ÖZGÜN ARAŞTIRMA ORIGINAL RESEARCH

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THE ASSOCIATION BETWEEN GRADE OF PERIODONTITIS AND GCF LEVELS OF TNF-α AND MIP-1α: A PRELIMINARY STUDY

PERİODONTİTİS DERECESİ İLE DİŞETİ OLUĞU SIVISI TNF-α VE MIP-1α SEVİYELERİ ARASINDAKİ İLİŞKİNİN DEĞERLENDİRİLMESİ: ÖN ÇALIŞMA

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Öz

Amaç

Periodontitisin patogenezindeki sitokin ve kemokinlerin rolü,periodontitisin başlaması ve ilerlemesinde önemli fonksiyonlara sahip olan ile Tümör Nekroz Faktör alfa (TNF-α) ve Makrofaj İnflamatuar Protein 1α ile (MIP 1α) gösterilmiştir. Bununla birlikte, farklı periodontitis derecelerinin sitokin ve kemokin profilleri hala belirsizdir ve periodontitisin ilerleme hızı ile ilişkili biyobelirteçler hakkında henüz kesin bilgiler bildirilmemiştir. Bu nedenle, bu çalışmanın amacı Derece A, B ve C'deki dişeti oluğu sıvısındaki (DOS) MIP-1α ve TNF-α'nın düzeylerini tahmin etmek ve periodontitis derecesini belirlemede güvenilir biyobelirteçler olarak rollerini değerlendirmektir.

Gereç ve Yöntem

Bireyler periodontitis derecelerine göre Evre IV periodontitis tanısı alan ve Derece A (Derece A, n = 21), Evre IV periodontitis tanısı alan ve Derece B (Derece B, n =21) ve Evre IV periodontitis tanısı alan Derece C bireyler (Derece C, n = 21) olmak üzere üç gruba ayrıldı.

Bulgular

Ortalama TNF-α seviyeleri açısından gruplar arasında anlamlı bir fark olmamasına rağmen, Derece C'deki ortalama MIP-1α seviyesi Derece B ve Derece A'dan anlamlı derecede yüksekti. Derece B'deki ortalama MIP-1α seviyesi Derece A'dan önemli ölçüde daha yüksek ($p < 0.05$, Kruskal–Wallis testi) bulundu. Sonuç: MIP-1α, periodontitis derecesi için tanımlayıcı bir biyobelirteç olarak klinik kullanıma sahip olabilir.

Anahtar Kelimeler: Dişeti Oluğu Sıvısı, Kemokin, Periodontitis, Sitokin

Abstract

Objective

The role of cytokines and chemokines in the pathogenesis of periodontitis indicates that tumor necrosis factor alpha (TNF-α) and macrophage inflammatory protein 1α (MIP1α) have crucial functions in the initiation and progression of periodontitis. However, the cytokine and chemokine profiles of different grades of periodontitis are still unclear, and no conclusive information has yet been reported on biomarkers associated with the progression rate of periodontitis. Thus, the aim of the present study was to estimate the gingival crevicular fluid (GCF) levels of MIP-1α and TNF-α in Grades A, B, and C and to evaluate their role as reliable biomarkers in determining the grade of periodontitis.

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Materials and Methods
Individuals were divided into three groups according to their grade of periodontitis: individuals diagnosed as Stage IV periodontitis with Grade A (Grade A, n = 21), individuals diagnosed as Stage IV periodontitis with Grade B (Grade B, n = 21), and individuals diagnosed as Stage IV periodontitis with Grade C (Grade C, n = 21).

Results
Although there were no significant differences between groups in terms of mean TNF-α levels, the mean MIP-1α level of Grade C was significantly higher than that of Grade B and Grade A. The mean MIP-1α level of Grade B was significantly higher than that of Grade A ($p < 0.05$, Kruskal–Wallis test).

Conclusion
MIP-1α could have clinical utility as a screening biomarker for the grade of periodontitis.

Keywords: Chemokines, cytokines, gingival crevicular fluid, periodontitis

Introduction

Periodontitis is a consequence of the interaction between the host immune response and subgingival microbial communities. This interaction promotes the release of inflammatory mediators that results in the destruction of tooth-supporting structures.1 Cytokines and chemokines, which are among the inflammatory mediators present in the diseased periodontium, have been implicated in the pathogenesis of periodontitis.2

Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine that has a wide range of biological effects, from stimulation of inflammatory responses to protection. TNF-α induces alveolar bone resorption and plays a critical role in the pathogenesis of periodontitis. Higher gingival crevicular fluid (GCF) levels of TNF-α are found in diseased periodontal sites.3,4

Macrophage inflammatory protein 1α (MIP1α) is a biologically active chemokine secreted by a variety of cell types and plays various biological roles, such as recruiting inflammatory cells and maintaining the effector immune response. MIP-1α induces bone destruction, and higher levels have been reported in the GCF of patients with periodontitis.5,6

Periodontitis classification has been modified several times in the last 30 years in accordance with emerging scientific findings. The 2017 classification included the rate of periodontitis progression and disease susceptibility in addition to the severity of periodontitis, which had been used as a main identifier of periodontitis for a long time. Thus, periodontitis was reclassified into four stages (I, II, III, and IV) according to severity of the disease, and three grades (A, B, and C) were used to differentiate disease susceptibility and rate of periodontitis progression.7-9

Understanding the role of cytokines and chemokines in the pathogenesis of periodontitis revealed that TNF-α and MIP-1α have a crucial function in the initiation and progression of periodontitis. However, similarities and dissimilarities between cytokine and chemokine profiles of different grades of periodontitis are still unclear, and no conclusive information has yet been reported on biomarkers associated with the progression rate of periodontitis. Thus, the aim of the present study was to estimate the GCF levels of MIP-1α and TNF-α in Grades A, B, and C and to evaluate their role as reliable biomarkers in determining the grade of periodontitis.

Materials and Methods

This study was conducted between August 2019 and February 2020 in Uşak University, Faculty of Dentistry, 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: 38-38-14, date: 03.02.2021).

Participants

Individuals 18 years of age or older woman and man were included in the study. Exclusion criteria included periodontal treatment in the previous six months, use of antibiotics or anti-inflammatory drugs in the previous six months, smoking, diabetes, lactation, pregnancy, or any systemic condition.

Clinical Periodontal Measurements

All clinical examinations were performed by one examiner, who was calibrated as previously reported. 10 Plaque index (PI),11 gingival index (GI),12 probing depth (PD), and clinical attachment loss (AL) were assessed at six sites of all teeth except third molars using a manual periodontal probe (Williams, Hu-Friedy, Chicago, IL).

Classification of Individuals
Patients were classified using the 2017 classification of periodontal and peri-implant diseases and conditions. Individuals were divided into three groups according to their grade of periodontitis: individuals diagnosed as Stage IV periodontitis with Grade A (Grade A, n = 21), individuals diagnosed as Stage IV periodontitis with Grade B (Grade B, n = 21), and individuals diagnosed as Stage IV periodontitis with Grade C (Grade C, n = 21).

GCF Sampling
Clinical examination was performed one week before GCF samples were collected. Four nonadjacent and deep periodontal pockets were selected for GCF sampling. After supragingival biofilm removal, sites were isolated and gently dried to avoid saliva contamination. Standard paper strips were inserted approximately 2 mm into the pocket/sulcus for 30 seconds to collect GCF. Blood-contaminated strips were discarded, and the strips were immediately transferred into sterile Eppendorf Tubes and stored for further analysis.

Cytokine/Chemokine Quantification
Enzyme-linked immunosorbent assay was used to analyze the GCF levels of TNF-α and MIP-1α with commercially available kits. The tubes were vortexed for 30 seconds and centrifuged for 5 minutes at 1500 g to elute. Assays were carried out according to the manufacturer's recommendations. The results were described as the total amount (pg/30sn) of cytokine.

Sample Size
The effect size (0.84), type 1 error (α = 0.05), and test power (1-β = 0.80) were determined for sufficient sample size. According to these calculations, a minimum of 19 individuals per group (total sample size of 57 individuals) was necessary.

Statistical Analysis
Normality of data was checked by using Kolmogorov–Smirnov and Shapiro–Wilk tests. As the normality assumption was violated, nonparametric Kruskal–Wallis and chi-squared tests were used in the comparison of the groups. The data were considered as mean and the standard deviation and statistical significance level were set at 0.05.

Results

A total of 37 (58.7%) male and 26 (41.3%) female participants were included in the study. The mean age of the participants was 51.95±8.34. The mean age of the individuals in Grade C was significantly lower than that of the individuals in Grade B and Grade A (p < 0.05, Kruskal–Wallis test) (Table 1). There was no significant difference between the groups in terms of gender distribution (p > 0.05, chi-squared test) (Table 2).

Table 1 The mean age of groups

	Grade	n	Mean±sd	p	Difference
Age	A	21	58.43±6.25	0.001*	1-2 1-3 2-3
	B	21	54.71±4.70		
	C	21	42.71±3.59		

Table 2 Gender distribution of groups

Gender		**Grade**			**Total**	**p**
		A	**B**	**C**		
Male	**n**	13	11	13	37	0.771
	% Row	35.1%	29.7%	35.1%	100.0%	
	% Column	61.9%	52.4%	61.9%	58.7%	
Female	**n**	8	10	8	26	
	% Row	30.8%	38.5%	30.8%	100.0%	
	% Column	38.1%	47.6%	38.1%	41.3%	

Table 3 The mean periodontal clinical parameters of groups

Clinical parameters	Grade	n	Mean±sd	p	Difference
PI	A	21	2.11±0.34	0.651	-
	B	21	2.10±0.38		
	C	21	2.14±0.31		
GI	A	21	2.32±0.29	0.639	-
	B	21	2.33±0.24		
	C	21	2.37±0.20		
AL (mm)	A	21	5.57±0.87	0.801	-
	B	21	5.62±0.71		
	C	21	5.67±0.65		
PD (mm)	A	21	4.60±0.75	0.351	-
	B	21	4.65±0.63		
	C	21	4.89±0.65		

Table 4 The mean TNF-α and MIP-1α levels of groups

Inflammatory mediators	Grade	n	Mean±sd	p	Difference
TNF-α (pg/30sn)	A	21	25.21±25.46	0.255	-
	B	21	25.67±25.55		
	C	21	32.72±30.50		
MIP-1α (pg/30sn)	A	21	12.16±5.09	0.005*	1-3 2-3 1-2
	B	21	15.05±5.53		
	C	21	19.49±4.07		

*: p<0.05, Kruskal-Wallis test

There was no significant difference between the groups in terms of mean PI, GI, AL, and PD ($p > 0.05$, Kruskal–Wallis test) (Table 3). Although there was no significant difference between groups in terms of mean TNF-α level, the mean MIP-1α level of Grade C was significantly higher than that of Grade B and Grade A. The mean MIP-1α level of Grade B was significantly higher than that of Grade A ($p < 0.05$, Kruskal–Wallis test) (Table 4).

Discussion

In the 2017 classification, the grade of periodontitis includes a retrospective analysis of the rate of progression of periodontitis, which provides additional information about the biological characteristics of the disease. Grading also features an assessment of the risk of further progression and is based on an assessment of bone loss at the worst-affected tooth in the dentition as a function of age.9,13,14 To date, a comparison of inflammatory mediators in the GCF of individuals with different grades of periodontitis has not been performed. This is the first study investigating the GCF levels of TNF-α and MIP-1α in individuals with different grades of periodontitis.

The current study confirmed that gender was not significantly associated with periodontal disease

progression rate, which did not agree with previous studies reporting that periodontitis is more prevalent in men than in women.15,16 A possible explanation for this is that males and females have the same susceptibility to future disease progression, but the disease is more seen frequently among males.

According to this study, the grade of periodontitis increased as the mean age of the groups decreased, which was expected because formula used in the grade calculation is inversely proportional to age.7-9

The lack of difference in periodontal clinical parameters (PI, GI, AL, and PD) between the groups can be explained by the fact that the individuals had the same disease severity. In addition, this result suggests that conventional clinical diagnostic measures fail to recognize individuals who are at risk of further progression.

The results of this study showed an increased GCF level of MIP-1α with an increase in the progression rate of periodontitis. As the progression rate of periodontitis increases, that is, as the grade of periodontitis progresses from A to C, the GCF level of MIP-1α increases. Our result was confirmed by a study showing that GCF levels of MIP-1α are elevated prior to bone loss in patients with aggressive periodontitis, suggesting that this chemokine can identify sites susceptible to bone loss.17 Another study stated that there is a correlation between periodontitis severity and MIP-1α level, while yet another study, by Emingil et al., stated that there was no relationship between periodontal disease severity and GCF level of MIP-1α.18,19 Our study was the first to examine the relationship between grade of periodontitis and GCF level of MIP-1α; these other studies we considered were conducted according to the 1999 classification, and the severity of periodontitis destruction was generally evaluated, not the rate of progression.

This result indicates that MIP-1α can be a candidate as a diagnostic biomarker for the grade of periodontitis, and we highlight some possible related hypotheses. First, as the grade of periodontitis increases, the composition of pathogenic bacteria associated with periodontitis can change, and the level of Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis can also increase, which can induce polymorphonuclear leukocytes and epithelial cells to produce MIP-1 α.6,18 Second, monocytes from different grade levels of periodontitis can show dissimilarity in mediator release, and activated monocytes may indirectly amplify monocyte functions by recruiting additional cells to inflammatory sites.19,20 Therefore distinct macrophage phenotypes might indicate differences in the release of MIP-1α.

In the current study, no association was found between levels of TNF-α in GCF and different grades of periodontitis. The reason for the lack of difference between the groups may be that the individuals have the same periodontal destruction severity. This result demonstrates that TNF-α may be a biomarker of periodontitis severity rather than periodontitis progression rate and that this molecule could be used to compare different stages of periodontitis.

A strength of the present study is that it was the first to investigate the impact of different periodontitis grades on the GCF levels of MIP-1α and TNF-α. However, this study has some limitations. First, this is a cross-sectional study that cannot determine causal relationships. Second, this study is limited to one specific point in time, and longer follow-up of individuals by a prospective cohort study should be performed.

Conclusion

In conclusion, these findings suggest that MIP-1α could have clinical utility as a screening biomarker for the grade of periodontitis, whereas TNF-α might aid in identifying periodontitis severity. For a better understanding of cytokine and chemokine factors associated with the grade of periodontitis, further analysis is essential.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Ethical Approval
This study was designed according to Helsinki declaration principles and approved by the Uşak University Faculty of Medicine Ethics Committee (decision no: 38-38-14 , date: 03.02.2021)

Consent to Participate and Publish
Written informed consent to participate and publish was obtained from all individual participants included in the study.

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