Journal of
PERIODONTAL RESEARCH

J Periodont Res 2013; 48: 475–482

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/jre.12029

Gingival crevicular fluid levels of human beta-defensins 1 and 3 in subjects with periodontitis and/or type 2 diabetes mellitus: a cross-sectional study

A. S. Ertugrul, A. Dikilitas, H. Sahin, N. Alpaslan, A. Bozoglan, Y. Tekin
Department of Periodontology, Faculty of Dentistry, Yuzuncu Yil University, Van, Turkey

A. S. Ertugrul, A. Dikilitas, H. Sahin, N. Alpaslan, A. Bozoglan, Y. Tekin. Gingival crevicular fluid levels of human beta-defensins 1 and 3 in subjects with periodontitis and/or type 2 diabetes mellitus: a cross-sectional study. J Periodont Res 2013; 48: 475–482. © 2012 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd

Background and Objective: Human β-defensins (hBDs) have a strong antibacterial action against various microorganisms, especially periodontal pathogens. The aim of this study was to compare the total levels of hBD-1 and hBD-3 in the gingival crevicular fluid of healthy patients with gingivitis (HG), healthy patients with chronic periodontitis (HP), patients with type 2 diabetes mellitus (DM) and gingivitis (DM2G) and patients with type 2 DM and chronic periodontitis (DM2P).

Material and Methods: A total of 80 patients were included: 20 HG, 20 HP, 20 DM2G and 20 DM2P. The levels of hBD-1 and hBD-3 in gingival crevicular fluid were measured using ELISA.

Results: The DM2P group had significantly higher periodontal clinical parameters at sites from which gingival crevicular fluid was collected compared with the other groups. The HG group had significantly lower periodontal clinical parameters within the gingival crevicular fluid-collected sites than did the HP, DM2G and DM2P groups. The gingival crevicular fluid of the DM2P group had significantly higher levels of total hBD-1 and hBD-3 than did that of the other groups; the hBD-1 and hBD-3 levels were significantly higher in the gingival crevicular fluid of the DM2G group than in that of the the non-DM type 2 groups (HG and HP). The gingival crevicular fluid of the HP group had significantly higher levels of total hBD-1 and hBD-3 in comparison with that of the HG group.

Conclusion: As a result of the observed vascular and cell activity changes that occur within patients diagnosed with DM, periodontal diseases become more severe. These changes hinder the migration and the ability of chemotactic factors and leukocytes to protect periodontal tissues from the effects of micro-

Abdullah Seckin Ertugrul, Department of Periodontology, Faculty of Dentistry, Yuzuncu Yil University, Van 65080, Turkey
Tel: +904322251764
Fax: +904322251747
e-mail: ertugrulseckin@yahoo.com, ertugrul@yyu.edu.tr

Key words: chronic periodontitis; diabetes mellitus type 2; gingivitis; human β-defensin-1; human β-defensin-3

Accepted for publication October 08, 2012

organisms. In order to eliminate microorganisms, the epithelial cells in patients with DM may release more hBD-1 and hBD-3 into the gingival crevicular fluid. Determining the amount of hBD-1 and hBD-3 in the gingival crevicular fluid of patients with and without DM will help to elucidate the relationship among hBD-1, hBD-3, DM and periodontal disease.

Introduction

Antimicrobial peptides are components of the innate immune system (1). The most important antimicrobial peptide group in humans is defensins. Defensins have the ability to inactivate many bacteria, fungi and protozoa, and some enveloped viruses (2). In humans, defensins can be subdivided into two families: α-defensins and β-defensins (3). Periodontal diseases destroy connective tissues as a consequence of bacteria–host interactions; thus, the amount of supportive tooth tissue decreases and tooth loss occurs. The development and the progression of periodontal disease occur in equilibrium with a patient's immune system and the presence and type of microorganisms (4). In periodontal tissues, the primary barrier against infection with microorganisms is the epithelium. Human β-defensin (hBD)-1 and hBD-3 play a significant role in the protection of periodontal tissues against microbes. Following contact of microorganisms with gingival epithelial cells, hBD-1, which is constitutively secreted, is activated by epithelial cells, which release hBD-3 (5). hBD-3 is expressed from epithelial cells following stimulation by a variety of microbial and host factors, such as periodontal pathogens (6, 7) or cytokines (8). hBD-1 shows microbicidal activity mainly against gram-negative bacteria, whereas hBD-3 shows microbicidal activity against many potentially pathogenic gram-positive bacteria and *Candida albicans* (3). hBD-1 and hBD-3 are thought to exert their antibacterial effects by creating permeability of the bacterial cellular membrane.

Diabetes mellitus (DM) is the common name for a group of metabolic diseases characterized by chronic hyperglycemia, which leads to dysfunction in proteins, fat metabolism- and carbohydrates as a result of the absolute deficiency of the release of insulin and/or its effect (10). DM is caused by a disorder in the production or utilization of insulin and is categorized into two main groups according to the type of insulin dependency (11). DM that is dependent on insulin is known as type 1 DM, and DM that is not dependent on insulin is named type 2 DM. The relationship between periodontal diseases and DM has been examined for many years (12, 13). Generally, it is known that periodontal diseases show rapid progress in patients who have been diagnosed with DM in comparison with patients without DM (14). It is also known that hyperglycemia has a detrimental effect on the normal functions of cells, such as chemotaxis, phagocytosis and the production and secretion of cytokines, in the periodontal tissues of patients with DM (15). Patients with DM also develop a thickening of the basal membrane in periodontal tissue and experience changes in vascularity (14). Vascular changes affect the distribution of nutrients in the gingival tissue and the migration of leukocytes. Depending on this situation, decreases in the elimination of metabolic waste and in the diffusion of oxygen occur, thus increasing the severity of periodontal diseases and decreasing the wound-healing capacity (16).

Increased levels of advanced glycation end-products (AGEs) in DM impart detrimental effects on fibroblasts and endothelial cells (17). The numbers of microorganisms increase as a result of the reduced secretion and production of leukocytes and cytokines, and the lower levels of chemotaxis and phagocytosis, in the periodontal tissues of patients with DM (14). It is known that higher levels of β-defensins are released in patients treated with insulin compared with patients who are not receiving such treatment. Additionally, it has been determined that high levels of hyperglycemia reduce the expression and function of hBD-3 in epithelial cells (18).

However, the roles of hBD-1 and hBD-3 against microorganisms in patients diagnosed with both DM and periodontal diseases are not yet fully understood. Few studies have analyzed the relationship among hBDs, DM and periodontal disease, and some questions remain unanswered. Therefore, we hypothesized that owing to the detrimental effects of DM on periodontal tissues, antimicrobial activity is decreased. For this reason, in patients with DM, increased amounts of hBD-1 and hBD-3 are released into the gingival crevicular fluid by epithelial cells in order to eliminate microorganisms. The effect of DM on the release of hBD-1 and hBD-3 is observed both in patients with gingivitis and in those with chronic periodontitis. The aim of this study was to compare hBD-1 and hBD-3 levels in the gingival crevicular fluid of four groups of subjects – healthy subjects with gingivitis, healthy subjects with chronic periodontitis, patients with type 2 DM and gingivitis and patients with type 2 DM and chronic periodontitis – using an ELISA.

Materials and methods

Patients

The 80 adult study subjects were classified into the following four groups: healthy subjects with gingivitis ($n = 20$; 11 men and nine women) (HG); healthy subjects with chronic periodontitis ($n = 20$; 10 men and 10 women) (HP); patients with type 2

DM and gingivitis ($n = 20$; nine men and 11 women) (DM2G); and patients with type 2 DM and chronic periodontitis ($n = 20$; nine men and 11 women) (DM2P) (Table 1). All measurements were performed by a single calibrated examiner (ASE). The periodontal status was evaluated by measuring the plaque index (19), the gingival index (20), probing depth, clinical attachment level (CAL) and bleeding on probing (BOP). Probing depth was defined, for each tooth, as the distance from the free gingival margin to the base of the periodontal pocket. The probing depth and CAL measurements were recorded at six points around each tooth.

The groups of patients with type 2 DM consisted of patients whose conditions are controlled in endocrinology clinics and who receive periodontal treatment in a periodontal clinic. We ensured that patients in the type 2 DM study groups had been diagnosed with DM at least 1 year before the study, in accordance with the American Diabetes Association 2009 criteria (21).

The patients with gingivitis and chronic periodontitis were selected in accordance with the clinical radiographic criteria proposed by the 1999 International Workshop for a Classification of Periodontal Diseases and Conditions (22). The criteria for patient selection consisted of the following: no medical complications (excluding DM in the appropriate groups); no smoking habits; no use of medications (excluding DM medications) that could affect periodontal status; no antibiotic use for at least 6 mo before the start of sampling; not pregnant; and at least 20 teeth in occlusion. The inclusion criteria for the patients with chronic periodontitis were as follows: inflammation in gingiva; supragingival vs. subgingival calculus and microbial dental-plaque formation; vertical and horizontal bone loss in radiographic examination; probing depth of $\geq$ 5 mm in at least six total sites of at least four teeth with one root; and CAL of $\geq$ 4 mm (23,24). The inclusion criteria for the gingivitis patients were as follows: BOP of at least 50% of the total gingiva; and no vertical or horizontal bone loss at radiographic examinations (bone crest at > 95% of the proximal tooth sites and < 3 mm between the cemento–enamel junction) (24,25). The Ethics Committee in Clinical Research of Yuzuncu Yil University approved the study protocols, including the periodontal examination and gingival crevicular fluid sampling (YYU-110411). Each subject read and signed an informed consent document and read the Helsinki Declaration before participating in the study.

Gingival crevicular fluid sampling

Four gingival crevicular fluid samples were obtained from four Ramfjord sample teeth (teeth 1.6, 2.4, 3.6 and 4.4; if not in the oral cavity, teeth 1.1 and 4.1) from all patients before periodontal treatment and periodontal probing were carried out. The gingival crevicular fluid samples were obtained from the mesiobuccal site of the tooth. Following isolation of the site with cotton rolls to prevent contamination with saliva, supragingival plaque was removed, the tooth was air dried and a 30-s gingival crevicular fluid sample was collected on filter-paper strips (Periopaper, Interstate Drug Exchange, Amityville, NY, USA). The gingival crevicular fluid samples were calibrated using a Periotron 8000 (Oraflow Inc., Plainview, NY, USA). The samples were immediately placed in Eppendorf tubes that contained 500 μL of buffer (50 mM Tris–HCl, pH 7.4, 200 mM NaCl, 10 mM $CaCl_2$ and 0.02% Triton X-100), transported to the laboratory and stored at −80°C.

Collection of DM diagnostic data

Venous blood samples were obtained from all individuals who participated in the study. The blood samples were stored at room temperature for approximately 30 min and then centrifuged. The glycosylated hemoglobin (HbA1c) and fasting plasma glucose levels were measuerd to evaluate glycemic control. Additionally, demographic information (i.e. age, gender, measurement of the circumference of the waist and the hip, and the body

Table 1. Demographic characteristics and clinical periodontal parameters of the study groups

Demographic characteristics	Study groups Healthy subjects with gingivitis(HG) ($n = 20$)	Healthy subjects with chronic periodontitis(HP) ($n = 20$)	Patients with gingivitis and diabetes mellitus type 2(DM2G)($n = 20$)	Patients with chronic periodontitis and diabetes mellitus type 2(DM2P) ($n = 20$)
Age (years)	39.42 ± 5.24	40.82 ± 7.24	42.74 ± 6.66	43.47 ± 8.75
Gender (female/male)	11/9	10/10	9/11	9/11
Gingival index	1.24 ± 0.13[bcd]	1.91 ± 0.19[acd]	1.6 ± 0.11[abd]	2.35 ± 0.24[abc]
Plaque index	1.51 ± 0.20[bcd]	2.12 ± 0.29[acd]	1.81 ± 0.22[abd]	2.53 ± 0.29[abc]
Probing depth (mm)	1.92 ± 0.26[bcd]	3.42 ± 0.19[acd]	2.31 ± 0.33[abd]	3.85 ± 0.41[abc]
Clinical attachment level (mm)	1.89 ± 0.24[bcd]	3.51 ± 0.27[acd]	2.22 ± 0.18[abd]	3.89 ± 0.47[abc]
Bleeding on probing	67.11 ± 4.42[bd]	82.32 ± 6.75[ac]	72.43 ± 5.23[bd]	91.51 ± 4.34[ac]

Values are given as mean ± standard deviation for the study sites in each group, unless indicated otherwise
[a]Significantly different from gingivitis, $p < 0.05$.
[b]Significantly different from chronic periodontitis, $p < 0.05$.
[C]Significantly different from gingivitis with diabetes mellitus type 2, $p < 0.05$.
[d]Significantly different from chronic periodontitis with diabetes mellitus type 2, $p < 0.05$.

mass index) was recorded for the entire study group. The HbA1c levels in the serum samples were measured using high-performance liquid chromatography. The duration of DM for individuals diagnosed with type 2 DM was calculated in terms of body mass index (kg/height in m^2). The circumference of the waist and the hip was measured using the measurement points for the circumference of the waist, as proposed by the World Health Organization, and the data were recorded.

hBD-1 and hBD-3 ELISAs

The total levels of hBD-1 and hBD-3 in the gingival crevicular fluid samples were assayed using a hBD-1 and hBD-3 sandwich ELISA kits (Alpha Diagnostic Inc., San Antonio, TX, USA). The minimum detection limits were 50 pg/mL for both hBD-1 and hBD-3. All assay procedures were conducted according to the manufacturer's instructions. The ELISA plates were then immediately assessed at 450 nm in an ELISA Reader (ChroMate® Microplate Reader Awareness, Palm City, FL, USA). The total amounts of gingival crevicular fluid hBD-1 and hBD-3 that were collected in 30 s.

Data analysis

The following clinical data were available for each tooth: visible plaque index; gingival index; probing depth; BOP; and CAL. The levels of hBD-1 and hBD-3 were measured in up to four gingival crevicular fluid samples per subject and expressed as pg/30 s. Different groups of patients were tested using a nonparametric test (the Mann–Whitney *U*-test with Bonferroni correction). The association between the periodontal parameters and the levels of hBD-1 and hBD-3 was tested for all sites by determining Spearman's rank correlation test coefficients. The level of significance was set at $p < 0.05$ with a 95% confidence interval.

Results

The ages and genders of the subjects in each study group are shown in Table 1. There were no significant differences in gender or age between the HG group and the DM2G group or between the HP group and the DM2P group ($p > 0.05$). The periodontal clinical parameters (plaque index, gingival index, probing depth, BOP and CAL) of the sites from which gingival crevicular fluid was collected are shown in Table 1. These clinical parameters were significantly higher in the DM2P group than in the other groups ($p < 0.05$) and significantly lower in the HG group than in the other groups ($p < 0.05$). Moreover, these clinical parameters were significantly higher in the DM2G group than in the HG group, and significantly higher in the HP group than in the DM2P group ($p < 0.05$).

Figure 1 shows the total gingival crevicular fluid hBD-1 levels in all groups. The total concentration of hBD-1 was measured in the gingival crevicular fluid samples obtained from the patients who participated in this study and was higher than 50 pg/mL (the minimum detection limit of the ELISA kit). The DM2P group had significantly higher levels of hBD-1 compared with the other groups ($p < 0.05$). The hBD-1 level was significantly higher in the DM2G group than in the non-type 2 DM groups (i.e. HG and HP groups) ($p < 0.05$). The HP group had significantly higher levels of hBD-1 in comparison with the HG group ($p < 0.05$).

Figure 2 shows the total amount of gingival crevicular fluid hBD-3 in all four study groups. The concentration of hBD-3 was measured in the gingival crevicular fluid samples obtained from all patients who participated in this study and was found to be higher than 50 pg/mL (the minimum detection limit of the ELISA kit). When these results were analyzed, the DM2P group had significantly higher levels of total hBD-3 in comparison with the other groups ($p < 0.05$), as found for gingival crevicular fluid total hBD-1 levels. The hBD-3 level was significantly higher in the DM2G group than in the non-type 2 DM groups (i.e. HG and HP groups) ($p < 0.05$). The HP group had significantly higher levels of total hBD-3 in comparison with the HG group ($p < 0.05$).

In the patients diagnosed with type 2 DM who participated in the study, the duration of DM was 5.7 ± 3.2 years in the DM2G group and 5.9 ± 1.9 years in the DM2P group; the body mass index in the DM2G and DM2P groups was 26.0 ± 2.0 kg/m^2 and 25.0 ± 1.8 kg/m^2, respectively; the fasting plasma glucose level in the DM2G and DM2P groups was 150.0 ± 6.4 mg/dL and 151.0 ± 7.3 mg/dL, respectively; and the glycated hemoglobin level in the DM2G and DM2P groups was 7.01% and 7.12%, respectively. When DM duration, body mass index, fasting plasma glucose and HbA1c data were compared between the DM2G and DM2P groups, no significant difference was observed ($p > 0.05$).

The correlations between periodontal clinical parameters and the total gingival crevicular fluid hBD-1 and hBD-3 levels are shown in Table 2. The total gingival crevicular fluid hBD-1 and hBD-3 levels were positively correlated with the periodontal clinical parameters of the gingival crevicular fluid-collected sites (i.e. gingival index, plaque index, probing depth, BOP and CAL).

Discussion

Studies that evaluate antimicrobial peptides in patients with periodontal disease and/or DM are available in the literature (26,27). However, there is a paucity of information about hBD-1 and hBD-3 expression in DM. The present study hypothesized that DM could play an important role in the secretion of hBD-1 and hBD-3 from the epithelial tissue to gingival crevicular fluid within patients who have gingivitis and chronic periodontitis. This is the first study to compare the hBD-1 and hBD-3 levels in gingival crevicular fluid with clinical periodontal parameters in patients with and without DM.

In this study, the hBD-1 and hBD-3 levels in the DM groups (DM2G and DM2P) were significantly higher than in the non-DM groups (HG and

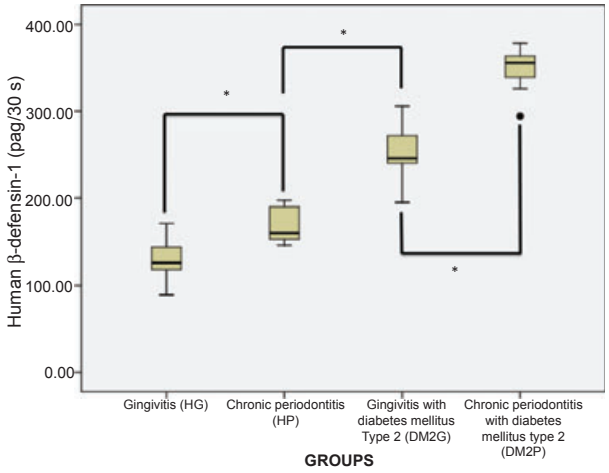

Fig. 1. Human β-defensin-1 level in the gingival crevicular fluid of gingivitis, chronic periodontitis, type 2 diabetus mellitus gingivitis and type 2 diabetus mellitus chronic periodontitis measured with ELISA-technique. *The groups are statistically different from each other, $p < 0.05$.

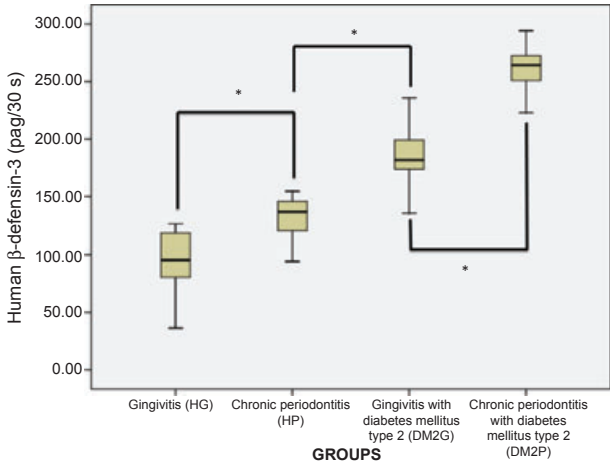

Fig. 2. Human β-defensin-3 level in the gingival crevicular fluid of gingivitis, chronic periodontitis, type 2 diabetus mellitus gingivitis and type 2 diabetus mellitus chronic periodontitis measured with ELISA-technique. * The groups are statistically different from each other, $p < 0.05$.

HP). These study results support our hypothesis. However, some limitations of the present study should be considered. The first limitation was the number of patients who participated in it: 80 in total (i.e. four groups of 20 patients). The reason why the patient numbers in the study were so low is that the inclusion criteria were very restrictive. According to the criteria, only nonsmoking patients were included in the study as cigarette smoking decreases the protective effect of the epithelium against microorganisms. Furthermore, the migration of inflammatory cells decreases, and hBD-1 and hBD-2 release is increased (28,29), in individuals who smoke. It has been found that the expression of hBD-1 and hBD-2 is higher in smokers than in nonsmokers (28,30). Another criterion for inclusion in this study was that patients with DM must have their condition under control as periodontal diseases are more severe in patients whose DM is not controlled than in patients whose DM is under control (14). Moreover, colonization of periodontopathogenic microorganisms in the

Table 2. Correlations between the levels of human β-defensin-1 and human β-defensin-3 in the gingival crevicular fluid and the clinical periodontal parameters (in the sample sites) of the study groups

Clinical periodontal parameters	Human β-defensin-1 (pg/30 s)	Human β-defensin-3 (pg/30 s)
Gingival index		
r	0.712	0.703
p	0.000	0.000
Plaque index		
r	0.665	0.621
p	0.005	0.007
Probing depth		
r	0.699	0.701
p	0.000	0.000
Clinical attachment level		
r	0.703	0.689
p	0.000	0.000
Bleeding on probing		
r	0.729	0.699
p	0.009	0.012

r, correlation coefficient; *p*, statistical significance. Correlation was significant at the 0.05 level.

periodontal pocket is higher in patients who do not have their DM under control than in those with controlled DM (31,32). The cytokine expression levels are higher in the gingiva in patients who do not have their DM under control than in those with controlled DM (32). The second limitation of the present study was that the patients in the present study did not have any systemic diseases, except for those associated with DM (i.e. hypertension, cardiovascular disease and kidney diseases). Systemic diseases both increase the severity of periodontal diseases and affect the amount of biomarkers in the gingival crevicular fluid (33). In addition to these criteria, patients who were not pregnant and had at least 20 teeth in occlusion were selected to participate in the study. We believe that restrictive inclusion criteria are important in studies that compare subjects with and without DM. It is difficult to find patients who comply with the aforementioned criteria, which is why the number of study participants was limited to 80.

This study used gingival crevicular fluid samples instead of saliva when

evaluating hBD-1 and hBD-3. Gingival crevicular fluid is an exudate that passes from the microcirculation in connective tissue through inflamed periodontal tissues to the gingival sulcus. Gingival crevicular fluid contains tissue-damaging enzymes, cytokines and bacterial- and tissue-destruction products (34).

In the literature, there are studies that have examined the relationship between hBD-1 and/or hBD-3 and periodontal diseases; however, in these studies the hBD-1 and/or hBD-3 values in patients with periodontitis and compromised periodontal health were compared using different methods, and inconsistent results were obtained. When such studies were examined, the levels of hBD1 and/or hBD3 mRNAs within the gingival biopsy samples were found to be lower than or similar to the levels in inflamed tissues compared with healthy tissues (4,35–38). Despite these results, some investigators have found higher levels of hBD-1 and/or hBD-3 in gingival biopsies of patients with periodontitis compared with periodontally healthy patients (39–41). In the present study, the gingival crevicular fluid hBD-1 level was found to be significantly higher in the HP group than in the HG group. We found that the hBD-3 levels in gingival crevicular fluid were significantly higher in patients with HP than in those with HG, and in those with DM2P than in those with DM2G. One reason why the results of the present study resemble or differ from those previously published could be that instead of utilizing gingival tissue samples, we analyzed gingival crevicular fluid; therefore, some bacteria might have developed resistance to hBD-1 and hBD-3. Furthermore, the levels of hBD-1 and hBD-3 may vary in the gingival crevicular fluid (42), and genetic polymorphisms (4) may have occurred.

Although there is currently no explanation for all the effects related to DM, type 1 DM is an autoimmune disease (43), and chronic inflammation is an effective mechanism in the pathogenesis of type 2 DM (44). Therefore, it was thought that the relationship between periodontal diseases and type 2 DM would be stronger than the relationship between periodontal diseases and type 1 DM. Consequently, we believed that it would be more appropriate to include patients with type 2 DM in the present study, and we excluded patients with type 1 DM. The primary treatment target seeks to prevent the organ damage caused by DM and other possible complications and to provide glycemic control (45). In order to provide and maintain glycemic control, the HbA1c levels of patients with DM have to be measured. The HbA1c level is an indicator that is used in evaluating the 2- to 3-mo metabolic glycemic control, and this indicator has been proven to be reliable (45). For good metabolic control, the HbA1c level should be below 7% (21,45). The serum HbA1c levels of the patients with type 2 DM who participated in the present study were measured and the results were included. When the HbA1c level and other values that are related to DM were compared for the two groups of patients with type 2 DM in the present study (i.e. DM2G and DM2P), no statistically significant differences were found. Thus, it is possible to compare the hBD-1 and hBD-3 levels of the two groups. DM and periodontal diseases affect each other.

The excessive increase in blood glucose levels can form compounds that are not enzymatically degraded within blood cells and tissues. As a result, AGEs are formed in tissues (46). When the concentrations of AGEs increase in the serum, more AGEs are secreted into the gingival crevicular fluid (14). AGEs usually form a macromolecule on collagen. This macromolecule sticks to the peripheral walls of the veins and causes the basal membrane to thicken and the vein lumen to become narrower. As a result, the feeding of tissue deteriorates. AGEs also lead to increased production of polymorphonuclear neutrophil superoxide (47,48). The polymorphonuclear neutrophils of patients with DM have been noted to have increased adherence to the endothelium and spontaneous activation that may render them ineffective to a microbial challenge. Hyperglycemia has been shown to be associated with reduced polymorphonuclear neutrophil degranulation (49), but not always with reduced polymorphonuclear neutrophil chemotaxis (50). In addition, the decrease in collagen production in the presence of hyperglycemia, the increase of collagenase activity and the formation of AGEs weakens the defense system of the periodontal tissue (10,46,51). Moreover, in the presence of hyperglycemia, increased apoptosis is observed of cells (e.g. fibroblasts and osteoblasts) that are responsible for the formation of the matrix between tissues (52). When apoptosis is triggered on the surface of monocytes, the signalling within the cell changes (i.e. oxidative stress and activation of the transcription factor, nuclear factor-kappa B, increases), which causes the monocyte/macrophage to produce more proinflammatory cytokines (53). It has been noted that the presence of high levels of AGEs in periodontal tissue leads to a decrease in the production of collagen and glycosaminoglycans (54). As a result of the changes in the oral microflora, a decrease in the production of collagen and an increase in collagenase activity occurs, which leads to an increase in periodontal tissue destruction (55). The tissue changes found in patients with DM as a result of metabolic instability decrease the ability of the host to cope with plaque pathogens, leading to severe gingival inflammation, which is not proportional to the amount of plaque present (51). Thus, tissue-production functions decrease as necrosis increases, which causes greater periodontal destruction in patients diagnosed with DM (14).

Our study hypothesis was that the total concentration of hBD-1 and hBD-3 would be higher in the gingival crevicular fluid of patients with DM than in patients without DM, and that the increased the total concentration of hBD-1 and hBD-3 are caused by complications within the periodontal tissues of DM patients. The results of the present study support this hypothesis. The reasons why the total

concentration of hBD-1 and hBD-3 were higher in the gingival crevicular fluid of patients diagnosed with DM were determined to be caused by the deterioration of the vascular structure as a result of the increased accumulation in AGEs in the periodontal tissues. Furthermore, cells, such as polymorphonuclear neutrophils, are unable to function properly and thus are incapable of mounting a sufficient defense against microorganisms. Correspondingly, there is an increase in the number of microorganisms within the dental plaque and increased amounts of hBD-1 and hBD-3 could have been released from the gingival tissue in order to prevent such an increase.

There are no studies in which the levels of hBD-1 and hBD-3 have been compared. Most studies have been conducted primarily on animal models. Page and Malik (56) found that the high mRNA levels of rat β-defensin 1 (rBD-1) are elevated in diabetic rats vs. Wistar control rats. In another study in which the expression levels of rBD-1 were compared between groups in which diabetic rats were treated with insulin, low expression levels of rBD-1 were found in the insulin-treated rats (57). Lan *et al.* (18) determined the molecular effects of a high-glucose environment on hBD-3 expression in their skin-lesion models; in their results, they found that treating keratinocytes with glycation end-products significantly decreased the levels of hBD3 produced, although hyperglycemic conditions inhibited constitutive expression of hBD-3 on keratinocytes and resulted in inadequate up-regulation of hBD-3 upon stimulation. Hiratsuka *et al.* (58) found that the levels of rBD-1 mRNA were significantly lower in the kidneys of several diabetic rodent models than they were among their lean littermates. In the present study, the gingival crevicular fluid samples from patients were compared. The varied results obtained in different studies are probably a result of the different types of models employed.

Therefore, within the limits of the present study, the decrease in new tissue formation within the periodontal tissues of patients with DM, and the tendency of such tissues to deteriorate, as well as the decrease in the defense system of periodontal tissues and vascular changes in patients with type 2 DM, may lead to the secretion of higher levels of hBD-1 and hBD-3 into the gingival crevicular fluid. Determining the total concentration of hBD-1 and hBD-3 in the gingival crevicular fluid of patients with different periodontal prognoses will help to define the response of soft tissues to periodontally important pathogenic microorganisms and elucidate the treatment prognosis. In addition, the present data support the concept that the pathogenesis of chronic periodontitis and that of diabetes-related periodontitis are distinct. Future studies will be directed toward the examination of the relevance of chronic periodontitis and DM, as well as the use of specific drugs that may moderate the host response to periodontal disease in patients with DM.

Acknowledgments

This study was self-funded by the authors and by the University of Yuzuncu Yil University (Committee of Research 2010-DF-B010). The authors thank the School of Dentistry at Yuzuncu Yil University for its support.

References

1. Zasloff M. Antimicrobial peptides of multicellular organisms. *Nature* 2002;**415**:389–395.
2. Froy O. Regulation of mammalian defensin expression by Toll-like receptor-dependent and independent signalling pathways. *Cell Microbiol* 2005;**7**: 1387–1397.
3. Scott MG, Hancock RE. Cationic antimicrobial peptides and their multifunctional role in the immune system. *Crit Rev Immunol* 2000;**20**:407–431.
4. Brancatisano FL, Maisetta G, Barsotti F *et al.* Reduced human beta defensin 3 in individuals with periodontal disease. *J Dent Res* 2011;**90**:241–245.
5. Gursoy UK, Kononen E. Understanding the roles of gingival beta-defensins. *J Oral Microbiol* 2012;**4**:1–10.
6. Feucht EC, DeSanti CL, Weinberg A. Selective induction of human beta-defensin mRNAs by Actinobacillus actinomycetemcomitans in primary and immortalized oral epithelial cells. *Oral Microbiol Immunol* 2003;**18**:359–363.
7. Vankeerberghen A, Nuytten H, Dierickx K, Quirynen M, Cassiman JJ, Cuppens H. Differential induction of human beta-defensin expression by periodontal commensals and pathogens in periodontal pocket epithelial cells. *J Periodontol* 2005;**76**:1293–1303.
8. Joly S, Organ CC, Johnson GK, McCray PB Jr, Guthmiller JM. Correlation between beta-defensin expression and induction profiles in gingival keratinocytes. *Mol Immunol* 2005;**42**:1073–1084.
9. Wimley WC, Selsted ME, White SH. Interactions between human defensins and lipid bilayers: evidence for formation of multimeric pores. *Protein Sci* 1994;**3**:1362–1373.
10. Pucher J, Stewart J. Periodontal disease and diabetes mellitus. *Curr Diab Rep* 2004;**4**:46–50.
11. Satman I, Yilmaz T, Sengul A *et al.* Population-based study of diabetes and risk characteristics in Turkey: results of the turkish diabetes epidemiology study (TURDEP). *Diabetes Care* 2002;**25**:1551–1556.
12. de Pommereau V, Dargent-Pare C, Robert JJ, Brion M. Periodontal status in insulin-dependent diabetic adolescents. *J Clin Periodontol* 1992;**19**:628–632.
13. Lakschevitz F, Aboodi G, Tenenbaum H, Glogauer M. Diabetes and periodontal diseases: interplay and links. *Curr Diabetes Rev* 2011;**7**:433–439.
14. Mealey BL, Oates TW. Diabetes mellitus and periodontal diseases. *J Periodontol* 2006;**77**:1289–1303.
15. Delamaire M, Maugendre D, Moreno M, Le Goff MC, Allannic H, Genetet B. Impaired leucocyte functions in diabetic patients. *Diabet Med* 1997;**14**:29–34.
16. Hirsch IB, Farkas-Hirsch R, Skyler JS. Intensive insulin therapy for treatment of type I diabetes. *Diabetes Care* 1990;**13**:1265–1283.
17. Alikhani Z, Alikhani M, Boyd CM, Nagao K, Trackman PC, Graves DT. Advanced glycation end products enhance expression of pro-apoptotic genes and stimulate fibroblast apoptosis through cytoplasmic and mitochondrial pathways. *J Biol Chem* 2005;**280**:12087–12095.
18. Lan CC, Wu CS, Huang SM, Kuo HY *et al.* High-Glucose Environment Inhibits p38MAPK Signaling and Reduces Human β-Defensin-3 Expression [corrected] in Keratinocytes. *Mol Med* 2011;**17**:771–779.
19. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between Oral Hygiene and Periodontal Condtion. *Acta Odontol Scand* 1964;**22**:121–135.
20. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and Severity. *Acta Odontol Scand* 1963;**21**:533–551.

21. Anonymous. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2009;**32** Suppl 1:S62–67.22.
22. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999;**4**: 1–6.
23. Ozturk A, Yildiz L. Expression of transient receptor potential vanilloid receptor 1 and toll-like receptor 4 in aggressive periodontitis and in chronic periodontitis. *J Periodontal Res* 2011;**46**: 475–482.
24. Pradeep AR, Raghavendra NM, Prasad MV, Kathariya R, Patel SP, Sharma A. Gingival crevicular fluid and serum visfatin concentration: their relationship in periodontal health and disease. *J Periodontol* 2011;**82**:1314–1319.
25. Sert T, Kirzioglu FY, Fentoglu O, Aylak F, Mungan T. Serum placental growth factor, vascular endothelial growth factor, soluble vascular endothelial growth factor receptor-1 and -2 levels in periodontal disease, and adverse pregnancy outcomes. *J Periodontol* 2011;**82**:1735–1748.
26. Gorr SU. Antimicrobial peptides of the oral cavity. *Periodontol 2000* 2009;**51**: 152–180.
27. Nakamura T, Honda K, Ishikawa S, Kitamura K, Eto T, Saito T. Plasma adrenomedullin levels in patients with non-insulin dependent diabetes mellitus: close relationships with diabetic complications. *Endocr J* 1998;**45**:241–246.
28. Wolgin M, Liodakis S, Pries AR, Zakrzewicz A, Kielbassa AM. HBD-1 and hBD-2 expression in HaCaT keratinocytes stimulated with nicotine. *Arch Oral Biol* 2012;**57**:814–819.
29. Chen L, Sun BB, Wang T *et al*. Cigarette smoke enhances {beta}-defensin 2 expression in rat airways via nuclear factor-{kappa}B activation. *Eur Respir J* 2010a;**36**:638–645.
30. Mahanonda R, Sa-Ard-Iam N, Eksomtramate M *et al*. Cigarette smoke extract modulates human beta-defensin-2 and interleukin-8 expression in human gingival epithelial cells. *J Periodontal Res* 2009;**44**:557–564.
31. Makiura N, Ojima M, Kou Y *et al*. Relationship of Porphyromonas gingivalis with glycemic level in patients with type 2 diabetes following periodontal treatment. *Oral Microbiol Immunol* 2008;**23**:348–351.
32. Casarin RC, Barbagallo A, Meulman T *et al*. Subgingival biodiversity in subjects with uncontrolled type-2 diabetes and chronic periodontitis. *J Periodontal Res* 2012. [Epub ahead of print].
33. Chen H, Zheng P, Zhu H *et al*. Platelet-activating factor levels of serum and gingival crevicular fluid in nonsmoking patients with periodontitis and/or coronary heart disease. *Clin Oral Investig* 2010b;**14**:629–636.
34. Golub LM, Kleinberg I. Gingival crevicular fluid: a new diagnostic aid in managing the periodontal patient. *Oral Sci Rev* 1976:49–61.
35. Dunsche A, Acil Y, Dommisch H, Siebert R, Schroder JM, Jepsen S. The novel human beta-defensin-3 is widely expressed in oral tissues. *Eur J Oral Sci* 2002;**110**:121–124.
36. Bissell J, Joly S, Johnson GK *et al*. Expression of beta-defensins in gingival health and in periodontal disease. *J Oral Pathol Med* 2004;**33**:278–285.
37. Dommisch H, Acil Y, Dunsche A, Winter J, Jepsen S. Differential gene expression of human beta-defensins (hBD-1, -2, -3) in inflammatory gingival diseases. *Oral Microbiol Immunol* 2005;**20**:186–190.
38. Hosokawa I, Hosokawa Y, Komatsuzawa H *et al*. Innate immune peptide LL-37 displays distinct expression pattern from beta-defensins in inflamed gingival tissue. *Clin Exp Immunol* 2006; **146**:218–225.
39. Kuula H, Salo T, Pirila E *et al*. Human beta-defensin-1 and -2 and matrix metalloproteinase-25 and -26 expression in chronic and aggressive periodontitis and in peri-implantitis. *Arch Oral Biol* 2008;**53**:175–186.
40. Lu Q, Samaranayake LP, Darveau RP, Jin L. Expression of human beta-defensin-3 in gingival epithelia. *J Periodontal Res* 2005;**40**:474–481.
41. Vardar-Sengul S, Demirci T, Sen BH, Erkizan V, Kurulgan E, Baylas H. Human beta defensin-1 and -2 expression in the gingiva of patients with specific periodontal diseases. *J Periodontal Res* 2007;**42**:429–437.
42. Brissette CA, Simonson LG, Lukehart SA. Resistance to human beta-defensins is common among oral treponemes. *Oral Microbiol Immunol* 2004;**19**:403–407.
43. Ludwig DS, Ebbeling CB. Type 2 diabetes mellitus in children: primary care and public health considerations. *JAMA* 2001;**286**:1427–1430.
44. Nishimura F, Iwamoto Y, Mineshiba J, Shimizu A, Soga Y, Murayama Y. Periodontal disease and diabetes mellitus: the role of tumor necrosis factor-alpha in a 2-way relationship. *J Periodontol* 2003;**74**:97–102.
45. Anonymous . Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33) UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998;**352**:837–853.
46. Mealey BL, Moritz AJ. Hormonal influences: effects of diabetes mellitus and endogenous female sex steroid hormones on the periodontium. *Periodontol 2000* 2003;**32**:59–81.
47. Karima M, Kantarci A, Ohira T *et al*. Enhanced superoxide release and elevated protein kinase C activity in neutrophils from diabetic patients: association with periodontitis. *J Leukoc Biol* 2005;**78**:862–870.
48. Schmidt AM, Weidman E, Lalla E *et al*. Advanced glycation endproducts (AGEs) induce oxidant stress in the gingiva: a potential mechanism underlying accelerated periodontal disease associated with diabetes. *J Periodontal Res* 1996;**31**: 508–515.
49. Gallacher SJ, Thomson G, Fraser WD, Fisher BM, Gemmell CG, MacCuish AC. Neutrophil bactericidal function in diabetes mellitus: evidence for association with blood glucose control. *Diabet Med* 1995;**12**:916–920.
50. Donovan RM, Goldstein E, Kim Y *et al*. A computer-assisted image-analysis system for analyzing polymorphonuclear leukocyte chemotaxis in patients with diabetes mellitus. *J Infect Dis* 1987;**155**:737–741.
51. Kiran M, Arpak N, Unsal E, Erdoğan MF. The effect of improved periodontal health on metabolic control in type 2 diabetes mellitus. *J Clin Periodontol* 2005;**32**:266–722.
52. Liu-DeRyke X, Collingridge DS, Orme J, Roller D, Zurasky J, Rhoney DH. Clinical impact of early hyperglycemia during acute phase of traumatic brain injury. *Neurocrit Care* 2009;**11**:151–157.
53. Lalla E, Lamster IB, Drury S, Fu C, Schmidt AM. Hyperglycemia, glycoxidation and receptor for advanced glycation endproducts: potential mechanisms underlying diabetic complications, including diabetes-associated periodontitis. *Periodontol 2000* 2000;**23**:50–62.
54. Willershausen-Zönnchen B, Lemmen C, Hamm G. Influence of high glucose concentrations on glycosaminoglycan and collagen synthesis in cultured human gingival fibroblasts. *J Clin Periodontol* 1991;**18**:190–195.
55. Firatli E. The relationship between clinical periodontal status and insulin-dependent diabetes mellitus Results after 5 years. *J Periodontol* 1997;**68**:136–140.
56. Page RA, Malik AN. Elevated levels of beta defensin-1 mRNA in diabetic kidneys of GK rats. *Biochem Biophys Res Commun* 2003;**310**:513–521.
57. Froy O, Hananel A, Chapnik N, Madar Z. Differential expression of rat beta-defensins. *IUBMB Life* 2005;**57**:41–43.
58. Hiratsuka T, Nakazato M, Date Y, Mukae H, Matsukura S. Nucleotide sequence and expression of rat beta-defensin-1: its significance in diabetic rodent models. *Nephron* 2001;**88**:65–70.