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Gingival crevicular fluid adrenomedullin level in individuals with and without diabetes mellitus type 2

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Background and Objective: Adrenomedullin, an antimicrobial peptide, has biological applications in many tissues, but its main attribute is its ability to lower arterial pressure. The plasma adrenomedullin level is elevated in pathophysiological conditions such as arterial hypertension, acute coronary syndrome, renal diseases, diabetes mellitus and periodontal diseases. The aim of this study was to compare the amounts of adrenomedullin in the gingival crevicular fluid of periodontally healthy individuals, individuals with chronic periodontitis, periodontally healthy individuals with diabetes mellitus type 2 and individuals with chronic periodontitis and diabetes mellitus type 2.

Material and Methods: Eighty-four individuals were included in this study: 21 periodontally healthy individuals; 21 individuals with chronic periodontitis; 21 periodontally healthy individuals with diabetes mellitus type 2; and 21 individuals with chronic periodontitis and diabetes mellitus type 2. An ELISA was performed to measure the adrenomedullin levels in gingival crevicular fluid.

Results: Groups with diabetes mellitus type 2 (periodontally healthy individuals and individuals with chronic periodontitis) had significantly higher periodontal clinical indices than did nondiabetes mellitus groups (periodontally healthy individuals and individuals with chronic periodontitis). The group of individuals with chronic periodontitis and diabetes mellitus type 2 had a significantly higher total adrenomedullin level compared with the other groups. Also, a significantly higher total adrenomedullin level was found in diabetes mellitus type 2 groups (periodontally healthy individuals and individuals with chronic periodontitis) compared with nondiabetes mellitus groups (periodontally healthy individuals and individuals with chronic periodontitis).

Conclusions: An increased adrenomedullin level was found in individuals with chronic periodontitis and also in individuals with diabetes mellitus. It is thought that the effect of diabetes mellitus on the pathogenesis of chronic periodontitis could have been achieved through antimicrobial peptides such as adrenomedullin, or that increased adrenomedullin was released in individuals with diabetes mellitus in order to ensure no further periodontal tissue loss.

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The oral bacterial flora is controlled by the innate immune system of the oral epithelia, saliva and gingival crevicular fluid, which is rich in antimicrobial proteins and peptides (1). Adrenomedullin (AM), which is expressed by surface epithelial cells in various tissues, is one such important antimicrobial peptide, described as having a potential role in the host defense of the body (2). AM has been shown to have antimicrobial properties against both gram-positive and gram-negative bacteria in the oral microflora and may also have a local bactericidal effect in the oral cavity (3). The AM level is elevated in many systemic diseases, including arterial hypertension (4,5), congestive heart failure (6,7), acute myocardial infarction (8), chronic renal failure (9), liver cirrhosis (10,11), sepsis (12), periodontal disease (13,14) and diabetes mellitus (DM) (15). The most common endocrine disease, DM, develops as a result of interactions among metabolic disorders characterized by chronic hyperglycemia, high-calorie diets, genetic transfer and environmental factors (16). DM has two variations: type 1 and type 2. Type 1 DM (T1DM) develops as a result of decreased insulin production caused by autoimmune destruction in pancreatic cells and is mostly seen in children and young adults (17). Type 2 DM (T2DM) is usually seen in adults and is characterized by decreased insulin resistance when pancreatic beta cells are unable to secrete sufficient amounts of insulin. Seventy to ninety-five per cent of DM patients have T2DM (16,17). AM is reportedly effective in complications associated with T2DM, and there is a correlation between AM plasma levels and diabetic retinopathy and neuropathy. An increase in the AM level is thought to be a defense mechanism against damage occurring in vessels during DM (18). In addition to other factors, DM has been shown to modify the host response to the bacterial challenge and, over time, DM may increase the risk for periodontal disease (19–22). Periodontal diseases develop as a response of periodontal tissues to alterations in oral bacteria. Bacterial biofilms are very important in gingival inflammation and tissue destruction in periodontal tissues (23). Evidence suggests that periodontal tissue destruction is mainly caused by the host's inflammatory response to the bacterial challenge (24). Upon contact with microorganisms, epithelial cells secrete, antimicrobial peptides (and especially AM) are expressed from epithelial cells (13,25,26). The lipopolysaccharides of the microorganisms trigger the secretion of AM from the tissues (27). It is also known that DM can change the responses of the tissues to microorganisms and that it constitutes a risk factor for periodontal diseases (20,21,28). We have tried to determine the AM level in gingival crevicular fluid in order to understand if there is an effect of DM on the amount of AM secreted to the gingival sulcus, with the knowledge that DM affects the tissue response to dental plaque and to microorganisms inside dental plaque. The role of AM in the pathogenesis of DM and periodontal diseases is not clear. AM may be related either to endothelial damage or to the existence of a generalized chronic inflammatory process. Our hypothesis is that protective AM is released in response to oral microorganisms in individuals with DM as a result of alterations in the immune response of such individuals to microorganisms and their toxins. We believe that if our hypothesis is verified, the role of AM in periodontal diseases and its relationship with DM in periodontal diseases would be clearly understood. The aim of this study was to compare the AM level in the gingival crevicular fluid of the following subject groups: periodontally healthy individuals; individuals with chronic periodontitis; periodontally healthy individuals with T2DM; and individuals with chronic periodontitis and T2DM.

Material and methods

Subjects

In total, 84 individuals were involved in this study: 21 periodontally healthy individuals with no T2DM; 21 individuals with chronic periodontitis and no T2DM; 21 periodontally healthy individuals with T2DM; and 21 individuals with chronic periodontitis and T2DM. Individuals were selected from patients referred to the Yuzuncu Yıl University Dentistry Faculty Department of Periodontology for treatment from May 2011 to November 2011. The following inclusion criteria were implemented: at least 10 teeth present in the oral cavity; no medical complications (excluding DM in the appropriate groups); no smoking habit; no use of medications that could affect periodontal status (excluding DM medications), such as anti-inflammatory agents, antibiotics, immunosuppressants or contraceptives; no evidence of microangiopathy or macroangiopathy; and no infections or renal/blood-pressure disorders. All individuals provided informed consent in accordance with the local Human Ethics Research Committee of Yuzuncu Yil University (YYU-110411). Each subject read and signed the Helsinki Declaration before inclusion in the study. A diagnosis of T2DM was established using the 2005 American Diabetes Association Criteria for the Diagnosis of Diabetes Mellitus (17). T2DM was diagnosed at a local clinic $\geq$ 3 years before the beginning of the study. All individuals were treated with diet (1400–1800 kcal/day) and exercise (12,000 steps/day walking).

Venous blood samples were taken from all individuals who participated in the study, and glycosylated hemoglobin (HbA1c) was measured using high-performance liquid chromatography. The duration of DM, the body mass index and the fasting plasma glucose level of the individuals diagnosed with T2DM were recorded.

Radiographic examination was performed, and clinical periodontal assessment, including periodontal status, was made by measuring the plaque index (PI) (29), the gingival index (GI) (30), the probing depth and the clinical attachment level (CAL) (31) at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual), and also by assessment of bleeding on probing

(BOP). These tests were performed by the same calibrated examiner (A.S.E.). The individuals were selected in accordance with the clinical and radiographic criteria proposed by the 1999 International Work Workshop (32).

Gingival crevicular fluid samples

All clinical and radiologic examinations and sampling site selections were performed the day before gingival crevicular fluid samples were collected. Four gingival crevicular fluid samples were taken from four Ramfjord sample teeth (teeth 1.6, 2.4, 3.6 and 4.4; if these teeth were missing from the oral cavity, teeth 1.1 and 4.1 were used instead) from patients with chronic periodontitis and from periodontally healthy individuals. After isolation of the tooth with a cotton roll, supragingival plaque was removed without touching the marginal gingiva to minimize plaque contamination of the strips. The crevicular site was dried with an air syringe. Gingival crevicular fluid was collected using a paper strip (Periopaper Interstate Drug Exchange, Amityville, NY, USA), which was inserted into the gingival crevice/pocket until mild resistance was encountered and then left in place for 30 s. The volume of gingival crevicular fluid was immediately determined using a calibrated Periotron 8000 (Oraflow, New York, NY, USA). The samples obtained from each individual were placed in separate eppendorf tubes; in other words, the paper strips used to collect gingival crevicular fluid from each of the Ramfjord sample teeth were placed in separately numbered for eppendorf tubes for each individual, were placed inside the separately numbered aliquots for each individual. To prevent mistakes, instead of taking only one gingival crevicular fluid sample from the Ramfjord teeth of each individual, four gingival crevicular fluid samples were taken from each tooth, and the mean of these samples was calculated. In total, 84 aliquots were used in the study, each of which contained four paper strips. The Periotron values of the four samples obtained from the Ramfjord sample teeth of individuals were summed and then divided by four to obtain an average Periotron value for each individual. As the results obtained following this ELISA represent the total of all four samples taken from each individual, the AM ELISA value were divided into four and AM ELISA value for one individual was calculated. The sample values were analyzed using a Microsoft Excel 2007 program (Microsoft Corporation, Redmond, WA, USA) to determine the AM concentrations. The AM concentration in gingival crevicular fluid was calculated by multiplying these ELISA values by 500 μL.

Laboratory measures of adrenomedullin

The AM levels in gingival crevicular fluid samples were measured using an ELISA kit (Uscn Life Science Inc., Wuhan, China). One-hundred microlitres of each dilution of standard blank or sample was added to the appropriate wells and covered with a plate sealer. The plate was incubated for 2 h at 37°C, and then the procedure was repeated. Next, 100 μL of each of prepared detection reagent A and prepared detection reagent B was added, along with 90 μL of substrate solution and the plate was incubated for 25–30 min at 37°C. Fifty microlitres of stop solution was then added, and the plate was immediately read at 450 nm in an ELISA Reader (Chro-Mate® Microplate Reader; FL, USA). The AM values (pg/mL) in gingival crevicular fluid were multiplied by the dilution (500 μL), and the total amount of AM collected in 30 s (pg/30 s) was determined. The AM concentrations in a unit volume (pg/μL) were determined by multiplying the gingival crevicular fluid AM values by 500 μL (to account for the dilution) and dividing by the gingival crevicular fluid volume (μL).

Statistical analyses

All data analyses were performed using a statistical program (SPSS version 17.0; SPSS, Chicago, IL, USA). Kolmogorov–Smirnov tests were used to measure normality. The data for AM and clinical periodontal indices were analyzed using nonparametric testing (the Mann–Whitney *U*-test with Bonferroni correction). Spearman rank correlation tests were used to determine the relationship between AM and clinical periodontal indices and the relationship between AM and percentage HbA1c (HbA1c%). $p < 0.05$ was considered to be statistically significant for all analyses.

Results

The ages and genders of the study groups are shown in Table 1. Periodontally healthy individuals with T2DM were younger than individuals in the other groups. There was no significant difference in age among the study groups ($p > 0.05$). Periodontal clinical index (PI, GI, probing depth, BOP and CAL) scores of the mouth are shown in Table 1. The groups with T2DM (periodontally healthy and chronic periodontitis) had significantly higher periodontal clinical index (PI, GI, probing depth, CAL and BOP) scores than non-T2DM groups (periodontally healthy and chronic periodontitis) ($p < 0.05$). T2DM individuals with chronic periodontitis had significantly higher periodontal clinical index (PI, GI, probing depth, CAL and BOP) scores than did individuals with chronic periodontitis but no T2DM. Periodontally healthy individuals with T2DM had significantly higher periodontal clinical index (PI, GI, probing depth, CAL and BOP) scores than did periodontally healthy individuals with no T2DM ($p < 0.05$). Also PI, GI, probing depth, CAL and BOP were significantly higher in the group of subjects with chronic periodontitis but no T2DM than in the periodontally healthy group with T2DM ($p < 0.05$) (Table 1).

The gingival crevicular fluid AM levels are presented in Table 1. The group with chronic periodontitis and T2DM had a significantly higher gingival crevicular fluid total AM level compared with the other groups (periodontally healthy with T2DM,

Table 1. Demographic characteristics, clinical periodontal parameters, adrenomedullin levels and the gingival crevicular fluid volume of study groups

	Periodontally healthy (non-T2DM) (n = 21)	Chronic periodontitis (non-T2DM) (n = 21)	Periodontal healthy with T2DM (n = 21)	Chronic periodontitis with T2DM (n = 21)	p value
Age (years)	43.4 ± 6.35	44.2 ± 5.28	41.4 ± 4.31	45.1 ± 8.21	NS
Gender (female/male)	11/10	10/11	9/12	11/10	–
PI	1.40 ± 0.09†,‡,§	2.05 ± 0.07*,‡,§	1.53 ± 0.14*,†,§	2.37 ± 0.29*,†,‡	< 0.05
GI	0.32 ± 0.07†,‡,§	2.04 ± 0.07*,‡,§	0.48 ± 0.11*,†,§	2.28 ± 0.29*,†,‡	< 0.05
Probing depth (mm)	1.37 ± 0.08†,‡,§	3.62 ± 0.31*,‡,§	1.54 ± 0.09*,†,§	4.01 ± 0.41*,†,‡	< 0.05
CAL (mm)	1.51 ± 0.16†,‡,§	3.50 ± 0.29*,‡,§	1.69 ± 0.18*,†,§	3.84 ± 0.42*,†,‡	< 0.05
BOP (%)	7.27 ± 2.43†,‡,§	61.14 ± 8.23*,‡,§	9.42 ± 1.72*,†,§	82.32 ± 11.25*,†,‡	< 0.05
Total adrenomedullin (pg/30s)	947 ± 78†,‡,§	1102 ± 114*,‡,§	1332 ± 187*,†,§	1599 ± 160*,†,‡	< 0.05
Adrenomedullin concentration (pg/μL)	5706 ± 927†,‡,§	4608 ± 367*,‡,§	4823 ± 245*,†,§	3299 ± 272*,†,‡	< 0.05
Gingival crevicular fluid volume (μL)	0.11 ± 0.04†,‡,§	0.21 ± 0.08*,‡,§	0.13 ± 0.02*,†,§	0.28 ± 0.20*,†,‡	< 0.05

Values are given as mean± standard deviation unless stated otherwise. BOP, bleeding on probing; CAL, clinical attachment level; GI, gingival index; NS, not significant; PI, plaque index; T2DM, type 2 diabetes mellitus.
*Significantly different from periodontally healthy individuals, $p < 0.05$.
†Significantly different from individuals with chronic periodontitis, $p < 0.05$.
‡Significantly different from periodontally healthy individuals with diabetes mellitus, $p < 0.05$.
§Significantly different from individuals with chronic periodontitis and diabetes mellitus, $p < 0.05$.

chronic periodontitis with non T2DM and periodontally healthy with no T2DM) ($p < 0.05$). The total AM level in the gingival crevicular fluid of individuals with chronic periodontitis but no T2DM was significantly higher than in periodontally healthy individuals with no T2DM ($p < 0.05$). Also, the total AM level of periodontally healthy individuals with T2DM was significantly higher than that of periodontally healthy individuals with no T2DM ($p < 0.05$).

When the data of AM concentration in gingival crevicular fluid were examined, the highest AM value was found in the periodontally healthy group with no T2DM and the lowest AM value was found in the chronic periodontitis group with T2DM ($p < 0.05$) (Table 1).

Laboratory and clinical data regarding DM of the individuals diagnosed with T2DM are presented in Table 2. The duration of DM, body mass index, fasting plasma glucose and HbA1c were evaluated for the two groups; between-group statistical differences for these data were not observed ($p > 0.05$).

The relationship between the HbA1c% and the total AM level is presented in Fig. 1. A positive-high correlation between the HbA1c% and total AM was found (correlation coefficient, $r = 0.880$; and $p = 0.000$). It has been observed that in individuals whose HbA1c% were determined as non-T2DM (HbA1c% < 6.5%) their total AM levels were < 1400 pg/30s. When the HbA1c% increases, the total AM level also increases to > 1400 pg/30 s. When the HbA1c% is > 7.5%, the total AM level also reaches its highest values. It has been observed that when the HbA1c% increases, the total AM level also increases.

Correlations between periodontal clinical indices and total gingival crevicular fluid AM levels of the study groups are shown in Table 3. a positive-weak correlation was found between the AM level in the non-T2DM periodontally healthy group and PI, GI, probing depth, CAL, BOP and gingival crevicular fluid volume (correlation coefficient, r, = 0.312, 0.380, 0.415, 0.402, 0.374, 0.414 respectively). Similarly, a positive-weak correlation was found between the AM level in the chronic periodontitis group with no T2DM and PI, GI, probing depth, CAL, BOP, and gingival crevicular fluid volume (correlation coefficient, r, = 0.412–0.404–0.396–0.388–0.386–0.478, respectively). The correlation was found to be stronger in DM groups. For example, a positive-medium correlation was found between the AM levels in the periodontally healthy group with T2DM and GI, probing depth, CAL and BOP data (correlation coefficient, r, = 0.622–0.608–0.596–0.602, respectively); a positive-medium correlation was found between probing depth,

Table 2. Laboratory characteristics obtained for periodontally healthy individuals with type 2 diabetes mellitus and for individuals with chronic periodontitis and type 2 diabetes mellitus

	Periodontally healthy individuals with type 2 diabetes mellitus	Chronic periodontitis individuals with type 2 diabetes mellitus
D-DM (years)	6.9 ± 2.7	7.3 ± 3.1
BMI (kg/m^2)	24 ± 1.1	26 ± 0.8
FPG (mg/dL)	145 ± 8.2	146 ± 10.5
HbA1c (%)	6.95	7.08

Values are given as mean± standard deviation or per cent. BMI, body mass index; D-DM, duration of diabetes mellitus; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin.

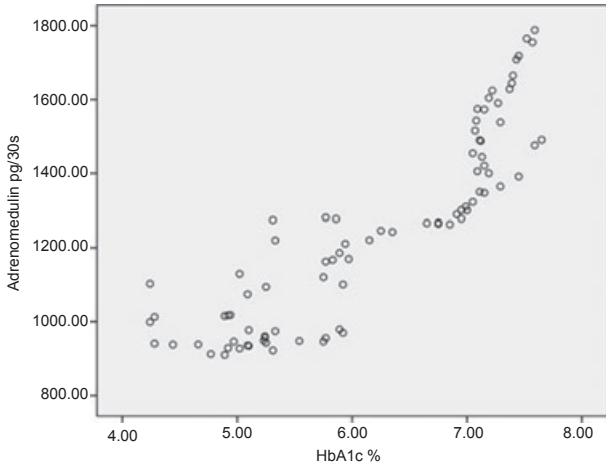

Fig. 1. Relationship between the percentage of glycosylated hemoglobin (HbA1c%) and the total adrenomedullin (AM) level in the gingival crevicular fluid from all individuals. HbA1c was measured using high-performance liquid chromatography. The AM level in gingival crevicular fluid was measured using an ELISA. The HbA1c critical level is higher than 7% in individuals with diabetes mellitus. Spearman rank correlation tests were used for AM levels and HbA1c% levels. A positive-high correlation between the HbA1c% level and the AM level was found (correlation coefficient, -*r*, = 0.88; and $p = 0.000$).

CAL and BOP data in the chronic periodontitis group with T2DM (correlation coefficient, *r*, = 0.686–0.625–0.614, respectively); and a positive-high correlation was found between GI and gingival crevicular fluid volume (correlation coefficient, *r*, = 0.714 –0.704, respectively).

Discussion

This is the first study to compare AM levels in the gingival crevicular fluid of individuals with chronic periodontitis and T2DM, periodontally healthy individuals with T2DM, periodontally healthy individuals with no T2DM and chronic periodontitis individuals with no T2DM. The total levels of AM in individuals with chronic periodontitis and T2DM were found to be significantly higher than in the other subject groups.

Studies with DM patients have shown that alterations in these patients include an elevation in the factors produced by and released from the endothelium, an increase in the permeability of blood vessels and enhanced platelet aggregation. Studies attributed this elevation to the vascular endothelial damage caused by advanced stages of diabetes (33–35). Hayaski *et al*. (15) assessed plasma AM levels and found that the AM levels in individuals with DM were significantly higher than in healthy control groups, and they emphasized that vascular endothelial damage observed in DM patients caused AM levels to increase. In our study, it was determined that the total AM levels in patients with T2DM were higher than those in the non-T2DM groups.

In the literature, the AM levels in individuals with T2DM are significantly higher than in systemically healthy individuals (15,36–38). Similarly, the AM levels in individuals with T1DM are known to be higher compared with those in healthy individuals (39). In our study, individuals with T2DM were compared with non-T2DM groups, and it was found that the total AM levels in gingival crevicular fluid were highest in the group with both T2DM and chronic periodontitis. The total levels of AM were lowest in the non-T2DM groups.

Kapas *et al*. (26) reported that AM is secreted from oral epithelial cells (keratinocytes) *in vitro*, and the AM level secreted increases in the presence of cytokines and steroid hormones. They (26) also indicated that alterations in antimicrobial activation affect the AM concentration in saliva. Lundy *et al*. (13) analyzed the AM levels in gingival crevicular fluid samples using radioimmunoassay; they found that AM was present in all gingival crevicular fluid samples and that the AM levels were significantly higher in individuals with periodontal diseases than in healthy individuals. Higher

Table 3. Correlations between the total adrenomedullin level in gingival crevicular fluid and the clinical periodontal parameters of the study groups

	Periodontally healthy	Chronic periodontitis	Periodontally healthy with diabetes mellitus	Chronic periodontitis with diabetes mellitus
	Adrenomedullin (pg/30 s)			
PI				
r	0.312	0.412	0.480	0.520
p	0.126	0.066	0.052	0.015
GI				
r	0.380	0.404	0.622	0.714
p	0.082	0.070	0.000	0.000
Probing depth (mm)				
r	0.415	0.396	0.608	0.686
p	0.064	0.076	0.000	0.000
CAL (mm)				
r	0.402	0.388	0.596	0.625
p	0.072	0.078	0.002	0.000
BOP (%)				
r	0.374	0.386	0.602	0.614
p	0.094	0.080	0.000	0.000
Gingival crevicular fluid volume (μL)				
r	0.414	0.478	0.865	0.704
p	0.076	0.042	0.000	0.000

BOP, bleeding on probing; CAL, clinical attachment level; GI, gingival index; PI, plaque index; *r*, correlation coefficient.
Correlation was significant at the 0.05 level.

AM levels in individuals with periodontal diseases are thought to be a result of the stimulation of AM secretion by lipopolysaccharides in compromised tissues. In our study, individuals were grouped and compared in terms of periodontal health, periodontal diseases and the presence of DM. Our observations were similar to those of Lundy *et al*. (13) and the total AM levels in individuals with chronic periodontitis were significantly higher than were those in periodontally healthy individuals. These higher levels of AM in individuals with chronic periodontitis are thought to be a consequence of increased secretion from endothelial cells as a result of damage in periodontally diseased tissues.

When the periodontal clinical indices (PI, GI, probing depth, CAL and BOP) were evaluated within the groups, the scores of the individuals diagnosed with chronic periodontitis were higher compared with those who were periodontally healthy. In the DM evaluation, the periodontal clinical index (PI, GI, probing depth, CAL and BOP) scores were highest in the chronic periodontitis group with T2DM. It is believed that individuals with DM have an altered tissue response to dental plaque and to the microorganisms inside the dental plaque, and, in connection with this, a higher degree of inflammation can be observed. The hyperglycemia present in DM distorts cellular functions (40). The reason for hyperglycemia is that the tissues are nonenzymatically glycated. The highly increased glucose concentration in the blood can lead to the nonenzymatic formation of compounds in the blood cells and the tissues (18,41). As a result, severe glycation end-products are established. The establishment of severe glycation end-products is one of the major mechanisms that leads to diabetic complications caused by hyperglycemia (42). Inflammatory cells, such as monocytes and macrophages, have receptors for severe glycation end-products. The accumulation of severe glycation end-products in these cells increases the sensitivity to periodontopathogens (20). The interference between the severe glycation end-products on the inflammatory cells and the receptors also leads to an increase in the production of pro-inflammatory cytokines. The actual effect of DM on periodontal tissue is the change in the immuno-inflammatory response and tissue homeostasis. When the concentration of the severe glycation end-products in serum increases, they can pass through the gingival crevicular fluid (42). The severe glycation end-products inside the gingival crevicular fluid may cause pathological changes in the periodontium by leading to oxidative stress, vascular injury and an increase in cytokines. Higher amounts of periodontal tissue loss can be observed in connection with these changes. This was found in the present study through the high clinical indices (PI, GI, probing depth, CAL and BOP) scores in the chronic periodontitis groups with periodontally healthy groups.

Total AM and the concentration of AM were used in our study. It has been reported that it would be more beneficial to use the total values instead of the concentration data as the volume of gingival crevicular fluid was very low and the total values were added to the correlation (41). Also, our study found a positive correlation between the volume of gingival crevicular fluid and the level of AM. The gingival crevicular fluid volume increases in serum exudates in parallel with an increase in gingival inflammation (41). The gingival crevicular fluid volume was highest in the individuals with T2DM, and some researchers obtained similar results to those obtained in our study (43). The gingival crevicular fluid volume was highest in the individuals with chronic periodontitis and T2DM. It is thought that the high AM levels in the two T2DM groups might not be caused the increase gingival crevicular fluid volume. The reason for this is that even though the gingival crevicular fluid volume was found to be lower in periodontally healthy individuals with T2DM than in chronic periodontitis individuals with no T2DM, the AM level was higher in the periodontally healthy individuals with T2DM than in the individuals with chronic periodontitis but no T2DM. This conclusion does not support the hypothesis that the amounts of AM are higher in individuals with a large volume of gingival crevicular fluid. It has been found, in the literature, that in individuals with chronic periodontitis and DM, the HbA1c level is reduced following periodontal treatment (44). As the data following periodontal treatment of the individuals were not recorded in our study, we did not make any comparisons before and after treatment. The HbA1c data of the individuals with T2DM who participated in our study were found to be statistically identical in both groups (periodontally healthy subjects with T2DM and subjects with chronic periodontitis and T2DM). When the total AM and HbA1c levels in gingival crevicular fluid were compared, a positive correlation between the HbA1c level and the AM level was found in all individuals. It was also determined, in our study, that the total AM levels were high in groups (periodontal healthy subjects with T2DM and subjects with chronic periodontitis and T2DM) whose HbA1c critical level was higher than 7% for DM. It is believed that the reason why the total AM level was higher, especially in the periodontally healthy group with T2DM compared with the chronic periodontitis group with non T2DM, is because of high HbA1c level in the periodontally healthy group with T2DM more than the AM level gingival inflammation and that the HbA1c level is high.

Türkoglu *et al*. (14) compared the AM levels in the gingival crevicular fluid of different individuals diagnosed with periodontitis. They (14) also found a positive correlation between the AM levels in gingival crevicular fluid and clinical periodontal parameters. Additionally, they (14) found that the total AM level in the gingival crevicular fluid of the chronic periodontitis group was significantly higher compared with the total AM level in the gingivitis and periodontally healthy groups. Moreover, Türkoglu *et al*. (14) found no

significant difference in total AM levels between gingivitis and periodontal healthy groups. We obtained results similar to those of Türkoglu *et al*. (14). We also found a positive correlation between periodontal parameters and AM levels. Higher AM levels were found in the chronic periodontitis group than in the periodontally healthy group. However, in contrast to Türkoglu *et al*. (14), we included individuals with T2DM in our study.

In conclusion, irreversible tissue damage occurs in individuals with DM. As a result, severe inflammation develops in periodontal tissues. Within the limits of the present data, it is suggested that DM might play a role in the elevated gingival crevicular fluid AM levels found in patients with chronic periodontitis. Determination of the levels of AM in gingival crevicular fluid is thought to help define the pathogenesis of both disease groups and to facilitate the understanding of possible treatment methods for these diseases. Further studies are needed to understand fully the effect of AM in periodontal diseases and DM.

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