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Original Research

The Effect of Smoking on Gingival Crevicular Fluid Sclerostin and TNF-A Levels in Patient with Periodontitis

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ABSTRACT

Objective: The objective of this study was to evaluate the impact of cigarette smoking on sclerostin and TNF-α levels in subjects exhibiting periodontally healthy conditions and those afflicted with periodontitis. The hypothesis tested was that sclerostin levels in smokers could serve as a diagnostic marker and a tool to assess the progression of the disease.

Methods: In the present study, gingival crevicular fluid samples were obtained from a total of 72 patients, who were divided into four distinct groups: a control group of 18 non-smoking individuals without periodontitis, a group of 18 non-smoking individuals with periodontitis, a group of 18 healthy individuals who smoked, and a group of 18 smokers with periodontitis. The levels of sclerostin and TNF-α were then evaluated using the enzyme-linked immunosorbent assay (ELISA) method.

Results: The findings indicated a positive correlation between sclerostin and TNF-α levels in the gingival fluid of individuals diagnosed with periodontal disease.Subsequent evaluations of the relationship between smoking and these biomarkers revealed a statistically significant increase in TNF-α levels, while the increase in sclerostin levels did not attain statistical significance. However, when the analyses were performed without taking into account age-related effects within groups (adjusted for age), a statistically significant increase in sclerostin levels due to smoking was observed.

Conclusion: In conclusion, it can be posited that the measurement of TNF-α levels in smokers may serve as a biomarker for the diagnosis and progression of the disease. Further studies are required to determine the role of sclerostin in this context.

Keywords: Gingival fluid, Periodontitis, Smoking, Sklerostin, TNF-α

INTRODUCTION

The periodontium consists of gums, periodontal ligament, cementum, and alveolar bone, and the purpose of the periodontium is to ensure the continuity and function of the tooth [1]. Periodontal diseases occur as a result of the disruption of the relationship between the host tissue and microbial plaque. Periodontitis, one of the periodontal diseases, can result in tooth and supporting tissue loss [2].

Local, environmental, systemic, and genetic factors are some of the main factors that play a role in the formation and spread of periodontal disease, especially periodontitis [3]. It is known that smoking is one of the effective environmental risk factors in the formation and progression of periodontal diseases [4].

It is known that the antimicrobial mechanism is impaired in smokers. As a result of impairment in this mechanism, the number of periodontal pathogens increases and the severity of periodontal disease increases accordingly. In addition, it is observed that bone and attachment loss increases in smokers due to the increased release of mediators that cause bone destruction [5].

In smokers; studies are showing increased prevalence and severity of periodontal destruction, pocket depth, attachment loss, and bone loss; Furthermore, suppression of clinical signs of periodontitis has been observed in smokers [6,7].

Although the effects of smoking on host response and its role in increasing the risk of periodontal disease are still being investigated, its effects on innate and acquired immune response cells have been examined separately. Accordingly, it has been reported that cigarette components suppress defense responses of the immune system, but exacerbate pathological responses [8].

Salvi et al. reported that smoking was a risk factor for periodontal diseases and the incidence of periodontitis was 2.5 to 7.0 times higher in smokers [9]. In another study comparing smokers and non-smokers, it was found that the risk of periodontitis was 3.9 times higher in the 19-30 age group and 2.8 times higher in the 31-40 age group [10]. Many studies show that gingivitis is more common in smokers [11,12]. Bergström et al. included smokers and non-smokers with good oral hygiene and similar PI values and found that GI and probing bleeding values were lower in smokers. They explained the reason for this as suppression of the normal inflammatory response against microbial dental plaque (MDP) by smoking [7]. Studies are showing that, in addition to local factors, host-related factors also change the course of periodontal disease [3]. It is thought that host-related factors affect the host tissue response. Biomarkers that are a reflection of the host tissue response, involved in bone metabolism and affecting bone metabolism may be clinical markers that are thought to provide information about the health or disease of periodontal tissues. Sclerostin and TNF-α are among the indicators known to be effective in bone metabolism [13,14].

Main Points

- The search for new biomarkers for the diagnosis of periodontal disease has been widespread in recent years.
- This study aims to demonstrate new biomarkers used in the diagnosis of periodontal disease and to evaluate their effects on smoking.
- It is expected that this study will guide future studies on the biomarkers used in this study.

Sclerostin is a kind of protein known to be cysteine-dependent and encoded by the Sost gene. It is known to be involved in bone metabolism by controlling the differentiation and functions of osteoblasts. It is a protein that inhibits the Wnt/β-catenin signaling pathway while exerting its effects on bone metabolism. It is known to strongly suppress bone growth [15,16]. Sclerostin is elevated in GCF in periodontitis patients [17,18]. There are also animal studies examining the activity of sclerostin in periodontal tissues. One of these studies is a study in which the treatment of bone defects caused by irritation with ligature and periodontitis in animals by applying sclerostin antibody (Scl-ab) was evaluated. In the study, it was observed that alveolar bone healing was significantly improved in the experimental groups treated with Scl-ab. Serum analysis revealed elevated levels of osteocalcin and procollagen type I N propeptide (PINP) in the experimental groups compared to the control groups. This finding indicates the efficacy of Scl-ab in cases involving the coexistence of periodontitis and osteopenia. The study utilised ligature-induced periodontitis in ovariectomised rats, observing that mineral apposition was significantly higher in the Scl-ab-treated group [19,20]. In a study conducted to evaluate sclerostin levels in the gingival groove fluid of patients with periodontitis, it was shown that sclerostin levels were statistically significantly higher in periodontitis patients [21]a glycoprotein, plays a key role in regulating bone mass. In this study, sclerostin levels in the gingival crevicular fluid (GCF. A study in mice has shown that sclerostin deficiency or functional inhibition of sclerostin facilitates bone formation by increasing Wnt/β-catenin signaling, suggesting a direct effect of sclerostin on bone metabolism [22]. TNF-α is one of the main mediators of the immune system. It increases neutrophil activity due to the increased release of matrix metalloproteinases (MMPs) and provides tissue and cell regeneration. It activates osteoclast development and provides

limited tissue repair through increased apoptosis in fibroblasts [23]. High levels of cytokines such as tumor necrosis factor-alpha and IL-1β in infected gingiva and the GCF of patients with periodontitis have revealed that these cytokines may be responsible for periodontal tissue destruction [24,25]. In a study evaluating the effects of receptors inhibiting these cytokines in periodontitis, a 60% decrease in bone resorption and a 67% decrease in osteoclast formation was found [14]. With the increase in gingival inflammation, the amount of TNF-α in GCF also increases. The presence of high levels of TNF-α has a direct effect on the development of periodontitis. This important effect of TNF-α is now known for certain. In many studies, TNF-α antagonist was applied to periodontal disease areas and it was found that the accumulation of inflammatory cells decreased by 80%. It was also found that alveolar bone loss was reduced by 60% in areas with periodontal disease [14,26,27].

Smoking is a well-known significant risk factor in the initiation and progression of periodontal diseases. Studies have demonstrated that sclerostin and TNF-α levels increase in individuals with periodontal diseases. However, the sclerostin level in gingival crevicular fluid and its relationship with clinical parameters in smoking patients with periodontitis has not been examined. In light of the information mentioned above, It is hypothesized that sclerostin and TNF-α levels in gingival fluid will be higher in smoking patients with periodontitis compared to non-smoking patients with periodontitis. This study aims to measure sclerostin and TNF-α levels in periodontally healthy smokers and patients with periodontitis, as well as to assess the association of clinical parameters with smoking.

MATERIALS AND METHODS

Participants and Clinical Evaluations

A total of 72 individuals, comprising 37 males and 35 females, with ages ranging from 23 to 54, who had applied to the Periodontology clinic at Gaziantep University Faculty of Dentistry, were included in this study. The research commenced after the study protocol was meticulously developed and approval was granted by the Gaziantep University Clinical Research Ethics Committee (see Annex-1), under decision number 2019/101 dated 13.03.2019. Before the commencement of the study, all participants were comprehensively informed about the study's objectives and methodology, and they willingly provided their consent by signing the voluntary consent form. All research was conducted according to the Declaration of Helsinki.

Exclusion and Inclusion Criteria

The study excluded individuals who were not between the ages of 18 and 65, pregnant or breastfeeding, had systemic diseases, had parafunctional habits, used antibiotics and oral contraceptives in the previous six months, had periodontal treatment in the previous six months, or had periodontal surgery in the previous six months. The study groups were not comprised of individuals who were using removable prostheses, had any substance addiction (except smoking), had restorative material in the tooth from which the gingival groove fluid sample was taken and in the adjacent teeth, or used a drug that affects periodontal tissues (cyclosporine, phenytoin, etc.). Systemically healthy individuals, who were not pregnant or breastfeeding, who had not undergone periodontal treatment within the previous six months, who had not used antibiotics or oral contraceptives within the previous six months, and who had at least 12 teeth in their mouths, except for third molars, were included in the study groups.

The experimental group comprised 18 individuals who were smokers and 18 who were non-smokers, all of whom were diagnosed with periodontitis following thorough clinical and radiological examinations. They exhibited an average clinical attachment loss of 4 mm or more, as well as a Probing Pocket Depth of 5 mm or more in at least 20 regions within the oral cavity. The control group consisted of 36 individuals who had overall periodontal health, with an equal distribution of 18 smokers and 18 non-smokers.

Within the smoker group, individuals who smoked a minimum of 10 cigarettes per day were assessed, while in the non-smoker group, participants who had never smoked in their lifetime or had abstained from smoking for at least two years were included. Smoking status was determined through direct inquiry with each individual [28,29].

Group A: Non-smoking periodontal healthy individuals (18 individuals, 8 males, and 10 females, aged between 27-46 [30].
Group B: Non-smoking individuals with periodontitis (18 individuals, 11 males and 7 females, aged between 29-51) Stage-3/4 degree A [30].

Group C: Periodontal healthy individuals who smoke (18 individuals, 9 males, and 9 females, aged between 23-54) [30].
Group D: Periodontitis individuals who smoke (18 individuals, 9 males, and 9 females, aged between 29-51) stage 4 degree C [30].

Clinical Parameters

Before collecting GCF samples, clinical measurements were conducted to assess the patients' periodontal status. The Williams (Williams, Hu-Friedy, Chicago, IL) probe was employed to make clinical measurements. During the clinical examination, several measurements were taken to evaluate the patients' periodontal health, including PI, PPD, GI, CAL, and bleeding on probing (BOP) [31–33]. PI, GI, and BOP were measured on four different surfaces of each tooth, encompassing the mesiobuccal, mid-buccal, distobuccal, and mid-palatal/lingual regions. These measurements were used to assess the patients' oral hygiene and gingival health. PPD and CAL measurements were taken from six different surfaces of each tooth. These surfaces included the mesio-buccal, mid-buccal, disto-buccal, mesiolingual/palatal, mid-lingual/palatal, and distolingual/palatal regions of the tooth. These measurements were utilized to evaluate the condition of the periodontal attachment and provide a more detailed assessment of periodontal health. These clinical measurements formed the foundation of this study and assisted in determining the patients' periodontal health status. The clinical measurements and collection of GCF samples were conducted by a single physician.

Collection of GCF Samples

Gingival crevicular fluid (GCF) samples were collected 24 hours after the periodontal clinical measurements of the patients. GCF samples were obtained from two sites during a single session, selected from the tooth in each quarter of the jaw with the highest probing pocket depth. Sterilized paper strips were used for GCF sample collection. Before sampling, the target areas were isolated using cotton tampons, and any plaque in these areas was removed with a probe. The areas were then meticulously dried using air spray, and applied from both the vestibular and palatinal regions, perpendicular to the tooth's long axis, to prevent saliva contamination. Each paper strip (Periopaper®, OraFlow Inc., Plainview, New York, USA) was gently placed into the gingival pocket until slight resistance was encountered (Utilizing the Brill Technique)[34] and left in the pocket for 30 seconds. Samples contaminated with blood or saliva were excluded from the evaluation. The volume of GCF on the strips was measured and recorded using a pre-calibrated Periotron 8000. The GCF volume was then converted to microliters (µl)[35]. This conversion was performed by transmitting the Periotron 8000 measurement values to a computer via a serial connection using the PERIOTRON (Orafow Inc., Plainview, New York, USA) program. After each volume determination, the device's poles were wiped with a dry gauze to prevent liquid contamination. Paper strips containing GCF samples were placed in Eppendorf tubes and stored at -80 degrees Celsius until the day of analysis.

Measurements of GCF Sclerostin, and TNF-α Levels

The sclerostin and TNF-α reagents, as well as the microplates containing GCF samples reserved for the study, were allowed to reach room temperature. Sclerostin and TNF-α levels in the GCF samples were measured using an ELISA method and Finetest ELISA kits (Finetest Sklerostine kit Batch No:H0599E108 E / FineTest TNF-α kit Batch No:H0302E108 E). These kits are based on the principle of a sandwich enzyme-linked immunosorbent assay. Measurement of sclerostin and TNF-α was performed with an ELISA reader (Biotek, ELx800, USA) at 450 nm. Sclerostin and TNF-α levels for both patients and controls were calculated with the assistance of a standard graph.

Statistical Analysis

The conformity of the data to a normal distribution was assessed using the Shapiro-Wilk test. For comparing non-normally distributed characteristics among more than two independent groups, Kruskal-Wallis and Dunn multiple comparison tests were employed. The Chi-square test was used to examine relationships between categorical variables, while the Spearman rank correlation coefficient was used to assess relationships between numerical variables. A linear mixed-effects model was employed to analyze SOST and TNF-α values, with age as a controlled variable. Statistical analyses were conducted using the SPSS for Windows version 22.0 software package, and a significance level of $P<0.05$ was considered statistically significant.

The minimum number of patients required in each group was determined to be 17 ($\alpha=0.05$, $1-\beta=0.80$) to detect a significant difference between the smoking and non-smoking groups concerning sclerostin and TNF-α values, with a Cohen's d effect size of 1.00. The analysis was performed using G Power version 3.1.

RESULTS

Demographic Findings

A total of 72 volunteers, 37 males and 35 females aged 23-54 years, were included in this study. Non-smoking periodontally healthy individuals were defined as "Group A" (n;18), non-smoking periodontally healthy individuals were defined as

"Group B" (n;18), smoking periodontally healthy individuals were defined as "Group C" (n;18), and smoking periodontally healthy individuals were defined as "Group D" (n;18). Among the individuals who participated in the study, 10 of the individuals in Group A were female and 8 were male; 7 of the individuals in Group B were female and 11 were male; 9 of the individuals in Groups C and D were female and 9 were male. The comparison of the demographic data of the individuals in the study between the groups is shown in Table 1.

The mean age ± standard deviation of the participants was 37.51±11.21 years. When the age medians were examined, a statistically significant difference was observed between the non-smoking healthy group and the smoking periodontitis (A and D) groups, but no statistically significant difference was found between all other groups. The groups were balanced in terms of gender distribution (P=0.788) (Chi-square test)

Clinical Periodontal and Biochemical Findings

Table 2 shows the intergroup changes and comparisons of clinical and laboratory findings including clinical data of the sample area (PI, GI, PPD), whole mouth kinetic data (PI(t), GI(t), PPD(t), CAL(t),), GCF volume measurements and GCF sclerostin and TNF-α levels.

According to the results of statistical analysis, whole mouth PI(t), PPD(t), GI(t), and CAL(t) values were found to be statistically significantly higher in the periodontitis group (B and D) than in the healthy group (A and C) ($p<0.001$). There was no statistically significant difference in whole mouth PI(t), PPD(t), and CAL(t) values between smoker periodontitis and non-smoker periodontitis groups (B and D). There was a statistically significant result in GI(t) values between smoker periodontitis and non-smoker periodontitis groups (B and D). ($p=0,015$).

There was no statistically significant difference in whole mouth GI(t), PPD(t) values between healthy smokers and healthy non-smokers (A and C). Statistically significant results were found in whole mouth PI(t), CAL(t) values between healthy smokers and healthy non-smokers (A and C) ($p<0,005$).

When the sample site PI, PPD, and GI values were analyzed, it was found that they were statistically significantly higher in the periodontitis group (B and D) than in the healthy group (A and C) ($p<0.001$). There was no statistically significant difference in sample site PI, PPD, and GI values between periodontitis smokers and non-smokers (B and D) and between healthy smokers and healthy non-smokers (A and C).

When GCF volume (µl) was analyzed, it was found that the mean GCF volume was statistically significantly higher in the periodontitis group (B and D) compared to the healthy group (A and C) ($p<0.001$) (Figure 1). There was no statistically significant difference in GCF volume (µl) between periodontitis smokers and non-smokers (B and D) and between healthy smokers and healthy non-smokers (A and C).

When the total amount of GCF sclerostin was evaluated, it was observed that the sclerostin values of the periodontitis group (B and D) were higher than the healthy group (A and C), and the participants in the smoking groups (C and D) were higher than the participants in the non-smoking groups (A and B), but the difference was not statistically significant.

When examining the age of the participants, a statistically significant difference was observed between the non-smoking healthy group and the smoking periodontitis (A and D) groups ($p<0.005$). When the group and age were included in the Mixed effect linear model to evaluate the factors affecting the sclerostin values, statistically significant results were found between the non-smoking healthy and smoking periodontitis (A and D) and non-smoking and smoking periodontitis (B and D) Groups. ($p<0,005$) (Figure 2) (Table 3)

When the total amounts of GCF TNF-α were evaluated, it was seen that the participants in the smoking groups (C and D) were higher than the participants in the non-smoking groups (A and B); TNF-α values of individuals with periodontitis were statistically significantly higher than healthy individuals. ($p<0.005$). When non-smoking healthy and smoking health groups were compared (A and C), it was observed that the GCF TNF-α values of smoking individuals were statistically significantly higher ($p<0.005$). In the comparison of non-smoking healthy and smoking periodontitis group (A and D), GCF TNF-α values of smokers with periodontitis were statistically significantly higher ($p<0.001$). In the comparison of smoker periodontitis and non-smoker periodontitis groups (B and D), GCF TNF-α levels of smoker periodontitis individuals were statistically significantly higher ($p<0,005$) (Figure 3).

Table 1. Comparison of demographic data between groups

		A (N=18) α	B (N=20) β	C (N=20) γ	D (N=20) Δ	Total	P
Age †		35,28 ± 6,2	37,5 ± 7,5	37,56±7,74	41,44±5,46	37,94±6,72	* αδ,
Min/Max		27/46	29/51	23/54	29/51	23/54	
Gender Ω	**M**	8 (44,4)	11 (61,1)	9(50)	9 (50)	37 (51)	
	W	10(55,6)	7 (38,9)	9(50)	9 (50)	35 (49)	

A (α): Non-smoker periodontally healthy group, B (β): Non-smoker periodontitis group, C (γ): Smoker periodontally healthy group, D (δ): Smoker periodontitis group

† mean ± std.deviation M:male W: Female

Ω n (%)

*Statistically significant ($p<0.05$)

**Statistically highly significant ($p<0.001$)

Table 2. Clinical periodontal parameters of all groups

	A (N=18) α	B (N=18) B	C (N=18) Γ	D (N=18) δ	P
PI(t)†	0,74±0,09	2,3 ±0,2	0,89 ± 0,05	2,32 ± 0,2	** αδ,βγ, γδ,αβ *αγ
PPD(t)(mm)†	2,32±0,42	6,35 ± 1,15	2,32 ± 0,11	6,84 ± 0,71	** αβ, αδ,βγ, γδ
GI(t)†	0,43±0,21	1,94 ± 0,11	0,24±0,12	1,61 ± 0,13	**γδ, βγ, αδ, αβ *βδ
CAL(t)(mm)†	0,11±0,13	7,07 ± 0,77	0,38 ±0,24	7,4 ± 0,83	** αβ, αδ, βγ, γδ *αγ
PI†	0,39 ± 0,5	1,83 ± 0,38	0,22±0,43	1,72 ± 0,46	** γδ, βγ, αδ,αβ
PPD(mm)†	2,67±0,59	6,5 ± 1,04	2,56±0,51	7,44 ± 0,98	**βγ, γδ,αβ,αδ
GI †	0,11±0,32	1,72 ± 0,46	0,17±0,38	1,44 ± 0,51	** αδ, αβ, γδ, Bγ
CAL (mm)†	0	6,72± 0,57	0	7,2± 0,64	** αβ, αδ, βγ, γδ *αγ
GCF Volüme (µl)†	0,36±0,11	1,13 ± 0,91	0,27±0,11	0,8 ± 0,17	** βγ, γδ, αβ,αδ
Sklerostin (ng/mL)†	29,57±6,23	30,63±4,95	34,43±8,16	39,29 ± 13,75	N
TNF-α (ng/mL)†	13,78 ± 5,18	14,51 ± 7,3	19,79 ± 9,12	24,91 ± 11,33	** αδ * αγ, βδ

A (α): Non-smoker periodontally healthy group, B (β): Non-smoker periodontitis group, C (γ): Smoker periodontally healthy group, D (δ): Smoker periodontitis group

CAL: Clinic attachment level PI(t):Plaque index total, PPD(t): Probing pocket depth total, GI(t): Gingival index total, CAL(t): Clinical attachment level total, PI: Plaque index sample site, PPD: Probing pocket depth sample site, GI: Gingival index sample site, GCF: Gingival Clevicular Fluid

† mean ± std.deviation

* Statistically significant ($p<0.05$)

**Statistically highly significant ($p<0.001$)

N: no statistically significant result

Table 3. Results when group and age are included in the mixed effect linear model (Adjusted by age)

Parameter	Prediction	standard deviation	P	%95 confidence interval	
				lower limit	upper limit
A vs D	-9,43	3,16	0,004*	-15,73	-3,13
B vs D	-8,47	3,06	0,007*	-14,59	-2,36
C vs D	-4,68	3,06	0,131	-10,79	1,43
Yaş	0,05	0,16	0,766	-0,27	0,37

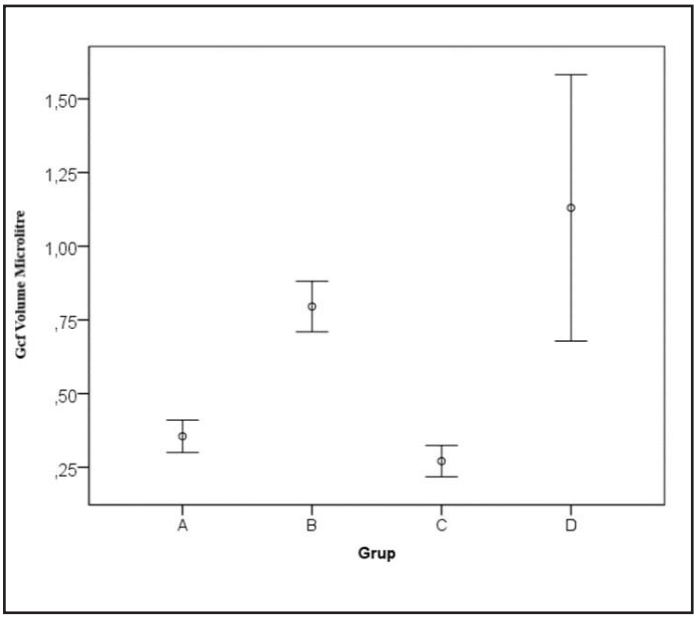

Figure 1. GCF volume (µl) values of the groups

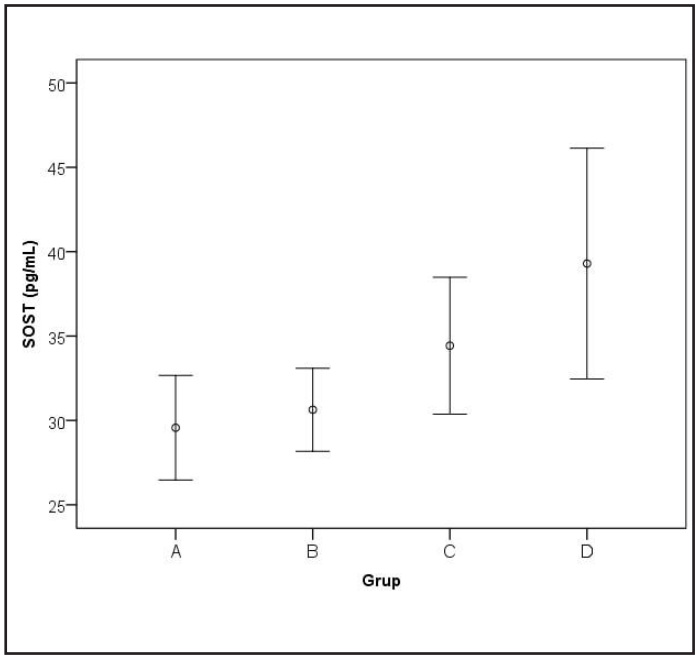

Figure 2. GCF TNF-α levels of the groups

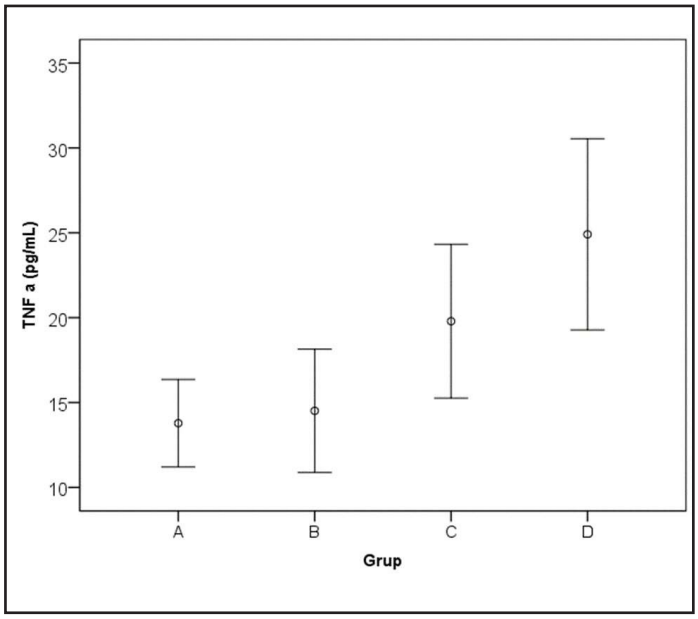

Figure 3. GCF sclerostin levels of the groups

DISCUSSION

Risk factors are as important as bacteria and the host tissue defense system in the initiation and progression of periodontal disease [5,36]. Numerous studies have examined the impact of smoking, one of the environmental risk factors, on periodontal tissues and periodontal diseases [7]. It is believed that antimicrobial defense mechanisms are impaired in smokers, leading to an increase in the number of pathogens, which in turn exacerbates the destruction and impact of periodontal disease [5,37,38]. In this study, conducted at Gaziantep University, Faculty of Dentistry, Department of Periodontology, a total of 72 individuals, 37 males and 35 females, aged between 23 and 54 years, were included. Among these, 36 patients were periodontally healthy and 36 were periodontitis patients. Patient selection was performed according to inclusion and exclusion criteria to support the diagnosis. By selecting patients following these criteria, we aimed to control parameters that might affect the study results and achieve standardization between the groups.

Following a thorough evaluation of the study's findings, it was ascertained that the sclerostin and TNF-α values were elevated in individuals diagnosed with periodontitis in comparison to those categorized as healthy. These observations signify the potential of these markers as diagnostic biomarkers for periodontal disease. Furthermore, the study revealed that individuals with periodontitis who smoke exhibited higher levels of sclerostin and TNF-α compared to non-smokers within the same group. This increase was found to be statistically significant for TNF-α, but not for sclerostin.

The study highlights the role of TNF-α as a critical inflammatory marker in periodontitis, with elevated levels observed in individuals with the disease compared to healthy controls. Notably, the highest TNF-α levels were detected in periodontitis patients who smoked, underscoring the exacerbating effect of smoking on periodontal inflammation. Furthermore, the analysis using linear mixed-effects modeling, which accounted for the variability in age distribution among the groups, identified a significant difference in sclerostin levels between non-smoker and smoker periodontitis groups. This suggests that smoking may influence sclerostin expression, potentially contributing to the pathophysiology of periodontitis in smokers. However, the Kruskal-Wallis test, applied to assess non-normally distributed data across multiple groups, did not show statistically significant differences in sclerostin levels, indicating that the relationship between sclerostin and periodontal disease may be more complex and influenced by factors beyond smoking status. These findings suggest that while TNF-α and sclerostin are relevant biomarkers in periodontitis, their interactions and influences are nuanced, particularly in the context of smoking, and warrant further investigation.

In the biochemical analyses performed in this study, it was observed that sclerostin values were higher in healthy smokers and smokers with periodontitis compared to non-smokers, though the difference was not statistically significant. Since the age distribution between the groups was not balanced, linear mixed effect modeling was used to compare the sclerostin and TNF-α values of the smoker periodontitis group and the other groups, adjusting for age. This analysis revealed that sclerostin levels in the smoker periodontitis group were significantly higher than those in the non-smoker periodontitis group. Additionally, sclerostin levels in the smoking periodontitis group were significantly higher compared to the non-smoking healthy group. These findings suggest that the apparent differences in sclerostin levels are not solely due to age distribution among individuals. Increasing the number of patients in future studies with more homogeneous groups could yield results that further support our hypothesis. This study also found that TNF-α levels were significantly higher in the healthy smoker group compared to the healthy non-smoker group.

A study conducted by Georgios et al. (2019) demonstrated the presence of the levels of sclerostin, WNT-5a, and TNF-α in the GCF of patients with and without periodontitis. WNT-5a gingival protein levels showed high diagnostic value for diffuse moderate to severe chronic periodontitis, while exhibiting low accuracy for localized chronic periodontitis [39].

TNF-α and IL-1b are known to induce SOST expression [40,41]. Baek et al. reported that TNF-α, a transcription activator for sclerostin, induces the synthesis of sclerostin in MLO-Y4 osteocytes. It was also demonstrated that the nuclear factor kappa-light-chain enhancer of activated B cells (NF-kB) directly binds to the NF-kB binding elements on the mouse SOST promoter. Therefore, the synthesis of sclerostin is upregulated due to NF-kB activation [42].

The study by Ashifa N et al. demonstrated that GCF sclerostin levels were elevated in patients with periodontitis in comparison to those with gingivally healthy periodontal conditions. Furthermore, the findings supported the hypothesis that this could be regarded as a potential biomarker of disease activity [21]a glycoprotein, plays a key role in regulating bone mass. In this study, sclerostin levels in the gingival crevicular fluid (GCF. In the study conducted by Ren Y et al., the SOST gene was removed in mice, effectively halting the synthesis of sclerostin. It was observed that the incidence of bone destruction was reduced, while bone formation was enhanced in mice where sclerostin synthesis was terminated. The hypothesis that osteocyte pathological alterations and sclerostin production from these cells are linked to periodontal defects has been substantiate [43]. With the increase in gingival inflammation, the amount of TNF-α in the GCF also increases and the presence of high levels of TNF-α has a direct effect on the formation of periodontitis. This important effect of TNF-α has been confirmed just like the effects of IL-1β and many studies have shown that the accumulation of inflammatory cells is reduced by 80% and alveolar bone loss is reduced by 60% in periodontal disease areas treated with tumor necrosis factor-alpha and IL-1β antagonists [26,27].

Moreover, TNF-α levels were higher in periodontitis smokers than in non-smoker periodontitis patients. These differences suggest that smoking increases the amount of mediators that initiate inflammation. The biochemical and statistical analyses demonstrated that smoking elevates the levels of TNF-α, a known pro-inflammatory mediator, and sclerostin, a strong suppressor of bone growth. While the difference in TNF-α levels was statistically significant, the significance in sclerostin levels was less pronounced, possibly due to the smaller sample size and unequal age distribution.

When the results of this study were evaluated, it was seen that the sclerostin levels of the periodontitis group were higher than the healthy group, but not statistically significant. Statistically significant results ($p<0.005$) were found between healthy non-smoker and smoker periodontitis (A and D) and healthy non-smoker and smoker periodontitis (B and D) groups. This data tells us that our hypothesis is statistically significant if the age distribution of the groups is more equal. The fact that the number of samples taken was close to the minimum value of the power analysis suggests the possibility of strengthening the results of the study with a larger number of samples.

In this study, the Brill technique was used during the collection of gingival groove fluid samples [34]. When other studies in the literature are examined, it is seen that samples are collected with similar techniques [44,45].

The assessment of smoking status was conducted through the utilisation of an individual questioning method. In this method, the responses provided verbally by the samples are of the utmost importance. While the individual questioning method is considered to be a safe approach, this particular situation does, however, present a limitation to the study [29]. This study has several limitations that may influence the generalizability of its findings. First, the small sample size and uneven age distribution among groups may have affected the statistical power of the analysis, particularly concerning sclerostin levels. Additionally, smoking status was assessed using self-reported data, which may be subject to reporting bias and inaccuracies. The study also relied on the Brill technique for GCF sample collection, which, while widely used, may have inherent variability in sample handling and processing. Finally, the cross-sectional design of the study limits its ability to infer causation between smoking, sclerostin levels, and periodontal disease progression. Future studies with larger, more demographically homogeneous samples and objective smoking verification methods are needed to address these limitations.

CONCLUSION

The findings of this study underscore the significant impact of smoking on periodontal inflammation and bone destruction, as evidenced by elevated levels of TNF-α and sclerostin in smokers with periodontitis. While the association between TNF-α and smoking was statistically significant, the relationship between sclerostin and smoking, though apparent, requires further investigation due to the small sample size and demographic imbalances. These results highlight the potential of sclerostin as a biomarker for periodontal disease activity, particularly in smokers. Further research with larger, well-balanced cohorts is essential to elucidate the complex interactions between smoking, sclerostin, and periodontal pathology. Addressing these gaps could provide valuable insights into targeted therapeutic strategies for smoking-related periodontal diseases.

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