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Evaluation of gingival crevicular fluid apelin levels in relation to periodontal status and type 2 diabetes

Canan Akdeniz[1], Arzum Güler Doğru[1], Revşa Evin Canpolat Erkan[2]

[1] Dicle University, Faculty of Dentistry, Department of Periodontology, Diyarbakır, Türkiye
[2] Dicle University, Faculty of Medicine, Department of Biochemistry, Diyarbakır, Türkiye

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Correspondence:

Dr. Canan AKDENİZ
Dicle University, Faculty of Dentistry, Department of Periodontology, Diyarbakır, Türkiye.
E-mail: canan.akdeniz@dicle.edu.tr

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Abstract

Aim: Adipokines in gingival crevicular fluid (GCF) may reflect systemic metabolic and inflammatory conditions, including diabetes and periodontitis. Apelin, a multifunctional peptide expressed in adipose tissue and various organs, is involved in both metabolic regulation and inflammatory pathways and therefore represents a biologically relevant mediator for exploring the link between diabetes mellitus and periodontitis. This study aimed to investigate GCF apelin levels in periodontally healthy individuals, patients with periodontitis, and those with periodontitis and Diabetes Mellitus (DM).

Methodology: Ninety participants were enrolled and equally distributed into three groups (n=30 each): Healthy, periodontitis, and periodontitis+DM. Clinical parameters were recorded, and GCF samples were collected for apelin measurement using ELISA. Data were analyzed using nonparametric and categorical statistical tests according to the distribution and type of variables.

Results: GCF apelin levels were comparable among the periodontally healthy, periodontitis, and periodontitis+DM groups, showing no statistically significant differences ($p = 0.991$). An inverse correlation was observed between GCF volume and apelin concentration ($r_s = -0.39$; $p < 0.001$). No significant differences were observed among the groups in terms of age, gender, or BMI ($p > 0.05$).

Conclusion: GCF apelin levels did not differ significantly among the study groups. The inverse correlation between apelin concentration and GCF volume, an indirect indicator of local inflammation, may reflect a relationship with local inflammatory status. These findings suggest that GCF apelin alone has limited clinical value for distinguishing periodontitis with DM. Therefore, measuring GCF apelin as a single biomarker is not recommended for identifying periodontal disease status or diabetic individuals within a periodontal population. Nevertheless, these results contribute to understanding the relationship between metabolic conditions and periodontal inflammation. Further longitudinal and mechanistic studies are needed to clarify the origin and regulation of apelin in periodontal tissues and to determine its potential role as a biomarker or therapeutic target.

Keywords: Apelin, periodontitis, diabetes mellitus, gingival crevicular fluid, adipokine, periodontal health

Introduction

Periodontitis is a long-standing inflammatory disease triggered by bacterial colonization, resulting in the breakdown of the structures that anchor the teeth (1). Although bacteria initiate gingival inflammation, its progression to periodontitis depends on the host response (2). Therefore, in addition to microbial pathogens, immunological and genetic mechanisms, as well as environmental factors such as diet and lifestyle, play roles in the onset and progression of periodontitis (3).

The diagnosis of periodontal disease relies on conventional parameters such as pocket depth (PD), bleeding on probing (BOP), clinical attachment loss (CAL), and alveolar bone status. Among these parameters, only BOP reflects the current periodontal condition, whereas the others indicate past periodontal destruction (4). In a site affected by gingivitis or periodontitis, a single-time clinical examination does not allow determination of whether active tissue breakdown is occurring. In this context, active tissue breakdown is defined as further clinical attachment loss or additional bone degradation (5). Since GCF is an inflammatory fluid, it reflects the current pathological activity within the periodontal tissues from which it is derived, and could be considered a possible marker for the detection and progression of periodontitis through changes in its components (6).

Periodontal disease and DM are two prevalent chronic conditions that influence each other through a bidirectional relationship (7). In individuals with uncontrolled diabetes, the risk of developing periodontitis is threefold higher, and severe periodontitis may further exacerbate poor metabolic control. Moreover, successful periodontal therapy can reduce HbA1c levels by an average of 0.4% in patients with type 2 DM, which, given the nature of the association between periodontitis and diabetes, suggests a causal relationship (8).

Apelin functions as a regulatory adipokine that interacts with the APJ receptor, which is part of the G protein-coupled receptor family. While APJ was first described in 1993, the apelin peptide was subsequently extracted from bovine stomach tissue by Tatemoto et al. in 1998 (9). In humans, the apelin gene is located on chromosome Xq25-q26.1 and encodes a 77-amino acid preproprotein, from which various apelin isoforms are derived through processing of its C-terminal region (10). Among these, apelin-36 is the most observed isoform, while apelin-13 is more potent and predominantly found in circulation. Although all isoforms of apelin can bind to APJ, they exhibit distinct biological activities (11). Apelin is highly expressed in adipose tissue, but it is also widely produced in multiple organs, including the lungs, mammary glands, reproductive organs, cardiovascular system, liver, placenta, skeletal muscle, brain, and heart (12, 13). Apelin plays a role in multiple physiological and pathological mechanisms, including fluid balance, cardiovascular regulation, glucose homeostasis, insulin production, cellular proliferation, oxidative stress, apoptosis and the formation of new blood vessels (11, 14).

Recent evidence indicates that apelin directly participates in periodontal tissue responses. In periodontal ligament cells, apelin has been shown to enhance Fusobacterium nucleatum–induced expression of COX-2, CCL2, CXCL8, TNF-α, and MMP-1, thereby promoting an inflammatory and proteolytic microenvironment associated with periodontal tissue breakdown. In addition, apelin has been reported to partially restore impaired migration of periodontal ligament cells, suggesting a context-dependent role in periodontal pathophysiology (15).

Apelin promotes the uptake of glucose by skeletal muscle and fat tissue in obese mice exhibiting insulin resistance (16). Apelin facilitates the transformation of white adipose tissue into brown fat, which is essential for boosting energy expenditure and optimizing insulin responsiveness (17). This effect of apelin treatment has been demonstrated in obese mice (18).

The interrelationship between chronic inflammatory diseases such as periodontitis and DM has long been recognized and accepted. These conditions—highly relevant for dental professionals—are linked through shared biological pathways, including systemic inflammation, insulin resistance, and metabolic dysfunction, as well as overlapping lifestyle-related risk factors. Consequently, unhealthy habits may increase systemic inflammation and insulin resistance, weaken immune responses, and elevate both the risk and coexistence of these diseases (19). The elevated or reduced levels of these adipokines, which can be detected in body fluids, may indicate the severity of metabolic diseases such as diabetes, as well as periodontitis. Moreover, adipokines and these diseases may mutually promote the development and progression of such multimorbidity (20). Considering that serum apelin levels are elevated in cases of DM, it is plausible that diabetes-related diseases may trigger the development and progression of periodontitis via apelin-mediated mechanisms. Additionally, it is possible that apelin secreted from the periodontium may also affect such systemic diseases (21).

Given elevated serum apelin in T2DM, we hypothesized that GCF apelin levels would be highest in the periodontitis+DM group. This study aims to determine apelin levels in GCF samples from periodontally healthy, periodontitis, and periodontitis+DM individuals, and to assess their associations with periodontal clinical parameters, considering apelin's potential role in the pathogenesis of diabetes and periodontitis. It was further hypothesized that GCF apelin levels might be associated with the presence of periodontitis and type 2 DM and with clinical periodontal parameters.

Material and Methods

The statistical power of the study was calculated using G*Power software (version 3.1.9.7, Heinrich Heine

University, Düsseldorf, Germany). A priori power analysis was performed for an F test (ANOVA: fixed effects, omnibus, one-way) with a significance level (α) of 0.05 and a desired power (1–β) of 0.95. Based on an effect size (f) of 0.475, derived from pilot data and comparable studies, the analysis indicated that a minimum total sample size of 72 participants (24 per group) was required to achieve an actual power of 0.9517. A secondary analysis using a slightly smaller effect size (f = 0.449) suggested a total sample size of 81 participants, corresponding to an actual power of 0.9531. In the present study, a total of 90 participants (30 per group) were included, exceeding the minimum sample size requirement and ensuring adequate statistical power (approximately 95%) to detect between-group differences.

A total of 90 individuals presenting to the Department of Periodontology, Faculty of Dentistry, Dicle University, were enrolled in the study. Ethical approval was granted by the Local Ethics Committee of Dicle University Faculty of Dentistry (Protocol No: 2024-01, dated 31.01.2024). This research adhered to the ethical guidelines set forth in the Declaration of Helsinki. All individuals included in the study provided written consent prior to participation, and demographic data were collected. The overall study design and workflow of clinical, biochemical, and statistical analyses are illustrated in Fig. 1.

Participants aged between 36 and 49 years and clinically diagnosed as periodontally healthy, with periodontitis, or with both DM and periodontitis were enrolled in the present investigation. Exclusion criteria comprised the use of immunosuppressive drugs; pregnancy or lactation; periodontal treatment within the previous six months; smoking; presence of additional systemic conditions besides diabetes; disorders affecting bone metabolism; antibiotic use in the last three months; history of head and neck radiotherapy within the past six months; and having fewer than 15 teeth.

Clinical examination

To ensure standardization in the evaluation of periodontal clinical parameters, a single examiner (C.A.) measured PI, gingival index (GI), PD, and CAL using a Williams periodontal probe (Hu-Friedy, Chicago, IL, USA). These parameters were recorded from six sites per tooth, excluding third molars: distobuccal, buccal, mesiobuccal, distolingual/distopalatal, lingual/palatal, and mesiolingual/mesiopalatal. PD and CAL were remeasured at all sites within 24 hours by the same calibrated examiner (C.A.). Intra-examiner reliability was assessed using intraclass correlation coefficients (ICC) based on a two-way random-effects model, yielding values of 0.956 for PD and 0.950 for CAL, which indicate excellent agreement between repeated measurements. BMI was calculated as body weight (kg) divided by height squared (m^2). All participants' BMI values were recorded. To minimize the confounding effect of obesity, only individuals with BMI values within the normal range according to the World Health Organization classification (18.5–24.9 kg/m^2) were included in the study (22). The periodontal health or disease status of the patients was evaluated based on the most recent classification proposed during the 2017 World Workshop focused on Periodontal and Peri-Implant Diseases and Conditions (23).

- **Group 1 (Periodontally healthy):** This group comprised 30 participants showing <10% bleeding on probing, probing depth ≤3 mm, no CAL, and no radiographic evidence of bone loss, with no systemic conditions reported.
- **Group 2 (Periodontitis):** This group included 30 systemically healthy participants diagnosed with stage II, III, or IV periodontitis, defined by CAL at interproximal sites of at least two non-adjacent teeth, or CAL ≥3 mm with PD ≥3 mm on the buccal or oral surfaces of at least two teeth, based on six-site-per-tooth measurements.
- **Group 3 (Periodontitis+DM):** According to the measurements taken from six sites per tooth, inclusion criteria were the presence of CAL at interproximal sites of at least two non-adjacent teeth, or PD ≥ 3 mm accompanied by CAL ≥ 3 mm on the buccal or oral surface of at least two teeth. This group comprised 30 patients diagnosed with stage II–III–IV periodontitis and type 2 DM. In general, individuals with HbA1c < 7 are considered to have controlled diabetes, whereas those with HbA1c ≥ 7 are regarded as poorly controlled (24). In the present study, to minimize the potential influence of diabetic complications and metabolic variability, only individuals with type 2 diabetes and HbA1c values between 7.0 and 8.5 (representing the poorly controlled group) were included.

Collection of GCF samples

GCF collection was performed one day following the recording of periodontal clinical indices. For consistency, samples in the periodontally healthy group were obtained from a standardized posterior tooth. In the periodontitis groups, sampling was performed from a periodontal site representative of disease involvement, based on probing depth measurements. If supragingival plaque was present, it was gently removed using a scaler (U 15/30, Hu-Friedy, Chicago, IL, USA). To avoid salivary contamination, cotton rolls were used for buccal isolation. The area was briefly air-dried without irritating the gingiva. Before GCF collection, the Periotron device was calibrated in accordance with the manufacturer's instructions, and a standard calibration curve was generated.

GCF was collected by placing PerioPaper® strips (Proflow Inc., Amityville, NY, USA) into the gingival sulcus until slight resistance was encountered. Following a 30-second interval, the strips were carefully withdrawn and immediately placed in a pre-calibrated Periotron

device (Periotron 8000; Oraflow Inc., Amityville, NY, USA) to record the volume. Each strip was then transferred into a clean 1.5 mL microcentrifuge tube and securely closed using parafilm. Samples contaminated with saliva or blood were discarded. Tubes were first stored at -20°C and later kept at -80°C until analysis.

The values obtained from each PerioPaper strip were converted into microliters (µL) using the PerioCalc software (Oraflow Inc., Amityville, NY, USA).

Biochemical analysis

The determination of apelin levels in GCF samples was performed using the ELISA method at the Medical Biochemistry Laboratory of the Faculty of Medicine, Dicle University. The apelin levels in GCF were determined using a commercially available Human Apelin ELISA kit (Elabscience Biotechnology Co., Ltd., Wuhan, China; Catalog No: E-EL-H0456, Lot No: WO1146443893) according to the manufacturer's instructions. The sensitivity of the kit was 37.5 pg/mL, with a detection range of 62.5–4000 pg/mL. According to the manufacturer, the intra-assay and inter-assay coefficients of variation (CV) were <10% (mean 5.1% and 4.9%, respectively), indicating high assay precision. To each sample, 200 µL of PBS-T (0.05% Tween 20 in phosphate-buffered saline) was added, followed by agitation at 300 rpm for 10 minutes using an orbital shaker. The samples were then centrifuged at 13,000 rpm for 10 minutes. After vortexing, the supernatants were transferred to a 96-well microplate template. The main steps of the ELISA procedure are shown in Figure 2. Reference standard solutions were prepared to generate a calibration curve for the ELISA assay. Finally, optical density was measured at a wavelength of 450 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA), and apelin concentrations were calculated based on the manufacturer-provided ELISA calibration curve, considering the individually measured GCF volumes of the samples. The obtained values were converted from picograms (pg) to nanograms (ng) for statistical analysis.

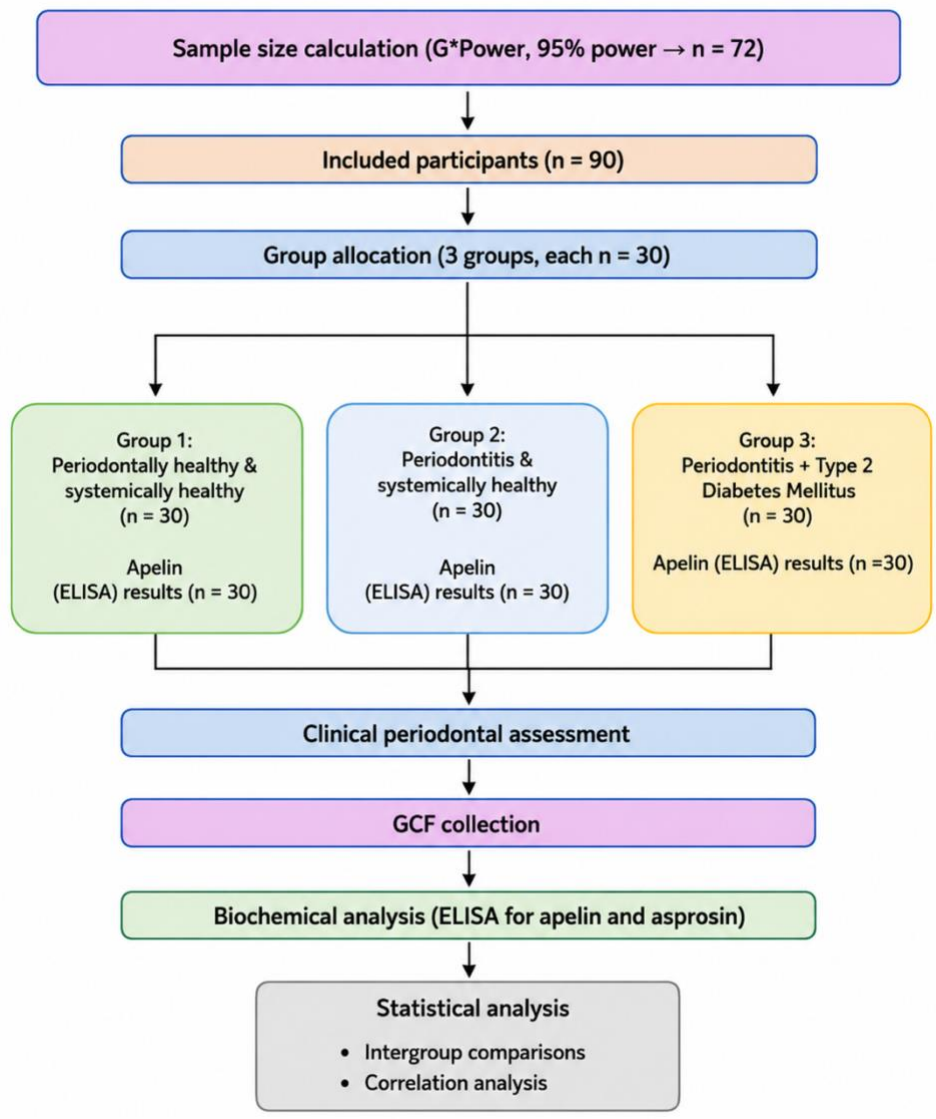

Figure 1. Study design and workflow of clinical, biochemical, and statistical analyses.

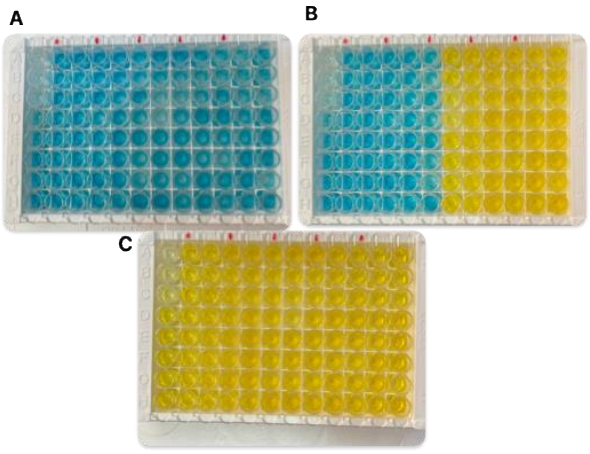

Figure 2. (A) Development of a blue color in the wells following the addition of the substrate reagent, (B) Color change of the wells from blue to yellow following the addition of the stop solution, (C) Complete color change of the wells to yellow following the addition of the stop solution.

Statistical analysis

All statistical evaluations were conducted using SPSS version 25.0 (IBM Corp., Armonk, NY, USA), and a p-value of <0.05 was considered statistically significant.

The Shapiro-Wilk test was applied to evaluate the normality of continuous variables. Variables with a normal distribution were reported as mean ± standard deviation, whereas those without were expressed as median (min-max). Categorical data were presented as frequencies and percentages (n, %). Group comparisons were conducted using the Mann-Whitney U test when the assumption of normality was not met. For comparisons among more than two groups, the Kruskal-Wallis test was utilized in the absence of normal distribution. If a significant result was found, post hoc pairwise comparisons were performed using the Dunn-Bonferroni test. Categorical variables were analyzed with Pearson's Chi-square test and the Fisher-Freeman-Halton test. Associations between continuous variables were assessed using Spearman's correlation analysis.

Results

The demographic characteristics and group distribution of the participants are summarized in Table 1. Table 2 presents the comparisons of gender, age, full-mouth clinical periodontal parameters, and BMI values between the groups.

No significant differences were observed among the groups in terms of age, gender, or BMI (p > 0.05) (Table 2). Full-mouth PI, GI, and PD values were markedly lower in the periodontally healthy individuals than in the other two groups (p < 0.001 for all). No significant differences were observed between the periodontitis and periodontitis+DM groups in terms of PI and GI (p > 0.99), as well as PD (p = 0.315). CAL values were significantly higher in the periodontitis+DM group compared to the periodontitis group (p = 0.019).

The comparison of clinical periodontal parameters, GCF volume, and apelin concentration at the sampled sites across the study groups is shown in Table 3. At the sampled sites, both PI and PD were found to be significantly lower in the periodontally healthy group than in the periodontitis and periodontitis+DM groups (p < 0.001 for both). The comparison of the periodontitis and periodontitis+DM groups revealed no statistically meaningful distinction in PI (p = 0.894) or PD (p > 0.99). At the sampled sites, GI values did not differ significantly between the groups (p = 0.276). The CAL at the sampled site was significantly greater in the periodontitis+DM group compared to the periodontitis group (p = 0.012). The median apelin level in GCF was measured as 0.12 ng/mL (min:0.07-max:0.22) in the healthy group, 0.12 ng/mL (min:0.07- max:0.33) in the periodontitis group, and 0.12 ng/mL (min:0.07-max:0.27) in the periodontitis+DM group, with no significant differences among the groups (p = 0.991). The distribution of GCF apelin concentrations in the study groups is shown in Figure 3. The GCF volume was significantly lower in the healthy group compared to the periodontitis and periodontitis+DM groups (p < 0.001 for both). However, no significant difference was found between the periodontitis and periodontitis+DM groups (p > 0.99).

Table 1. Demographic data and periodontal status of the participants.

	n (%)
Group / Variable	
Periodontally Healthy	30 (33.33)
Periodontitis	30 (33.33)
Periodontitis - Stage	
Stage II	14 (46.70)
Stage III	10 (33.30)
Stage IV	6 (20)
Periodontitis - Grade	
Grade A	4 (13.30)
Grade B	14 (46.70)
Grade C	12 (40)
Periodontitis+DM	30 (33.33)
Periodontitis+DM - Stage	
Stage II	7 (23.30)
Stage III	16 (53.30)
Stage IV	7 (23.30)
Periodontitis+DM - Grade	
Grade A	1 (3.30)
Grade B	16 (53.30)
Grade C	13 (43.30)
Gender	
Male	42 (46.7)
Female	48 (53.3)
Age (years), (Mean ± SD)	41.9 ± 3.2
BMI (kg/m²), (Mean ± SD)	24.14 ± 1.28

Data are expressed as mean ± standard deviation (minimum: maximum) and n (%). DM: Diabetes Mellitus, BMI: Body Mass Index.

Table 2. Comparison of the demographical data, BMI, full mouth clinical periodontal parameters between the study groups.

Parameter	Healthy (n=30)	Periodontitis (n=30)	Periodontitis+DM (n=30)	p-value
Gender				
Male (n=42)	14 (46.7)	14 (46.7)	14 (46.7)	1.000[a]
Female (n=48)	16 (53.3)	16 (53.3)	16 (53.3)	
Age (years)	42 (36-46)	41 (36-49)	43 (38-47)	0.158[b]
BMI (kg/m^2) (median (Q1-Q3))	23.85 (23.5-24.68)	24.40 (23.7-24.68)	24.35 (23.53-24.80)	0.645[b]
PI	0.66 (0.03-1)	1.74 (1-2.58)	1.73 (0.82-2.73)	*<0.001[b]
GI	0 (0-0.36)	1.43 (1.08-3)	1.59 (0.84-3)	*<0.001[b]
PD (mm)	1.44 (1.15-2)	2.19 (1.39-5.32)	2.70 (1.68-4.90)	*<0.001[b]
CAL (mm)	-	3.37 (2.44-7.35)	4.22 (2.52-5.57)	*0.019[d]

Data are presented as median (minimum: maximum) and n (%).
a: Chi-square test, b: Kruskal-Wallis test, c: Fisher-Freeman-Halton test, d: Mann-Whitney U test
DM: Diabetes Mellitus, BMI: Body Mass Index, PI: Plaque Index, GI: Gingival Index, PD: Pocket depth, CAL: Clinical attachment loss

Table 3. Comparison of clinical periodontal parameters of the GCF sampling sites, GCF apelin and GCF volume between the study groups.

	Healthy (n=30)	Periodontitis (n=30)	Periodontitis+DM (n=30)	p-value
Sampling site PI	1 (0-1)	1 (0-3)	2 (1-3)	*<0.001[b]
Sampling site GI	-	1 (1-3)	1.50 (1-3)	0.276[d]
Sampling site PD (mm)	1.50 (1-2.50)	2.50 (1.50-6)	2.50 (1.50-6)	*<0.001[b]
Sampling site CAL (mm)	-	3.50 (2.50-9)	4.50 (2-8)	*0.012[d]
GCF volume (µL)	0.48 (0.28-0.93)	0.81 (0.34-1.41)	0.69 (0.41-1.41)	*<0.001[b]
Apelin (ng/mL)	0.12 (0.07-0.22)	0.12 (0.07-0.33)	0.12 (0.07-0.27)	0.991[b]

Data are presented as median (minimum: maximum).
b: Kruskal-Wallis test, d: Mann-Whitney U test
DM: Diabetes Mellitus, PI: Plaque Index, GI: Gingival Index, PD: Pocket depth, CAL: Clinical attachment loss, GCF: Gingival crevicular fluid

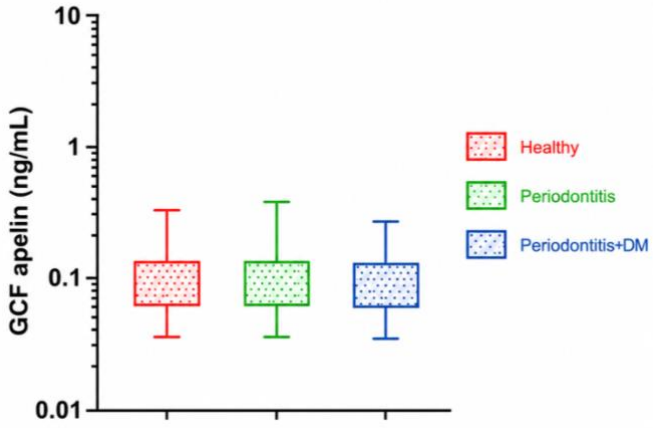

Figure 3. Box-and-whisker plots showing GCF apelin levels in healthy individuals, patients with periodontitis, and patients with periodontitis accompanied by diabetes mellitus (DM). Data are presented on a logarithmic scale as median and interquartile range (IQR), while whiskers indicate minimum-maximum values. Intergroup comparisons were performed using the Kruskal–Wallis test.

The results of the Spearman correlation analysis among full-mouth clinical indices, sample site clinical indices, GCF volume, apelin concentration, and BMI are presented in Table 4. When the correlations among the full-mouth clinical indices, sample-site clinical indices, and GCF volume were examined, all pairwise comparisons showed significant positive correlations ($p < 0.001$). An inverse relationship was identified between GCF volume and apelin concentrations, which was statistically significant ($r_s = -0.39$; $p < 0.001$). No significant associations were found between apelin concentration and the other parameters.

Table 4. Correlation of clinical periodontal parameters, GCF volume, and Apelin concentration.

	Full mouth PI		Full mouth GI		Full Mouth PD		Full mouth CAL		Apelin con-centration		Sampling site PI		Sampling site GI		Sampling site PD		Sampling site CAL		GCF Volume	
	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p
Full mouth PI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Full mouth GI	0.87	<0.001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Full mouth PD	0.70	<0.001	0.79	<0.001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Full mouth CAL	0.76	<0.001	0.85	<0.001	0.88	<0.001	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Apelin concentration	0.03	0.761	-0.11	0.325	-0.09	0.372	-0.07	0.509	-	-	-	-	-	-	-	-	-	-	-	-
Sampling site PI	0.74	<0.001	0.67	<0.001	0.53	<0.001	0.53	<0.001	-0.05	0.662	-	-	-	-	-	-	-	-	-	-
Sampling site GI	0.84	<0.001	0.91	<0.001	0.82	<0.001	0.86	<0.001	-0.09	0.380	0.64	<0.001	-	-	-	-	-	-	-	-
Sampling site PD	0.64	<0.001	0.68	<0.001	0.74	<0.001	0.71	<0.001	-0.01	0.951	0.43	<0.001	0.71	<0.001	-	-	-	-	-	-
Sampling site CAL	0.70	<0.001	0.78	<0.001	0.82	<0.001	0.91	<0.001	-0.04	0.684	0.51	<0.001	0.78	<0.001	0.74	<0.001	-	-	-	-
GCF Volume	0.52	<0.001	0.58	<0.001	0.49	<0.001	0.49	<0.001	-0.39	<0.001	0.28	<0.001	0.53	<0.001	0.42	<0.001	0.44	<0.001	-	-

PI: Plaque Index GI: Gingival Index
PD: Pocket depth CAL: Clinical attachment loss
GCF: Gingival crevicular fluid r_s: Spearman's correlation coefficient

Discussion

As far as we are aware, no previous research has investigated GCF apelin levels in participants categorized as periodontally healthy, diagnosed with periodontitis, or presenting with both periodontitis and DM. Our findings indicated that GCF apelin concentrations did not differ significantly among these groups.

Beyond its role as a lipid storage depot, adipose tissue is currently acknowledged as an endocrine organ with metabolic activity that secretes numerous molecules, including proinflammatory cytokines. Among these, adipokines contribute to insulin sensitivity regulation, energy balance, immune-inflammatory responses, wound repair, and various physiological and pathological mechanisms (25). Apelin, a peptide-based adipokine, has emerged as a promising therapeutic target in DM and obesity (26). Obesity has been associated with low-grade systemic inflammation, which may exacerbate periodontal inflammation and tissue destruction through shared mechanisms involving cytokines and adipokines (27–29). BMI is known to be related to diabetes and insulin resistance, and adipokines secreted from adipose tissue have been implicated in this link (30, 31). However, while BMI is an important systemic factor, the present study primarily focuses on the potential role of apelin as an inflammatory mediator in periodontitis and diabetes.

Previous research has indicated that apelin administration confers metabolic protection in diabetic and/or obese mouse models (16). Moreover, differences in serum apelin concentrations between diabetic and/or obese patients and healthy controls have been reported. These findings have been interpreted by the authors as suggesting that apelin may play a role in the pathogenesis of diabetes and obesity and could serve as a potential biomarker for these conditions (32). The association between serum/salivary apelin levels and diabetes reported in various cross-sectional human studies has been inconsistent. While some studies have demonstrated that serum/salivary apelin levels are elevated in individuals with diabetes (33–36), others have shown decreased levels in diabetic individuals (37–39). In our study, apelin levels in GCF were found to be similar between diabetic and non-diabetic participants, with no statistically significant difference, which differentiates our findings from previous research. This discrepancy may be attributed to our use of GCF instead of serum samples, as employed in other studies. It may be hypothesized that apelin detected in GCF could partly originate from local periodontal tissues rather than solely reflecting systemic circulation. Periodontal ligament fibroblasts, gingival epithelial cells, and other resident periodontal cells may therefore represent potential local sources of apelin. However, this possibility cannot be confirmed by the present study. If local production contributes substantially to GCF apelin levels, systemic alterations associated with metabolic diseases such as T2DM may not be directly reflected in the GCF compartment. In addition, potential differences in the expression or activity of the apelin receptor (APJ) in periodontal tissues may influence local apelin signaling. Further mechanistic studies are required to clarify the cellular sources and regulation of apelin in periodontal tissues.

A cohort study aiming to determine whether differences in plasma apelin levels precede the onset of type 2 DM suggested that circulating apelin may serve as a predictor of diabetes development independently of insulin resistance (40). The elevated plasma apelin levels observed in those affected by type 2 DM are thought to represent a compensatory mechanism aimed at counteracting insulin resistance (41). Indeed, apelin has been shown to enhance insulin sensitivity and improve glucose uptake, both in obese mice with insulin resistance and in healthy men following intravenous apelin injection (16, 41). Onalan et al. reported that serum apelin levels were significantly lower in individuals with type 2 DM compared to the healthy group, while those with impaired fasting glucose exhibited no notable difference in apelin levels relative to healthy subjects (39). In this regard, their findings align with ours, and the observed outcome may be attributed to the role of apelin in enhancing insulin sensitivity.

Apelin has been suggested to be involved in the inflammatory processes of periodontitis. Experimental studies have indicated that apelin may enhance the synthesis of pro-inflammatory and proteolytic molecules, pointing to a potential role in periodontal tissue responses. In this context, elevated apelin levels reported in conditions such as diabetes and obesity have been proposed to be associated with increased susceptibility to periodontal tissue destruction. Conversely, apelin produced within periodontal tissues has also been suggested to influence systemic metabolic conditions. Taken together, these observations have led to the suggestion in the literature that a bidirectional relationship may exist between apelin and diabetes/obesity (21). Yoldaş et al. evaluated GCF apelin levels in three groups including periodontally healthy, gingivitis, and periodontitis participants, and reported markedly elevated concentrations in the periodontitis group compared to the others (42). In this respect, our study differs from the existing literature, and although the study designs are not identical, our results show partial differences. Differences between the findings of Yoldaş et al. and the present study may be partly explained by methodological variations, including the lack of BMI assessment and the inclusion of smokers in their study population, both of which may influence local inflammatory and adipokine profiles in GCF.

Currently, the available evidence regarding apelin levels in gingival crevicular fluid is limited, and existing studies show considerable methodological heterogeneity. As a result, it remains unclear whether apelin can be considered a reliable biomarker in the pathogenesis of periodontitis and diabetes. Given these limitations, cross-sectional studies primarily allow the assessment of associations at a single time point and do

not permit evaluation of temporal changes or causal relationships. Therefore, to better elucidate the role of apelin in periodontal and diabetic conditions, future studies should adopt longitudinal or prospective cohort designs, ideally with larger sample sizes and careful control of potential confounding factors such as obesity, smoking, and metabolic status.

In the present study, a strong negative correlation was observed between apelin levels and GCF volume. However, GCF volume represents an indirect and non-specific indicator of periodontal inflammation and is influenced by multiple local and systemic factors. Therefore, the observed inverse association should be interpreted with caution. Although parallels have been drawn with other inflammatory markers previously investigated in periodontal research (43–46), direct functional inferences regarding apelin cannot be made based solely on these comparisons. Given that apelin levels were comparable among periodontitis with diabetes, periodontitis without diabetes, and periodontally healthy groups, the proposed anti-inflammatory role of apelin lacks direct empirical support. Accordingly, any potential anti-inflammatory effect of apelin should not be considered a definitive conclusion but rather a hypothesis that requires further experimental validation.

A review of the literature reveals that overweight and obese individuals have a higher prevalence of periodontitis (47, 48). The underlying mechanisms by which obesity exacerbates periodontal breakdown remain unclear, and the pathological connection between obesity and periodontitis has yet to be fully elucidated. This may be attributed to elevated levels of proinflammatory cytokines in the GCF of obese individuals and a higher microbial load of periodontal pathogens in obese individuals with periodontitis compared to those of normal weight (49, 50). In this study, participants with similar BMI values were intentionally included to minimize the confounding effect of obesity on both periodontal parameters and apelin levels. This design allowed a more accurate assessment of the relationship between apelin and periodontal status, independent of obesity-related metabolic influences. Additionally, to avoid the potential influence of diabetic complications and to reduce variability, HbA1c levels were maintained between 7.0 and 8.5 in the diabetic participants. However, restricting HbA1c levels to this range may limit the generalizability of the findings to the broader diabetic population, and it remains unclear whether similar results would be observed in individuals with well-controlled diabetes.

This study has several limitations. First, its observational and cross-sectional design prevents inference of causality or temporal relationships. In addition, the relatively small sample size may limit the generalizability of the findings. Factors such as the sensitivity of the ELISA kit, the method of sample analysis, and the geographical origin of the participants might also have influenced the results. Furthermore, apelin levels were measured only in GCF and not in serum or saliva, which restricts the ability to make systemic comparisons.

Strengths of our study include the well-defined study groups, meticulous adherence to the protocol for GCF collection and biochemical analysis, and the blinding of the researcher conducting the biochemical laboratory analyses. Additionally, the study design was carefully structured to minimize confounding effects by ensuring comparable BMI, age, and gender distributions among the groups.

Conclusion

Within the limitations of this study, GCF apelin levels did not differ significantly among the healthy, periodontitis, and periodontitis+DM groups. Nevertheless, the significant inverse correlation observed between GCF volume and apelin concentration is noteworthy. Its clinical applicability and potential importance in periodontal disease should be clarified through larger, longitudinal studies that include histological and microbiological evaluations. Additionally, future studies should consider evaluating apelin in conjunction with other adipokines (e.g., leptin, adiponectin) or inflammatory cytokines (e.g., IL-1ß, TNF-α) within a multiplex biomarker panel.

Disclosures

Ethical Approval: The study was approved by the Dicle University Faculty of Dentistry Local Ethics Committee, in accordance with the ethical standards of the Declaration of Helsinki (approval number: 2024-01).

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