ORIGINAL RESEARCH

The Impact of Nonsurgical Periodontal Therapy on Serum Levels of Dickkopf-Related Protein-1 in Smokers and Nonsmokers with Periodontitis: A Prospective Comparative Study

Ehab Azab [1], Alaa Attia[1,2], Wael Yaghmoor[1], Salwa Aldahlawi [1], Abdel-Rahman Youssef[1,3]

[1]Department of Basic and Clinical Oral Sciences, Faculty of Dentistry, Umm Al-Qura University, Makkah, Saudi Arabia; [2]Department of Oral Medicine and Periodontology, Faculty of Dentistry, Al-Azhar University, Assiut, Egypt; [3]Department of Microbiology and Immunology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt

Correspondence: Ehab Azab, Department of Basic and Clinical Oral Sciences, Faculty of Dentistry, Umm Al-Qura University, Prince Sultan Road, Makkah, 21421, Saudi Arabia, Tel +966 12 527 0000, Email etazab@uqu.edu.sa

Purpose: This study aimed to evaluate and compare Dickkopf-related protein-1 (DKK1) serum levels and periodontal clinical parameters of smokers and nonsmokers with periodontitis at baseline and after nonsurgical periodontal treatment.

Patients and Methods: A prospective comparative study was conducted among 24 patients with periodontitis who were divided according to the smoking habits into two groups: nonsmokers (G1) and smokers (G2). All the participants were assessed clinically by recording the probing depth (PD), clinical attachment loss (CAL), plaque index (PI), and bleeding index (BI), and immunologically by measuring the DKK1 serum levels at baseline and six weeks after nonsurgical periodontal therapy.

Results: The two groups showed a significant decrease in PI, BI, and CAL after periodontal therapy ($p < 0.05$), while PD was significantly reduced in G1 ($p = 0.005$). The PI mean value was significantly higher at the baseline in G2 versus G1 ($p = 0.050$), while PD, BI, and CAL values were not significantly different between the groups ($p = 0.056$, $p = 0.241$, and $p = 0.381$, respectively). For DKK1 serum levels, there was a statistically significant decrease after treatment compared to the baseline for both groups (G1: $p < 0.001$; G2: $p < 0.001$) but no significant difference before ($p = 0.131$) and six weeks after treatment ($p = 0.334$) between the two groups.

Conclusion: Although nonsurgical periodontal treatment effectively improved periodontal clinical parameters and reduced DKK1 serum levels, there were no significant differences in the DKK1 serum levels among the smokers and nonsmokers with periodontitis.

Keywords: periodontitis, dickkopf-related protein-1, periodontal therapy, Wnt signaling, bone cells

Introduction

Periodontitis is a form of inflammatory disease that affects tooth surrounding structures as a result of interactions between the host's immune cells and periodontal pathogenic bacteria.[1] These interactions involve releasing many substances that provoke inflammation, connective tissue degradation, and alveolar bone destruction, which may result in tooth loss.[2,3] The host susceptibility to periodontal pathogens has been reported to be primarily responsible for the disease initiation and progression.[4] Smoking and other conditions, including hereditary diseases, immunological diseases, and systemic diseases, have been reported to induce the risk of periodontitis in patients.[5]

Smoking is a well-recognized environmental risk factor for periodontal disease initiation and progression.[6] Studies have found that smoker patients are more susceptible to periodontitis than nonsmoker controls, and show deeper probing depth (PD) and greater attachment and alveolar bone loss.[7] Smoking modifies the periodontal microbial colonies and the host immune response.[8,9] These modifications lead to changes in vascular permeability, reduction in antibody production, impairment in neutrophil chemotaxis and phagocytosis, increase in reactive oxidative stress, and disturbance in the other

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immune cells' activities, causing an increase in proinflammatory mediators' formation.[9,10] Moreover, smoking affects periodontal healing capacity and posttreatment tissue response.[11]

Periodontal pathogenic bacteria have many virulence factors that trigger the host immune cells to release numerous proinflammatory cytokines, inducing connective tissue degradation and causing an imbalance in bone metabolism that favors bone resorption.[3,12,13] Bone homeostasis is maintained by balanced activity between osteoblastic bone-forming cells and osteoclastic bone-resorbing cells. Bone metabolism is a complex process that utilizes many transduction pathways.[14,15] Understating the different mechanisms of bone metabolism could help in the management of periodontal disease.[16]

Osteocytes have been shown as a central regulator cell that organizes both osteoblast and osteoclast activities either directly by cell connection or indirectly by factors secretion.[17,18] Osteocytes produce Dickkopf-related protein-1 (DKK1), which blocks the Wnt/ß-catenin signaling pathway required for osteoblast differentiation and bone formation.[19–23] DKK1 interferes with the Wnt signaling pathway by binding to the osteoblast's extracellular region of lipoprotein receptor-related protein 5/6 (LRP5/6), inducing ß-catenin degradation by preventing its translocation to the nucleus. This leads to the inhibition of the Wnt/β-catenin signaling pathway and the suppression of bone formation.[24,25]

Several studies have demonstrated that DKK1 is implicated in periodontal disease. A significant increase in the expression of DKK1 mRNA was found in the gingival tissue of patients with chronic periodontitis compared to the gingival tissue of healthy individuals, while DKK1 serum levels did not change significantly in patients with chronic periodontitis compared to healthy individuals.[26] In addition, it has been found that smokers with chronic periodontitis had higher DKK1 serum levels compared to nonsmokers with chronic periodontitis.[27] Moreover, systemic administration of the DKK1 antibody enhanced alveolar bone regeneration in the experimental molar extraction model in rats.[28]

Early detection of periodontitis plays a crucial role in its prevention and progression.[2,29] Clinical and radiographical examinations are still the best available tools for the diagnosis of periodontal disease; however, they do not carefully measure the disease activity or evaluate the patient's treatment response or predict future disease progression.[30] Hence, the search is ongoing to discover a reliable diagnostic tool such as biomarkers for early detection and monitoring of periodontal disease.

There are scarce data regarding the impact of nonsurgical periodontal therapy on DKK1 serum levels in smoker and nonsmoker patients with periodontitis. Therefore, we hypothesized that there is no difference in the DKK1 serum levels in smoker and nonsmoker patients with periodontitis after nonsurgical periodontal therapy.

Materials and Methods
Study Design and Participants
This study was approved by the Umm Al-Qura University Faculty of Dentistry Institutional Review Board Committee (IRB: 157–19). Twenty-four patients with periodontitis were recruited from the Dental Teaching Hospital, Umm Al-Qura University, Makkah, Saudi Arabia, after obtaining their informed consent. Participants who were 25–55 years old, systemically healthy, had ≥ 15 remaining teeth (excluding third molars) and had periodontitis (interproximal clinical attachment loss (CAL) ≥ 3 mm in at least two nonadjacent teeth, probing depth (PD) ≥ 5 mm, and radiographic bone loss ≥ 20%[31]) were included in this study. In addition, smokers were defined as regular users of any smoking habit, including cigarettes, electronic cigarettes, cigars, water pipes, and smokeless tobacco.

Participants who received periodontal treatment in the last six months; were pregnant or lactating women; were diagnosed with osteoporosis; used antibiotics in the previous six months; needed antibiotic premedication; used glucocorticoids, bisphosphonates, or denosumab; were regularly using nonsteroidal anti-inflammatory drugs; or were unable to sign the consent form were excluded from this study.

Study Procedures
Periodontal clinical parameters, including PD, plaque index (PI), bleeding index (BI), and CAL, and the smoking habits of the participants were assessed and recorded. Peripheral blood samples were collected in serum separator tubes, kept for clotting for up to 30 minutes at room temperature, and then centrifuged at 3000 rpm for 10 minutes. Serum was

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collected and stored at −80°C. The participants were asked to visit the clinic one week later to receive nonsurgical periodontal treatment, including oral hygiene instructions and scaling and root planing. Six weeks after nonsurgical periodontal treatment, the participants' periodontal clinical parameters were assessed, and serum samples were obtained as described above.

Enzyme-Linked Immunosorbent Assay

DKK1 serum levels at baseline and after periodontal therapy were measured using an enzyme-linked immunosorbent assay (ELISA) (Human DKK1 Quantikine ELISA Kit; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. The microplate was precoated with human DKK1 monoclonal antibody. Standard or diluted serums (8-fold dilution) were added per well and incubated for 2 hours. After washing, human DKK1 conjugate was added to each well, incubated for 2 hours, and then washed. Substrate was added for 30 minutes, and then the reaction was stopped. The optical density of each well was determined at 450 nm using an ELISA spectrophotometric reader (SPECTROstar; BMG LABTECH, Offenburg, Germany). The total amounts of DKK1 were displayed as picogram/ milliliter (pg/mL).

Statistical Analysis

Statistical Package for the Social Sciences (version 26) and GraphPad Prism 7 (GraphPad Software, San Diego, CA) were utilized for analytical statistics and data presentation. The data were represented using mean and standard error (SE). Paired *t*-test was utilized to analyze the effect of periodontal treatment in the same group, while unpaired *t*-test was used to compare the means of the smoker and the nonsmoker groups. The difference was considered statistically significant at $p \leq 0.05$.

Results

Twenty-four patients with periodontitis were selected for this study and divided based on their smoking history into group 1 (G1: 12 nonsmokers) and group 2 (G2: 12 smokers).

Evaluation of Clinical Parameters

The descriptive analysis of the periodontal clinical parameters for both the nonsmokers (G1) and the smokers (G2) was expressed in mean, standard error, and t- and p-values as shown in Tables 1 and 2.

At baseline, G2 (smokers) showed a worse clinical finding as a higher mean PD and CAL and lower BI; however, this difference was not statistically significant. The PI was significantly higher in G2 compared to G1 ($p < 0.05$) (Table 1).

After nonsurgical periodontal treatment, both groups showed significant improvements in all clinical parameters (PI, BI, and CAL) (Table 2). Although the reduction in PD was significant in G1 ($p = 0.005$), the change in PD in G2 was not statistically significant ($p = 0.287$) (Table 2). Comparing the clinical findings of both groups six weeks after nonsurgical therapy revealed that both groups had a similar presentation, with the only difference being a deeper residual PD in G2 ($p < 0.001$) (Table 1).

Serum DKK1 Levels

Descriptive statistical findings of serum DKK1 levels in G1 and G2 are presented in Table 3. Despite the increase in the mean values of DKK1 levels in the smokers versus the nonsmokers, there was no statistically significant difference in the DKK1 serum levels at the baseline ($p = 0.131$) and six weeks following the nonsurgical periodontal treatment ($p = 0.334$) (Table 3). However, the intragroup comparison showed that the DKK1 serum level was significantly reduced following nonsurgical treatment compared to the baseline for both groups ($p < 0.001$) (Figure 1).

Discussion

Periodontal pathogens and smoking induce the production of several mediators that can be detected in the saliva, serum, and gingival crevicular fluid (GCF) of patients with periodontitis, which could be used as biomarkers to diagnose and monitor periodontal tissue degradation and bone resorption.[32] Biomarkers are widely used nowadays in the medical field.

Table 1 Comparisons of Clinical Parameters Between Nonsmoker and Smoker Patients with Periodontitis at the Baseline and Six Weeks After Nonsurgical Periodontal Therapy

Parameters	Comparison	Mean	SE	t-Value	p-value
PI	G1(Baseline)	56.03	8.78	− 2.204	0.050*
	G2(Baseline)	81.20	7.52		
BI	G1(Baseline)	64.29	6.75	1.238	0.241
	G2(Baseline)	52.69	6.86		
PD	G1(Baseline)	2.79	0.12	− 0.912	0.056
	G2(Baseline)	3.36	0.21		
CAL	G1(Baseline)	3.11	0.31	− 2.141	0.381
	G2(Baseline)	3.57	0.29		
PI	G1 (6 weeks)	31.84	4.83	− 1.077	0.304
	G2 (6 weeks)	41.90	6.56		
BI	G1 (6 weeks)	28.15	3.36	− 0.222	0.828
	G2 (6 weeks)	29.14	2.65		
PD	G1 (6 weeks)	2.32	0.11	− 6.293	< 0.001**
	G2 (6 weeks)	3.18	0.15		
CAL	G1 (6 weeks)	2.60	0.26	− 1.294	0.222
	G2 (6 weeks)	3.12	0.29		

Note: *Significant (P≤0.05), **Highly significant (P≤0.01).
Abbreviations: BI, bleeding index; PI, plaque index; PD, pocket depth; CAL, clinical attachment loss; G1, nonsmokers; G2, smokers.

With advances in genomic and proteomic analysis, biomarkers are likely to succeed in disease diagnosis and treatment. Several proinflammatory and bone homeostasis molecules have been studied and evaluated for use as biomarkers for periodontitis, such as interleukin-1β (IL-1β), tumor necrosis factor-alpha, IL-6, IL-8, IL-17, C-reactive protein, matrix metalloproteinases, prostaglandins, osteocalcin, receptor activator of nuclear factor κB ligand (RANKL), and osteoprotegerin (OPG).[33,34] Although using these mediators might improve the diagnosis and prognosis of periodontal disease, none has been confirmed until now as a definitive biomarker.[35]

Bone homeostasis is maintained by balanced activity between bone-forming cells and bone-resorbing cells. Two major pathways are involved in this process. The first pathway is mediated via the balance between RANKL and OPG. Osteoclast differentiation and activation are stimulated by the interaction of RANK with its ligand, RANKL. Periodontal inflammation causes an imbalance in the osteoblast—osteoclast axis that favors osteoclastic bone resorption.[36] The second pathway is the Wnt/ß-catenin signaling, which is essential for bone formation, and its related inhibitors produced by osteocytes such as DKK1 have been involved in several inflammatory and bone disorders.[37] The DKK1 serum levels reflect the suppression of bone formation.[38] In addition, high DKK1 expression reduces alkaline phosphatase activity and endogenous β-catenin, while silencing Wnt receptor mRNAs inhibited alkaline phosphatase activity, which is essential for osteoblast differentiation.[39] However, the role of Wnt/ß-catenin signaling and its regulators in periodontal disease needs to be further elucidated.

The present study explored the effect of nonsurgical periodontal therapy on DKK1 levels in smoker and nonsmoker patients with periodontitis. Periodontal treatment positively improved the clinical parameters and significantly reduced

Table 2 Comparisons of Clinical Parameters Within Nonsmoker and Smoker Patients with Periodontitis Patients at Baseline and Six Weeks After Nonsurgical Periodontal Therapy

	Parameters	Comparison	Mean	SE	t-Value	p-value
G1= Non-Smokers	PI	Baseline	56.03	8.78	2.302	0.042*
		6 weeks	31.84	4.83		
	BI	Baseline	64.29	6.75	5.169	<0.001**
		6 weeks	28.15	3.36		
	PD	Baseline	2.79	0.12	3.470	0.005**
		6 weeks	2.32	0.11		
	CAL	Baseline	3.11	0.31	2.859	0.016*
		6 weeks	2.60	0.26		
G2= Smokers	PI	Baseline	81.20	7.52	6.011	<0.001**
		6 weeks	41.90	6.56		
	BI	Baseline	52.69	6.86	3.588	0.004**
		6 weeks	29.14	2.65		
	PD	Baseline	3.36	0.21	1.119	0.287
		6 weeks	3.18	0.15		
	CAL	Baseline	3.57	0.29	2.743	0.019*
		6 weeks	3.12	0.29		

Note: *Significant (P≤0.05), **Highly significant (P≤0.01).
Abbreviations: BI, bleeding index; PI, plaque index; PD, pocket depth; CAL, clinical attachment loss; G1, nonsmokers; G2, smokers.

Table 3 Comparisons of DDK1 Levels (Pg/Ml) Before and After Nonsurgical Periodontal Treatment for Both Groups

Groups	Comparison	Mean	SE	t-test	p-value
G1	Baseline	5069.00	504.58	4.803	<0.001**
	6 weeks	4086.17	487.91		
G2	Baseline	6102.67	296.68	5.649	<0.001**
	6 weeks	4794.92	410.47		
G1	Baseline	5069.00	504.58	−1.630	0.131
G2	Baseline	6102.67	296.68		
G1	6 weeks	4086.17	487.91	−1.011	0.334
G2	6 weeks	4794.92	410.47		

Note: **Highly significant (P≤0.01).
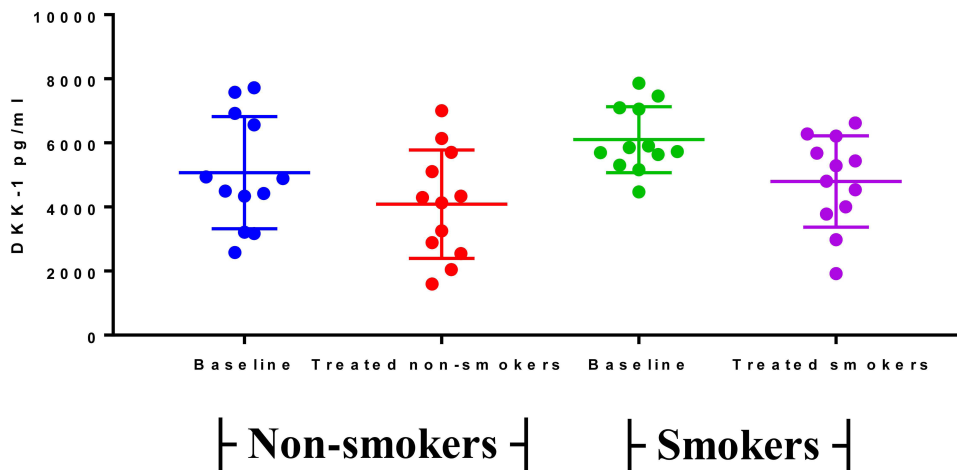**Abbreviations**: G1, nonsmokers; G2, smokers.

Figure 1 Comparative evaluation of the serum levels of DKK1 in nonsmokers periodontitis (n=12) and smokers periodontitis patients (n=12) before and after treatment. The serum levels of DKK1 are reduced in nonsmokers (p <0.001) and smokers patients (p <0.001) after treatment.

the DKK1 serum levels in both groups when compared to the baseline. The smokers in the present study had worse periodontal clinical parameters than the nonsmokers, which is consistent with the findings of several studies.[40,41] In the current study, there was an improvement in periodontal outcomes after periodontal therapy in both the smokers and the nonsmokers, but the response was higher in the nonsmokers than the smokers. When comparing periodontal parameters six weeks after nonsurgical periodontal therapy in the nonsmokers versus the smokers, there was a considerable reduction in PI and PD in the nonsmokers compared to the smokers. These results are in line with a previous report, reflecting the significant impact of smoking on periodontal tissues and the response to periodontal therapy.[41]

We hypothesized that there would be no significant difference in the DKK1 serum levels in smokers and nonsmokers with periodontitis after nonsurgical periodontal therapy. However, there was a significant decrease in DKK1 serum levels after nonsurgical periodontal therapy at six weeks in both the smokers and the nonsmokers, but there was no significant difference between the two groups. Despite the lack of statistically significant differences between the groups, the DKK1 levels were still greater in the smokers compared to the nonsmokers. These results demonstrate the beneficial effects of nonsurgical periodontal therapy on DKK1 reduction in patients with periodontitis.

Previous studies have shown that the mRNA expression and protein levels of DKK1 are significantly increased in the gingival specimen as well as the serum of patients with chronic periodontitis compared to those without periodontitis.[26,28] Similarly, DKK1 has been reported to be upregulated in patients with chronic periodontitis presenting with type II diabetes and/or smoking.[27] Moreover, GCF-DKK1 levels have been found to be associated with periodontal bone loss, periodontitis, and its severity in patients with early rheumatoid arthritis.[42] Both DKK1 and smoking were found to impact bone homeostasis negatively, DKK1 could block the expression of Wnt signaling, consequently decreasing OPG expression and resulting in bone loss.[43] Additionally, long lifetime smoking exposure could negatively impact local OPG production, increasing RANKL/OPG ratio and resulting in bone resorption.[44]

This study has some limitations including the small sample size and only assessed the effect of nonsurgical periodontal therapy on systemic levels of DKK1. Further studies are needed to evaluate the effect of nonsurgical periodontal therapy on levels of DKK1 in gingival crevicular fluid and saliva. Despite these limitations, the main strength of this study is that it provides insight into the effect of nonsurgical treatment on DKK1 levels in patients with periodontitis.

Conclusion

Although nonsurgical periodontal treatment effectively improved the periodontal clinical parameters and reduced the DKK1 serum levels, there were no significant differences in the DKK1 serum levels between the smokers and nonsmokers with periodontitis.

Abbreviations

DKK1, Dickkopf-Related Protein-1; PD, probing depth; CAL, clinical attachment loss; PI, plaque index; BI, bleeding index; LRP5/6, Lipoprotein receptor-related protein 5/6; ELISA, enzyme-linked immunosorbent assay (ELISA); SE, standard error; GCF, Gingival crevicular fluid; IL-1β, Interleukin-1β; TNF-α, tumor necrosis factor -α; OPG, osteopro-tegerin; RANKL, receptor activator of nuclear factor κB ligand.

Ethics Approval and Consent to Participate

This study was approved by Umm Al-Qura University Faculty of Dentistry Institutional Review Board Committee (IRB: 157-19) and followed the guidelines of Helsinki declaration. The signed consent form was acquired from all participants before enrollment in this study.

Disclosure

The authors report no conflicts of interest in this work.

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