test and control group showed a significant difference ($p < 0.001$).

Comparison of blood vitamin D and calcium values at baseline and 6 months are shown in Table 2.

At baseline, the mean blood vitamin D level values (12.73 ± 2.25 and 17.81 ± 4.03) showed a significant statistical difference between the two groups ($p < 0.001$). At 6 months, the mean vitamin D level values (24.53 ± 1.98 and 17.8 ± 4.14) revealed a highly significant statistical difference between the test and the control groups ($p < 0.001$).

At baseline, the mean blood calcium level values (9.46 ± 0.27 and 17.81 ± 4.03) showed statistical difference between the two groups which was not significant ($p = 0.935$). At 6 months, the mean

Table 1: Comparison of the clinical parameters in the test group and control groups

		Test group	*Control group*	*Intergroup comparison*
Clinical parameters	*Time interval*	*Mean ± SD*	*Mean ± SD*	*p-value*
Plaque index	Baseline	2.05 ± 0.57	1.63 ± 0.67	$p = 0.02$[a]
	3 months	1.15 ± 0.63	0.82 ± 0.580	$p = 0.048$[a]
	6 months	0.46 ± 0.4	0.26 ± 0.35	$p = 0.07$
Gingival index	Baseline	2.1 ± 0.51	1.39 ± 0.45	$p < 0.001$[a]
	3 months	1.18 ± 0.56	0.86 ± 0.39	$p = 0.022$[a]
	6 months	0.42 ± 0.28	0.48 ± 0.3	$p = 0.487$
Pocket probing depth	Baseline	5.61 ± 0.75	2.84 ± 0.36	$p < 0.001$[a]
	3 months	4.53 ± 0.94	2.53 ± 0.5	$p < 0.001$[a]
	6 months	3.3 ± 0.54	2.3 ± 0.47	$p < 0.001$[a]
Clinical attachment level	Baseline	5.61 ± 0.75	2.84 ± 0.36	$p < 0.001$[a]
	3 months	4.53 ± 0.94	2.53 ± 0.5	$p < 0.001$[a]
	6 months	3.3 ± 0.94	2.3 ± 0.5	$p < 0.001$[a]

[a]$p < 0.05$ – significant value

Table 2: Comparison of the blood parameters in the test and control groups

		Test group	*Control group*	*Intergroup comparison*
Blood parameters	*Time interval*	*Mean ± SD*	*Mean ± SD*	*p-value*
Vitamin D	Baseline	12.73 ± 2.25	17.81 ± 4.03	$p < 0.001$[a]
	6 months	24.53 ± 1.98	17.8 ± 4.14	$p < 0.001$[a]
Calcium	Baseline	9.46 ± 0.27	9.47 ± 0.39	$p = 0.935$
	6 months	9.55 ± 0.3	9.46 ± 0.44	$p = 0.386$

[a]$p < 0.05$ – significant value

values for the test and control groups (9.55 ± 0.3 and 9.46 ± 0.44) also did not show a significant statistical difference between the two groups ($p = 0.386$).

Discussion

The mean plaque index scores revealed a statistically significant difference in the values between the control and test groups at baseline 3 months; however, the difference in the values at 6 months did not show a statistically significant difference. This result is expected as at baseline the test group where the participants had periodontitis was likely to have high plaque scores as compared to healthy controls. This difference remained statistically significant even at 3 months. This could be attributed to poor motivation to maintain dental hygiene measures among the test group patients which could also be a major factor predisposing to poor plaque control and development of periodontitis. The difference in the mean plaque scores between the two groups were statistically significant at 6 months. This shows that the improvement in dental hygiene was similar in the two groups, where the test group participants, after undergoing repeated oral hygiene evaluations, developed motivation to maintain good dental hygiene and demonstrated improved oral hygiene and thus reduced mean plaque score.

The mean plaque scores comparison between baseline and 6 months values showed a significant statistical difference for both the test and control groups with the test group showing a highly significant statistical difference ($p < 0.001$). By these results, we can infer that NSPT benefited both the groups with the test groups showing a greater benefit.

The results of the study revealed that at baseline, the statistical difference in the mean values of gingival index scores between the test and control groups was highly significant. This can be attributed to higher gingival index scores due to inflammation in the gingiva in the patients suffering from periodontal disease in comparison to the control subjects, as periodontitis itself being an inflammatory pathology is highly likely to have advanced from gingivitis. At 3 months, the statistical difference in the same values was significant. However, at 6 months, the statistical difference in the same values became non-significant. The results reflect an improvement in gingival inflammation following NSPT in both the test and control groups with the test group showing a greater improvement in gingival inflammation, and thus resulting in gingival index scores similar to the healthy controls, thus possibly explaining the non-significant statistical difference in gingival index scores between the two groups.

Both PPD and CAL were used to assess the degree of tissue destruction and monitor disease progression. While PPD measured the distance from the gingival margin to the bottom of the gingival sulcus/periodontal pocket, CAL was measured as the distance from the cementoenamel junction (CEJ) to the bottom of the periodontal pocket/gingival sulcus. CAL gives a more accurate measure of tissue destruction as PPD can give false readings as gingival margin position is subject to changes as in gingival enlargement or recession, whereas CEJ location remains comparatively constant throughout life. In this study, the PPD and CAL measurements were the same for the patients as none of the patients who were a part of the study presented with gingival enlargement or recession.

The results revealed that at baseline, the statistical difference between the mean values of both PPD and CAL for both the groups was highly significant at baseline, 3 months, and 6 months. This again shows that the test group gained greater benefit from NSPT as compared to the control group.

The results also demonstrated that at baseline, the statistical difference in the mean blood vitamin D levels was highly significant between the test and control groups due to lower Vitamin D levels

in the test participants as compared to the healthy controls. This is expected as lower Vitamin D levels in periodontitis patients could be either due to a deficiency resulting in lower calcium absorption and thus greater predisposition to bone loss or due to consumption of Vitamin D by the body owing to its role as an anti-inflammatory and immunomodulatory agent to fight the inflammation seen in periodontitis. At 6 months, the intergroup comparison in the vitamin D levels again revealed a highly significant statistical difference. This shows that following NSPT, Vitamin D levels improved in the periodontitis patients of the test group, whereas NSPT did not show any effect in patients without periodontitis.

This could be attributed to reduced inflammation and improvement in clinical parameters, such as plaque index, gingival inflammation, PPD, and CAL, following NSPT and the possible role vitamin D as an anti-inflammatory, antimicrobial, and immune-modulatory agent in addition to playing an important role in bone metabolism. Vitamin D is known for its ability to downregulate the proliferation of T cells and the production of cytokines and its role in the production of cathelicidins and defensins from gingival epithelium against infection. Vitamin D is proven to play a role in the inhibition of periodontal inflammation by decreasing the expression of various pro-inflammatory mediators, such as Interleukin-6, Interleukin-8 IL-17A, matrix metalloproteinases, such as MMP-1 and MMP-3, plasminogen activator inhibitor 1 and tumor necrosis factor- α. Antimicrobial properties of vitamin D include selective inhibition of *P. gingivalis* growth and reduction of virulence factor gene expression. Certain antibacterial properties of vitamin D itself might be another function of 1,25(OH)2D. Vitamin D is known to attenuate nuclear factor κB (NFκB) activation by *P. gingivalis* in human monocytic cell line, which modulates the inflammatory response. Also, vitamin D is actively involved in potentially downregulating the expression of monocyte TLR2 and TLR4, thereby suppressing the inflammatory responses that are activated by these receptors. Vitamin D might also encourage an appropriate innate immune response and prevent an over-expression of the innate immune responses and thus prevent the tissue damage associated with these responses. Vitamin D promotes the transformation from a T1 helper (Th1) cell activity to a more tolerant Th2 response which results in a less destructive tissue response to inflammation and thus prevention of inflammation-associated tissue destruction.[11]

This explains the reduced levels of Vitamin D in subjects with periodontitis in comparison to healthy controls at baseline. Lower vitamin D levels and thus lower anti-inflammatory, immune-modulatory, and antimicrobial response of the test group is likely to contribute to the progression of periodontal disease.

Following NSPT, a decrease in inflammation as evidenced by an improvement in clinical parameters resulted in a reduced consumption of Vitamin D as an anti-inflammatory agent and thus improved Vitamin D levels in the group with periodontitis.

There was statistically no significant difference in the mean calcium levels between both the group with periodontitis and group with healthy subjects at baseline and 6 months. This shows that although Vitamin D levels improved for the test group, they remained in the insufficient range and were not enough to raise the blood calcium level.

Thus, it is fair to advise Vitamin D supplementation in addition to traditional NSPT in periodontitis patients to not only improve the therapeutic outcome but also to possibly raise the blood calcium levels.

A similar result was seen by Madi et al.[2] who found a correlation between reduced Vitamin D levels and periodontitis, where periodontitis patients with low Vitamin D levels suffered greater bone loss as compared to periodontitis patients with comparatively higher Vitamin D levels.

Similar results were also seen in the study done by Laky et al.[12] who found that patients with periodontitis had lower Vitamin D levels as compared to subjects without periodontal disease.

In a study done on Puerto Rican adults, Abreu et al.[13] found lower Vitamin D levels in patients with periodontitis as compared to the healthy controls.

Bonnet et al.[7] found moderate evidence correlating low plasma Vitamin D levels with periodontitis as measured with clinical parameters such as gingival index (GI) and loss of attachment (LOA).

Antonoglou et al.[14] in their study also found a correlation between low Vitamin D levels and periodontitis; however, they could not confirm an association between Vitamin D levels and periodontal health.

Dietrich et al.,[15] in their study reported that individuals with high serum vitamin D presented with lower probing pocket depth and CAL.[10]

Similar results were also found by Jimenez et al.[16] in his study, whereas Millen et al.[17] found that women with higher Vitamin D levels of >50 ng/mL were 33% at a lower risk of periodontal diseases as compared to women with inadequate levels of Vitamin D.

The study done by Teles et al.[18] supported the proposed anti-inflammatory role of Vitamin D and found that subjects with adequate vitamin D levels had a lower tendency of bleeding on probing.

A study done by Ketharanathan et al.[19] among the Tamil and Norwegian populations found a correlation between reduced Vitamin D levels and periodontal disease as evidenced by radiographic bone loss.

However, according to a report by Pinto et al.[20] the current data in literature is inconclusive to link Vitamin D levels with periodontal disease.

In addition to its role in periodontal disease, low Vitamin D levels have also been implicated in poor glycemic control in diabetics as evidenced by the results of the study by Agrawal et al.[21] who found that low Vitamin D and calcium levels are correlated with increased random blood sugar and glycated hemoglobin levels in addition to an increase in probing pocket depth and clinical attachment loss, thus contributing toward an increase in periodontal disease severity.

Similarly, Joseph et al.[22] found a lower level of blood Vitamin D level in individuals suffering from chronic periodontitis (CP) and patients with both CP and diabetes mellitus.

Vitamin D deficiency has also been proven to play a role in aggressive periodontitis (AgP) according to the study done by Anbarcioglu et al.[23] which revealed that patients with AgP presented with lower serum Vitamin D levels as compared to controls and CP patients.

However, Liu et al.[24] found contradictory results in their study with patients with AgP presenting with higher Vitamin D levels.

Isola et al.[25] found that individuals suffering from CP and CP along with chronic heart disease (CHD) had significantly lower levels of blood vitamin D as compared to individuals with only CHD and healthy controls. Moreover, the presence of CP resulted in lower blood vitamin D levels. Thus, low Vitamin D levels were directly correlated with periodontal disease.

In addition to the above-mentioned studies, the results of the study done by Pai et al.[10] showed that NSPT improved the Vitamin D levels in CP patients.

Thus, this study along with the aforementioned studies can be extrapolated to conclude that low Vitamin D levels are associated with periodontitis with NSPT improving the Vitamin D levels in periodontitis and having no effect on calcium levels. The results of the present study can further be used to justify the use of Vitamin D and calcium supplementation as an adjunctive treatment modality along with NSPT to improve the therapeutic outcome and prevent further disease progression.

Strength

- Evaluated serum Vitamin D levels in a varied age-group.
- Evaluated the effect of NSPT on calcium and Vitamin D levels.

Limitations

- Relatively shorter follow-up time.
- Small sample size.
- No microbiological analysis done.

Future Direction

- The role of Vitamin D supplementation as an adjunct to conventional periodontal therapy can be explored.
- The effect of surgical periodontal therapy in improving Vitamin D and calcium levels can be investigated.

Conclusion

This study confirmed a correlation between serum Vitamin D levels and periodontitis with periodontitis patients presenting with lower Vitamin D levels as compared to their healthy controls. All the patients who participated in the study received NSPT as a treatment modality. Further, the results of the study showed that NSPT significantly improved the Vitamin D levels in periodontitis patients by reducing the bacterial bioburden and the subsequent inflammatory response.

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