Examination of the Effect of Periodontal Disease on Salivary Gas6 and MFG-E8 Proteins

Periodontal Hastalığın Tükürük Gas6 ve MFG-E8 Proteinleri Üzerindeki Etkisinin İncelenmesi

Melis YILMAZ https://orcid.org/0000-0003-3435-1856
Emrah TURKMEN https://orcid.org/0000-0001-5166-2109
Nur BALCI https://orcid.org/0000-0001-7986-7085
Hilal TOYGAR https://orcid.org/0000-0001-7409-1484

Istanbul Medipol University, Faculty of Dentistry, Department of Periodontology, Istanbul

Citation: Yılmaz M, Türkmen E, Balcı N, Toygar H. Examination of the Effect of Periodontal Disease on Salivary Gas6 and MFG-E8 Proteins. *Int Arc Dent Sci.* 2025; 46(2): 99-103.

ABSTRACT

INTRODUCTION: Periodontal disease is a chronic inflammatory process whose primary etiological factor is microbial dental plaque. MFG-E8 is known to play a role in the regulation of apoptotic pathways, angiogenesis, and the maintenance of tissue homeostasis. Gas-6 protein is reported to have effects on cell proliferation and adhesion, as well as the phagocytosis of apoptotic cells and platelet aggregation. The aim of this study is to elucidate the role of Gas6 and MFG-E8 molecules in the pathogenesis of periodontal disease.

MATERIAL and METHODS: The study included 20 healthy individuals and 20 patients with stage 3 grade B periodontitis who applied to clinic for dental treatment. Clinical periodontal parameters were recorded, and the levels of Gas6 and MFG-E8 in saliva samples were evaluated using ELISA kits.

RESULTS: Salivary MFG-E8 level in the periodontitis group was significantly lower than healthy participants (p: 0.021). Salivary MFG-E8 level showed a negative correlation with SCD among the clinical parameters (r: -0.082, $p < 0.05$). There was no significant difference in salivary Gas-6 levels between the control and periodontitis groups (p: 0.282).

CONCLUSION: The lower amount of salivary MFG-E8 in individuals with periodontal disease compared to healthy individuals suggests that MFG-E8 is an effective molecule for tissue homeostasis.

Keywords: *Periodontal disease, saliva, cytokine*

ÖZ

GİRİŞ: Periodontal hastalık primer etiyolojik faktörü mikrobiyal dental plak olan kronik inflamatuvar bir süreçtir. MFG-E8 apoptotik yolakların düzenlenmesi, anjiyogenez ve doku homeostazının sağlanmasında görev aldığı bilinmektedir. Gas-6 proteini ise hücre proliferasyonu ve adezyonunun yanı sıra apoptotik hücrelerin fagositozunda ve platelet agregasyonunda etkisi olduğu bildirilmiştir. Her iki molekülün çeşitli kronik sistemik hastalıklarla ilişkisi gösterilmiş olsa da periodontal hastalık ile ilgili sınırlı sayıda çalışma bulunmaktadır. Bu çalışmanın amacı Gas6 ve MFG-E8 moleküllerinin periodontal hastalık patogenezindeki yerinin aydınlatılmasıdır.

YÖNTEM ve GEREÇLER: Çalışmaya dişeti tedavisi amacıyla kliniğe başvuran sistemik ve periodontal olarak sağlıklı 20 kişi ve evre 3 derece B periodontitise sahip 20 hasta dahil edilmiştir. Klinik periodontal parametreler (SCD, SKİ, DÇ, KAS) kaydedilmiştir. Hastalardan elde edilen tükürük örneklerindeki Gas6 ve MFG-E8 seviyeleri ELISA kitleri ile değerlendirilmiştir.

BULGULAR: Klinik periodontal parametreler periodontitis grubunda sağlıklı gruba göre anlamlı seviyede yüksek bulunmuştur ($p: 0.001; p<0.05$). Periodontitis grubuna ait tükürük MFGE-8 seviyesi sağlıklı katılımcılara göre anlamlı düzeyde düşüktür (p: 0.021). Tükürük MFG-E8 seviyesi klinik parametrelerden SCD ile negatif korelasyon göstermektedir (r: -0.082 $p < 0.05$). Kontrol ve periodontitis gruplarına ait tükürük gas-6 seviyeleri arasında anlamlı bir fark bulunmamıştır (p: 0.282).

SONUÇ: Periodontitis bireylerdeki tükürük MFG-E8 miktarı sağlıklılara göre daha düşük bulunması MFG-E8'in doku homeostazı için etkili bir molekül olduğunu düşündürmektedir.

Anahtar Kelimeler: *Periodontal hastalık, tükürük, sitokin*

Corresponding author: yilmazmelis9@gmail.com
Received Date: 04.06.2024
Accepted Date: 01.07.2024

INTRODUCTION

Periodontal disease is a chronic inflammatory condition initiated by a dysbiotic microbiota in susceptible individuals. The complex interaction between the host's inflammatory response and the dysbiotic microbiota leads to the progressive destruction of periodontal supporting structures, including the gingiva, cementum, periodontal ligament, and alveolar bone, ultimately resulting in tooth loss over time.[1] Upon activation of the host immune response, the inflammatory process is triggered, releasing various chemokines and cytokines (such as IL-1beta, TNF-alpha, and IL-17), which contribute to tissue destruction.[2] Simultaneously, certain anti-inflammatory mediators play a role in suppressing inflammation, aiming to restore tissue homeostasis.[3]

Milk fat globule-epidermal growth factor–8 (MFG-E8), also known as lactadherin, is produced in various organs and tissues, including macrophages, fibroblasts, dendritic and epithelial cells, as well as mammary glands.[4] Studies have shown that MFG-E8 plays a crucial role in multiple processes, such as enhancing apoptotic cell clearance, reducing neutrophil infiltration and tissue fibrosis, promoting angiogenesis, maintaining homeostasis, and mediating various anti-inflammatory responses.[5,6] In a study conducted by Abe et al. in 2014, MFG-E8 was reported to regulate osteoclast function by preventing their excessive resorptive activity.[5]

Growth arrest-specific gene 6 (Gas-6) is a 75-kDa molecule produced by various cell types, including immune cells, vascular cells, and endothelial cells.[7,8] In addition to its roles in cell proliferation and adhesion, platelet aggregation, and adipocyte development regulation, Gas-6 also influences the phagocytosis of apoptotic cells, similar to MFG-E8.[7] In human umbilical vein endothelial cells stimulated with lipopolysaccharides from Porphyromonas gingivalis, a key periodontal pathogen, Gas-6 has been reported to inhibit the release of inflammatory factors.[9]

Studies have demonstrated that MFG-E8 and Gas-6 play significant roles in biological systems. Although these molecules are known to be involved in the pathogenesis of chronic diseases such as obesity, diabetes, and atherosclerosis, no study has yet evaluated them together in individuals with periodontal disease.[10-15] Therefore, the aim of this study is to assess salivary MFG-E8 and Gas-6 protein levels in healthy individuals and those with stage 3 grade B periodontitis, thereby elucidating their role in the pathogenesis of periodontal disease.

MATERIAL and METHODS

The study group consisted of 20 patients who were systemically healthy, met the inclusion criteria, and were diagnosed with stage 3 grade B periodontitis (P) according to the 2017 World Workshop on Periodontology. These patients were selected from those who applied to the Periodontology Clinic of Istanbul Medipol University Faculty of Dentistry. The control (C) group included 20 systemically and periodontally healthy participants. The inclusion criteria were as follows: individuals aged between 18 and 65 years, without any systemic disease, having at least 20 natural permanent teeth in occlusion (excluding third molars), not using any orthodontic appliances, non-smokers, not having used antimicrobial and/or anti-inflammatory drugs in the last 3 months, not having undergone periodontal treatment in the last 6 months, and not having received surgical periodontal treatment in the last year.

The socio-demographic data and periodontal indices (PD, BOP, PI, CAL) of all participants in the study were recorded by a single researcher (M.Y.) using a William's periodontal probe at six sites per tooth.

Unstimulated saliva samples were collected from the patients in the morning. After comfortably seating the patients, they were instructed to rinse their mouths with distilled water and then spit into a plastic tube. The saliva samples were centrifuged at 2800 g for 10 minutes, transferred into Eppendorf tubes, and stored at -80°C until the day of the experiment.[16]

Biochemical Analysis

The levels of Gas-6 and MFG-E8 in the collected samples were determined using ELISA kits (human ELISA immunoassay, Shanghai Sunred Biological Technology, Shanghai, China) and analyzed according to the manufacturer's instructions. Colorimetric evaluation was performed using a microplate reader (Thermo Scientific, MA, USA) at 450 nm. The corresponding standard curve was used to calculate the concentrations. Each sample was analyzed in duplicate, and the average values of the results were used.

Statistical Analysis

Statistical calculations were performed using the GraphPad Prism 10 (Boston, MA) statistical software package. The Shapiro-Wilk test was used to assess data normality. For comparisons between groups, the Student's t-test was applied to normally distributed data, while the Mann-Whitney U test was used for non-normally distributed data. The Spearman correlation test was conducted to determine the relationship between salivary Gas-6 and MFG-E8 levels and clinical periodontal parameters. All tests were performed at a significance level of $\alpha = 0.05$.

RESULTS

An analysis of demographic and clinical data showed no significant differences between the groups in terms of age and gender ($p = 0.1012$ and $p = 0.956$, respectively).

However, periodontal parameters, including plaque index, probing pocket depth, bleeding on probing index, and clinical attachment level, were significantly higher in the periodontitis group compared to healthy participants ($p < 0.001$ for all) (Table 1).

Table 1. Demographic and clinical parameters of the control and periodontitis groups

	Control (C) n=20	Periodontitis (P) n=20	*p*
Age (year)	38.9 ± 5.6	41.7 ± 6.4	0.1012
Gender F/M	9/11	10/10	0.956
PI	0.49 ± 0.30	1.69 ± 0.31	<0.001*
PD (mm)	1.35 ± 0.16	3.04 ± 0.72	<0.001*
BOP (%)	7.55 ± 4.94	48.05 ± 16.43	<0.001*
CAL (mm)	1.21 ± 0.16	3.47 ± 0.66	<0.001*

PI, plaque index; PD, probing depth; BOP, bleeding on probing index; CAL, clinical attachment level.
*Statistically significant difference compared to the control group ($p < 0.05$).

The salivary MFG-E8 level in the periodontitis group was significantly lower compared to healthy participants ($p = 0.021$). However, there was no significant difference in salivary Gas-6 levels between the control and periodontitis groups ($p = 0.282$) (Figure 1).

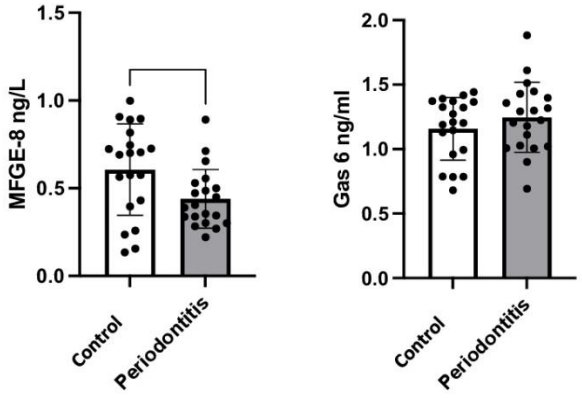

Figure 1: Salivary MFG-E8 and Gas-6 protein levels in healthy individuals and patients with stage 3 grade B periodontitis.

*Statistically significant difference compared to the control group ($p < 0.05$).

The salivary MFG-E8 level showed a weak but significant negative correlation with pocket depth ($r = -0.082$, $p < 0.05$). Additionally, a weak but significant positive correlation was observed between salivary Gas-6 and MFG-E8 levels ($r = 0.373$, $p < 0.05$) (Table 2).

Table 2. Correlation between clinical parameters and GAS-6, MFG-E8 proteins

	PI	BOP	PD	CAL	GAS-6
GAS-6	0.119	0.058	0.019	0.265	
MFGE-8	-0.216	0.114	-0.082*	-0.082	0.373*

(Spearman correlation coefficients, r) Spearman correlation test. Significance values of $p < 0.05$ are marked with (*).

DISCUSSION

Periodontal disease is the sixth most common disease worldwide, and its prevalence increases with age. As periodontal disease progresses, individuals may experience tooth loss, leading to both aesthetic and functional problems, ultimately reducing their quality of life. A comprehensive understanding of the disease pathogenesis and the elucidation of its unresolved mechanisms are crucial for early diagnosis and treatment.[1] For this reason, in our study, we evaluated the salivary levels of Gas-6 and MFG-E8 proteins, which are thought to play a role in the disease mechanism, in both healthy individuals and those with periodontal disease.

MFG-E8 is a peripheral membrane glycoprotein known to be involved in various processes, including apoptotic cell clearance, anti-inflammatory effects, and angiogenesis.[17] Recent studies have demonstrated that MFG-E8 plays a significant role in the development of autoimmune diseases such as systemic lupus erythematosus, age-related diseases, and inflammatory conditions.[17,18] However, there are only a limited number of studies investigating its role in periodontal disease, a chronic inflammatory condition. In a study conducted by Yavuz et al. in 2019, MFG-E8 and IL-1β levels in saliva and gingival crevicular fluid (GCF) were evaluated in healthy individuals, and those with gingivitis and chronic periodontitis. While salivary MFG-E8 levels did not show a significant difference among the groups, GCF MFG-E8 levels were found to be significantly higher in healthy individuals compared to those with gingivitis and chronic periodontitis. IL-1β, a known pro-inflammatory mediator, was found at lower levels in the serum and GCF samples of healthy participants compared to the other two groups.[19] In an animal study, experimental periodontitis was induced in MFG-E8-deficient mice, and greater bone loss was observed compared to the control group. The authors reported that osteoclasts are produced and regulated by MFG-E8.[5] When MFG-E8 levels were evaluated in gingival crevicular fluid (GCF) samples obtained from healthy individuals and patients with varying degrees of periodontal disease, higher levels were found in healthy individuals compared to those with periodontal disease. Furthermore, after periodontal treatment, an increase in MFG-E8 levels was observed in patients with periodontal disease.[20] In our study, the salivary MFG-E8 level was found to be significantly lower in the periodontitis (P) group compared to the control (C) group. Considering that MFG-E8 exhibits anti-inflammatory properties, its higher levels in the

saliva samples of healthy participants suggest that our findings are consistent with the literature. Additionally, the significant negative correlation between pocket depth and MFG-E8 levels further supports the notion that this molecule is secreted in greater amounts under healthy conditions (Table 2). The composition of saliva is influenced not only by the secretions of major and minor salivary glands but also by hormones, food residues, gingival crevicular fluid (GCF), and other sources.[21] Although our study did not evaluate MFG-E8 levels in GCF, previous studies have reported higher GCF MFG-E8 levels in healthy individuals compared to those with periodontal disease.[19,20] Given that saliva composition is also affected by GCF, our findings further support these previous studies. In light of our results, it is suggested that the potential role of MFG-E8 in periodontal disease is to mediate the transition to homeostasis by suppressing the inflammatory response.

Gas-6 belongs to the vitamin K-dependent protein family and is known to be involved in various pathophysiological processes, including thrombosis, phagocytosis of apoptotic cells, inhibition of inflammation, and vascular calcification.[7] In an animal study, the absence of Gas-6 was shown to cause increased production of inflammatory cytokines and reactive nitrogen species in mice. Additionally, it was reported that the increase in IL-6 production led to the induction of Th17 cells. With the rise in inflammatory burden, the oral microbiota transitioned towards dysbiosis, and the authors suggested that Gas-6 serves as a key immunological regulator in host-commensal interactions.[22] When the GAS6/AXL signaling pathway was evaluated in human periodontal ligament cells stimulated with P. gingivalis lipopolysaccharides, a decrease in Gas-6 and AXL levels was observed.[23] Moreover, when serum Gas-6 levels were assessed in 50 patients with type 2 diabetes, they were found to be lower compared to healthy individuals.[24] To the best of our knowledge, only one study in the literature has evaluated salivary Gas-6 levels in periodontitis patients. However, in that study, Gas-6 was not detected in the ELISA raw data of either the healthy or periodontitis salivary samples.[25] In our study, although Gas-6 was detectable in the salivary samples of the periodontitis group, it did not differ significantly from the levels in healthy individuals. However, its positive correlation with MFG-E8 suggests that this molecule may play a role alongside MFG-E8 in the suppression of inflammation.

Periodontal disease is a complex process involving various inflammatory pathways. Clarifying the missing aspects of its pathogenesis is essential for disease prevention and early diagnosis. Therefore, in our study, we investigated the MFG-E8 and Gas-6 proteins, which, to our knowledge, have not been previously evaluated together in salivary samples, in healthy individuals and patients with stage 3 grade B periodontitis. According to our results, the lower levels of MFG-E8 in the periodontitis group suggest that it may serve as a potential indicator of periodontal disease. However, to determine the possible role of MFG-E8 and Gas-6 in periodontal disease, further long-term studies with larger sample sizes, including serum and gingival crevicular fluid (GCF) samples, are needed.

CONCLUSION

In conclusion, research on the mechanism of periodontal disease has predominantly focused on pro-inflammatory molecules. However, considering the significant role of anti-inflammatory mediators in the disease process, increasing studies on these molecules may contribute to the identification of new therapeutic targets that could aid in periodontal treatment.

.

REFERENCES

1. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. *Nat Rev Dis Primers* 2017; 3: 17038.
2. Graves D. Cytokines that promote periodontal tissue destruction. *J Periodontol.* 2008;79(8 Suppl):1585-91.
3. Freire MO, Van Dyke TE. Natural resolution of inflammation. *Periodontol 2000.* 2013; 63(1): 149-64.
4. Aziz M, Jacob A, Matsuda A, Wang P. Review: milk fat globule-EGF factor 8 expression, function and plausible signal transduction in resolving inflammation. *Apoptosis.* 2011;16(11):1077-86.
5. Abe T, Shin J, Hosur K, Udey MC, Chavakis T, Hajishengallis G. Regulation of osteoclast homeostasis and inflammatory bone loss by MFG-E8. *J Immunol.* 2014; 193(3): 1383-91.
6. Deroide N, Li X, Lerouet D, et al. MFGE8 inhibits inflammasome-induced IL-1β production and limits postischemic cerebral injury. *J Clin Invest.* 2013; 123(3): 1176-81.
7. Laurance S, Lemarié CA, Blostein MD. Growth arrest-specific gene 6 (gas6) and vascular hemostasis. *Adv Nutr.* 2012; 3(2): 196-203.
8. Manfioletti G, Brancolini C, Avanzi G, Schneider C. The protein encoded by a growth arrest-specific gene (gas6) is a new member of the vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade. *Mol Cell Biol.* 1993; 13(8): 4976-85.
9. Liu YJ, Ouyang XY, Wang YG, Lv PJ, An N. Role of vitamin K-dependent protein Gas6 in the expression of endothelial cell adhesion molecule-1

and chemokines induced by Porphyromonas gingivalis lipopolysaccharide. *Beijing Da Xue Xue Bao Yi Xue Ban.* 2018; 50(1): 20-5.

10. Huang W, Jiao J, Liu J, et al. MFG-E8 accelerates wound healing in diabetes by regulating "NLRP3 inflammasome-neutrophil extracellular traps" axis. *Cell Death Discov.* 2020;6:84.
11. Li Y, Ran W, Zhang J, et al. Elevated serum milk fat globule-epidermal growth factor 8 levels in type 2 diabetic patients are suppressed by overweight or obese status. *IUBMB Life.* 2017; 69(2): 63-71.
12. Zhao H, Zhang H, Qin X. Age-related differences in serum MFG-E8, TGF-β1 and correlation to the severity of atherosclerosis determined by ultrasound. *Mol Med Rep.* 2017; 16(6): 9741-8.
13. Maquoi E, Vörös G, Carmeliet P, Collen D, Lijnen HR. Role of Gas-6 in adipogenesis and nutritionally induced adipose tissue development in mice. *Arterioscler Thromb Vasc Biol.* 2005; 25(5): 1002-7.
14. Maree AO, Jneid H, Palacios IF, Rosenfield K, MacRae CA, Fitzgerald DJ. Growth arrest specific gene (GAS) 6 modulates platelet thrombus formation and vascular wall homeostasis and represents an attractive drug target. *Curr Pharm Des.* 2007; 13(26): 2656-61.
15. Saltiel AR, Olefsky JM. Inflammatory mechanisms linking obesity and metabolic disease. *J Clin Invest.* 2017; 127(1): 1-4.
16. Yilmaz M, Yay E, Atalay N, et al. Do arginine metabolites have a role in periodontitis due to smoking? A new perspective. *Oral Dis.* 2024; 30(2): 743-753.
17. Li BZ, Zhang HY, Pan HF, Ye DQ. Identification of MFG-E8 as a novel therapeutic target for diseases. *Expert Opin Ther Targets.* 2013; 17(11): 1275-85.
18. Ushikubo M, Saito S, Kikuchi J, et al. Milk fat globule epidermal growth factor 8 (MFG-E8) on monocytes is a novel biomarker of disease activity in systemic lupus erythematosus. *Lupus.* 2021; 30(1): 61-9.
19. Yavuz MC, Pekbağriyanik T, Sağlam M, Köseoğlu S. Evaluation of milk fat globule-epidermal growth factor-factor VIII and IL-1β levels in gingival crevicular fluid and saliva in periodontal disease and health. *Odontology.* 2019; 107(4): 449-56.
20. Kajikawa T, Meshikhes F, Maekawa T, et al. Milk fat globule epidermal growth factor 8 inhibits periodontitis in non-human primates and its gingival crevicular fluid levels can differentiate periodontal health from disease in humans. *J Clin Periodontol*. 2017; 44(5): 472-83.
21. Kaufman E, Lamster IB. The diagnostic applications of saliva--a review. *Crit Rev Oral Biol Med.* 2002; 13(2): 197-212.
22. Nassar M, Tabib Y, Capucha T, et al. GAS6 is a key homeostatic immunological regulator of host-commensal interactions in the oral mucosa. *Proc Natl Acad Sci U S A.* 2017; 114(3): E337-E46.
23. Zhang S, Liu Y, Wang X, An N, Ouyang X. STAT1/SOCS1/3 Are Involved in the Inflammation-Regulating Effect of GAS6/AXL in Periodontal Ligament Cells Induced by. *J Immunol Res.* 2021; 2021: 9577695.
24. Fouad NA, Eltaher SM, Abdullah OA, Metwally RA. Serum Level of Growth Arrest-Specific 6 (Gas6) Protein and Genetic Variations in the Gas6 Gene in Patients with Type 2 Diabetes Mellitus. *Egypt J Immunol.* 2015; 22(1): 41-7.
25. Balcı N, Dyrmishi A, Çetin M, Çekici A. Periodontal Durumun Tükürük Growth Arrest-Specific Protein 6(Gas6) Düzeyi Üzerine Etkisinin İncelenmesi. *Ata Diş Hek Fak Derg.* 2020; 30(3): 379-385.