Decreased Salivary Gremlin-1 Levels in Periodontitis Patients

Periodontitis Hastalarında Azalan Tükürük Gremlin-1 Düzeyleri

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

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

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ABSTRACT

INTRODUCTION: Gremlin-1, a member of the Transforming Growth Factor-Beta protein superfamily, is an antagonist of Bone Morphogenetic Proteins involved in processes such as angiogenesis, inflammation, fibrosis, and osteogenesis. The aim of this study is to compare salivary Gremlin-1 levels in individuals with periodontitis to those in periodontally healthy individuals and to evaluate the relationship of these levels with clinical parameters.

METHODS: A total of 40 systemically healthy individuals were included, comprising 20 stage III/grade B periodontitis and 20 periodontal healthy individuals. Clinical periodontal parameters (plaque index (PI), probing depth (PD), clinical attachment loss (CAL) and bleeding on probing (BOP)) were recorded. Salivary Gremlin-1 levels were analyzed using an ELISA.

RESULTS: All periodontal measurements were significantly higher in the periodontitis group rather than controls ($p<0.0001$). Salivary Gremlin-1 levels were significantly lower in the periodontitis compared to the control group ($p<0.0001$). Negative correlations were found between Gremlin-1 levels and PI ($r=-0.592$, $p=0.011$), PD ($r=-0.452$, $p=0.003$), and CAL ($r=-0.453$, $p=0.003$). No significant correlation was observed between Gremlin-1 levels and BOP ($p>0.05$).

CONCLUSION: Decreased saliva Gremlin-1 levels in periodontitis patients and their negative correlation with clinical periodontal parameters suggest that saliva Gremlin-1 might modulate the inflammation and limit tissue remodeling as a systemic compensation mechanism in periodontitis.

Keywords: *Gremlin-1, Periodontitis, Saliva*

ÖZ

GİRİŞ ve AMAÇ: Gremlin-1, Transforming Growth Faktör-Beta protein süper ailesinin bir üyesi olup, Bone Morfogenetik Protein'lerin antagonistidir ve anjiyogenez, inflamasyon, fibrozis ve osteogenez gibi süreçlerde rol oynar. Bu çalışmanın amacı, periodontitisli bireylerdeki tükürük Gremlin-1 seviyelerini periodontal olarak sağlıklı bireyler ile karşılaştırmak ve bu seviyelerin klinik parametrelerle ilişkisini değerlendirmektir.

YÖNTEM ve GEREÇLER: Çalışmaya 40 sistemik olarak sağlıklı birey dahil edildi; bunlardan 20'si evre III/derece B periodontitisli ve 20'si periodontal olarak sağlıklı bireylerdi. Klinik periodontal parametreler (plak indeksi (Pİ), sondalama derinliği (SD), klinik ataşman kaybı (KAK) ve sondalamada kanama (SKİ)) kaydedildi. Tükürük Gremlin-1 seviyeleri ELISA yöntemi kullanılarak analiz edildi.

BULGULAR: Tüm periodontal ölçümler, kontrol grubuna kıyasla periodontitis grubunda anlamlı derecede daha yüksek bulundu ($p<0.0001$). Tükürük Gremlin-1 seviyeleri, periodontitis grubunda kontrol grubuna göre anlamlı derecede daha düşük bulundu ($p<0.0001$). Gremlin-1 seviyeleri ile Pİ ($r=-0.592$, $p=0.011$), SD ($r=-0.452$, $p=0.003$) ve KAK ($r=-0.453$, $p=0.003$) arasında negatif korelasyonlar gözlendi. Gremlin-1 seviyeleri ile SKİ arasında anlamlı bir korelasyon bulunmadı ($p>0.05$).

SONUÇ: Periodontitis hastalarında azalmış tükürük Gremlin-1 seviyeleri ve klinik periodontal parametrelerle olan negatif korelasyonu, tükürük Gremlin-1'in sistemik bir kompansasyon mekanizması olarak inflamasyonu modüle edebileceğini ve doku yeniden şekillenmesini sınırlayabileceğini düşündürmektedir.

Anahtar Kelimeler: *Gremlin-1, Periodontitis, Tükürük*

Corresponding author: emrahturkmen@medipol.edu.tr
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INTRODUCTION

Periodontitis is a chronic inflammatory condition marked by the progressive destruction of the supporting tissues of the teeth, including the alveolar bone. It is primarily caused by the host's immune response to a dental biofilm and influenced by various factors, including genetic predisposition, environmental factors, and systemic conditions.[1] Numerous studies have identified key molecules and signaling pathways, such as Transforming Growth Factor-beta (TGF- β), Bone Morphogenetic Proteins (BMPs) and Nuclear Factor Kappa B (NF-κB) that play a critical role in periodontal inflammation-induced alveolar bone loss.[2] As periodontitis progresses, NF-κB serves as a central regulator of inflammatory responses through orchestrating a network of mediators influencing the body's reaction to pathogenic organisms.[3]

Gremlin-1 is a member of the TGF-β protein superfamily and has recently been identified as a antagonists for BMPs and is involved in various metabolic processes including angiogenesis, inflammation, adipogenesis, fibrosis, and osteogenesis.[4] Previous studies have shown that Gremlin plays a role in the pathological processes of inflammation-related diseases.[5,6] It has been reported that Gremlin-1 exacerbates osteoarthritis by activating the NF-κB pathway.[7] Additionally, Gremlin-1's pro-inflammatory function has been associated with renal inflammation and thrombo-inflammation.[8] Gremlin-1, through its interaction with BMPs, regulates various cellular processes critical for tissue homeostasis and repair. By antagonizing BMPs, Gremlin-1 can modulate bone formation and resorption,[5] which is particularly relevant in the context of periodontal disease where bone loss is a hallmark feature. The dual role of Gremlin-1 in promoting inflammation through the NF-κB pathway and regulating bone metabolism makes it a molecule of significant interest in understanding the pathogenesis of periodontitis.

Recently, Gremlin-1 has been associated with oral related diseases. Gremlin-1 has been shown to contribute to the formation of dental hard tissues and the pathological development of cleft lip and palate.[9] It has been reported that Gremlin-1 is related in oral squamous cell carcinoma (OSCC)[10] and the development of apical periodontitis[11] through the NF-κB signaling pathway, and its inhibition can suppress the progression of these lesions. Also, Gremlin-1 has been shown to be distributed in periodontal tissues and to play a site-specific role in regulating cell growth and differentiation.[12] Gremlin-1 has been detected in the gingival tissue of patients with periodontitis and has been shown to mediate the activity of the NF-κB signaling pathway through the TGF-β1/Smad3/β-catenin and JNK signaling pathways, thereby regulating the development of periodontitis.[13]

To our knowledge, there are no studies evaluated that salivary Gremlin-1 levels in individuals with periodontitis. We hypothesized that Gremlin-1 levels in saliva reflect its systemic role in regulating inflammatory responses and tissue remodeling in periodontal disease, potentially decreasing as a compensatory mechanism to prevent excessive inflammation and tissue destruction in periodontitis. Therefore, the aim of this study is to compare the salivary Gremlin-1 levels in individuals with periodontitis to those in periodontally healthy individuals and to evaluate the relationship of these levels with clinical parameters.

MATERIAL AND METHODS

Study population

This study is a cross-sectional study conducted on individuals who applied to the departments of periodontology at Medipol University, Istanbul, from October 2023 to April 2024. A total of 40 subjects were included, including a systematically healthy control group with a healthy periodontium and systematically healthy periodontitis group with stage III grade B generalized periodontitis. The Ethics Committee of Istanbul Medipol University approved the study (No: E-10840098-202.3.02-1081, on 08.02.2024), which was conducted in accordance with the Declaration of Helsinki. All participants provided informed consent.

Inclusion criteria required participants to be (1) between 18 and 65 years old, (2) have at least 20 natural teeth, excluding third molars, (3) be systemically healthy, and (4) provide consent to participate in the study. Exclusion criteria included (1) the use of antibiotics, nonsteroidal anti-inflammatory drugs, steroids, immunosuppressants, beta-blockers, calcium channel blockers, anticoagulants, or hormonal contraceptives within the 3 months prior to the study, (2) received periodontal treatment in the last 6 months, (3) smoking, (4) pregnancy or lactation, (5) have any systemic conditions that might impact immune response, (5) using orthodontic appliances.

Clinical periodontal examination and diagnosis

The periodontal status was determined based on the 2017 AAP/EFP classification for periodontal and peri-implant diseases.[14] A probing depth (PD) of 3 mm or less, along with less than 10% of sites exhibiting bleeding on probing and with intact periodontitum, was defined as a healthy periodontium.[14] Individuals were diagnosed with periodontitis if they showed clinical attachment loss (CAL) of 2 mm or more at two or more non-adjacent teeth. The highest loss interdental clinical attachment loss was recorded for each tooth, and a clinical attachment loss of 5 mm or more and periodontitis-related tooth loss of 4 or fewer teeth were defined as stage III periodontitis.[14] Periodontitis grade was determined based

on the ratio of radiographic bone loss to the patient's age.[14] Patients with a ratio between 0.25 and 1.00 were classified as Grade B.

Two calibrated periodontal experts (E.T, N.B.) recorded all clinical periodontal measurements, including plaque index (PI), pocket depth (PD), bleeding on probing (BOP) and CAL by measuring six regions of all teeth using a William's periodontal probe. The two examiners were calibrated by training on 10 non-study volunteers.[15] The probing depth scores showed high reliability, as confirmed by inter-examiner analysis (κ = 0.896) conducted prior to the study. The assessment revealed that the mean of repeated probing measurements was within 1 mm for 90% of the sites.

Samples collection and laboratory analysis

Unstimulated saliva samples were collected between 9:00 and 11:00 in the morning after an overnight fast to facilitate the analysis of selected markers. Participants avoided any oral hygiene procedures on the morning of sample collection. They were instructed to sit in a relaxed position, rinse with distilled water, and then spit into a sterile plastic tube for 10 minutes. Then saliva samples were centrifuged at 2800 g for 10 minutes. The flow rate of saliva (SFR) was measured by dividing the volume of saliva collected by the duration of the collection period.[15] All samples were transferred to Eppendorf tubes and stored at −80 °C until the day of analysis.

Saliva samples were analyzed for Gremlin-1 using commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits (Elabscience, Houston, Texas, USA) following the manufacturer's instructions. All samples were tested in duplicate, and the values were averaged.

Statistical analyses

Statistical analyses were conducted using GraphPad Prism 10 software. The Shapiro-Wilk test was used to evaluate the normality of the parameters. Group comparisons were conducted using the t-test for normally distributed data and the Mann-Whitney test for data that were not normally distributed. Spearman correlation analysis was performed to determine correlations between biochemical and clinical periodontal parameters. A significance level of $p<0.05$ was considered statistically significant.

RESULTS

Table 1 presents the demographic and clinical findings of the study groups. A total of 40 systemically healthy subjects were included in this study. The control group (C) consisted of 20 individuals with healthy periodontium (11 females and 9 males; mean age: 38.2 ± 6.43 years), and the periodontitis group (P) included 20 patients diagnosed with Stage III, Grade B periodontitis (12 females and 8 males; mean age: 41.5 ± 7.1 years).

There were no significant differences according to age and gender between the periodontitis and control groups ($p>0.05$). There was no significantly difference was found between the periodontitis and control groups for SFR *($p>0.05$).* Periodontal measurements (PI, BOP, PD, CAL) were significantly higher in the periodontitis group rather than control *($p < 0.001$)*.

Gremlin-1 levels were significantly lower in the periodontitis group compared to the control group *($p<0.0001$).* (Table 2, Figure 1).

Table 3 presents the correlations between Gremlin-1 levels and clinical periodontal parameters. Gremlin-1 was showed a significantly moderate negative correlation with PI (r=-0.592, *$p=0.011$*), PD (r=-0.452, *$p=0.003$*), and CAL (r=-0.453, *$p=0.003$*), however was not correlated with BOP (*$p>0.05$*).

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

Parameters	Control (C) n=20	Periodontitis (P) n=20	*p*
Age (year)	38.2 ± 6.43	41.5 ± 7.1	*0.107**
Gender F/M	11/9	12/8	*0.215*
SFR	0.41 ± 0.08	0.40 ± 0.10	*0.633**
PI	0.93 ± 0.28	2.40 ± 0.19	***<0.001****
PD (mm)	1.33 ± 0.17	2.95 ± 0.54	***<0.001****
BOP (%)	3.8 ± 2.39	57.19 ± 17.36	***<0.001****
CAL (mm)	1.33 ± 0.17	3.34 ± 0.69	***<0.001****

*Student-t test was used. Data shown as mean ± standard deviation

Abbreviations: SFR, saliva flow rate; PI, plaque index; PD, probing depth; BOP, bleeding on probing; CAL, clinical attachment lost.. Statistical difference with the control group $p<0.05$. Significantly different values are shown in boldface type.

Table 2: Saliva levels of Gremlin-1 in periodontitis and control groups

Biochemical	Control (C) n=20	Periodontitis (P) n=20	*p*
Gremlin-1 (ng/ml)	3.36 (1.01-9.68)	1.12 (0.17-3.38)	***<0,0001****

* Mann Whitney test was used. Data shown as median (min-max).

Statistical difference with the control group $p<0.05$. Significantly different values are shown in boldface type.

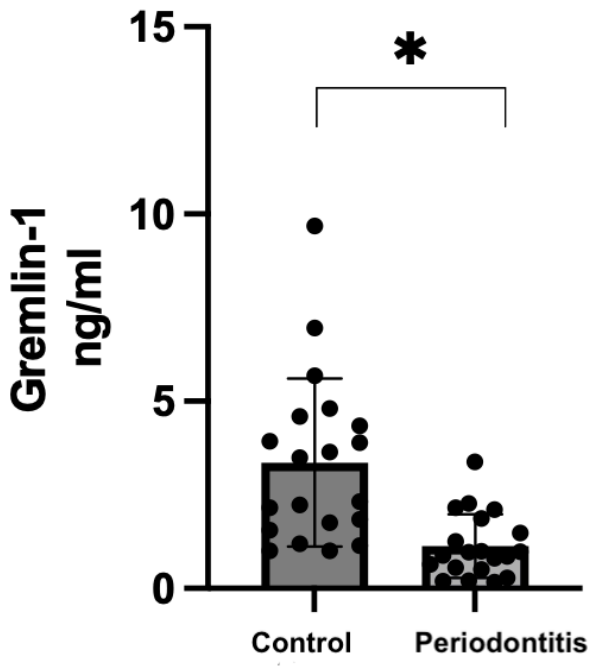

Fig 1: Saliva levels of Gremlin-1 in control volunteers and periodontitis patients. Box-and-whisker plots with the median (horizontal line), interquartile range (box) and outlier (circles) values are shown. *Significantly different *($p < 0.05$)* from the control group.

Table 3: Correlations between biomarkers and periodontal clinical parameters (Spearman correlation coefficients, *r values*) ($n = 40$).

Variables	SFR	PI	BOP	PD	CAL
PI	-0.150	-	-	-	-
BOP	-0.115	0.266	-	-	-
PD	-0105	0.854	0.014	-	-
CAL	-0.148	0,742	0,034	0,824	-
Gremlin-1	0.123	**-0.592***	-0.192	**-0.452***	**-0.453***

Significantly different values are shown in boldface type, * *$p<0.05$;*

DISCUSSION

Periodontitis is defined as a chronic inflammatory disease in which TGF-β regulates tissue repair and inflammation, while NF-κB contributes to tissue destruction and disease progression by promoting the production of pro-inflammatory cytokines.[16] Gremlin-1, a member of the TGF-β superfamily, has been shown to exacerbates periodontitis by stimulating NF-κB signaling pathway.[13] However, no studies have assessed the saliva levels of Gremlin-1 in periodontitis patients. Consequently, we investigted saliva levels of Gremlin-1 in patients with stage III/grade B periodontitis.

In this study, no differences were observed between the groups in terms of age, gender, and SFR. Saliva flow rate is known to be associated with individuals' overall health status and systemic conditions.[17] The fact that all participants in our study were systemically healthy might have contributed to the stability of SFR. The total unstimulated saliva flow rate is typically around 0.3-0.4 ml/min. During sleep, this rate drops to approximately 0.1 ml/min, whereas it rises to about 4.0-5.0 ml/min during activities such as eating, chewing, and other forms of stimulation.[17] The wide range of variation in saliva flow rate under normal physiological conditions and natural variations among individuals may also influence this result. The balanced distribution of age and gender between the two groups suggests that these demographic factors do not have a significant impact on saliva flow rate. Additionally, the similarity in age and gender between the groups emphasizes that the study included a homogeneous population in terms of demographic characteristics, and these factors are not significant variables in the evaluation of periodontitis.

The present study shown that lower saliva Gremlin-1 levels in stage III/grade B periodontitis patients compared to the healthy controls and negatively correlated with plaque index, probing depth, and clinical attachment loss. We have demonstrated for the first time lower Gremlin-1 levels in saliva were associated with periodontitis . Gremlin-1 is known to crucial regulator of the TGF-β1/Smad3/NF-κB axis and JNK/NF-κB. It has been shown that Gremlin-1 modulates inflammation in a tissue-dependent manner.[18] Gremlin-1 has been shown to aggravate osteoarthritis in human models by activating the NF-κB pathway.[7] Corsini et al. (2013), showed that in endothelial cells, Gremlin-1 triggers the production of pro-inflammatory chemokines and adhesion molecules through activating NF-κB pathways.[7] Besides, Gremlin-1 plays a protective role in vascular inflammation and atherosclerotic plaque progression. Various studies have demonstrated that Gremlin-1 Various studies have demonstrated that Gremlin-1 suppresses macrophage migration inhibitory factor (MIF)-dependent monocyte activation and movement, and it reduces leukocyte infiltration, thereby limiting the development of atherosclerotic plaques,[19,20] also it promotes angiogenesis both in vitro and in vivo by binding to and activating vascular endothelial growth factor receptor 2.[21] It is known that periodontitis is a chronic inflammatory disease marked by enhanced macrophage infiltration and activation.[22] The decreased salivary Gremlin-1 levels in our study may suggest that it may have a similar protective role during inflammation in periodontitis. Muller et al. (2021), highlighted the protective role of Gremlin-1 in myocardial function, demonstrating its involvement in regulating fibrosis and wound healing across various tissues, including the heart.[23] Gremlin-1 interacts with BMP signaling pathways to modulate collagen production and fibrosis.[23] This suggests that

Gremlin-1 acts as a BMP antagonist, blocking BMP signaling to precisely control BMP gradients essential for tissue repair and fibrosis regulation. Our findings align with these insights, indicating that decreased salivary levels of Gremlin-1 in periodontitis may be relate to its regulatory role in tissue remodeling and inflammatory response modulation.

Gremlin-1's involvement in periodontitis might also extend to its interaction with BMP and TGF-β pathways. Gremlin-1 has been shown to inhibit BMP-dependent apoptosis of myofibroblasts and cause extracellular matrix accumulation, impacting fibrotic processes in chronic diseases such as kidney and liver fibrosis.[24] The presence of Gremlin-1 in various inflammatory and fibrotic conditions suggests a complex role in mediating tissue responses to chronic injury and inflammation. Interestingly, while Gremlin-1 promotes fibrosis in some tissues, it appears to have an inhibitory effect on TGF-β-induced collagen production in myocardial fibroblasts, as shown by Muller et al. (2021).[23] This dual role may depend on the specific tissue environment and the balance of signaling pathways involved. A recent short report was showed that Gremlin-1 is essential for M2-like polarization of macrophages, enhancing this process in response to Th2 cytokines IL4 and IL13, and its depletion inhibits M2 polarization, highlighting a novel mechanism in fibrosis and remodeling in lung diseases.[25] The decreased salivary Gremlin-1 levels in periodontitis observed in our study might reflect a tissue-specific regulatory mechanism where Gremlin-1 modulates the inflammatory response and remodelling in periodontitis.

Additionally, Nagatomo et al. (2008), reported that transgenic overexpressing Gremlin-1 in mice caused defects in enamel and dentin, and they argued that this may also affect periodontal disease involving both bone and soft tissues.[9] A study by Ghuman et al. (2019), revealed that gingival fibroblasts express Gremlin-1, which inhibits BMP-mediated osteoblastic differentiation. They have been reported that the inhibitory effect of Gremlin-1 on osteoblastic differentiation may contribute to the impaired bone regeneration observed in periodontitis.[12] A recent study investigated the role of Gremlin-1 in inflammatory apical periodontitis and showed that Gremlin-1 expression was significantly increased in inflamed periapical tissues.[11] Guan et al. (2022), reported that Gremlin-1 expression was increased in inflamed periodontal tissues, contributing to the activation of the NF-κB signaling pathway and interleukin-1β (IL-1β).[13] Also they showed that Gremlin-1 regulated the osteogenesis ability of human periodontal ligament stem cells and that blocking Gremlin-1 suppressed alveolar bone loss and reduced inflammation by affecting ICAM-1, VCAM-1, and IL-1β levels.[13] On the contrary to these studies, our study found decreased saliva Gremlin-1 levels in patients with stage III/grade B periodontitis. This discrepancy may be suggests that while Gremlin-1 is upregulated in local tissues to manage inflammation and bone remodeling, its systemic levels in saliva may decrease as a compensatory mechanism to prevent excessive inflammation and tissue destruction. This could highlight the possibility of complex regulatory mechanisms of Gremlin-1, reflecting different roles in local tissue environments versus systemic circulation. However, examining it together with saliva samples, especially gingival crevicular fluid (GCF) samples, which better reflect the local response, may elucidate the role of Gremlin-1 in periodontal disease and its precise regulatory mechanisms.

Additionally, our study revealed negative correlations between salivary Gremlin-1 levels and PI, PD and CAL. Guan et al. reported increased Gremlin-1 expressions in gingival samples of patients with periodontitis, but the severity of periodontitis was not specified and they did not examine the correlation in terms of clinical periodontal parameters.[13] Our study findings indicate that higher Gremlin-1 levels are associated with better periodontal health outcomes in in these parameters. This may be because, at the local tissue level, Gremlin-1 helps to regulate inflammation and facilitate tissue repair. The lack of correlation with BOP, however, suggests that Gremlin-1's role might be more related to the structural aspects of periodontal health rather than the acute inflammatory response that causes bleeding. This could indicate that while Gremlin-1 is involved in longer-term tissue remodeling and inflammation regulation, it does not directly affect the acute inflammatory response measured by bleeding on probing. BOP is a more immediate indicator of gingival inflammation and might be influenced by other factors that do not involve Gremlin-1.

This study has some limitations. Main limitations can be considered the lack of an analysis of GCF. GCF composition largely reflects the inflammatory state of periodontal tissues, making it essential for understanding periodontal pathogenesis. Analyzing GCF offers the advantage of site-specific information regarding periodontal health. While saliva analysis can also provide insights into the periodontal condition by reflecting GCF composition,[26] evaluating together with GCF directly could further validate and compared our findings. Other limitation is the relatively small sample size. Nevertheless, salivary analysis can alone still offer substantial insights into the role of Gremlin-1 in periodontitis. Additionally, the cross-sectional design of our study limits our ability to monitor the progression of the disease over time.

In the current study, the results highlight the significance of Gremlin-1 in periodontitis and its correlation with periodontal status. These findings suggest that decreased salivary Gremlin-1 levels may serve as a systemic compensatory mechanism to regulate inflammation and limit tissue destruction in severe

periodontitis, highlighting its potential as a biomarker for disease progression. However, further analysis and larger sample size are required to fully clarify the role of Gremlin-1 in the pathogenesis of periodontitis.

CONCLUSION

This is the first study investigating saliva levels of Gremlin-1 in stage III grade B periodontitis. With the limitations, the present study demonstrated that decreased salivary Gremlin-1 levels in patients with periodontitis compared to healthy controls and negatively correlated with PI, PD and CAL. This study suggest that salivary Gremlin-1 might modulate to the inflammation and limit tissue remodelling by acting as a systemic compensation mechanism in periodontitis in the future with using different sample analysis methods and large-cohort studies.

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