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KAHRAMANMARAŞ SÜTÇÜ İMAM ÜNİVERSİTESİ
BİLİMSEL ARAŞTIRMA PROJELERİ KOORDİNASYON BİRİMİ
PROJE YAYIN DİLEKÇESİ

Proje Adı		
Raftlin ve Presepsin Periodontal hastalığın değerlendirilmesinde bir biomarker olarak kullanılabilir mi?		
Proje No	**Başlama Tarihi**	**Bitiş Tarihi**
2021/7-18 M	31-12-2021	30-12-2022
Yayın Türü	**Yayın / Makele Başlığı**	
Makale	*Association between raftlin and presepsin levels with periodontal healthy and disease conditions*	
Dergi ISSN	**DOI**	**Cilt / Sayfa / Yıl**
105880	https://doi.org/10.1016/j.archoralbio.2024.105880	0003-9969 / 1-8 / 2024
Yayınlandıgı Dergi Kısa Ad	**Yayınlandıgı Dergi**	
Belirtilmemiş	Archives of Oral Biology	

İLGİLİ MAKAMA

Yukarıda bilgileri verilen ilgili otomasyona girilmiş yayın bilgileri içerisinde ; *"Söz konusu çalışma/yayın/sunum/poster/bildiri/* ***KAHRAMANMARAŞ SÜTÇÜ İMAM ÜNİVERSİTESİ Bilimsel Araştırma Projeleri birimi*** *tarafından 2021/7-18 M proje numaralı "Raftlin ve Presepsin Periodontal hastalığın değerlendirilmesinde bir biomarker olarak kullanılabilir mi?" konusu ile ilgili olup, ilgili birimce desteklenmiştir." ("This work is supported by the* ***Scientific Research Project Fund of KAHRAMANMARAŞ SÜTÇÜ İMAM ÜNİVERSİTESİ*** *under the project number 2021/7-18 M")* ifadesi yer almaktadır.

PROJE YÜRÜTÜCÜSÜNÜN

Ünvanı, Adı-Soyadı : Dr.Öğr.Üyesi Eda ÇETİN ÖZDEMİR

Tarih : 17-05-2024

Bu belge, güvenli elektronik imza ile imzalanmıştır.

Belge Doğrulama Kodu :b50b4f48de

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Archives of Oral Biology 160 (2024) 105880

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Research paper

Association between raftlin and presepsin levels with periodontal healthy and disease conditions

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ARTICLE INFO

Keywords:
Raftlin
Presepsin
Gingival crevicular fluid
Periodontitis
Periodontal disease
Gingivitis

ABSTRACT

Objective: The aim of this study was to examine the association between Raftlin and Presepsin levels in periodontal healthy/diseases, hypothesizing a change in their levels. Also, the study aimed to determine their potential role in diagnosing and predicting the prognosis of periodontal diseases.
Design: A cross-sectional study design was used, including 20 periodontally healthy individuals, 21 gingivitis patients, and 21 periodontitis patients. Clinical measurements and gingival crevicular fluid (GCF) sample collection were conducted, and the levels of Raftlin and Presepsin were analyzed. Statistical analysis was performed to evaluate the differences and correlations among the groups.
Results: Raftlin and Presepsin levels displayed significant variations among groups in both total amount (mean values for Raftlin in periodontitis, gingivitis, and healthy were 33.42, 17.45, 7.70 pg/30 s, respectively; for Presepsin, values were 3.98, 3.01, 1.92 pg/30 s, respectively) ($p < 0.001$) and concentration levels (pg/μl) ($p = 0.007$ for Raftlin, $p = 0.026$ for Presepsin). Particularly noteworthy were the concentration distinctions observed exclusively between the periodontitis and healthy groups.
Conclusions: The present study offers preliminary insights into the presence and variations of raftlin and presepsin in the GCF across different periodontal conditions. While these findings hint at a potential role for these markers in periodontal disease, further research is essential to fully understand their diagnostic and prognostic capabilities.

1. Introduction

Periodontal diseases are inflammatory chronic diseases characterized by the disruption of the complex and specific balance between pathogenic bacteria within the dental biofilm and the host defense system, leading to tissue destruction (Chapple et al., 2018). The primary etiological factor in the development of periodontal disease is microbial dental plaque. The main sources of tissue destruction in periodontal tissues are the pathogenic bacteria in the microbial dental plaque and the chemical mediators produced by host defense system cells. Additionally, the severity of tissue destruction varies according to the response of the host (Preshaw, 2008).

Early detection and treatment of periodontal diseases are important for preventing disease progression and preventing tooth/tissue loss. Recent research has demonstrated that the mediators found in the gingival crevicular fluid (GCF), which is the fluid in the periodontal pocket, play a valuable role in the diagnosis of periodontal diseases (Gürlek, Gümüş, Nile, Lappin, & Buduneli, 2017). Throughout the course of periodontal disease, changes in the content of GCF have been shown due to the effects of inflammatory processes. These changes can manifest as increases or decreases in the levels of biochemical substances called mediators. The most commonly investigated mediators include prostaglandins, cytokines, enzymes, and inflammatory markers (Ghallab, 2018).

The detection of these mediators can be used as a clinical tool to diagnose and determine the prognosis of periodontal diseases. For example, high levels of prostaglandins found in GCF can indicate the presence of inflammation and help predict the severity of periodontal disease (Basegmez, Yalcin, Yalcin, Ersanli, & Mijiritsky, 2012). Similarly, the levels of cytokines and enzymes can provide information about

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https://doi.org/10.1016/j.archoralbio.2024.105880
Received 21 August 2023; Received in revised form 28 December 2023; Accepted 2 January 2024
Available online 12 January 2024

the intensity of the inflammatory process and the response to treatment. The biochemical analysis of GCF is a non-invasive method and can be an important step in the early detection and treatment of periodontal diseases (Barros, Hefni, Nepomuceno, Offenbacher, & North, 2018).

Presepsin is a protein used as an inflammatory marker. It is formed by the breakdown of pro-kallikrein 1, a precursor protein present in the bloodstream. Presepsin can be found at increased levels in systemic inflammatory conditions, especially septic shock and infections. Presepsin is being evaluated as a potential indicator for early diagnosis and prognosis (Dierikx et al., 2023). Clinically, measuring presepsin levels can be used to diagnose septic shock early and monitor the response to treatment. Therefore, presepsin can play an important role in the diagnosis and treatment process of many critical illnesses (Okamura, 2015).

Raftlin, also known as lipopolysaccharide-binding protein receptor (LPBRR), is a protein identified in humans and other mammals (Bilgen, Ural, Kurutas, & Bekerecioglu, 2019). It is primarily found in lipid raft domains, specialized regions of the cell membrane enriched with cholesterol and sphingolipids. Raftlin is believed to play a role in various cellular processes, including signal transduction and immune responses (Hursitoglu et al., 2023). One notable function attributed to raftlin is its role in innate immunity. It has been suggested that raftlin interacts with lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, and may participate in the recognition and response to bacterial infections (Lee, Yoo, Ku, Kim, & Bae, 2014). Additionally, raftlin has been implicated in regulating Toll-like receptor signaling, which is crucial for initiating immune responses against pathogens. While the exact mechanisms and all functions of raftlin are still under investigation, it is considered an interesting protein with potential effects in immune-related processes (Watanabe et al., 2011). Additionally, Bayliss' findings revealed that raftlin is recruited by neuropilin-1 to the activated VEGFR2 complex, thus regulating proangiogenic signaling in endothelial cells (Bayliss, Sundararaman, Granet, & Mellor, 2020). Tatematsu's investigation showcased the role of raftlin in mediating LPS-induced TLR4 internalization and TICAM-1 signaling in human monocyte-derived dendritic cells and macrophages (Tatematsu et al., 2016). These collective results strongly indicate that raftlin contributes to the development of inflammation by participating in the activation of TLR3 and TLR4. Further research is needed to deepen our understanding of raftlin, its cellular biology, and its roles in human health.

The aim of this study is to investigate the relationship between Raftlin and Presepsin levels in periodontal diseases. We hypothesize that the levels of these markers will differ significantly between diseased and healthy conditions. This study seeks to determine whether these mediators can serve as potential indicators in the diagnosis of periodontal diseases.

2. Materials and methods

2.1. Study population and design

This study was designed as a single-blind, cross-sectional study and included 20 periodontally healthy individuals, as well as 21 patients with gingivitis and 21 patients with periodontitis who were referred to the Department of Periodontology Clinic at Kahramanmaraş Sütçü İmam University with different periodontal complaints. The criteria for determining periodontal health, gingivitis, and chronic periodontitis were based on the 2018 European Federation of Periodontology (EFP) workshop criteria based on periodontal examinations and radiographic records (Caton et al., 2018). All participants provided written informed consent, and the study protocol was approved by the Ethics Committee of Kahramanmaraş Sütçü İmam University (number:2021/28, No:06).

2.2. Inclusion and exclusion criteria

The inclusion criteria for the study required participants to be systemically healthy, not be pregnant or lactating, not be taking medications that could affect periodontal tissues such as beta blockers, not have used antibiotics or nonsteroidal anti-inflammatory drugs in the previous six months, and not be smokers or alcohol drinkers (either never started or quit at least 10 years ago). Additionally, participants needed to have at least 18 teeth present. Patients with local periapical pathologies, caries or restorations detected through periapical or panoramic x-ray examination were excluded from the study. Likewise, individuals with a history of orthodontic or periodontal treatment (surgical or non-surgical) were also excluded.

2.3. Clinical examination and radiological examination

A single calibrated examiner conducted all clinical measurements. These measurements included probing pocket depth (PPD), clinical attachment level (CAL), plaque index (PI), gingival index (GI), and bleeding on probing (BOP). The measurements were recorded on a computer using a specially prepared chart form. A Williams-type periodontal probe with a 0.5-mm diameter was used for the measurements. The clinical parameters were recorded for six different sites on all teeth. In the periodontally healthy group, total BOP was below 10%, while in the gingivitis group, it was 30%. After full mouth clinical periodontal parameter measurements were made, single tooth calculations were also performed for the sampled teeth. While the whole mouth parameters (PI, GI, PPD) were recorded, the clinical data of the sampled teeth were recorded as (T-PI, T-GI, T-PPD).

2.4. GCF sample collection and sample analysis

GCF samples were collected from all patients before performing periodontal measurements. The selected areas for each tooth were isolated with cotton rolls to prevent contamination by saliva. After careful removal of supragingival plaque, the area was dried, and sterile prefabricated paper strips (Periopaper) were gently inserted into the periodontal pocket and left there for 30 s. No pooling was performed for the GCF samples; each was obtained individually from a single tooth using a paper strip. Mechanical irritation was avoided during this process. The volume of GCF samples was measured using a calibrated Periotron 8000 device. The samples were then stored at − 80ºC until analysis. The analysis of GCF samples was conducted at Biochemistry Department of Medical Faculty of Kahramanmaras Sutcu Imam University, using a ELISA. The levels of raftlin and presepsin in the samples were measured at 450 nm using commercially available ELISA kits specifically designed for raftlin (Lot: MBS7241519, Mybiosource, CA, USA) and presepsin (Lot: MBS773009, Mybiosource, CA, USA). The level of Raflin and Presepsin were presented as total amount (pg/30 s) and concentration (pg/μl).

2.5. Statistical analysis

Jamovi software (Version 2.3.21) was used for statistical analysis. Data were presented as mean and standart deviation values. The normality of distribution was checked with the Kolmogorov-Smirnov test. Due to normal distribution, ANCOVA analysis was conducted to detect the differences between groups and age was added as a covariate to control the effect of confounding factor. Tukey post-hoc was conducted for multiple comparisons analysis. Demographic differences between groups were calculated using a chi-squared test. The relationship between periodontal and inflammatory variables was analyzed with the Pearson Correlation test. A value of $p < 0.05$ was accepted as statistically significant. Additionally, ROC analysis was performed to determine specificity and sensitivity.

3. Results

Significant differences were observed between the groups in terms of

age range and education frequency ($p < 0.05$). Specifically, a higher number of subjects with periodontitis were found in the 40–60 age range compared to other groups. Furthermore, the healthy group had a higher proportion of individuals with a university education compared to the gingivitis and periodontitis groups ($p < 0.05$). However, no significant differences were observed in terms of sex and tooth brushing frequency ($p > 0.05$) (Table 1).

Significant differences between case-control groups were found for all oral health indices the periodontitis group was found to be statistically higher in all clinical parameters. ($p < 0.05$). For MT, no significance was found among all groups, ($p > 0.05$), and for T-CAL and GCF volume, no significance was found between the gingivitis and healthy groups ($p > 0.05$) (Table 2).

A significant difference was observed between the groups in terms of Raftlin total amount (pg/30 s) levels ($p < 0.001$) (Fig. 1). Similarly, a significant difference was found between the groups in regard to Presepsin total amount (pg/30 s) levels ($p < 0.001$) (Fig. 1). In terms of concentration levels of raftlin(pg/μl) and presepsin(pg/μl); A statistical difference was found only between the periodontitis and healthy groups. P values are $p = 0.007$, $p = 0.026$ for Raftlin and Presepsin, respectively. (Fig. 2).

Furthermore, a significant positive correlation was observed between Presepsin levels and all oral health indices ($p < 0.05$). Similarly, a significant positive correlation was found between Raftlin levels and all oral health indices ($p < 0.05$), except for MT ($p > 0.05$) (Fig. 3).

In the ROC analysis, the optimal cut-off value of 0.99 for the Raftlin (pg/30 s) value in the diagnosis of periodontitis and gingivitis was seen to have 100% sensitivity and 100% specificity (Fig. 4). In terms of Presepsin (pg/30 s), with the optimal cut-off value of 0.54, %75 sensitivity and %76.2 specificity was obtained for the diagnosis of gingivitis. In addition, in regard to Presepsin (pg/30 s), with the optimal cut-off value of 0.73, %95 sensitivity and %100 specificity was obtained for the diagnosis of periodontitis (Fig. 4).

Table 1
Demographic attributes of the included subjects in the study according to case-control groups.

	Gingivitis (N = 21)	Periodontitis (N = 21)	Healthy (N = 20)	Total (N = 62)	p-value
Sex					0.254[1]
Female	10.0 (47.6%)	10.0 (47.6%)	14.0 (70.0%)	34.0 (54.8%)	
Male	11.0 (52.4%)	11.0 (52.4%)	6.0 (30.0%)	28.0 (45.2%)	
Age range					< 0.001[1]
20-30	11.0 (52.4%)	2.0 (9.5%)	15.0 (75.0%)	28.0 (45.2%)	
30-40	6.0 (28.6%)	7.0 (33.3%)	4.0 (20.0%)	17.0 (27.4%)	
40-60	4.0 (19.0%)	12.0 (57.1%)	1.0 (5.0%)	17.0 (27.4%)	
Education					0.034[1]
Elementary	1.0 (4.8%)	4.0 (19.0%)	0.0 (0.0%)	5.0 (8.1%)	
High-school	3.0 (14.3%)	4.0 (19.0%)	0.0 (0.0%)	7.0 (11.3%)	
University	17.0 (81.0%)	13.0 (61.9%)	20.0 (100.0%)	50.0 (80.6%)	
Tooth brushing					0.133[1]
None	2.0 (9.5%)	0.0 (0.0%)	0.0 (0.0%)	2.0 (3.2%)	
1 per day	7.0 (33.3%)	10.0 (47.6%)	3.0 (15.0%)	20.0 (32.3%)	
2 per day	11.0 (52.4%)	9.0 (42.9%)	14.0 (70.0%)	34.0 (54.8%)	
3 per day	1.0 (4.8%)	2.0 (9.5%)	3.0 (15.0%)	6.0 (9.7%)	

[1] Chi-squared test

Table 2
Relationship between oral health indices and case-control groups, median (min-max).

Oral Health Index	Gingivitis (N = 21)	Periodontitis (N = 21)	Healthy (N = 20)	p-value
T-PI	1.43 ± 0.525^a	1.771 ± 0.474^b	0.664 ± 0.399^c	< .001[1]
T-GI	1.191 ± 0.603^a	1.772 ± 0.218^b	0.471 ± 0.443^c	< .001[1]
T-PPD (mm)	1.957 ± 0.269^a	2.701 ± 0.376^b	1.544 ± 0.276^c	< .001[1]
T-BOP	37.923 ± 26.363^a	63.901 ± 21.973^b	1.933 ± 3.312^c	< .001[1]
PI	1.049 ± 0.605^a	1.704 ± 0.325^b	0.543 ± 0.356^c	< .001[1]
GI	1.015 ± 0.446^a	1.651 ± 0.245^b	0.331 ± 0.28^c	< .001[1]
PPD (mm)	1.86 ± 0.336^a	3.292 ± 0.722^b	1.415 ± 0.204^c	< .001[1]
BOP	33.095 ± 22.718^a	59.957 ± 21.769^b	1.442 ± 2.567^c	< .001[1]
MT	1.095 ± 1.375^a	2.524 ± 2.909^a	0.65 ± 0.988^a	0.020[1]
GCF volume (μl)	0.293 ± 0.117^a	1.571 ± 0.902^b	0.142 ± 0.087^a	< .001[1]
T-CAL	0.075 ± 0.216^a	3.091 ± 0.526^b	0 ± 0^a	< .001[1]

[1] ANCOVA Tukey-post hoc comparison. Different superscripts indicate a significant difference between groups ($p < 0.05$). Age was added as a covariate to control the effect of confounding factor. Abbreviations; PI: Full Mouth Plak Index, GI: Full Mouth Gingival Index, PPD: Full Mouth Periodontal Pocket Depth, BOP: Full Mouth Bleeding on Probing, T-PI: Sampled Teeth Plak Index, T-GI: Sampled Teeth Gingival Index, T-PPD: Sampled Teeth Periodontal Pocket Depth, T-BOP: Sampled Teeth Bleeding on Probing MT: Missing Teeth, GCF: Gingival Crevicular Fluid, T-CAL: Sampled Teeth Clinical Attachment Level

In the ROC analysis for consantration data, the optimal cut-off value of 0.52 for the Raftlin (pg/μl) value in the diagnosis of periodontitis was seen to have 75% sensitivity and 76.2% specificity (Fig. 5) The optimal cut-off value of 0.44 for the Raftlin (pg/μl) value in the diagnosis of gingivitis was seen to have 50% sensitivity and 47.6% specificity. Also, the optimal cut-off value of 0.54 for the Presepsin (pg/μl) value in the diagnosis of periodontitis was seen to have 85% sensitivity and 85.7% specificity (Fig. 5). The optimal cut-off value of 0.43 for the Presepsin (pg/μl) value in the diagnosis of gingivitis was seen to have 55% sensitivity and 57.1% specificity.

3.1. Power analysis

A post-hoc power analysis was calculated with G-Power 3.1 (University Kiel, Germany) software. 99% power was found with 0.59 eta square, 0.05 type 1 error, and 62 sample sizes.

4. Discussion

The present study aimed to investigate the differences between periodontitis, gingivitis, and periodontally healthy individuals by examining raftlin and prepsepsin levels in the GCF. In our knowledge this is the first study to investigate the levels of raftlin and prepsepsin in the GCF. Therefore, this study sought to fill the gap by comparing periodontitis and gingivitis to periodontally healthy individuals in terms of these markers and also, mediators that may play a potential role in the diagnosis and prognosis of periodontal diseases. Our findings show that Raftlin and Prepsepsin levels differ significantly in periodontal disease sites.

Raftlin has been shown to play a significant role in signal transmission through the B cell receptor (BCR). It is also involved in the formation of the nucleocapture complex, which triggers autoimmune responses, and in the activation of Toll-like receptor 3 (TLR3)

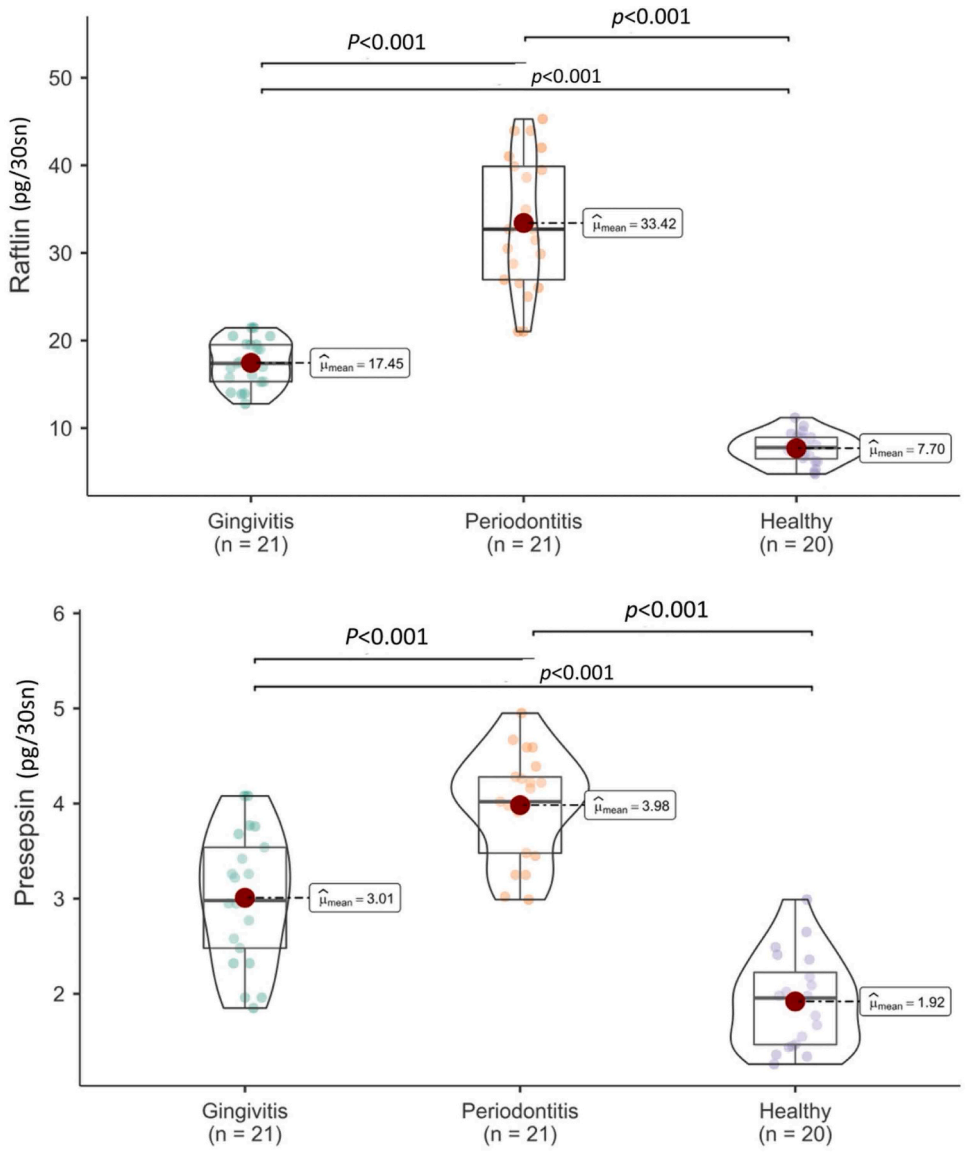

Fig. 1. Violin plot of Total Amount Raftlin (pg/30 s) and Presepsin (pg/30 s) in GCF that presents the relationship between case-control groups (ANCOVA, Tukey post-hoc).

(Hajishengallis, Wang, Liang, Triantafilou, & Triantafilou, 2008; Watanabe et al., 2011). Researchers have suggested that raftlin could be a novel parameter for understanding the pathophysiology of vascular inflammatory responses, diagnosing inflammatory diseases, and evaluating immune responses. In our study, we evaluated raftlin in individuals with periodontal health, gingivitis, and periodontitis. Although the results showed that total raftlin levels were higher in individuals with periodontitis, raftlin concentration in GCF was lower in the periodontitis group compared to the healthy group. It was observed that both the total amount and concentration levels of raftlin showed significant differences between healthy and periodontitis GCF samples. This study is the first study to investigate raftlin in the GCF, reveals its potential as a key biomarker in distinguishing between periodontal health and disease.

Prepsepsin, also known as sCD14-ST, is a biomarker that has the potential to aid in the early detection of systemic infections. In a study conducted by Chenevier-Gobeaux in 2016, it was observed that the levels of prepsepsin increase in peripheral mononuclear cells and a specific type of white blood cell lineage when exposed to a bacterial stimulant called lipopolysaccharide (LPS) (Chenevier-Gobeaux et al., 2016). Currently, researchers are assessing the potential of presepsin as an early diagnostic and prognostic indicator (Dierikx et al., 2023). In summary, these papers suggest that both prepsepsin and Prep1 are significant contributors to the immune system, while the precise role of PrP in immune function requires further clarification. The present study identified statistically significant levels of total amount presepsin in the periodontitis group. Although similar results were found in the current study due to the lack of data on the changes in these markers after periodontal treatment, we cannot assert that these parameters serve as diagnostic markers. It is more appropriate to suggest that these markers may contribute to the pathogenesis of periodontal disease rather than directly indicating inflammatory destruction or the need for wound healing.

GCF originates from periodontal tissues, combining serum with local substances like inflammatory mediators, antibodies, and breakdown products from dental plaque (Subbarao et al., 2019). Researchers are exploring GCF for diagnosing periodontal disease, focusing on markers like prostaglandin E2, neutrophil elastase, and β-glucuronidase (Lamster, 1997). Analyzing inflammatory markers in GCF could also aid in understanding how certain systemic conditions, like diabetes mellitus, can affect periodontal disease, and how periodontal disease or inflammation can impact systemic disorders like cardiovascular or

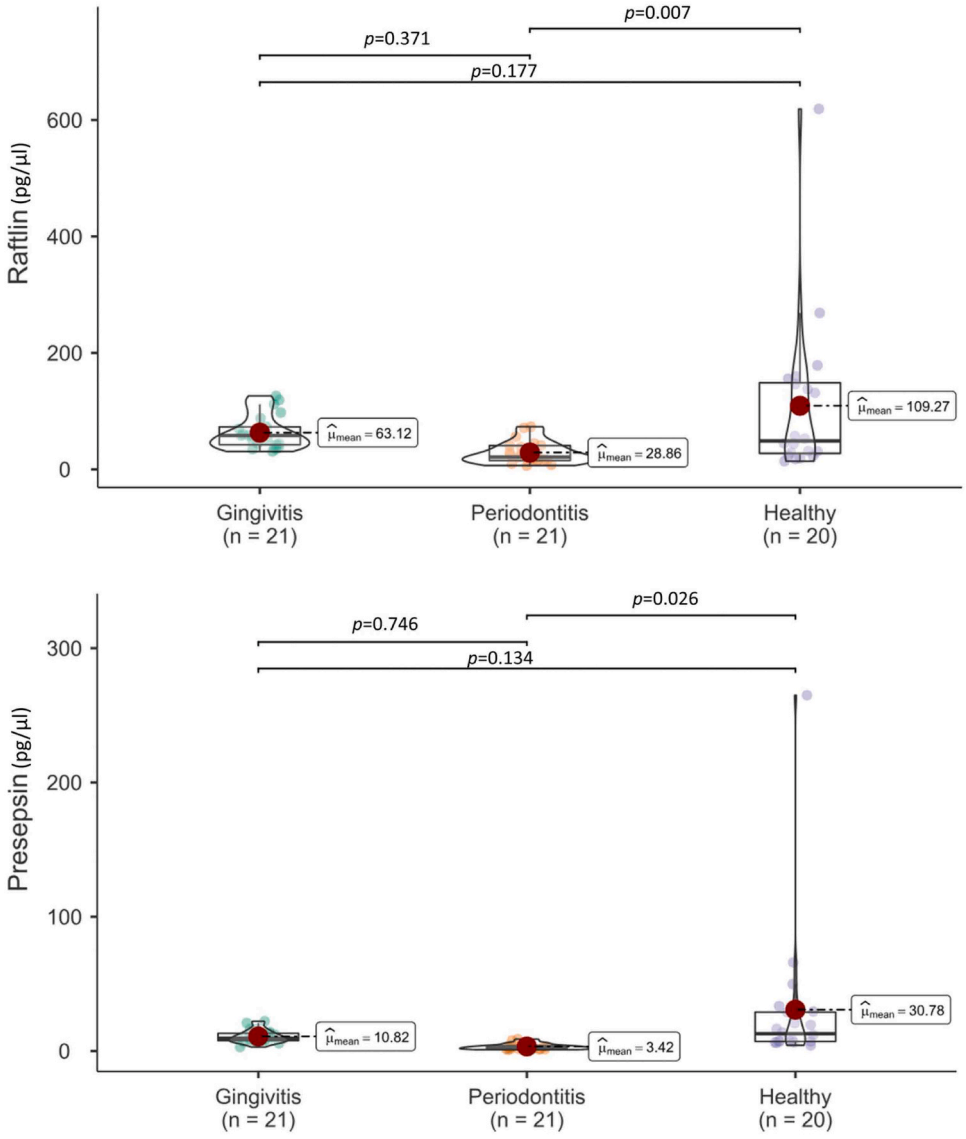

Fig. 2. Violin plot of Raftlin Consentration (pg/µl)and Presepsin Consentration (pg/µl)) in GCF that presents the relationship between case-control groups (ANCOVA, Tukey post-hoc).

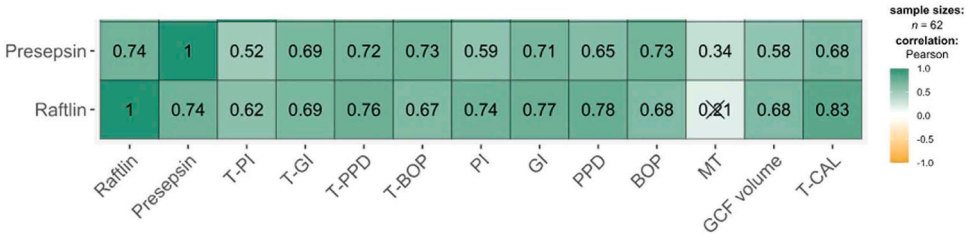

Fig. 3. Correlation matrix boxes: green and orange boxes indicate positive and negative correlations, respectively (Colour tones indicate the strength of the correlation). Boxes without "X" indicate significance ($p < 0.05$). The numbers on the boxes are spearmen correlation coefficients. PI: Full Mouth Plak Index, GI: Full Mouth Gingival Index, PPD: Full Mouth Periodontal Pocket Depth, BOP: Full Mouth Bleeding on Probing, T-PI: Sampled Teeth Plak Index, T-GI: Sampled Teeth Gingival Index, T-PPD: Sampled Teeth Periodontal Pocket Depth, T-BOP: Sampled Teeth Bleeding on Probing MT: Missing Teeth, GCF: Gingival Crevicular Fluid, T-CAL: Sampled Teeth Clinical Attachment Level.

cerebrovascular diseases (Lamster & Ahlo, 2007). The research papers indicate that GCF has the potential to serve as a diagnostic tool for periodontitis. Overall, these studies suggest that GCF can be a valuable diagnostic tool for assessing periodontitis. The differences in total amount and concentrations in the data are attributed to the very low volume of GCF. This is because a change of just one microliter in GCF volume can alter concentrations in terms of ratio/proportion. Additionally, while it is known that providing total amounts in studies is important for standardization, both concentration and total amounts of biochemical parameters were reported in this study, as it is among the

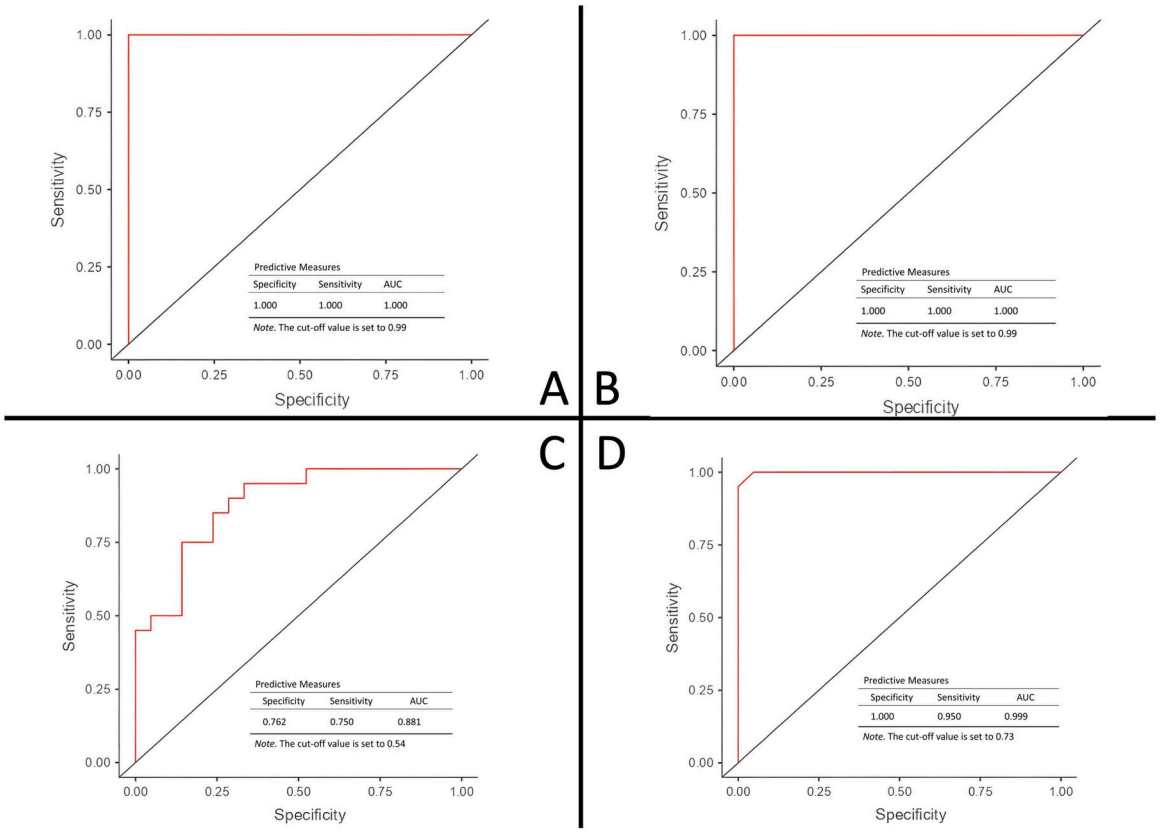

Fig. 4. ROC graph of Total Amounts of Raftlin (pg/30 s) and Presepsin (pg/30 s) in GCF. **A:** Raftlin, Gingivitis vs Healthy, **B**: Raftlin, Periodontitis vs Healthy, **C:** Presepsin, Gingivitis vs Healthy, **D:** Presepsin, Periodontitis vs Healthy.

first in its field.

The study had a relatively small sample size, with 20 periodontally healthy individuals, 21 patients with gingivitis, and 21 patients with periodontitis. A larger sample size would provide more robust and representative results. The study utilized a cross-sectional design, which limits the ability to establish causal relationships or determine temporal associations. Longitudinal studies would be beneficial in assessing changes in prepsepsin and raftlin levels over time.The study participants were selected from individuals referred to the Department of Periodontology Clinic at a specific university, which may introduce selection bias. The findings may not be generalizable to the wider population. Also, the study was conducted at a single center, which may limit the generalizability of the findings to other settings or populations. It is important to acknowledge these limitations when interpreting the findings of the study and consider them as areas for further research and improvement in future studies. Besides that, the study's design, and population selection present inherent limitations. Specifically, the study exclusively involved non-smoking, systemically healthy adults. This demographic does not encompass the entire spectrum of individuals affected by periodontitis, particularly those with systemic health issues or smoking habits, who may exhibit different disease progressions or marker levels. Consequently, the findings might not be generalizable to all populations with periodontal disease. Additionally, the study's observational nature only allows for the identification of associations between marker levels and disease stages, without establishing a causal relationship or diagnostic capability. This limitation restricts the applicability of the results in clinical diagnostic settings for periodontal disease. Also, the present study acknowledges the significant age difference between the periodontitis and healthy control groups as a major limitation. To mitigate this, we employed ANCOVA analysis with age as a covariate, aiming to control its potential confounding impact on our findings.

5. Conclusion

In conclusion, our study shed light on the role of raftlin in various oral conditions, including periodontal health, gingivitis, and periodontitis. The findings indicate that raftlin levels are elevated in individuals with periodontitis, suggesting its potential as a diagnostic marker for this inflammatory condition. Moreover, this study is significant as it represents the first investigation of raftlin within the GCF. The identification of raftlin as a novel parameter holds promise for understanding the underlying mechanisms of vascular inflammatory responses, diagnosing inflammatory diseases, and assessing immune responses. Further research in this field could provide valuable insights into the pathophysiology of inflammatory conditions and potentially lead to the development of targeted therapeutic approaches.

Statement of Ethics

The current study was conducted at Kahramanmaraş Sütçü İmam University, and the research protocol was submitted to the Non-Interventional Clinical Research Ethics Committee for approval. Subsequently, the study was granted permission under decision number 06 during session 2021/28.

Disclosure Statement

The authors have no conflicts of interest to declare.

Funding

We thank Kahramanmaraş Sütçü İmam University Scientific Research Projects Coordination Unit for supporting our work through project number 2021/7–18M.

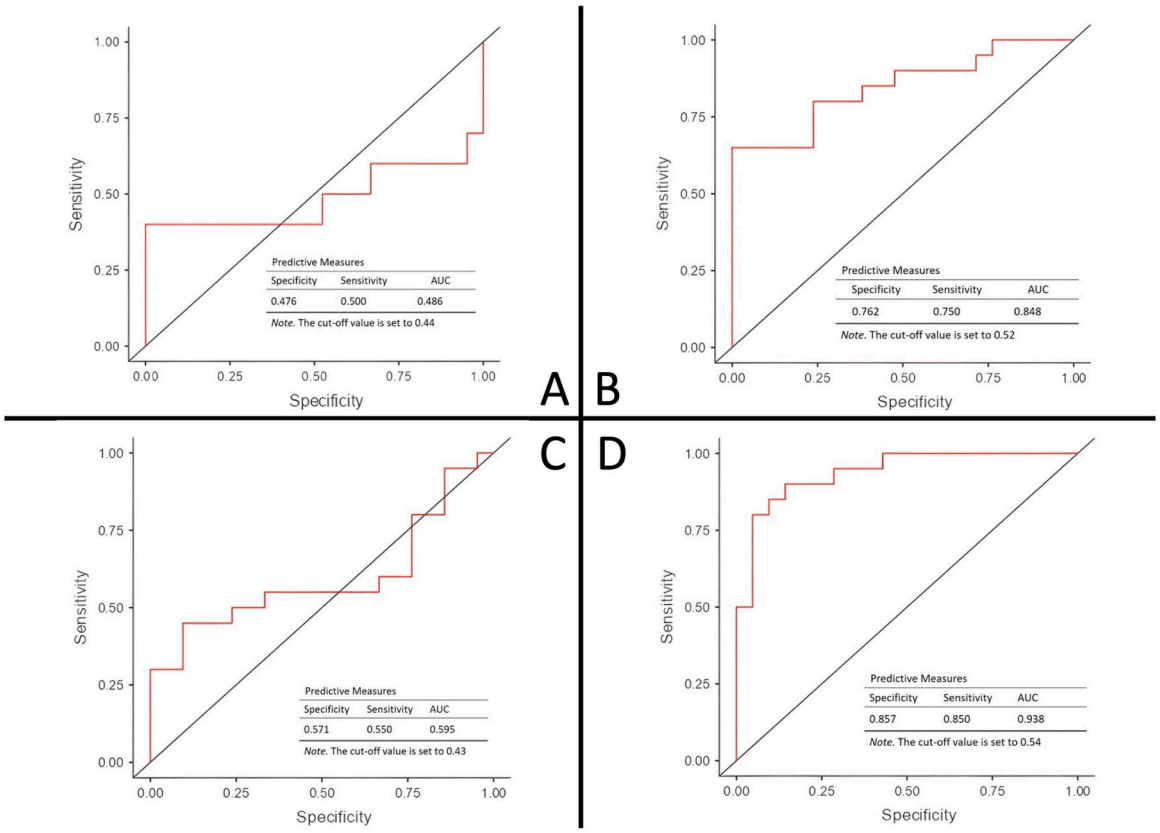

Fig. 5. ROC graph of Concentration of Raftlin (pg/µl) and Presepsin (pg/µl) in GCF. **A:** Raftlin, Gingivitis vs Healthy, **B**: Raftlin, Periodontitis vs Healthy, **C:** Presepsin, Gingivitis vs Healthy, **D:** Presepsin, Periodontitis vs Healthy.

CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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