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Is there a relationship between periodontal conditions and asprosin levels in gingival crevicular fluid, saliva and serum?

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Abstract

Objectives This study aimed to investigate the presence of asprosin hormone in the biological fluids of patients with periodontal inflammation and compare it to those with periodontal healthy.

Materials and methods Seventy-five individuals between the ages of 18 to 45, 25 periodontal healthy, 25 with gingivitis, and 25 with periodontitis, were included in the study. Gingival crevicular fluid (GCF), blood serum and saliva were obtained from individuals in each group. Tumour necrosis factor-alpha (TNF-α) and asprosin levels in these fluids were determined using the ELISA. Clinical periodontal measurements were recorded and body mass index was calculated. One-way ANOVA and Bonferroni tests were performed for statistical analysis. Spearman test was used to evaluate correlations. The significance level was determined as $p < 0.05$.

Results Body mass index values were not different between the groups ($p = 0.446$). Clinical periodontal measurements were significantly higher in the periodontitis group. Concentrations of TNF-α in GCF, serum and saliva increased significantly in patients with gingivitis and periodontitis ($p < 0.001$). The higher TNF-α levels were obtained in patients with periodontitis than in individuals with gingivitis ($p = 0.001$). While asprosin levels were found to be significantly higher in patients with gingivitis and periodontitis ($p < 0.001$), no significant difference was observed between both groups ($p > 0.05$). GCF-asprosin levels were positively correlated with the concentrations in serum and saliva in all individuals included in the study ($p < 0.05$).

Conclusion The periodontal inflammation caused an increase in asprosin hormone in gingival crevicular fluid independently of the type of periodontal disease.

Trial registration This study is registered with number of "NCT06627972" in ClinicalTrials.gov website from the date of October 3, 2024.

Keywords Gingival crevicular fluid, Asprosin hormone, Tumour necrosis factor alpha, Gingivitis, Periodontitis

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Introduction

Periodontal health is a sustainable situation of oral and dental health without any sign of inflammation and destruction of periodontal tissue [1]. In contrast, periodontal diseases, such as gingivitis and periodontitis, are chronic infectious conditions characterised by inflammation in the supporting tissues surrounding the tooth, which develop as a result of the host's response to various microorganisms and microorganism products with microbial dental plaque accumulation. Eventually, the destruction of alveolar bone, gingival recession, loss of attachment, and even tooth loss can be observed [2].

In the process of inflammation, the gingiva loses its firm consistency due to oedema or capillary dilatation and the stippling structure of the surface turns into a shiny and smooth form. Moreover, there is an increase in the temperature of the gingival sulcus and the flow rate of gingival crevicular fluid (GCF) [3, 4]. In addition to clinical symptoms, many histological and immunological events also occur. In an unhealthy periodontium, high levels of the inflammatory response products of the host can be detected in the content of GCF, and these components are important in obtaining information regarding the pathogenesis of periodontal disease [5, 6]. It is known that local inflammatory events triggering by periodontal diseases cause damage or adversely affect the functioning of many distant tissues or organs via the circulatory system. In the literature, many studies prove that periodontal diseases are associated with various systemic diseases, such as cardiovascular diseases, diabetes mellitus, obstructive pulmonary disease, and rheumatoid arthritis [7–9]. Various cytokines and virulence factors involved in inflammatory processes play a role in the association of periodontal diseases with systemic diseases. Moreover, the changes in mediator levels due to inflammation cause local and systemic effects [10].

Adipokines are substances produced from adipose tissue and have numerous important functions, such as intake, regulation of blood pressure, lipid and carbohydrate metabolism, and inflammation. In 2016, Romere et al. [11] reported that asprosin is encoded by two exons of the Fibrillin-1 gene during fasting. In the fasting state, plasma asprosin level increases, and it decreases after eating [11] Asprosin has been shown to be directly effective in metabolic processes like the glucose metabolism [12]. After crossing the blood–brain barrier, asprosin hormone stimulates the orexigenic (appetite-stimulating) AgRP neuron group through a cAMP-dependent pathway activity. Simultaneously, gamma aminobutyric acid-dependent proopiomelanocortin neurones are suppressed, thus increasing appetite, adiposity and finally body weight [11, 13].

Asprosin increases B-cell-dependent inflammation by inducing hepatic glucose synthesis and also increases glucose levels in the blood by inhibiting insulin secretion [14]. Many studies revealed that the serum asprosin concentration of Type 2 diabetes mellitus patients was higher than that of systemically healthy controls [15, 16]. Asprosin hormone belongs to the class of adipokines increasing in obese individuals and exacerbating the destructive effects of periodontal diseases [17]. The relationship between periodontal diseases and systemic diseases is based on the fact that various mediators secreted in periodontal diseases have the potential to produce systemic effects and that biochemical events observed in systemic diseases may exacerbate periodontal diseases [18]. This bidirectional relationship currently exists between rheumatoid arthritis and periodontitis as well as diabetes mellitus and periodontitis [8, 19]. The aim of this study was to investigate the level of asprosin hormone in the gingival crevicular fluid of patients with periodontal disease and to compare it with that of the periodontal healthy individuals, thus determining whether asprosin is a diagnostic marker for diagnosis of the periodontal diseases.

Materials and methods

The study protocol was registered in ClinicalTrials.gov (NCT06627972). The obligatory permission for this study was obtained from Adıyaman University Clinical Research Ethics Committee (2021-13-2). Moreover, this study was conducted in accordance with the principles of the Declaration of Helsinki in 1975, as revised 2013. All participants signed informed consent form.

Study groups

This study was carried out with 75 volunteers aged 18 to 45 years old, periodontal healthy, with gingivitis and periodontitis, and who applied to the Periodontology Clinics of Adıyaman University Faculty of Dentistry between January 2022 and July 2022. The groups were formed in the following manner:

Group H (periodontal healthy) ($n = 25$) Twenty-five individuals with a probing bleeding percentage of < 10%, probing pocket depth ≤ 3 mm, and no attachment loss.

Group G (with gingivitis) ($n = 25$) Twenty-five individuals with a probing bleeding percentage of > 10%, a maximum probing pocket depth of ≤ 3 mm, and no attachment loss.

Group P (with periodontitis) ($n = 25$) Twenty-five individuals (Stage I or II, Grade A) with at least 4 mm of probing depth (PD) on the surface of at least two non-adjacent teeth with the presence of bleeding on probing (BOP), attachment loss, and the presence of horizontal bone loss not exceeding < 33% radiographically and no tooth loss because of periodontal causes [20].

Exclusion criteria

Individuals were excluded from the study in case of the having the following criteria.

- any systemic and genetic disease or condition.
- obesity.
- irregular dietary habits.
- taking medication regularly.
- pregnancy, lactating and menopause.
- undergoing any periodontal treatment in the last six months.
- smoking.

Clinical measurements

While PD, clinical attachment loss (CAL) and BOP were measured on six surfaces of each tooth, plaque index (PI) [21] and gingival index (GI) [22] were measured on four surfaces of tooth, except third molars. All measurements were performed by a single examiner who was a specialist in the field. A periodontal probe (PCPUNC15, Hu-Friedy) with an interval of 1 mm was used for the measurements.

The distance from the gingival margin to the base of the pocket was recorded as pocket depth. In the evaluation of clinical attachment loss, the distance from the enamel-cementum border to the base of the pocket was measured. Average values of PD and CAL were obtained by dividing the sum of the values obtained from six surfaces of all teeth by the number of tooth surfaces examined. For the bleeding index in probing, probing was performed on six surfaces of each tooth. The bleeding percentage was calculated as dividing the number of the bleeding surfaces by the number of tooth surfaces examined and then multiplying by 100.

Obtaining the fluid samples

The sampling was conducted between 9 and 11 am. Individuals were informed not to eat anything, brush their teeth or exercise for at least two hours prior to the appointment time. All the samples were kept in a -80 °C freezer until the day of analysis.

Collection of GCF

In the Group H and the Group G, GCF samples were collected from the gingival crevice of the first molars. If the first molar was missing, the second premolar was selected for the collection of GCF. Whereas, the deepest periodontal pocket in the mouth was selected for the collection of GCF in the Group P. Prior to sampling, a cotton roll was placed on the vestibular sulcus to prevent saliva and dental plaque contamination. The area was then dried with an air spray. Two paper strips (Periopaper®, OraFlow Inc., Amityville, NY, USA) were placed 1 mm into the sulcus and the papers were allowed to absorb the GCF. Samples contaminated with blood or saliva were excluded. The paper strip was left in the sulcus for 30 s and then placed in an Eppendorf tube.

Collection of saliva

All participants were allowed to rinse with plenty of water before saliva samples were collected. The patient was seated in a comfortable position. For 10 min and without any stimulus, the patient was asked to accumulate saliva in the mouth and spit into a cup. The sample was centrifuged at 10,000 rpm for 5 min and the final supernatant was stored in a -80 °C deep freezer.

Collection of serum

Five ml of blood was collected from the antecubital fossa using hemogram tubes (Vacutainer®, UK). After centrifugation at 3000 rpm for 8 min, the serum was separated from the blood. Thereafter, serum samples were transferred to Eppendorf tubes.

Biochemical analysis

The ELISA method was used to determine the levels of asprosin and TNF-α in GCF, saliva, and serum samples. Human Asprosin ELISA Kit (Cloud-Clone, Cat No: SEA332 Hu, Wuhan, China) and TNF-α ELISA Kit (Cloud-Clone, Cat No: SEA133 Hu, Wuhan, China) were used in the analyses performed in Adıyaman University, Medical Faculty, Medical Biochemistry Laboratory. While the asprosin kit has a detection range of 0.156-10 ng/mL and the minimum detectable dose of this kit is typically less than 0.064ng/mL, the detection range of Human-TNF-α kit is 15.6-1,000 pg/mL and the minimum detectable dose of this kit is typically less than 6.2pg/mL. The analyses were performed as specified in the manufacturer's instructions for use.

Preparation of GCF samples for ELISA

GCF samples were removed from the deep freezer the night before the analysis; 200 µl of PBS-T (a mixture of 0.05% Tween 20® and phosphate-buffered saline) was added to the samples and stirred in an orbital shaker at 175 rpm for 15 min. Following the day, the mixture was centrifuged at 13.000 rpm for 2 min. Then, the standard and sample were placed in a 96-well plate template.

Preparation of serum and saliva samples for ELISA

Serum and saliva samples were removed from the deep freezer and kept at +4 °C for one night. Each sample was mixed with the help of a vortex and the standard and samples were placed on the 96-well plate template in the ELISA kit.

For standardization, asprosin hormone was diluted with 1 ml standard and TNF-α was diluted with 0.5 ml standard and gently shaken for 10 min without foaming.

The prepared asprosin standard concentration was 40 ng/mL and TNF-α was 1,000 pg/mL. Serial dilutions were made from the standard concentrations prepared as stock solutions. For asprosin, 7 wells were prepared containing standard solution with 750 µl in the first well and 500 µl in the other wells, and for TNF-α, 7 wells were prepared containing standard solution with 250 µl in the wells, and then serial dilutions were made. Separate pipette tips were used to ensure concentrations in the transfer of each well and the liquids in the wells were thoroughly mixed during transfers. The concentration of the standard dilution in the last well was 0 pg/mL and 0 ng/mL for TNF-α and asprosin, respectively. If a sample was outside the calibration curve, a dilution was made and re-evaluated to obtain a value within the curve and the result was multiplied by the dilution factor.

Asprosin and TNF-α concentrations were calculated with the help of an absorbance evaluation programme (Curve Expert, Version 1.4, USA); protein concentrations were measured using a non-linear regression model.

Statistical analysis

Statistical analysis was performed using a package programme (SPSS, Version 25, USA). As descriptive statistics, mean ± standard deviation values for numerical variables and number and % values for categorical variables are given. The conformity of the data to normal distribution was analysed by Shapiro-Wilk test. Further, one-way analysis of variance ANOVA was used to compare the data among the groups. If the F-value was significant in the ANOVA analysis, the Bonferroni post-hoc test was conducted to determine which groups the difference was between. In addition, the differences among categorical variables were analysed using the Chi-square test. The relationship between quantitative variables was calculated by Spearman correlation coefficient. A correlation coefficient between 0.8 and 1 indicated a very strong relationship, values between 0.6 and 0.8 indicated a strong relationship, values between 0.4 and 0.6 indicated a moderate relationship and values between 0.2 and 0.4 indicated a weak relationship. Significance level was determined as $p < 0.05$ at the 95% confidence interval.

Results

In our study, in which the asprosin concentrations obtained from the biological fluids (GCF, serum and saliva) of patients in the groups were evaluated, the post-hoc power of the statistical analysis performed with a sample size of 75 and a 5% margin of error was determined to be over 95%. From 75 participants, all samples of gingival crevicular fluid, serum and saliva were readable in the biochemical analysis.

This study included 75 individuals aged between 19 and 45 years. The mean age of the participants was 27.89 ± 5.28 years old; 36 of the participants were female and 39 were male. There was no statistically significant difference between the groups in terms of age ($p = 0.987$) and gender ($p = 0.852$) (Table 1). Body mass index (BMI) values are shown in Table 1. These could not show a significant difference among the groups ($p = 0.446$).

The mean CAL value in the Group P was 1.03 ± 0.34 mm. When PD, BOP, PI, and GI data were compared at the group level, it was observed that the Group P had the highest scores and the Group H had the lowest ones ($p < 0.05$) (Table 2). In addition, serum-, saliva-, and GCF-asprosin levels were statistically higher in the Group P and the Group G compared to those in the Group H ($p < 0.001$). No significant difference was found between the Group P and the Group G in terms of asprosin levels in all the fluid samples ($p > 0.05$) (Table 3).

Serum, saliva, and GCF-TNF-α levels were statistically higher in the Group P and the Group G compared to those in the Group H ($p < 0.001$). In addition, the TNF-α values measured from all three fluids were statistically significantly higher in the Group P than those in the Group G ($p = 0.001$) (Table 4).

The correlation of asprosin and TNF-α levels are shown the Table 5. The serum-asprosin level was positively correlated with the concentrations in the saliva and the GCF. Also, TNF-α levels in the fluids showed a positive correlation with the level of asprosin in the serum, saliva and GCF ($p < 0.05$) (Table 5).

Table 1 Comparison of demographic characteristics and BMI values between the groups

Variables		H	G	*P*	Total	*p*
Gender n(%)	**Female**	12 (48)	11 (44)	13 (52)	36 (48)	0.852
	Male	13 (52)	14 (56)	12 (48)	39 (52)	
Age (mean ± SD)		27.76 ± 5.37	28.00 ± 5.66	27.92 ± 5.01	27.89 ± 5.28	0.987
BMI (kg/m^2) (mean ± SD)		24.24 ± 2.39	24.96 ± 2.00	24.79 ± 1.76	24.66 ± 2.06	0.446

BMI; body mass index, Groups- H; periodontal healthy, G, gingivitis, P, periodontitis, SD; standard deviation, $p < 0.05$; statistically significant

One-way ANOVA test for comparison of BMI

Chi-Square (X^2) test; for comparison of gender

Table 2 Comparison of clinical parameters of the participant to the study groups

Parameters	Groups (mean ± SD)			*p*
	Healthy	Gingivitis	Periodontitis	
PD (mm)	1.40 ± 0.14^{c}	1.70 ± 0.13^{b}	3.16 ± 0.48^{a}	**0.001**
BOP (%)	3.68 ± 1.59^{c}	48.23 ± 5.70^{b}	73.19 ± 6.51^{a}	**0.001**
PI	0.50 ± 0.17^{c}	1.23 ± 0.20^{b}	1.99 ± 0.25^{a}	**0.001**
GI	0.22 ± 0.11^{c}	0.98 ± 0.19^{b}	1.85 ± 0.26^{a}	**0.001**

PD; probing depth, BOP; bleeding on probing, PI; plaque index, GI; gingival index, n; participant number, mm: millimeter, %: percentage, SD; standard deviation, *$p < 0.05$; statistically significant. One-way ANOVA test used for comparison of parameters. Bonferroni Post-Hoc was used for the pairwise comparison. Different superscript letters (a, b and c) in a row show difference between the groups

Table 3 Asprosin levels in the biological fluids of the body

Biological fluids (ng/mL)	Groups	mean ± SD	*p*
Serum	Healthy (H)	0.025 ± 0.026^{a}	**< 0.001***
	Gingivitis (G)	0.268 ± 0.049^{b}	
	Periodontitis (P)	0.271 ± 0.064^{b}	
Saliva	Healthy (H)	0.027 ± 0.023^{a}	**< 0.001***
	Gingivitis (G)	0.170 ± 0.032^{b}	
	Periodontitis (P)	0.182 ± 0.048^{b}	
GCF	Healthy (H)	0.029 ± 0.026^{a}	**< 0.001***
	Gingivitis (G)	0.110 ± 0.031^{b}	
	Periodontitis (P)	0.123 ± 0.039^{b}	

GCF; gingival crevicular fluid, ng/mL; nanogram/mililiter, $p < 0.05$; statistically significant,

One-way ANOVA test; multiple comparison

Bonferroni Post-Hoc; pairwise comparison

Different superscript letters ("a" and "b") in the column show difference between the groups in the relevant fluid

Discussion

Periodontal pathogens cause destruction in the periodontium directly or indirectly via their own virulence factors and host-derived enzymes. In their study conducted in 2016, Dede et al. [23] reported that PI, GI, PPD, BOP, and CAL values obtained from individuals with periodontitis and gingivitis were higher compared to those obtained from the periodontal healthy individuals. Further, in a study conducted with 90 participants, Shojaee et al. [24] found that GI, CAL, PD, and BOP values were higher in individuals with periodontitis than those with gingivitis and periodontal healthy, as well as they were higher in individuals with gingivitis than in periodontal healthy ones. In addition to the abovementioned clinical parameters, which are useful for us to establish the diagnosis of periodontal disease, the presence of host mediators in GCF can be used for diagnostic purposes in determining the severity of the disease [25].

The increased levels of TNF-α in tissue fluids indicate that the destructive effects of the periodontal diseases are dimensionally exacerbated. This induces osteoclast activation and subsequent alveolar bone loss by inducing receptor activator nuclear Kappa-B ligand (RANKL-B) expression. It has been reported that TNF-α levels are increased in the serum, saliva, and GCF of individuals with periodontitis [26, 27]. Therefore, it has been suggested that TNF-α expression levels could be used as a biomarker for the diagnosis of periodontal diseases [28, 29]. A study conducted by Singh et al. [30] was reported that patients with periodontitis had higher levels of TNF-α in saliva compared to periodontal healthy individuals. In another study, Gümüş et al. [31] found that TNF-α levels in serum and saliva were higher in the periodontitis groups than those in the healthy group. In addition, it was reported that TNF-α levels were higher in the gingivitis group compared to the healthy group [32]. According to our results, the differences among the

Table 4 TNF-α levels in different biological fluids

Biological fluids (pg/ml)	Groups	mean ±SD	*p*
Serum	Healthy (H)	3.509 ± 1.626[a]	**< 0.001***
	Gingivitis (G)	14.591 ± 7.998[b]	
	Periodontitis (P)	22.011 ± 9.069[c]	
Saliva	Healthy (H)	3.164 ± 0.633[a]	**< 0.001***
	Gingivitis (G)	7.445 ± 3.366[b]	
	Periodontitis (P)	11.506 ± 4.674[c]	
GCF	Healthy (H)	2.424 ± 0.214[a]	**< 0.001***
	Gingivitis (G)	5.561 ± 2.253[b]	
	Periodontitis (P)	8.053 ± 3.194[c]	

GCF; gingival crevicular fluid, pg/mL; picogram / mililiter SD; standard deviation, $p < 0.05$; statistically significant

One-way ANOVA test; multiple comparison

Bonferroni Post-Hoc; pairwise comparison

There is a statistical significant difference between Group P and Group G ($p < 0.001$)

Different superscript letters ("a", "b" and "c") in the column show difference between the groups in the relevant fluid

Table 5 Correlations of asprosin and TNF-α levels in the biological fluids of all participants

	Correlation	Serum-A	Saliva-A	GCF-A
Serum-A	r	1	0.809*	0.710*
	p		< 0.001	< 0.001
	n		75	75
Saliva-A	r	0.809*	1	0.718*
	p	< 0.001		< 0.001
	n	75		75
GCF-A	r	0.710*	0.718*	1
	p	< 0.001	< 0.001	
	n	75	75	
Serum TNF- α	r	0.695*	0.755*	0.668*
	p	< 0.001	< 0.001	< 0.001
	n	75	75	75
Saliva-TNF- α	r	0.683*	0.709*	0.690*
	p	< 0.001	< 0.001	< 0.001
	n	75	75	75
GCF-TNF- α	r	0.672*	0.656*	0.629*
	p	< 0.001	< 0.001	< 0.001
	n	75	75	75

GCF; gingival crevicular fluid, A; asprosin, TNF- α; tumour necrosis factor alpha, r; Spearman correlation coefficient, n; number of participant, *$p < 0.05$; statistically significant

concentrations of TNF-α in the saliva, serum and GCF of the groups are in concordance with the above-mentioned studies.

Asprosin is an adipokine-derived hormone that is closely associated with obesity [14] It is stated that the obesity is defined above 30 kg/m^2 in many guidelines [33]. A prospective study including 117 obese subjects with a BMI > 35 kg/m2 and 57 normal participants found fasting asprosin levels were markedly higher in obese participants than in controls [34]. None of the patients employed in the study was associated with obesity. BMI values were not statistically different among the groups. In the literature, there are many studies that the asprosin hormone could be detected in the blood serum even in non-obese individuals. Wang et al. [35] found serum-asprosin level in a non-obese group was 0.96 0.48 ng/mL. In another study, the serum asprosin level in individuals with polycystic ovary syndrome and in healthy ones were

compared, and it was found that the asprosin level in healthy individuals was 3.69 ± 1.22 ng/mL [36]. In addition, Zhang et al., they compared the serum asprosin levels of diabetic individuals with healthy ones— observed that the asprosin level in healthy individuals was 1.77 ng/mL [16]. In our study, the serum asprosin level was found to be 0.025 ng/mL in the periodontal and systemically healthy group. The fact that serum asprosin levels were detectable in systemically healthy individuals is in accordance with the literature. However, the differences in serum-asprosin levels can be explained with biochemical analysis, kit and sample size.

It was observed that the serum and salivary asprosin levels could increase in the presence of periodontal disease. Salivary-asprosin level was 0.182 ng/mL in the periodontitis group, 0.170 ng/mL in the gingivitis group, and 0.027 ng/mL in the periodontal healthy group. A study demonstrated the asprosin in the human salivary gland is produced and secreted by acinar cells, as are other salivary proteins [37]. For this reason, the presence of asprosin hormone in the secreted saliva even in the periodontally healthy group can be associated with the natural process of the salivary glands. Moreover, it has been reported that the concentration of asprosin hormone showed an increase in the presence of periodontitis, independently of overweight [38]. The results of this study showed that serum and salivary asprosin concentrations were increased especially as the periodontitis phase changes from Stage I to Stage IV [38]. In our study, the levels of serum- and salivary-asprosin hormone in periodontitis and/or gingivitis, which are characterised by gingival redness, bleeding, and even bone destruction, were higher compared to the periodontal healthy individuals. This was in accordance with a recent study by Gül et al. [38].

To the best of our knowledge, there is no study investigated asprosin hormone level in the gingival crevicular fluid in the literature. In this respect, this is the first study to analyse asprosin level in gingival crevicular fluid of individuals with periodontal disease including gingivitis and periodontitis. The GCF-asprosin levels were found to be statistically significantly higher in the periodontitis and gingivitis groups when compared to the periodontal healthy group. Further, there was no statistically significant difference between the periodontitis and gingivitis groups in terms of asprosin levels in the GCF, serum and saliva. Recent evidence support that asprosin has a role in inflammatory process that leads to an increase in local and systemic circulating levels of pro-inflammatory mediators, including interleukin- 6 and TNF-α [39]. The high levels of the asprosin hormone may be due to the increase in TNF-α concentration, which is known to reach high level in the biological fluids during the inflammation of periodontal tissue [31, 40, 41]. A study, in which the effect of asprosin on the release of TNF-α was evaluated in co-cultures with lipopolysaccharide stimulation, have shown an enhancing expression of TNF- α [42]. In the present study, the levels of TNF-α may have been affected from the levels of asprosin in the serum, saliva and GCF. Asprosin has been shown to impair insulin secretion from pancreatic beta cells under hyperglycemic condition by activating pro-inflammatory mechanisms related to the tool-like receptor pathway [43]. Moreover, it has been reported that the elevated circulating TNF levels are associated with insulin resistance [41]. Considering the bi-directional relationship between diabetes and periodontitis, a possible synergistic effect of TNF- α with the asprosin hormone may raise more dramatic results for insulin resistance in diabetic patients.

In the present study, serum asprosin concentration in the participants was highly correlated with that of the saliva and the GCF. Recently, a correlation of saliva with serum asprosin was already demonstrated and seems to be BMI-dependent [37]. Another study showed that saliva asprosin concentration was also increased upon fasting which strongly correlated with serum asprosin levels. Also, this study proved the acute running exercise asprosin release into the blood was induced and remains stable for at least 2 h afterwards [44]. But, so far it is not understood by which mechanism running exercise leads to increased asprosin levels. Among the various biological fluids, GCF has a special place not only as it is capable of providing site-specific information about the periodontal lesion but it also reflects components within serum [45]. The evidence support that the content of gingival crevicular fluid will be affected by that of the serum. The positive correlations among asprosin concentrations in the serum asprosin, saliva and GCF are strongly possible to be associated with above-mentioned mechanisms.

The present study had several limitations. One of them was not to specify the periodontitis according to the staging of the disease, because the distribution of patients in the periodontitis group to the stages was no homogenous (Stage I, $n = 20$, Stage II, $n = 5$). Another limitation of this study was that asprosin levels in the biological fluids were examined in a single time period and regardless of daily dietary.

Conclusion

The outcomes of the present study revealed that there was an increased concentration of asprosin hormone in the GCF, saliva and serum of patients with periodontal diseases. In general aspect, asprosin levels were no affected by the type of the periodontal diseases, which they had similar results in both gingivitis and periodontitis groups. There is needed for more investigations with long-term follow-up and the larger population to

examine GCF-asprosin levels according to the different stages of periodontitis.

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Author contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Sedat Tutuş], [Abdulsamet Tanık] and [Muhittin Önderci]. The first draft of the manuscript was written by [Osman Fatih Arpağ] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability
The datasets generated and/or analyzed during the current study are not publicly available due to privacy concerns but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was performed in accordance with the principles of the Declaration of Helsinki. The Clinical Research Ethics Committee of the Adıyaman University granted approval (Ethics approval number: 2021-13-2). Informed consent was obtained from all the participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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