ORAL DISEASES

Oral Diseases (2010) 16, 476–481. doi:10.1111/j.1601-0825.2010.01659.x

http://www.blackwellmunksgaard.com

ORIGINAL ARTICLE

Crevicular fluid matrix metalloproteinase-8, -13, and TIMP-1 levels in type 2 diabetics

L Kardeşler[1], B Bıyıkoğlu[1], Ş Çetinkalp[2], M Pitkala[3], T Sorsa[3], N Buduneli[1]

[1]*Department of Periodontology, School of Dentistry, Ege University, İzmir, Turkey;* [2]*Department of Metabolic Diseases and Endocrinology, School of Medicine, Ege University, İzmir, Turkey;* [3]*University of Helsinki, Institute of Dentistry and Department of Oral and Maxillofacial Diseases, Helsinki University Hospital, Helsinki, Finland*

OBJECTIVES: To evaluate whether type 2 diabetes mellitus (DM) enlarged and if so the quantum of such increase in the gingival crevicular fluid (GCF) levels of matrix metalloproteinase-8 (MMP-8), MMP-13 and tissue inhibitor of metalloproteinases-1 (TIMP-1).

METHODS: Subjects (*n* = 73) were divided into five groups as follows: 12 DM patients with gingivitis (DM-G), 12 DM patients with periodontitis (DM-P), 12 systemically healthy patients with gingivitis (H-G), 13 systemically healthy patients with periodontitis (H-P) and 24 periodontally, systemically healthy volunteer subjects (H-C). Full-mouth clinical periodontal measurements were performed at six sites per tooth. Gingival crevicular fluid samples were obtained from two sites representing the clinical periodontal diagnosis in single-rooted teeth. Gingival crevicular fluid levels of MMP-8, MMP-13 and TIMP-1 were analysed by immunofluorometric MMP assay (IFMA), enzyme-linked immunosorbent assay (ELISA). Data were tested statistically by parametric tests.

RESULTS: All clinical periodontal measurements were similar in both diabetic and systemically healthy patients with periodontal disease (all *P* > 0.05). Total amounts of MMP-8 in GCF samples were significantly lower in H-C group than DM-G, DM-P, H-P groups (all *P* < 0.05). Matrix metalloproteinase-13, TIMP-1 total amounts were similar in study groups (*P* > 0.05). Diabetes mellitus patients exhibited similar levels of MMP-8, MMP-13, TIMP-1 with systemically healthy gingivitis/periodontitis patients (*P* > 0.05).

CONCLUSIONS: Within the limits of this study, DM does not seem to significantly affect GCF levels of MMP-8, MMP-13, TIMP-1 or clinical periodontal status.

Oral Diseases (2010) **16**, 476–481

Keywords: diabetes mellitus; ELISA; gingival crevicular fluid; IFMA; MMP-8; MMP-13; periodontal disease; TIMP-1

Correspondence: Dr. Nurcan Buduneli, Department of Periodontology, School of Dentistry, Ege University, 35100 - Bornova, İzmir, Turkey. Tel: +90 232 388 11 05, Fax: +90 232 388 03 25, E-mail: nurcan.buduneli@ege.edu.tr
Received 25 October 2009; revised 19 November 2009; accepted 29 November 2009

Introduction

Type 2 diabetes mellitus (DM) represents a metabolic disorder of multiple aetiology, characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion (β-cell dysfunction), insulin action (insulin resistance) or both (Gerich, 2003). A progressive deterioration of β-cell function coupled with the addition of acquired insulin resistance for which the β-cell can not compensate causes this chronic metabolic disease. Fifty-four percent of type 2 DM patients present both insulin resistance and low insulin secretion (Gerich, 2003). Type 2 DM patients were 2.8 times more likely to have destructive periodontal disease (Emrich *et al*, 1991) and 4.2 times more likely to have alveolar bone loss progression (Taylor *et al*, 1998). Effects of diabetes on the periodontium have been discussed in a review paper (Mealey and Oates, 2006). In fact, periodontal disease has been considered to be another complication of DM (Löe, 1993) and evidence also supports the hypothesis of poor glycaemic control contributing to poor periodontal health (Ainamo *et al*, 1990; Ünal *et al*, 1993). Moreover, decreased salivary levels of reduced salivary glutathione were detected in diabetics suggesting a role in periodontal tissue destruction by predisposing tissues to oxidative stress (Gümüş *et al*, 2009).

Diabetes mellitus patients have elevated levels of advanced glycation end products (AGEs) in their gingival tissues that may be associated with a state of enhanced oxidative stress, a potential mechanism for accelerated tissue injury (Ryan *et al*, 2003). Advanced glycation end products can interact with specific receptors on cells, such as macrophages, stimulating the production of enzymes like MMPs and cytokines like IL-1β (Vlassara, 1992).

Periodontal disease is a common chronic infection-induced inflammatory disease, characterised by loss of the tooth supporting structures; connective tissue attachment and alveolar bone. Periodontopathogens are capable of triggering tissue destruction; induction of host cells to increase their matrix metalloproteinase

(MMP) release is among the mechanisms of this indirect tissue destruction. Matrix metalloproteinases are enzymes acting not only in physiological development and tissue remodelling but also in pathological tissue destruction. Matrix metalloproteinases can be divided into five major groups: collagenases (MMP-1, -8, -13), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, -10, -11), membrane-type MMPs (MMP-14, -15, -16, -17) and others (Sorsa *et al*, 2004).

Matrix metalloproteinase-8 is the major destructive MMP abundantly present in periodontally diseased gingiva and gingival crevicular fluid (GCF) (Ingman *et al*, 1996). The major cells producing MMP-8 are polymorphonuclear neutrophils (PMN), while gingival fibroblasts, endothelial cells, sulcular epithelial cells, plasma cells and odontoblasts can also express this enzyme (Hanemaaijer *et al*, 1997; Wahlgren *et al*, 2001). Matrix metalloproteinase-13 is another interstitial collagenase important in periodontal tissue destruction, and expressed by sulcular epithelial cells, macrophage-like cells, plasma cells and gingival fibroblasts in periodontitis lesion (Tervahartiala *et al*, 2000; Kiili *et al*, 2002; Hernandez *et al*, 2006). Matrix metalloproteinase-13 is also expressed in osteoblastic cell lines located adjacent to the osteoclasts at sites of active bone resorption (Fuller and Chambers, 1995). Tissue inhibitors of MMPs (TIMPs) regulate the activities of these enzymes. An imbalance between MMPs and TIMPs result in the pathological tissue destruction in periodontitis (Hannas *et al*, 2007). Tissue inhibitor of metalloproteinase-1 is more effective on interstitial collagenases.

Matrix-degrading MMPs, such as MMP-2, -8, and -9, have been implicated in plaque rupture through their capacity to thin the protecting fibrous cap of the plaque, thus rendering it more vulnerable (Dollery *et al*, 1995). Arteriosclerotic lesions in patients with diabetes mellitus are usually more vulnerable than lesions from non-diabetic subjects, thus potentially explaining their increased cardiovascular risk, but hitherto, nothing is known about the role of MMP serum levels in diabetic patients with coronary artery disease (Moreno *et al*, 2000).

Our hypothesis was that type 2 DM-related alterations in GCF levels of MMPs and/or their inhibitors may be part of the mechanisms by which diabetes affects periodontal health. Therefore, the aim of this study was to evaluate GCF levels of MMP-8, MMP-13, and TIMP-1 in type 2 DM patients with inflammatory periodontal disease.

Materials and methods

Study population

A total of 73 subjects were recruited for the present cross-sectional study. Twenty-four type 2 diabetic patients with periodontal disease (DM group) (9 males and 15 females, with a mean age of 49.00 ± 8.1 years), 25 otherwise healthy periodontally diseased patients (PD group) (14 males and 11 females, with a mean age of 50.00 ± 7.62 years), and 24 systemically and periodontally healthy control subjects (H group) (10 males and 14 females, with a mean age of 49.12 ± 6.64 years) were included in the study conducted during the period between May 2006 and June 2008. Diabetic patients were those consecutively referred during routine medical care visits from an outpatient diabetes clinic (Department of Metabolic Diseases and Endocrinology, School of Medicine, Ege University). All diabetic patients were diagnosed as having type 2 DM at least 1 year prior to the study using American Diabetes Association (2007), and were being treated with stable doses of oral hypoglycaemic agents and/or insulin by the same physician (ŞÇ). Patients were excluded if they had any known systemic diseases other than diabetes that can influence the periodontal status, a history of antibiotic therapy within the preceding 3 months or periodontal treatment within the last 6 months. Moreover, patients having less than 14 teeth were also excluded from the study. Smoking history was recorded but smokers were not excluded. The numbers of smokers in the study groups were as follows: 8, 14, and 14, respectively in the diabetic group, systemically healthy periodontally diseased group, and healthy control group. The patients in PD group were recruited from those patients seeking dental treatment in the School of Dentistry, Ege University. These patients were matched with the DM group with regard to age, smoking status and clinical periodontal diagnosis. These patients were free of any known systemic disease, were not taking any medication and had not received any antibiotics within the last 3 months and any periodontal treatment within the last 6 months. Volunteer subjects in the healthy control group drawn from the staff of Dental School were systemically and periodontally healthy and had neither history nor sign of periodontal disease, i.e., probing depths <3 mm with no attachment loss, no obvious clinical inflammation, and no bleeding on probing (BOP). The study was conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki. The study protocol was explained and written informed consent was received from each individual before their enrolment in the study.

Gingival crevicular fluid sampling

Gingival crevicular fluid samples were obtained from buccal aspects of two interproximal sites in single-rooted teeth in each individual participating in the study. Prior to GCF sampling, dichotomous plaque recording was performed as present or absent and supragingival plaque was then removed carefully by sterile curettes and the surfaces were dried and isolated by cotton rolls. Filter paper strips (Periopaper; ProFlow Inc., Amityville, NY, USA) were placed in the orifices of gingival sulcus/pocket for 30 s. Care was taken to avoid mechanical trauma and strips visually contaminated with blood were discarded. The absorbed GCF volume was estimated by a calibrated instrument (Periotron 8000; Oraflow Inc., Plainview, NY, USA). Then, the two strips from each patient were placed into one polypropylene tube before freezing at −40°C. The Periotron readings were converted to an actual volume (μl) by

reference to the standard curve. All GCF samples were stored at −40°C until the laboratory analyses.

Clinical periodontal measurements
Subsequent to the recording of dichotomous plaque index (PI) and GCF sampling, full-mouth clinical periodontal measurements were recorded at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual, mesio-lingual) including probing depth (PD), clinical attachment level (CAL), and dichotomous BOP score as present or absent in 20 s. Williams periodontal probe was used for clinical periodontal measurements and all measurements were performed by two precalibrated researchers (BB and LK) who were blinded to the systemic condition of the individual. Twelve of the 24 DM patients were diagnosed as having gingivitis, whereas 12 had chronic periodontitis. In the systemically healthy periodontal disease group, 12 patients had gingivitis and 13 patients had chronic periodontitis. The clinical periodontal diagnoses were assigned using the criteria defined at the International Workshop for a Classification of Periodontal Diseases and Conditions (Armitage, 1999).

Gingival crevicular fluid enzyme immunoassay

MMP-8 analysis by immunofluorometric assay (IFMA)
Collagenase-2 (MMP-8) levels in the GCF samples were determined by a time-resolved IFMA as described by Hanemaaijer *et al* (1997). The monoclonal MMP-8-specific antibodies 8708 and 8706 (Medix Biochemica Oy Ab, Kauniainen, Finland) were used as a catching and tracer antibody respectively. The tracer antibody was labelled using europium chelate. The assay buffer contained 20 mM Tris-HCl, pH 7.5, 0.5 M NaCl, 5 mM $CaCl_2$, 50 μM $ZnCl_2$, 0.5% bovine serum albumin, 0.05% sodium azide and 20 mg l^{-1} diethylene triamine penta-acetic acid (DTPA). Samples were diluted in assay buffer and incubated for 1 h, followed by incubation for 1 h with the tracer antibody. Enhancement solution was added, and after 5 min fluorescence was measured using a 1234 Delfia Research Fluoremeter (Wallac, Turku, Finland). The specificity of the monoclonal antibodies (Hanemaaijer *et al*, 1997) against MMP-8 was the same as that of polyclonal MMP-8 antibodies (Lauhio *et al*, 1994). The total amounts of MMP-8 were expressed as ng/2 samples and the concentrations were expressed as ng ml^{-1}.

MMP-13 and TIMP-1 analysis
Matrix metalloproteinase-13 and TIMP-1 concentrations were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocol (Biotrak ELISA Systems, Amersham Biosciences UK Ltd, Buckinghamshire, UK). All samples were analysed in duplicate. The so called secondary antibody in each kit was conjugated with horseradish peroxidise and tetra methyl benzidine (TMB) was used as a substrate. The absorbance was measured at 450 nm using Labsystems Multiskan RC (Thermo Bioanalysis Corporation, Santa Fe, NM, USA). The total amounts of matrix metalloproteinase-13 and TIMP-1 were expressed as ng/2 samples and concentrations were expressed as ng/ml.

Laboratory markers of diabetes mellitus
Venous blood samples were taken from each DM patient and analysed for fasting plasma glucose (FPG), glycated haemoglobin (HbA1c), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL). Moreover, duration of type 2 DM, body weight and heights of the DM patients were recorded and body mass index (BMI) was calculated.

Statistical analysis
ANOVA and Brown-Forsyth tests followed by *post hoc* Tukey and Dunnett C-tests were used with Bonferroni correction when appropriate for the group comparisons of the biochemical data as well as the clinical periodontal measurements. Pearson correlations were utilised to look at the relationships between GCF levels of the assayed inflammatory mediators and the clinical parameters of type 2 DM and periodontal disease. Pearson chi-square tests and Mann–Whitney tests were used to compare the data of the DM patients exhibiting gingivitis with those exhibiting periodontitis. As a result of the high standard deviations, logarithmic transformation was performed to MMP-8 total amount, MMP-8 concentration, TIMP-1 total amount and concentration, as well as MMP-8/TIMP-1 ratio.

Results

Clinical analyses
There were no differences in mean age, gender distribution and smoker ratios of the diabetic and non-diabetic periodontal disease groups ($P > 0.05$). The healthy control group was significantly younger than the other groups ($P < 0.05$). Clinical periodontal measurements were analysed in two steps: first, the full-mouth recordings at six sites per tooth were compared between the study groups, and second, the clinical recordings at the GCF sampling sites were tested statistically. The mean values of clinical periodontal measurements are outlined in Table 1. The diabetic and non-diabetic groups with gingivitis or periodontitis were similar in PD, CAL, PI, and BOP measurements in terms of both whole-mouth recordings and sample-site recordings ($P > 0.05$). The healthy control group exhibited significantly lower values in all clinical periodontal measurements than the DM and PD groups ($P < 0.05$).

Biochemical analyses
The values of the systemic markers of DM are given in Table 2. The diabetic periodontitis group revealed significantly lower blood triglyceride levels than the diabetic gingivitis group ($P < 0.05$). All other variables were similar in diabetic gingivitis and diabetic periodontitis patients ($P > 0.05$). Table 3 presents the biochemical data obtained in GCF samples. There were no significant differences between the diabetic and non-dia-

Table 1 Clinical periodontal characteristics (mean ± s.d.) of the study groups

Clinical variable	*DM-gingivitis* (n = *12*)	*DM-periodontitis* (n = *12*)	*Healthy-gingivitis* (n = *12*)	*Healthy-periodontitis* (n = *13*)	*Healthy control* (n = *24*)
PD (mm) (full-mouth)	2.2 ± 0.3	3.4 ± 0.7	2.4 ± 0.2	3.5 ± 0.7	1.2 ± 0.2*
CAL (mm) (full-mouth)	2.4 ± 0.5	4.1 ± 1.2	2.6 ± 0.5	4.3 ± 0.8	0.4 ± 0.7*
BOP (%) (full-mouth)	52 ± 16	73 ± 22	52 ± 19	80 ± 17	8.5 ± 7*
PI (%) (full-mouth)	93 ± 8	93 ± 11	89 ± 6	96 ± 6	15 ± 4*
PD (mm) (sample sites)	2.9 ± 0.6	4.3 ± 1.5	2.5 ± 0.5	4.5 ± 1.7	1.2 ± 0.4*
CAL (mm) (sample sites)	2.8 ± 0.9	4.1 ± 2.3	2.1 ± 0.8	5.1 ± 2.2	0*
BOP (%) (sample sites)	46 ± 26	79 ± 33	42 ± 42	85 ± 32	8 ± 2*
PI (%) (sample sites)	73 ± 3	74 ± 9	88 ± 31	96 ± 14	0*

PD, probing depth; CAL, clinical attachment level; BOP, bleeding on probing; PI, plaque index; DM, diabetes mellitus.
*Significantly lower than the other groups; $P < 0.05$

Table 2 Diabetes mellitus (DM)-related variables (mean ± s.d.) of the DM patients ($n = 24$)

DM-related variables	*DM-gingivitis* (n = *12*)	*DM-periodontitis* (n = *12*)
Duration of DM (years)	7.17 ± 5.08	5.92 ± 5.05
HbA1c (%)	7.67 ± 2.46	6.81 ± 2.11
Fasting plasma glucose (mg dl^{-1})	148.25 ± 90.34	128.05 ± 65.52
Total cholesterol (mg dl^{-1})	207.42 ± 42.74	202.92 ± 35.61
Triglyceride (mg dl^{-1})	245.67 ± 258.14	136.75 ± 43.32*
LDL-cholesterol (mg dl^{-1})	126.15 ± 17.22	137.05 ± 33.92
HDL-cholesterol (mg dl^{-1})	44.83 ± 14.14	42.17 ± 12.66
Weight (kg)	70.42 ± 11.55	76.33 ± 15.05
Height (m)	1.65 ± 0.09	1.64 ± 0.09
BMI	25.64 ± 2.77	28.34 ± 4.11

HbA1c, glycated haemoglobin; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BMI, body mass index; DM, diabetes mellitus.
*Significantly lower than the DM-gingivitis group ($P = 0.028$).

betic gingivitis or periodontitis groups in GCF total amount or concentration of MMP-8, MMP-13, TIMP-1 and MMP-8/TIMP-1 ratio ($P > 0.05$). The situation was the same after logarithmic transformations. The healthy control group exhibited significantly lower GCF sample volume, MMP-8 total amount and concentration, MMP-13 total amount, and MMP-8/TIMP-1 ratio than the diabetic and non-diabetic gingivitis or periodontitis patients ($P < 0.05$). Matrix metalloproteinase-8/TIMP-1 ratio in the healthy control group was significantly lower than the DM-gingivitis, DM-periodontitis and healthy-gingivitis groups ($P < 0.05$).

Discussion

Diabetes mellitus is a complex metabolic syndrome that affects both the quality and length of life with major complications. Diabetes mellitus-induced changes in immune cell function produce an inflammatory immune cell phenotype (up regulation of pro-inflammatory cytokines from monocytes/polymorphonuclear leucocytes and down regulation of growth factors from macrophages). Eventually, this predisposes to chronic inflammation, progressive tissue breakdown, and diminished tissue repair capacity (Iacopino, 2001). Progression of periodontal disease is very much dependent on host response. The nature and extent of diabetes effects on periodontal disease have not been fully clarified. In this study, we analysed the clinical condition as well as the GCF levels of MMP-8, -13, and TIMP-1 in 24 type 2 DM patients, 25 systemically healthy patients with inflammatory periodontal disease, and 24 systemically and periodontally healthy individuals.

It is well known that the volume of GCF is greatly dependent on the degree of inflammation in periodontal tissues. The increase in the GCF volume results in pseudo decreases in the concentrations of GCF ingredients. Therefore, it has been stated that evaluation of total amounts rather than the concentrations gives more reliable results (Kardeşler *et al*, 2008). For this reason, we based our evaluations on the total amount, and additionally we tested the concentration results statistically.

Clinical and epidemiological studies have reported higher prevalence and increased severity of periodontitis in diabetic patients than non-diabetic controls (Cianciola *et al*, 1982; Safkan-Seppala and Ainamo, 1992; Collin *et al*, 1998). Arrieta-Blanco *et al* (2003) reported higher gingivitis index and gingival recession in diabetic patients when compared with the controls. Similarly, Lu and Yang (2004) compared the periodontal condition of 72 type 2 diabetic patients with that of 92 non-diabetic controls and reported higher gingival index and attachment loss in diabetic patients which were also associated with HbA1c levels. Lim *et al* (2007) evaluated the relationship between markers of

Table 3 Biochemical data (mean ± s.d.) obtained in gingival crevicular fluid (GCF) samples

Biochemical variable	*DM-gingivitis* (n = *12*)	*DM-periodontitis* (n = *12*)	*Healthy-gingivitis* (n = *12*)	*Healthy-periodontitis* (n = *13*)	*Healthy control* (n = *24*)
GCF volume (μl)	0.76 ± 0.28†	0.66 ± 0.2†	1.27 ± 0.42†	0.87 ± 0.4†	0.15 ± 0.08‡
MMP-8 total amount (ng/2 samples)	56.04 ± 46.16	106.47 ± 148.92	44.52 ± 62.93	53.65 ± 66.27	1.03 ± 2.6‡
MMP-8 concentration (ng μl^{-1})	67.7 ± 56.8†	74.86 ± 79.7†	58.5 ± 59.9†	52.5 ± 45.7†	4.58 ± 8.15‡
MMP-13 total amount (ng/2 samples)	0.78 ± 0.39	1.23 ± 0.46	0.71 ± 0.34	0.82 ± 0.37	0.14 ± 0.09‡
MMP-13 concentration (ng μl^{-1})	1.03 ± 0.41	0.98 ± 0.23	1.06 ± 0.22†	0.95 ± 0.14	0.93 ± 0.20
TIMP-1 total amount (ng/2 samples)	1.88 ± 2.54	1.70 ± 3.09	1.34 ± 2.50	0.74 ± 1.83	0.41 ± 0.64‡
TIMP-1 concentration (ng μl^{-1})	2.89 ± 3.59	1.91 ± 3.55	1.72 ± 3.14	0.89 ± 2.17	2.57 ± 3.43
MMP-8/TIMP-1	10.39 ± 9.3†	9.93 ± 5.9†	5.34 ± 1.75	2.92 ± 1.07	1.25 ± 1.88§

GCF, gingival crevicular fluid; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; DM, diabetes mellitus.
†Significantly higher than the healthy control group ($P < 0.05$).
‡Significantly lower than the other groups ($P < 0.05$).
§Significantly lower than DM-gingivitis, DM-periodontitis, and healthy-gingivitis groups ($P < 0.05$).

metabolic control and inflammation on severity of periodontal disease in 181 patients with type 1 or 2 DM. They reported positive correlations between HbA1c and percentage of sites with BOP and also percentage of sites with probing depths ≥5 mm. Very recently, we suggested that non-surgical periodontal treatment in type 2 diabetic chronic periodontitis patients may help to improve glycaemic control, as we observed significant decreases in HbA1c levels 3 months after completion of non-surgical periodontal treatment (Kardeşler *et al*, 2010). In this study, no significant correlation was found between HbA1c value and clinical periodontal measurements or GCF levels of the MMPs, TIMP-1. This discrepancy could be attributable to the rather small size of our study population and/or the rather mild nature of periodontal disease. In fact, the well-controlled diabetes in our patients can also at least in part explain this finding. Accordingly, the well-controlled diabetics with chronic periodontitis in our recent study (Kardeşler *et al*, 2010) did not reveal any change in HbA1c levels after non-surgical periodontal treatment. Moreover, within the limits of this study, it can be suggested that periodontitis does not worsen blood concentrations of lipids when compared with gingivitis. On the other hand, one limitation of the study is the lack of laboratory analysis in systemically healthy periodontal disease group, as there might be undiagnosed diabetes patients and/or prediabetic patients with slightly increased glucose intolerance influencing their host susceptibility to periodontal disease. Furthermore, a larger study population would probably have reduced the standard deviations and thereby increased the statistical strength of the report and may even have revealed significant differences between the study groups.

Persson *et al* (2003) evaluated radiographic signs of alveolar bone loss in 1101 older (60–75 years of age) subjects and concluded that probing depth differences between type 1/type 2 diabetes *vs* non-diabetic subjects may reflect the presence of pseudo-pockets rather than progressive periodontitis in many subjects with DM and reported that periodontitis is not a predominant coexisting disease in older subjects with DM. The best predictor for severe periodontal disease in subjects with type 2 DM was reported to be smoking followed by HbA1c level. In this study, not only the age but also the smoker/non-smoker ratio was matched between the DM and PD groups. Moreover, the clinical periodontal measurements were very similar in the diabetic and non-diabetic periodontal disease groups. We believe that controlling for age, smoking and clinical periodontal status would enable evaluation of the sole effect of type 2 DM on GCF levels of the investigated MMPs and TIMP-1.

Poor metabolic control of diabetes reflected as higher levels of HbA1c has been associated with increased severity of periodontitis (Tervonen and Knuuttila, 1986; Seppala and Ainamo, 1994). Diabetes-associated susceptibility traits for periodontitis include neutrophil dysfunction, abnormal cross-linking and glycosylation of collagen, defective secretion of growth factors, cytokines and subsequent impaired healing (Verma and Bhat, 2004; Mealey and Oates, 2006). However, it is uncertain which of the hypothesised mechanisms or their combinations are directly responsible for the detrimental effects of diabetes on periodontal health. Accumulation of glucose-mediated AGEs in diabetic patients impairs chemotactic and phagocytic function of polymorphonuclear leucocytes (Wilson and Reeves, 1986; Marhoffer *et al*, 1992). According to our present findings, it may be suggested that the increases in GCF levels of MMP-8, MMP-13 and TIMP-1 are related to the clinical periodontal parameters rather than the presence type 2 DM.

In conclusion, within the limits of this study, type 2 DM alone does not seem to have a significant influence on GCF levels of MMP-8, MMP-13 and TIMP-1. Therefore, it is likely that type 2 DM may affect periodontal disease via mechanisms other than changing MMP-8, -13, TIMP-1 content in GCF locally. Longitudinal clinical studies on larger scale are required to better clarify the nature as well as possible mechanisms of diabetes effects on periodontium.

Acknowledgements

The study was supported by Academy of Finland, Helsinki University Central Hospital Research Foundation.

References

Ainamo J, Lahtinen A, Uitto V (1990). Rapid periodontal destruction in adult humans with poorly controlled diabetes. A report of 2 cases. *J Clin Periodontol* **17:** 22–28.

American Diabetes Association (2007). Diagnosis and classification of diabetes mellitus. *Diabetes Care* **30**(Suppl. 1)**:** S42–S47.

Armitage GC (1999). Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* **4:** 1–6.

Arrieta-Blanco JJ, Bartolome-Villar B, Jimenez-Martinez E, Saaverda-Vallejo P, Arrieta-Blanco FJ (2003). Dental problems in patients with diabetes mellitus (II): gingival index and periodontal disease. *Medicina Oral* **8:** 233–247.

Cianciola LJ, Park BH, Bruck E, Mosovich L, Genco RJ (1982). Prevalence of periodontal disease in insulin-dependent diabetes mellitus (juvenile diabetes). *JADA* **104:** 653–660.

Collin HL, Uusitupa M, Niskanen L *et al* (1998). Periodontal findings in elderly patients with non-insulin dependent diabetes mellitus. *J Periodontol* **69:** 962–966.

Dollery CM, McEwan JR, Henney AM (1995). Matrix metalloproteinases and cardiovascular disease. *Circ Res* **77:** 863–868.

Emrich LJ, Schlossman M, Genco RJ (1991). Periodontal disease in non-insulin dependent diabetes mellitus. *J Periodontol* **62:** 123–131.

Fuller K, Chambers TJ (1995). Localisation of mRNA for collagenase in osteocytic, bone surface and chondrocytic cells but not osteoclasts. *J Cell Sci* **108:** 2221–2230.

Gerich JE (2003). Contributions of insulin-resistance and insulin-secretory defects to the pathogenesis of type 2 diabetes mellitus. *Mayo Clin Proc* **78:** 447–456.

Gümüş P, Buduneli N, Çetinkalp Ş *et al* (2009). Salivary antioxidants in patients with type 1 or 2 diabetes and inflammatory periodontal disease: a case–control study. *J Periodontol* **80:** 1440–1446.

Hanemaaijer R, Sorsa T, Konttinen YT *et al* (1997). Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells. Regulation by tumor necrosis factor-a and doxycycline. *J Biol Chem* **272:** 31504–31509.

Hannas AR, Pereira JC, Granjeşro JM, Tjaderhane L (2007). The role of matrix metalloproteinases in the oral environment. *Acta Odontol Scand* **65:** 1–13.

Hernandez M, Valenzuela MA, Lopez-Otin C *et al* (2006). Matrix metalloproteinase-13 is highly expressed in destructive periodontal disease activity. *J Periodontol* **77:** 1863–1870.

Iacopino AM (2001). Periodontitis and diabetes interrelationships: role of inflammation. *Ann Periodontol* **6:** 125–137.

Ingman T, Tervahartiala T, Ding Y *et al* (1996). Matrix metalloproteinases and their inhibitors in gingival crevicular fluid and saliva of periodontitis patients. *J Clin Periodontol* **23:** 1127–1132.

Kardeşler L, Buduneli N, Bıyıkoğlu B, Çetinkalp Ş, Kütükçüler N (2008). Gingival crevicular fluid PGE_2, IL-1ß, t-PA, PAI-2 levels in type 2 diabetes and relationship with periodontal disease. *Clin Biochem* **41:** 863–868.

Kardeşler L, Buduneli N, Çetinkalp Ş, Kinane DF (2010). Adipokines and inflammatory mediators following initial periodontal treatment in type 2 diabetic chronic periodontitis patients. *J Periodontol* **81:** 24–33. DOI: 10.1902/jop.2009.090267.

Kiili M, Cox SW, Chen HY *et al* (2002). Collagenase-2 (MMP-8) and collagenase-3 (MMP-13) in adult periodontitis: molecular forms and levels in gingival crevicular fluid and immunolocalisation in gingival tissue. *J Clin Periodontol* **29:** 224–232.

Lauhio A, Salo T, Ding Y *et al* (1994). *In vivo* inhibition of human neutrophil collagenase (MMP-8) activity during long-term combination therapy of doxycycline and non-steroidal anti-inflammatory drugs (NSAID) in acute reactive arthritis. *Clin Exp Immunol* **98:** 21–28.

Lim LP, Tay FBK, Sum CF, Thai AC (2007). Relationship between markers of metabolic control and inflammation on severity of periodontal disease in patients with diabetes mellitus. *J Clin Periodontol* **34:** 118–123.

Löe H (1993). The sixth complication of diabetes mellitus. *Diabetes Care* **16:** 476–480.

Lu HK, Yang PC (2004). Cross-sectional analysis of different variables of patients with non-insulin dependent diabetes and their periodontal status. *Int J Periodontics Rest Dent* **24:** 71–79.

Marhoffer W, Stein M, Maeser E, Federlin K (1992). Impairment of polymorphonuclear leukocyte function and metabolic control of diabetes. *Diabetes Care* **15:** 256–260.

Mealey BL, Oates TW (2006). Diabetes mellitus and periodontal diseases. AAP-Commissioned review. *J Periodontol* **77:** 1289–1303.

Moreno PR, Murcia AM, Palacios IF (2000). Coronary composition and macrophage infiltration in atherectomy specimens from patients with diabetes mellitus. *Circulation* **102:** 2180–2184.

Persson RE, Hollender LG, MacEntee MI, Wyatt CC, Kıyak HA, Persson GR (2003). Assessment of periodontal conditions and systemic disease in older subjects. *J Clin Periodontol* **30:** 207–213.

Ryan ME, Oana C, Kamer A (2003). The influence of diabetes on the periodontal tissues. *JADA* **134:** 34S–40S.

Safkan-Seppala B, Ainamo J (1992). Periodontal conditions in insulin-dependent diabetes mellitus. *J Clin Periodontol* **19:** 24–29.

Seppala B, Ainamo J (1994). A site by site follow-up study on the effect of controlled versus poorly controlled insulin-dependent diabetes mellitus. *J Clin Periodontol* **21:** 161–165.

Sorsa T, Tjaderhane L, Salo T (2004). Matrix metalloproteinases (MMPs) in oral diseases. *Oral Dis* **10:** 311–318.

Taylor G, Burt B, Becker M *et al* (1998). Non-insulin dependent diabetes mellitus and alveolar bone loss progression over 2 years. *J Periodontol* **69:** 76–83.

Tervahartiala T, Pirila E, Ceponis A *et al* (2000). The *in vivo* expression of the collagenolytic matrix metalloproteinases (MMP-2, -8, -13, and -14) and matrilysin (MMP-7) in adult and localized juvenile periodontitis. *J Dent Res* **79:** 1969–1977.

Tervonen T, Knuuttila M (1986). Relation of diabetes control to periodontal pocketing and alveolar bone level. *Oral Surg Oral Med Oral Pathol* **61:** 346–349.

Ünal T, Fıratlı E, Sivas A, Meriç H, Öz H (1993). Fructosamine as a possible monitoring parameter in non-insulin dependent diabetes mellitus patients with periodontal disease. *J Periodontol* **64:** 191–194.

Verma S, Bhat KM (2004). Diabetes mellitus-a modifier of periodontal disease expression. *J Int Academy Periodontol* **6:** 13–20.

Vlassara H (1992). Receptor-mediated interactions of advanced glycosylation end products with cellular components within diabetic tissues. *Diabetes* **41**(Suppl. 2)**:** 52–56.

Wahlgren J, Maisi P, Sorsa T *et al* (2001). Expression and induction of collagenases (MMP-8 and -13) in plasma cells associated with bone-destructive lesions. *J Pathol* **194:** 217–224.

Wilson R, Reeves W (1986). Neutrophil phagocytosis and killing in insulin-dependent diabetes. *Clin ExpImmunol* **63:** 478–484.