Original Article / Özgün Araştırma

Evaluation of the Relationship between Diabetes and Smoking and IL-6, IL-17 and IL-23 Levels in Individuals with Stage II, IV Periodontitis

Evre II, IV Periodontitis Olan Bireylerde Diyabet ve Sigara ile IL-6, IL-17 ve IL-23 Düzeyleri Arasındaki İlişkinin Değerlendirilmesi

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Abstract

Objective: The purpose of the present investigation was to examine the relationship between diabetes mellitus (DM) and smoking on gingival crevicular fluid (GCF) levels of interleukin (IL)-6, IL-17, and IL-23 in patients with stage II and stage IV periodontitis.

Materials and Methods: Individuals were divided into nine groups according to their periodontal diagnosis, smoking, and DM status: periodontally and systemically healthy individuals (PH-H, n=14), periodontally healthy smokers (PH-S, n=14), periodontally healthy diabetics (PH-D, n=14), systemically healthy individuals with stage II periodontitis (SII-H n=14), smokers with stage II periodontitis (SII-S, n=14), diabetics with stage II periodontitis (SII-D, n=14), systemically healthy individuals with stage IV periodontitis (SIV-H, n=14), smokers with stage IV periodontitis (SIV-S, n=14), and diabetics with stage IV periodontitis (SIV-D, n=14). GCF samples were collected and analyzed.

Results: The mean GCF levels of IL-6, IL-17, and IL-23 in patients with stage IV periodontitis were significantly higher than those in patients with stage II periodontitis and in periodontally healthy individuals.

Conclusion: As the stage of periodontitis increases, the levels of IL-6, IL-17, and IL-23 increase.

Öz

Amaç: Bu araştırmanın amacı, evre II ve IV periodontitisli hastalarda diyabetes mellitus (DM) ve sigara ile dişeti oluğu sıvısındaki (DOS) interlökin (IL)-6, IL-17 ve IL-23'ün seviyeleri arasındaki ilişkiyi incelemektir.

Gereç ve Yöntemler: Bireyler periodontal tanı, sigara ve DM durumlarına göre periodontal ve sistemik olarak sağlıklı (PH-H, n=14), periodontal sağlıklı sigara içen (PH-S, n=14), periodontal sağlıklı diyabetik (PH-D, n=14), evre II periodontitisli sistemik sağlıklı (SII-H n=14), evre II periodontitisli sigara içen (SII-S, n=14), evre II periodontitisli diyabetik (SII-D, n=14), evre IV periodontitisli sistemik sağlıklı (SIV-H, n=14), evre IV periodontitisli sigara içen (SIV-S, n=14) ve evre IV periodontitisli diyabetik (SIV-D, n=14) olarak dokuz gruba ayrılarak DOS örnekleri analiz edildi.

Bulgular: evre IV periodontitisli hastalarda IL-6, IL-17 ve IL-23'ün ortalama DOS seviyeleri, evre II periodontitisli hastalardan ve periodontal olarak sağlıklı bireylerden önemli ölçüde daha yüksekti.

Sonuç: Periodontitis evresi arttıkça IL-6, IL-17 ve IL-23 seviyeleri yükselir.

Introduction

Periodontitis is a multifactorial inflammatory disease characterized by loss of tooth-supporting tissues, and clinical expression is determined by genetic, environmental, and behavioral factors (1).

Smoking and having diabetes mellitus (DM) are major risk factors for periodontitis. In the most recent classification of periodontal diseases, smoking and DM were identified as modifying risk factors in determining the progression rate of periodontitis (2). Smoking alters the host's defense by causing changes in vascular function, neutrophil/monocyte activity, and cytokine and inflammatory mediator release. DM increases the risk of the initiation and progression of periodontitis by contributing to inflammation in the periodontal supporting tissues (3). Although the effects of DM and smoking on gingival crevicular fluid (GCF) cytokine levels have been investigated, most of these studies were conducted according to the 1999 classification system, and the effects of smoking and DM were not evaluated comparatively (4). In the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions, periodontitis was categorized in 4 different stages and 3 different grades. Stage II periodontitis (SII) individuals reflect the clinical findings of periodontitis more than stage I periodontitis individuals. As a result, they apply to our clinic more frequently with complaints such as pain, swelling and bleeding. Stage IV periodontitis (SIV) is the terminal phase of the periodontitis (2). Interleukin (IL)-6 is a cytokines associated with inflammatory periodontal diseases. Increased IL-6 level is associated with osteoclastic activity (5). IL-17 is a proinflammatory cytokine. It has been reported that IL-17 supports osteoclastogenesis by increasing receptor activator of nuclear factor-B ligand production in osteoblastic cells (6). IL-23 is a member of the IL-12 family. IL-23 increases IL-17 release in T cells (7). In this context, the aim of this study is to examine the effects of DM and smoking on GCF levels of IL-6, IL-17, and IL-23 in patients with Stage II and SIV.

Materials and Methods

This study was conducted between May 2019 and January 2020 at Uşak University in the Faculty of Dentistry of the Department of Periodontology. The individuals were informed about the study, and written consent was obtained. This study was designed according to Helsinki declaration principles and approved by the Uşak University Faculty of Medicine Ethics Committee (decision no: 13-13-14, date: 05.01.2022).

Sample Size

A sufficient sample size was determined for IL-6 using a one-way ANOVA test (8). The f-type effect size (0.42), type 1 error (α=0.05), and test power (1-β=0.95) were determined. According to these calculations, a minimum of 11 individuals per group (total sample size of 99 individuals) would be necessary. Considering the possible setbacks at the clinical stage, the number of people in each group was determined as 14, and the total number of samples was determined as 126.

Participants

Clinically, 526 people over the age of 18 were examined. When the extrusion criteria were applied, 126 people were reached. Smokers (S) had smoked more than five packs of cigarettes during their lifetime and continued to smoke. Diabetics (D) had been diagnosed with type 2 DM by a physician and were on insulin supplementation or oral hypoglycemic agents (8).

Exclusion Criteria

Exclusion criteria included periodontal treatment in the previous six months, use of antibiotics or anti-inflammatory drugs in the previous six months, lactation, pregnancy, or any systemic condition that could affect periodontal disease progression (impaired lipid metabolism or hypercholesterolemia).

Clinical Periodontal Measurements

Individuals were diagnosed according to the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions (2). Plaque index (9), gingival index (10), probing depth (PD), and clinical attachment loss were assessed at six sites of all teeth except third molars using a manual periodontal probe (Williams, Hu-Friedy, Chicago, IL).

Classification of Individuals

Individuals were divided into nine groups according to their periodontal diagnosis, smoking history, and DM status: periodontally (PH) and systemically healthy (PH-H, n=14), PH-S, n=14, PH-D, n=14, systemically healthy individuals with SII-H, n=14, S with SII-S, n=14, D with SII-D, n=14, systemically healthy individuals with SIV-H, n=14, S with SIV-S, n=14, and D with SIV-D, n=14.

GCF Sampling

Clinical examination was performed one week before GCF samples were collected. In patients with Stage II and SIV, four nonadjacent and deep periodontal pockets were selected for GCF sampling. In PH patients, four nonadjacent and non-inflamed sites were selected.

Cytokine Quantification

Enzyme-linked immunosorbent assay was used to analyze the GCF levels of IL-6, IL-17, and IL-23 with commercially available kits. Assays were carried out according to the manufacturer's recommendations.

Statistical Analysis

Data analysis was performed using the IBM SPSS V23 and R program. Shapiro-Wilk ve Kolmogorov-Smirnov tests were used to check the normality of the data. Two-way analysis of variance was used to compare the normally distributed data according to the group and subgroups, and multiple comparisons were examined with the Tukey test. Two-way Robust test was used using the WRS2 1 package of data that were not normally distributed according to the group and subgroups, and multiple comparisons were examined with the Bonferroni test. Pearson's chi-square test was used to compare gender according to group and subgroups. One-way analysis of variance was used to compare normally distributed data according to groups of 3 and above, and multiple comparisons were examined with the Tukey test. Kruskall-Wallis test was used to compare data that were not normally distributed according to groups of 3 and above, and multiple comparisons were examined with Dunn's test. Analysis results were presented as mean ± standard deviation and median (minimum-maximum) for quantitative data. Statistical significance level were set at $p<0.05$.

Results

Demographic Data

A total of 126 individuals (73 male, 53 female) were included in the study. The mean age of the Stage IV group was significantly higher than that of the Stage II and PH groups ($p<0.001$) (Table 1).

There was no significant difference between the PH, Stage II, and Stage IV groups in terms of gender distribution ($p>0.05$) (Table 1).

Smoking and DM Status

In individuals with DM, years of DM and Hemoglobin A1c (HbA1c) levels in the SIV-D group were significantly higher than in the SII-D and PH-D groups ($p<0.001$). In S, years of smoking in the SIV-S group was significantly higher than in the SII-S and PH-S groups ($p<0.001$). The number of cigarettes smoked per day in the SIV-S and SII-S groups was significantly higher than in the PH-S group ($p<0.001$) (Table 2).

Periodontal Clinical Parameters

The mean PD of the Stage IV group were significantly higher than those of the Stage II and PH groups ($p=0.001$) (Table 3).

Table 1. The gender distribution of groups

Groups		Gender n (%)			Age (Mean ± SD)	p-value
		Female	Male	p-value		
PH	PH-H	18 (42.9)	24 (57.1)	0.798	33.64±7.02[c]	<0.001
	PH-S					
	PH-D					
SII	SII-H	17 (40.5)	25 (59.5)		51.31±5.13[b]	
	SII-S					
	SII-D					
SIV	SIV-H	20 (47.6)	22 (52.4)		58.24±7.03[a]	
	SIV-S					
	SIV-D					

Pearson's chi-square test

Analysis of variance test statistic

[a-c]No difference between groups and subgroups with the same letter

PH: Periodontal health, SII: Stage II periodontitis, SIV: Stage IV periodontitis, H: Health, D: Diabetes, S: Smoking

GCF Levels Of IL-6, IL-17, And IL-23

The mean IL-6 level of the Stage IV group was significantly higher than that of the Stage II and PH groups (p<0.001). IL-17 and IL-23 levels of the Stage IV group were significantly higher than those of the Stage II and PH groups (p<0.001). IL 23 level of the SIV-D and SII-D groups were higher than the other subgroups (p<0.001) (Table 3).

Discussion

According to this study, the extent and severity of periodontitis increased as the mean age of the groups increased, which was expected because periodontitis is often seen as a cumulative disease (11). The current study confirmed that gender was not significantly associated with periodontal disease but previous studies have reporting that periodontitis more prevalent in men than in women (12).

Table 2. The smoking and DM status of individuals

Groups		Years of diabetes	p-value	HbA1c level	p-value	Years of smoking	p-value	Number of cigarettes/day	p-value
PH	PH-S		<0.001		<0.001	6±0.96[c]	<0.001	8.21±3.73[b]	<0.001
	PH-D	4.14±0.77[c]		6.88±0.27[c]					
SII	SII-S					13.14±3.46[b]		18.93±4.46[a]	
	SII-D	8.21±1.37[b]		7.89±0.32[b]					
SIV	SIV-S					24.07±5.55[a]		22.5±5.8[a]	
	SIV-D	11±2.48[a]		8.19±0.29[a]					

One-way analysis of variance, [a-c]No difference between groups with the same letter.
PH: Periodontal health, SII: Stage II periodontitis, SIV: Stage IV periodontitis, H: Health, D: Diabetes, S: Smoking

Table 3. Probing depth and GCF cytokine levels of groups

	Alt grup	Grup					
		PH		SII		SIV	
PD	D	1.99±0.18	1.95 (1.77-2.44)[A]	3.07±0.29	3.08 (2.44-3.66)[C]	4.91±0.65	4.97 (3.95-6.13)[D]
	H	1.99±0.17	1.98 (1.74-2.31)[A]	2.68±0.39	2.82 (2.06-3.23)[BC]	4.44±0.9	4.48 (2.99-6.33)[D]
	S	2.27±0.19	2.3 (1.96-2.56)[B]	3.22±0.39	3.17 (2.56-3.88)[C]	5.26±0.82	5.12 (4.13-7.56)[D]
	Total	2.08±0.22	2.01 (1.74-2.56)[c]	2.99±0.42	3 (2.06-3.88)[b]	4.87±0.85	4.96 (2.99-7.56)[a]
IL-6	D	0.52±0.09	0.51 (0.41-0.71)	0.88±0.35	1.04 (0.42-1.38)	1.08±0.14	1.09 (0.83-1.33)
	H	0.69±0.16	0.69 (0.35-0.94)	0.73±0.13	0.68 (0.54-0.94)	0.89±0.41	0.71 (0.43-1.83)
	S	0.49±0.1	0.47 (0.35-0.68)	0.69±0.14	0.7 (0.43-0.91)	0.97±0.42	0.83 (0.45-2.19)
	Total	0.57±0.15[c]	0.55 (0.35-0.94)	0.77±0.24[b]	0.71 (0.42-1.38)	0.98±0.35[a]	1 (0.43-2.19)
IL-17	D	6.14±0.25	6.14 (5.76-6.66)	7.17±0.87	7.31 (5.97-9.12)	8.26±0.56	8.46 (7.24-8.98)
	H	5.84±0.63	5.87 (4.44-6.95)	6.57±0.59	6.49 (5.24-7.8)	7.44±0.83	7.48 (5.97-8.83)
	S	6.07±0.25	6.03 (5.76-6.53)	6.99±0.92	6.65 (6.11-9.12)	7.57±0.71	7.34 (6.53-8.98)
	Total	6.02±0.43[c]	6.04 (4.44-6.95)	6.91±0.83[b]	6.72 (5.24-9.12)	7.75±0.78[a]	7.76 (5.97-8.98)
IL-23	D	4.15±0.59	4 (3.22-5.23)[A]	5.92±0.68	5.92 (4.67-6.99)[C]	6.87±2.43	6.47 (4.31-12.49)[BC]
	H	4.06±0.18	4.03 (3.69-4.4)[A]	5.07±0.67	5.22 (3.52-6.2)[BC]	5.63±1.68	5.65 (3.51-9.26)[ABC]
	S	4.36±0.69	4.18 (3.44-5.8)[AB]	5.24±0.98	5.21 (3.87-7.18)[ABC]	5.83±1.74	5.51 (4.22-11.46)[C]
	Total	4.19±0.54	4.04 (3.22-5.8)[b]	5.41±0.86	5.4 (3.52-7.18)[a]	6.11±2.01	5.7 (3.51-12.49)[a]

PH: Periodontal health, SII: Stage II periodontitis, SIV: Stage IV periodontitis, PD: Probing depth, H: Health; D: Diabetes; S: Smoking, [A-D]No difference between interactions with the same letter, [a-c]No difference between groups and subgroups with the same letter

In diabetic individuals, the stage of periodontitis was associated with DM duration and HbA1c levels. This result is in line with studies reporting that elevated HbA1c levels and longer DM durations increase inflammation in periodontal tissues, which is associated with a higher risk of periodontitis (13). The number of cigarettes smoked per day by the PH-S group was significantly lower than that of the SII-S and SIV-S groups, which is in line with studies demonstrating that increased dosage and intensity of smoking are risk factors for worsened periodontal health (14).

The results of the current study demonstrated that IL-6 was upregulated as the severity of periodontitis increased. The results obtained from this study are in accordance with those reported by Becerik et al. (15) S in the PH, Stage II and SIV groups presented IL-6 levels similar to those of the healthy and diabetic individuals in those groups. In contrast, other studies have shown higher or similar GCF levels of IL-6 in S in comparison with non-S (16). This indicates that smoking seems to modulate IL-6 levels in healthy sites; however, as periodontitis becomes established, the level of IL-6 is regulated by periodontal infection rather than by smoking.

This study addressed the significant increase in the GCF levels of IL-17 with an increase in the amount of periodontal destruction. This can be explained by the role of IL-17 in the functional impairment of polymorphonuclear leukocytes and the activation of fibroblasts to produce inflammatory mediators (17). In accordance with this result, higher GCF levels in periodontitis patients compared to healthy controls and role of IL-17 in the severity of periodontal inflammation were reported (18).

This present finding indicates that S and non-S patients with periodontitis and those who were PH did not exhibit different IL-17 levels. The present IL-17 data showed that smoking did not affect GCF levels of IL-17 in PH and diseased individuals, which is in accordance with Buduneli et al. (19). The fact that smoking did not affect GCF IL-17 levels in this study can be explained as follows: S in the PH, Stage II, and Stage IV groups had deeper PDs than non-S in those groups. According to Johnson et al. (20) GCF levels of IL-17 increase as the PD increases that requires higher IL-17 levels in S than in non-S according to this study. However, considering the suppressing effect of smoking on proinflammatory cytokines, the elevated level of IL-17 caused by higher PD comes to a level similar to that of non-S. In other words, the GCF level of IL-17 that increases with increasing PD decreases with the suppressive effect of smoking.

Although periodontitis patients with diabetes tend to have higher GCF levels of IL-17, PH individuals with and without DM have similar IL-17 levels. Explanation can be offered regarding this result. The glycemic control of the patients with DM may modulate the level of IL-17, as the HbA1c levels of the PH-D group were significantly lower than those of the SII-D and SIV-D groups.

This study implies a strong association between GCF IL-23 level and periodontal health and disease. IL-23 levels increased significantly as the severity of periodontal destruction increased. This result is in accordance with that of Lester et al. (21) and DM and smoking exerted no considerable impact on IL-23 levels in patients with periodontitis or PH individuals. This result is not accordance with previous studies that reported smoking causes cytokine expression downregulation and that diabetes stimulates the production of proinflammatory cytokines (21). The lack of effect of smoking and diabetes on IL-23 levels may be because S and D who are PH or have periodontitis have microbial loads similar to those of non-S and non-D who are PH or have periodontitis. This similar microbial load stimulates macrophages and dendritic cells, thereby coincidently stimulating similar production of IL-23.

A strength of the present study is that it was the first to investigate the impact of DM and smoking on the levels of IL-6, IL-17, and IL-23 in patients with different stages of periodontitis. However, the study has some limitations. First, this is a cross-sectional study that cannot determine causal relationships. Second, smoking status and DM duration were determined by self-report.

Conclusions

This study implies an association between GCF levels of IL-6, IL-17, and IL-23 and periodontal health and disease. As the stage of periodontitis increases, the levels of IL-6, IL-17, and IL-23 increase. Also, periodontal status worsens as the duration and frequency of smoking and years of diabetes and HBa1c level. As the stage of periodontitis increases, the

time and cost spent for the treatment of the disease also increases. To prevent this negative situation, periodontal treatment and follow-up appointments of S and diabetic patients should be increased.

Ethics

Ethics Committee Approval: This study was designed according to Helsinki declaration principles and approved by the Uşak University Faculty of Medicine Ethics Committee (decision no: 13-13-14, date: 05.01.2022).

Informed Consent: The individuals were informed about the study, and written consent was obtained.

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Authorship Contributions

Concept: U.Y., Design: U.Y., F.K., A.D., Data Collection or Processing: F.K., Analysis or Interpretation: U.Y., Literature Search: U.Y., F.K., A.D., Writing: U.Y., F.K., A.D.

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