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Evaluation of Vitamins C, D, and E in Periodontal Health Before and After Therapeutic Intervention

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Abstract:
Periodontal diseases characterized by chronic inflammation and tissue destruction have been linked to oxidative stresses and the role of the body's antioxidant defence systems. In this reports, we investigated the impact of antioxidant vitamins C, D, and E on periodontal health before, and after non-surgical periodontal treatment.
In a clinical study with 120 participants diagnosed with moderate to severe periodontitis, we developed a control group where participants received scaling and root planing (SRP) only, and a test group where participants received scaling and root planing (SRP) along with daily supplementation of vitamins C (500 mg), D (1000 IU), and E (400 IU) for 3 months. Clinical measurements (probing depth (PD), clinical attachment level (CAL), bleeding on probing (BOP) were recorded at baseline and 3 months of treatment. Serum levels of vitamins C, D, and E were also determined using an enzyme-linked immunosorbent assay (ELISA).
There was a statistically significant improvement in clinical parameters in both SRP only and daily supplementation of vitamins C, D, and E groups. The test groups showed significantly greater difference in PD (SRP group: 3.5 ± 0.5 mm vs. SRP + C, D, E group: 4.1 ± 0.6 mm, $p<0.01$); CAL (SRP group: 3.7 ± 0.6 mm vs. C, D, E: 4.3 ± 0.7 mm; $p<0.05$), and BOP (SRP group: 20.3 ± 7.2% vs. SRP + C, D, E: 30.4 ± 8.5%, $p<0.01$).
Serum levels of vitamins C, D, and E improved significantly in the test groups (vitamin C: 1.5 ± 0.4 mg/dL, $p<0.001$; vitamin D: 35.6 ± 6.1 ng/mL, $p<0.01$; vitamin E: 12.3 ± 2.0 mg/L, $p<0.001$); while the control group did not exhibit any statistically significant difference regarding serum levels of vitamins C, D, and E.A considerable negative correlation was found between serum antioxidant levels and clinical parameters. Specifically, higher antioxidant levels were associated with better periodontal health. In summary, these findings support the interpretive assumption that antioxidant vitamins C, D, and E played a positive and meaningful role in reducing oxidative stress and promoting a healthy periodontal response. As a result, vitamins C, D, and E, either alone or in combination, may represent a key supplement to conventional periodontal therapy, providing a significant opportunity for periodontal disease patients and improving clinical outcomes. Future studies should identify the long-term benefits of antioxidant supplementation and appropriate dosing regimens in the management of periodontal disease.

Key words: Vitamin C, D, E, Periodontal

Introduction

Periodontal diseases, such as gingivitis and periodontitis, are among the most common chronic inflammatory diseases in the world that affect tooth-supporting structures, including the gingiva, periodontal ligament, and alveolar bone (1). The genesis of periodontal diseases is influenced by accumulating biofilm (dental plaque) on teeth and the immune-inflammatory response of the host to the disease-causing microbes (2). The reaction of microbial pathogens and the host immune response causes the release of pro-inflammatory cytokines, matrix metalloproteinases (MMPs), and reactive oxygen species (ROS), all of which contribute to tissue destruction and bone loss. Of these numerous factors, oxidative stress appears to play an important role in the initiation and pathogenesis of periodontal diseases.(3)

Oxidative stress is generated as a result of an imbalance between the production of ROS and the antioxidant defense mechanisms of the host.ROS, including superoxide anions, hydrogen peroxide and hydroxyl radicals, are highly reactive species which can damage a host of cellular elements including lipids, proteins and DNA (RRR). In the setting of periodontal diseases, ROS may be produced by microbes as well as host immune cells, such as neutrophils and macrophages, during the inflammatory reaction (4). While ROS are involved when responding to microbes' infections, too much ROS may also exacerbate disease by harming periodontal tissues when persistent inflammation and tissue damage are taking place. That is, antioxidant vitamins, which can neutralize ROS and reverse damage due to oxidative damage, are crucial for periodontal health (RRR) (5).

Antioxidant vitamins, particularly C, D and E, have gained interest for their role in reducing oxidative damage and ultimately promoting periodontal health. Vitamin C (ascorbic acid), which is a water-soluble antioxidant, is capable of scavenging free radicals and regeneration of other antioxidants, such as vitamin E. Additionally, Vitamin C is critical for collagen synthesis, which is essential for the integrity and repair of periodontal tissues (6). Vitamin D, which is a fat-soluble secosteroid, is best known for calcium homeostasis and bone metabolism (RRR); however, D also has potent immunomodulatory and anti-inflammatory actions by ultimately downregulating pro-inflammatory cytokines and increasing the production of anti-microbial peptides such as cathelicidin, which can assist with the prevention of periodontal pathogens. Vitamin E, which is a lipid-soluble antioxidant, prevents the lipid peroxidation of membranes; also, vitamin E cooperatively interacts with vitamin C to scavenge free radicals (RRR) (7).

The relationship with vitamin antioxidants and periodontal health has been extensively reviewed, and associations with vitamin deficiencies and risk and severity of periodontal disease have been shown (RRR) (8). For instance, it was noted that Vitamin C deficiency is used as a diagnostic tool for scurvy, which presents with bleeding gums and

tooth loss (9). For Vitamin D, low levels are associated with increasing periodontal inflammation and loss of bone. Vitamin E deficiency increases oxidative stress and impairs healing of periodontal disease (RRR). The role of antioxidant vitamins with respect to periodontal treatment has been minimally explored. For example, although SRP is the gold standard for non-surgical treatment of periodontal disease, it may not be effective due to continuing inflammation and oxidative stress. Therapies that deal with inflammation and oxidative stress may enhance the effectiveness of SRP therapy (RRR) (10).

The aim of this study primarily will be to measure vitamins C, D, and E levels in periodontal disease participants prior to and after receiving non-surgical periodontal therapy. And secondarily, to study the effects of antioxidant supplementation on clinical measures of periodontal disease activity. All participants will be the same. Serum, vitamin levels will be collected and correlated with measures of periodontal disease activity including probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP). This research study will provide insight for antioxidant vitamins as potential therapeutic agents for clinical practice and in this clinical setting. Findings from this research study may lead to more innovative and adjunctive therapies that use these antioxidants to enhance effects of conventional periodontal treatment in the future.

Methodology

Study Design

This prospective clinical study was conducted over 12 months at different dentistry clinics in Baghdad city. The Institutional Ethics Committee approved the study protocol, and written informed consent was obtained from all participants.

Participants

One hundred twenty systemically healthy adults (aged 25–55) with moderate to severe chronic periodontitis were recruited. Participants were excluded if they had a history of systemic diseases, smoking, or antioxidant supplementation within the past six months.

Intervention

Participants were divided into two groups:

Control Group (n=60): Received scaling and root planing (SRP) alone.

Test Group (n=60): Received SRP followed by daily supplementation with vitamins C (500 mg), D (1000 IU), and E (400 IU) for 3 months.

Clinical and Biochemical Assessments

Clinical Parameters: Probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP) were recorded at baseline and 3 months post-treatment.

Serum Antioxidant Levels: Blood samples were collected to measure serum levels of vitamins C, D, and E using enzyme-linked immunosorbent assay (ELISA) kits purchased from Mybiosource company.

Statistical Analysis

Data were analyzed using SPSS software (version 25.0). Paired t-tests and independent t-tests were used to compare within-group and between-group differences, respectively. A p-value <0.05 was considered statistically significant.

Results

The results of this study are presented in three tables, highlighting the clinical and biochemical outcomes before and after treatment in both the control and test groups.

Table 1: Clinical Parameters at Baseline and 3 Months Post-Treatment

Parameter	Control Group (Baseline)	Control Group (3 Months)	Test Group (Baseline)	Test Group (3 Months)	p-value (Within Group)	p-value (Between Groups)
Probing Depth (PD, mm)	5.2 ± 0.8	4.1 ± 0.6	5.3 ± 0.7	3.5 ± 0.5	<0.01	<0.01
Clinical Attachment Level (CAL, mm)	4.8 ± 0.9	4.3 ± 0.7	4.9 ± 0.8	3.7 ± 0.6	<0.05	<0.05
Bleeding on Probing (BOP, %)	45.6 ± 10.2	30.4 ± 8.5	46.2 ± 9.8	20.3 ± 7.2	<0.01	<0.01

Interpretation: Both groups showed significant improvements in clinical parameters after treatment, but the test group (SRP + antioxidant supplementation) demonstrated more substantial reductions in PD, CAL, and BOP compared to the control group (SRP alone). The between-group differences were statistically significant ($p<0.01$ for PD and BOP, $p<0.05$ for CAL).

Table 2: Serum Antioxidant Levels at Baseline and 3 Months Post-Treatment

Parameter	Control Group (Baseline)	Control Group (3 Months)	Test Group (Baseline)	Test Group (3 Months)	p-value (Within Group)	p-value (Between Groups)
Vitamin C (mg/dL)	0.8 ± 0.2	0.9 ± 0.3	0.8 ± 0.2	1.5 ± 0.4	<0.05	<0.001
Vitamin D (ng/mL)	25.3 ± 5.4	26.1 ± 5.6	24.8 ± 5.2	35.6 ± 6.1	<0.05	<0.01

Vitamin E (mg/L)	8.2 ± 1.5	8.4 ± 1.6	8.1 ± 1.4	12.3 ± 2.0	<0.05	<0.001

Interpretation: Serum levels of vitamins C, D, and E increased significantly in the test group after antioxidant supplementation ($p<0.001$ for vitamins C and E, $p<0.01$ for vitamin D). In contrast, the control group showed no significant changes in antioxidant levels, except for a marginal increase in vitamin C ($p<0.05$).

Table 3: Correlation Between Serum Antioxidant Levels and Clinical Parameters

Parameter	Vitamin C (r-value)	Vitamin D (r-value)	Vitamin E (r-value)	p-value
Probing Depth (PD)	-0.72	-0.65	-0.68	<0.01
Clinical Attachment Level (CAL)	-0.61	-0.58	-0.63	<0.05
Bleeding on Probing (BOP)	-0.75	-0.70	-0.73	<0.01

Interpretation: A strong negative correlation was observed between serum antioxidant levels and clinical parameters. Higher levels of vitamins C, D, and E were associated with lower PD, CAL, and BOP scores, indicating a protective role of these antioxidants in periodontal health.

Discussion:

The results we found in this study offer strong support for the role of antioxidants vitamins C, D, and E in improving periodontal health and outcomes of non-surgical periodontal treatment. While both groups demonstrated significant differences in clinical parameters, the greater changes were observed in PD, CAL and BOP for the test group, compared to the control group, suggest their potential as adjunct therapies to conventional periodontal treatment. Our finding parallel the growing literature supporting the role of oxidative stress in the etiology of periodontal disease, and potential protective effect of antioxidants in preventing tissue damage.

Oxidative stress, defined as an imbalance between reactive oxygen species (ROS) and antioxidants is a significant modulator in periodontal tissue destruction. ROS induce damage to tissues by oxidizing lipids, proteins, and DNA. Antioxidants counteract ROS damage by scavenging free radicals to prevent cell and tissue from oxidative damage. Compared to control group receiving scaling and root planing (SRP) alone, the test group receiving SRP and antioxidant supplementation demonstrated greater improvement in clinical parameters and serum antioxidant levels. Therefore antioxidant vitamins C, D, and E may play a critical role in reducing oxidative stress and enhance periodontal healing.

Vitamin C is a water soluble vitamin and a powerful antioxidant. It scavenges free radicals, regenerates other antioxidants (e.g. vitamin E), and synthesizes collagen required in tissue repair.

The notable and substantial increase in serum vitamin C concentrations in the intervention group ($p<0.001$) has been associated with the improved clinical parameters that were observed in the study. Collectively these results affirm that the vitamin C can serve to provide improvements in clinical periodontal parameters. Furthermore, evidence has been shown regarding the positive effects of vitamin C and oxidative stress related to periodontal health: (11) outcome study of patients showed chronic periodontitis showed the significant effects of vitamin C on several oxidative stress markers. Likewise (12) ultimate conclusions and possible mechanism behind vitamin C supplementation positively impacted the periodontal healing of patients who are undergoing periodontal care non-surgically. Vitamin D, which possesses immunomodulatory and anti-inflammatory effects, likely also contributed to the improved clinical outcomes observed in this present study. This immunomodulatory action antagonized the pro-inflammatory cytokines in the development of periodontal disease (i.e. interleukin-6 [IL-6], TNF-α); (13) information supports that vitamin D provides a systemic anti-inflammatory effect (via inhibited IL-6 and TNF-α production) that may be influential in reducing inflammation in periodontitis. Vitamin D supplementation also enhances the creation of antimicrobial peptides, like cathelicidin, that combat pathogens implicated in periodontal disease. The effect noted in the test groups change in serum vitamin D levels ($p<0.01$) supports significant contribution of the vitamin D contribution to the success of the periodontal healing. The findings of the present study corroborate previous findings that identified specific benefits of vitamin D for periodontal health. For example, (13) showed that vitamin D supplementation significantly inhibited the amount of inflammatory markers amongst a group of participants with periodontal disease. Similarly, (14) concluded that those participants who had higher vitamin D levels were likely to have lower prevalence of periodontitis as compared to participants with lower vitamin D levels.Vitamin E, a fat-soluble antioxidant, is a component that protects cell membranes from lipid peroxidation, and it works in concert with vitamin C to quench free radicals. The dramatic increases in serum vitamin E ($p<0.001$) in the test group relate to less oxidative damage and more favorable periodontal parameters. The outcomes in this study were further supported by previous research addressing the role of vitamin E and its protective role against tissue damage associated with oxidative stress. For example, (15,16) found that vitamin E supplementation positively influenced periodontal status in patients with chronic periodontitis and non-insulin dependent diabetes mellitus. In another example, (17,18) demonstrated that the vitamin E supplementation improved clinical outcomes and decreased oxidative stress for patients with periodontal disease.

For any given sample of patients with chronic periodontitis, the strongest negative association between serum antioxidant levels and clinical parameters consisted of PD, CAL, and bleeding on probing. Increased levels of serum antioxidant profiles suggest greater periodontal health. These results also correlate with previous studies demonstrating the protective effects of antioxidants against oxidative stress related tissue damage. The

contribution of the synergistic action of vitamins C, D, K, and E likely further contributed to the overall improved clinical outcomes in the test group. Together the vitamins likely worked to quench free radicals, down-regulate inflammation, and up-regulate tissue repair should be considered as possible adjunctive therapy in conventional periodontal treatment.

While this study offers valuable insight, there were some limitations to consider. The duration of the study was limited to 3 months only, which may reflect the one-time effect of the antioxidant supplementation rather than global health effects. It would have been advantageous to follow the outcomes over a sufficiently longer time frame. The sample population within the study consisted of individuals who were systemically healthy, which limits the applicability of results to the current population of patients with systemic conditions that exacerbate periodontal diseases, such as diabetes or cardiovascular diseases. Future research should evaluate the long-term effects of antioxidant supplementation in beard healthy patients and consider patients with systemic comorbidities. Future studies should study dosing regimens and combinations of antioxidants to determine the best dose and maximize clinical and protective effectiveness are warranted.

Overall, this research demonstrated the promise of antioxidant vitamins C, D, and E to bolster periodontal health, as well as improve non-surgical periodontal therapy outcomes; overall antioxidant supplementation drove down oxidative stress levels, clinical outcomes improved, and healing of the periodontal tissues were observed. The vitamin supplementation offered a possible adjunctive avenue to better manage periodontal diseases. More longitudinal studies will be needed to investigate the long-term outcomes of vitamins D, C, and E through antioxidant supplementation, as well as effective dosing strategies and regimens regarding their effectiveness for periodontal management.

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