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Gingival advanced glycation end-products in diabetes mellitus-associated chronic periodontitis: an immunohistochemical study

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Background and Objective: The accumulation of advanced glycation end-products (AGEs) seems to play an important role in the development of diabetes mellitus (DM)-associated periodontitis; however, some aspects of this issue are still scarcely known, such as the expression of AGEs in type 1 DM-associated periodontitis and the clinical factors able to affect their accumulation. This study aimed to clarify these points by evaluating the expression of AGEs in DM-associated periodontitis.

Material and Methods: Sixteen systemically and periodontally healthy subjects and 48 subjects suffering from generalized, severe, chronic periodontitis (16 with type 1 DM, 16 with type 2 DM and 16 systemically healthy subjects) were studied clinically, periodontally and metabolically. The immunohistochemical expression of AGEs in gingival tissues was also evaluated.

Results: Subjects affected with type 1 DM presented a significantly higher percentage of AGE-positive cells than did subjects affected with type 2 DM, not only in the epithelium, but also in vessels and fibroblasts. A positive and significant correlation was found between gingival expression of AGEs and length of time affected with DM both in type 1 and type 2 DM; glycated hemoglobin, lipid profile, body mass index and age did not correlate significantly with gingival AGEs in any of the classes of subjects studied.

Conclusions: Gingival AGEs are increased in both type 1 and type 2 DM-associated periodontitis; however, the clinical parameter that determines their accumulation, and therefore their degree of influence on the development of DM-associated periodontitis, may be the duration of DM.

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Key words: advanced glycation end-products; diabetes mellitus; immunohistochemistry; periodontitis

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The relationship between diabetes mellitus (DM) and chronic periodontitis has been extensively studied (1,2). Some studies have suggested that the increased severity of diabetic periodontal disease is associated with an enhanced inflammatory response to periodontal pathogens (3). In diabetic subjects, accelerated alveolar bone loss is mediated by several potential pathogenic mechanisms, including impaired recruitment and function of neutrophils in response to infection with pathogenic bacteria (4,5), diminished generation of

collagen and exaggerated collagenolytic activity (6,7), as well as genetic predisposition (8).

Diabetic complications caused by chronic hyperglycemia seem to have a multifactorial origin, and the biochemical process of advanced glycation is one of the major pathways involved in the development and progression of both microvascular and macrovascular disease (9). In fact, sporadically elevated levels of blood glucose contribute to the generation of largely irreversible advanced glycation end-products (AGEs) (10) and impair kidney function, the major site of AGE clearance (9). AGEs are a complex group of compounds formed via a nonenzymatic reaction between reducing sugars and amine residues on proteins, lipids or nucleic acids. The rate of turnover of proteins for glycoxidation, the degree of hyperglycemia and the extent of oxidant stress in the environment are considered key factors in the formation of AGEs (11), and both intracellular and extracellular proteins may be glycated and oxidized. AGE-modified proteins may be more resistant to enzymatic degradation (12), promoting further accumulation of AGE in local tissue (9) and a range of cellular responses, such as osteoclast-induced bone resorption, vascular complications and stimulation of secretion of inflammatory cytokines, collagenase and several growth factors (13).

AGEs may exert their biologic effects in tissues by receptor-independent or receptor-dependent pathways. Through receptor-independent pathways, AGEs may directly impact on the structural integrity of the vessel wall and underlying basement membrane; in particular, excessive cross-linking of matrix molecules (i.e. collagen) can lead to an alteration of matrix–matrix and matrix–cell interactions (9). Regarding instead the receptor-dependent pathway, it is well established that AGEs may also exert their pathogenic effects by engagement of cellular binding sites/receptors. To date, the most extensively studied is receptor for AGE (RAGE), but other binding proteins include AGE receptors 1, 2 and 3 and the ezrin, radixin and moesin family (9). These receptors are responsible for a range of functions in diabetic tissues, including modulation of cellular properties by receptor-triggered signal transduction on AGE engagement, as well as removal and detoxification of AGEs.

AGE accumulation seems to play an important role also in the development of DM-associated periodontitis; in fact, increased immunoreactivity of AGEs has been demonstrated in the gingiva of diabetic patients affected by chronic periodontitis (14), indicating an involvement of these molecules in the periodontal destruction that occurs in DM (14,15). Moreover, in diabetic patients the serum levels of AGEs were found to be associated with severity of periodontal degeneration (16). However, available information on this is still scarce and, to our knowledge, some important aspects have not yet been examined: first, as shown in Table 1, most studies on the role of the AGE-RAGE system in the development of periodontitis in humans have prevalently addressed RAGE rather than AGE (Table 1), thus disregarding the receptor-independent effects of AGEs; second, no studies have been published that include, in their case records, subjects affected by type 1 DM, the type of DM most frequently found in young persons (Table 1) (17) and whose periodontitis can be different from that present in type 2 DM (18,19); and, third, clinical factors that affect gingival AGE levels have not been analyzed.

To clarify these points we carried out an immunohistochemical study for AGE in the gingival tissue of healthy subjects and of patients affected by chronic periodontitis, with and without type 1 and type 2 DM.

Material and methods

Subjects

The study was carried out between 2005 and 2011. Sixteen systemically and periodontally healthy subjects (CT) and 48 subjects suffering from generalized, severe, chronic periodontitis [16 systemically healthy individuals (PD-S), 16 with DM1 (PD-DM1) and 16 with DM2 (PD-DM2)] were studied; subjects affected by DM were recruited from among patients attending a clinical appointment at the Endocrinology Clinic, Department of Clinical and Molecular Sciences of the Polytechnic University of Marche, while CT and PD-S subjects were obtained among a series of patients treated in the Division of Periodontology, Department of Odontostomatological and Specialized Clinical Sciences of the Polytechnic University of Marche.

General inclusion criteria were: (i) age > 35 years; and (ii) the presence of at least 20 teeth.

Specific inclusion criteria were: (i) for periodontitis subjects, a diagnosis of generalized, severe, chronic periodontitis made on the basis of the presence of more than 30% of measured sites with > 5 mm of clinical attachment loss (20,21) (this diagnosis was made by evaluating whether the

Table 1. Studies that evaluated the advanced glycosylation end-product/receptor for advanced glycosylation end-product (AGE-RAGE) system in diabetes mellitus (DM)-associated periodontitis in humans

Author (reference)	Number of diabetic subjects studied	Type of DM studied	Molecule studied
Schmidt *et al.* (14)	4	Type 1 (one subject) Type 2 (three subjects)	Gingival AGEs
Katz *et al.* (15)	8	Type 2	Gingival RAGEs
Takeda *et al.* (16)	97	Type 2	Serum AGEs
Amir *et al.* (49)	20	Type 2	Gingival RAGEs
Rajeev *et al.* (50)	19	Type 2	Gingival RAGEs
Yu *et al.* (51)	12	Type 2	Gingival RAGEs

periodontal destruction was commensurate with plaque levels or other local contributing etiological factors); (ii) for diabetic subjects, a diagnosis of type 1 or type 2 DM (17) established at least 12 mo before the study; (iii) for nondiabetic subjects, glycated hemoglobin (HbA1c) in the nondiabetic range (< 6.1%) and plasma glycemia lower than 100 mg/dL; and (iv) for CT subjects, probing depth < 3 mm, gingival index = 0 (absence of clinical inflammation) and clinical attachment loss < 2 mm.

Exclusion criteria were as follows: (i) the presence of any important disease other than DM in the groups of diabetic subjects; (ii) being a smoker; (iii) having taken antibiotics, corticosteroids or nonsteroidal anti-inflammatory drugs within the 6 mo before treatment; and (iv) having undergone periodontal treatment within the previous 2 years.

This study was carried out in compliance with an informed protocol approved by our institution. The research objectives were explained to the patients, who then signed an informed consent form.

Determination of periodontal status

All participants were evaluated clinically and radiographically. The following clinical and periodontal parameters were assessed by the same examiner (S.D.A.): (i) plaque index (22); (ii) gingival index (22); (iii) sulcus bleeding index (23); (iv) probing depth, measured to the nearest millimetre from the gingival margin to the bottom of the pocket; and (v) clinical attachment loss, measured from the cemento–enamel junction to the bottom of the pocket. Probing depth and clinical attachment loss were assessed at six sites around each tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual locations) for the whole mouth, excluding third molars.

The same type of calibrated periodontal probe (15 mm, probe tip diameter = 0.5 mm, University of North Carolina; UNC-15; Hu Friedys, Chicago, IL, USA) was used for all measurements.

Periapical radiographs were performed by the same investigator (S.D.A.) using the long-cone paralleling technique with the same radiographic equipment, film, exposure and development conditions. Callipers were used for the measurements, which were expressed in millimetres and corrected to account for the magnification factor of the equipment used. Bone loss was expressed as a fraction of the total root length at two sites (mesial and distal) for every tooth.

Biochemical parameters

Fasting blood samples were collected. Total, high-density lipoprotein cholesterol and triglycerides were determined. All biochemical parameters were measured in the clinical laboratory of our hospital. Plasma glucose was measured by photometric determination using the hexokinase method. HbA1c was measured in whole blood using ion-exchange high-performance liquid chromatography with the Bio-Rad Variant Haemoglobin Testing System (Bio-Rad Laboratories, Hercules, CA, USA). Total cholesterol, high-density lipoprotein cholesterol and triglycerides were assayed enzymatically with a final Trinder reaction (ADVIA 2400 SIEMENS; Bayer Diagnostics, Tarrytown, NY, USA).

Gingival biopsies

For CT subjects, gingival biopsies were collected during crown-lengthening surgery or during extraction of a fractured tooth, while, for subjects suffering from periodontitis, gingival biopsies were collected during periodontal surgery. All surgical procedures were performed by one surgeon (S.D.A.). After local anesthesia using 40 mg/mL of articaine hydrochloride and 0.012 mg/mL of (R)-adrenalin hydrochloride (Ubistesin; 3M ESPE AG, D-82229 Seefeld, 3M Italy), an intrasulcular incision was made with a 15C blade on the palatal/lingual part of the tooth and two oblique releasing incisions were made from the mesial and distal extremities to remove the coronal portion of the gingiva (3 mm × 3 mm) during the surgical treatment.

Immunohistochemistry

To examine the expression of AGEs, immunohistochemistry was performed on serial paraffin tissue sections using the avidin–biotinylated enzyme complex method. After heat drying, the sections were dewaxed in xylene and rehydrated through a graded series of ethanol. For more effective unmasking of antigenic sites, two antigen-retrieval solutions were applied by incubating sections with a Dako Target Retrieval solution (DakoCytomation, Carpinteria, CA, USA), pH 6.0, at 750 kW for 20 min. Endogenous peroxidase activity was quenched by incubating the sections in 3% (volume by volume) hydrogen peroxide for 7 min at room temperature. Tissue sections were then incubated for 60 min with monoclonal anti-AGE Ig G1 (clone 6D12, diluted 1:500; Trans-Genic Inc., Tokyo, Japan). The antigen–antibody complex was subsequently visualized using the Envision^{TM} Detection System kit peroxidase/DAB (DakoCytomation). Sections were then incubated with 3,3′-diaminobenzidine (0.05% diaminobenzidine in 0.05 M Tris buffer, pH 7.6, and 0.01% hydrogen peroxide; Sigma-Aldrich, Milan, Italy), counterstained with Mayer's hematoxylin (Bio-Optica SPA, Milan, Italy) and coverslipped.

Tissue sections of the early stage of human atherosclerotic lesions of the aorta were immunohistochemically processed as positive controls. Negative controls were processed by substituting the primary antibody with nonimmune serum.

Semiquantitative analysis of AGE immunostaining was carried out in epithelial, dermal and endothelial cells of subepithelial connective tissue vessels of gingival tissues. Immunoreactivity of the antibodies was evaluated independently by two observers (L.G. and A.Z.), blinded to the clinical parameters, and agreement between the observers was always > 95%. The number of AGE-positive cells was

counted among at least 1000 cells in the more representative fields using a light microscope at ×250 magnification; the values obtained were expressed as a percentage of the total cells counted. Moreover, staining intensity was classified using a five-point scale: no staining (negative), faint staining, moderate staining, good staining and strong staining.

Statistical analysis

The Shapiro–Wilk test was applied to verify the normal distribution of the continuous variables. Continuous variables were expressed as mean ± standard error of the mean if normally distributed and as median (interquartile range) if not normally distributed.

The chi-square test was used to compare the frequency of some variables in different groups. For the variables not normally distributed, comparisons among the four groups were carried out using the Kruskal–Wallis test followed by the Mann–Whitney *U*-test (if significant differences were detected); for normally distributed values, comparisons among groups were made using analysis of variance followed by Fisher's least significant difference *post-hoc* test. *p* values were corrected using the Bonferroni–Holm method (24). In the case of statistical comparison between only two groups, the Mann–Whitney *U*-test was used for data not normally distributed and the Student's unpaired test was used for normally distributed data.

Spearman correlations were performed. Significance was set at $p < 0.05$. Statistical analyses were performed using the SPSS 16 package (SPSS Inc., Chicago, IL, USA).

Results

Histological and clinical evaluation

To define the histological features of the gingival samples, paraffin sections were evaluated histologically using hematoxylin and eosin staining.

Healthy gingival mucosa showed parakeratinized stratified squamous epithelium with rete pegs and connective tissue papillae. The stroma was fibrous with some fibroblasts and with a rare inflammatory cell infiltrate, mainly in the marginal gingival zone (Fig. 1A).

Mucosa from patients with chronic periodontitis exhibited an epithelium lining the pockets that varied in thickness and sometimes was hyperplastic and acanthotic. In the underlying connective tissue, high vascularization and a high-grade inflammatory infiltrate (consisting mainly of lymphocytes, macrophages and plasma cells, in varying proportions) were present (Fig. 1B).

Significant statistical differences were observed for clinical parameters among subject groups (Table 2). As expected, PD-DM1 patients were younger than PD-DM2 patients; moreover, higher values of body mass index (BMI), HbA1c and blood glucose were found in diabetic patients compared with nondiabetic patients.

The periodontal features of the patients included in our study are shown in Table 3: we observed no statistical differences among periodontitis subjects.

Immunohistochemical evaluation

Immunohistochemical results are summarized in Table 4.

Immunostaining of AGEs was observed in the cytoplasm of epithelial and endothelial cells of all gingival samples. Fibroblasts and gingival inflammatory cell infiltrates were found to be AGE-positive only in PD-DM1 (Fig. 2A) and PD-DM2 (Fig. 2B) subjects.

In gingival tissue from PD-DM1 subjects (Fig. 2A) we found a significant increase in the number of AGE-positive cells in the epithelium and in vessels compared with the other study groups. Moreover, we observed a statistically significant increase of AGE-positive vessels in gingival tissue from PD-DM2 patients (Fig. 2B) compared with CT subjects (Fig. 2D). Furthermore, in gingival tissue from PD-DM1 subjects we also found an increase in the numbers of AGE-positive cells in fibroblasts compared with PD-DM2 patients (Fig. 2B), while there was no significant difference in the number of AGE-positive cells in inflammatory infiltrates between PD-DM1 subjects and PD-DM2 subjects.

Concerning intensity diabetic subjects (Fig. 2A,B) presented AGE immunostaining higher than PD-S subjects (Fig. 2C) who in turn had greater intensity than CT ones (Fig. 2D): in particular PD-DM1 showed a strong AGE immunostaining, PD-DM2 showed good AGE immunostaining, PD-S moderate AGE immunostaining and CT subjects presented faint AGE immunostaining. However, no differences in the intensity of inflammatory infiltration were found between PD-DM1 subjects and PD-DM2 subjects.

Table 5 shows the statistical correlations between clinical parameters and the percentage of AGE-positive cells in epithelium, vessels, fibroblasts

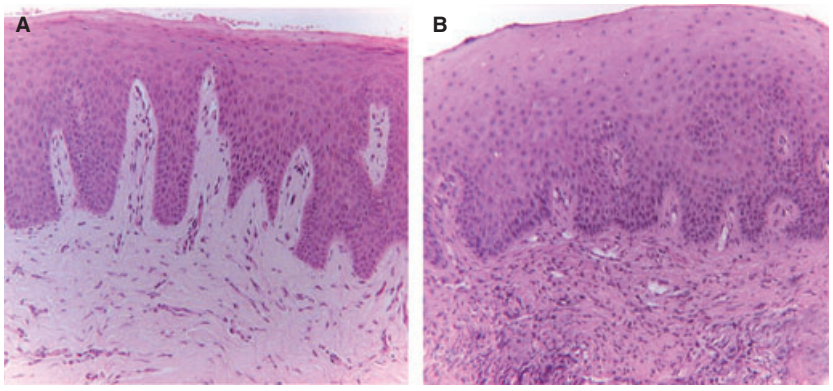

Fig. 1. Hematoxylin and eosin staining in (A) healthy gingiva and (B) chronic periodontitis tissue. Original magnification: ×400.

Table 2. General, clinical and biochemical characteristics of the subjects in each study group

Characteristic	CT	PD-S	PD-DM1	PD-DM2
Male/female	12/4	9/7	11/5	13/3
Age (years)	55 ± 1.76 [a]	56.5 ± 1.32 [a]	46.1 ± 0.70	59 ± 1.25 [a]
DM duration (months)	–	–	80 (27–98.2)	18 (13–54)[a]
BMI (kg/m^2)	23.4 (22.2–24) [abc]	26 (24.3–27) [ab]	30 (25.3–35.5)	34.7 (26.5–46.7)
HbA1c (%)	5 (4.9–5.2) [abc]	5.3 (5.2–5.5) [ab]	8.3 (7.4–8.7)	7.9 (7.2–8.2)
Glycemia (mg/dL)	82.5 (76.2–89) [ab]	87.5 (78.7–92) [ab]	138 (122.7–186.5)	157 (140.5–163)
Triglycerides (mg/dL)	100.5 (96.2–120)	108.5 (95–125.7)	104.5 (80.2–127.2)	179.5 (70.5–215)
Total cholesterol (mg/dL)	167.8 ± 5.17	152.9 ± 9.32	181.7 ± 7.47	163 ± 10.7
High-density lipoprotein cholesterol (mg/dL)	68.5 (53.2–82.2) [b]	67 (49.5–90.7) [b]	67 (58.2–90.7) [b]	46.5 (37.2–49.7)

Continuous variables were expressed as mean ± standard error of the mean if normally distributed and as median (interquartile range) if not normally distributed.
BMI, body mass index; CT, periodontally healthy subjects; DM, diabetes mellitus; HbA1c, glycated hemoglobin; PD-DM1, subjects affected by periodontitis and type 1 diabetes mellitus; PD-DM2, subjects affected by periodontitis and type 2 diabetes mellitus; PD-S, systemically healthy individuals affected by periodontitis.
Statistical comparison among the four groups of subjects: [a]$p < 0.05$ vs. PD-DM1; [b]$p < 0.05$ vs. PD-DM2; [c]$p < 0.05$ vs. PD-S; not significant if not specified otherwise.

Table 3. Full-mouth periodontal parameters of the subjects in each study group

Parameter	CT	PD-S	PD-DM1	PD-DM2
Gingival index	0 (0–0)	1.6 (1.4–2.3) [a]	1.9 (1.4–2.6)[a]	2 (1.5–2.7)[a]
Sulcus bleeding index	0 (0–0)	1.9 (1.1–3)[a]	1.9 (1.6–3.4)[a]	2 (1.4–3)[a]
Probing depth (mm)	2.6 (2.2–2.8)	7.1 (7–7.3)[a]	6.9 (6.9–7.1)[a]	7 (7–7.2)[a]
Clinical attachment loss (mm)	1.1 (0.8–1.3)	6.6 (6.4–6.7)[a]	6.6 (6.2–6.7)[a]	6.4 (6.3–6.6)[a]
Plaque index	0 (0–0)	1.6 (1.3–1.7)[a]	1.5 (1.3–1.6)[a]	1.4 (1.3–1.6)[a]
Bone loss (%)	5 (4–6.7)	60.5 (58.2–62)[a]	59 (58.2–62.7)[a]	59.5 (54.2–62.7)[a]

Continuous variables are expressed as median (interquartile range) as they were not normally distributed.
CT, periodontally healthy subjects; PD-DM1, subjects affected by periodontitis and type 1 diabetes mellitus; PD-DM2, subjects affected by periodontitis and type 2 diabetes mellitus; PD-S, systemically healthy individuals affected by periodontitis.
Statistical comparison among the four groups of subjects; [a]$p < 0.05$ vs. CT; not significant if not specified otherwise.

Table 4. Percentage of advanced glycosylation end-product (AGE)-positive cells in epithelium, vessels, fibroblasts and gingival inflammatory cell infiltrates of the four groups of study subjects

Location of AGEs	CT	PD-S	PD-DM1	PD-DM2
Epithelium (AGE %)	62.5 (46.2–73.7) [a]	70 (61.2–70) [a]	90 (75–93.7)	75 (65–80) [a]
Vessels (AGE %)	51.8 ± 2.88 [a]	58.7 ± 4.19[a]	74 ± 2.38	63.4 ± 1.97 [ab]
Fibroblasts (AGE %)	*	*	43.1 ± 3.97	24.3 ± 4.46 [a]
Gingival inflammatory cell infiltrates (AGE %)	*	*	20 (10–30)	20 (11.25–63.75)

Continuous variables were expressed as mean ± standard error of the mean if normally distributed and as median (interquartile range) if not normally distributed.
CT, periodontally healthy subjects; PD-DM1, subjects affected by periodontitis and type 1 diabetes mellitus; PD-DM2, subjects affected by periodontitis and type 2 diabetes mellitus; PD-S, systemically healthy individuals affected by periodontitis.
Statistical comparison among the four groups of subjects; [a]$p < 0.05$ vs. PD-DM1; [b]$p < 0.05$ vs. CT; not significant if not specified otherwise.
*AGE positivity not present in any of the samples.

and gingival inflammatory cell infiltrates; in both PD-DM1 and PD-DM2 subjects we found a significant, positive correlation between the duration of DM and the percentage of AGE-positive cells in epithelium, vessels and fibroblasts. No other significant correlation was present.

Discussion

The aim of this study was to evaluate the expression of gingival AGEs in DM-associated periodontitis in order to clarify some relatively unknown aspects of this relationship, such as the expression of these molecules in type 1 DM-associated periodontitis and the clinical factors able to influence their accumulation. Healthy and periodontitis subjects (with and without DM) were studied, selecting, for the groups with periodontitis, only those affected by severe, chronic, generalized periodontitis, in order to

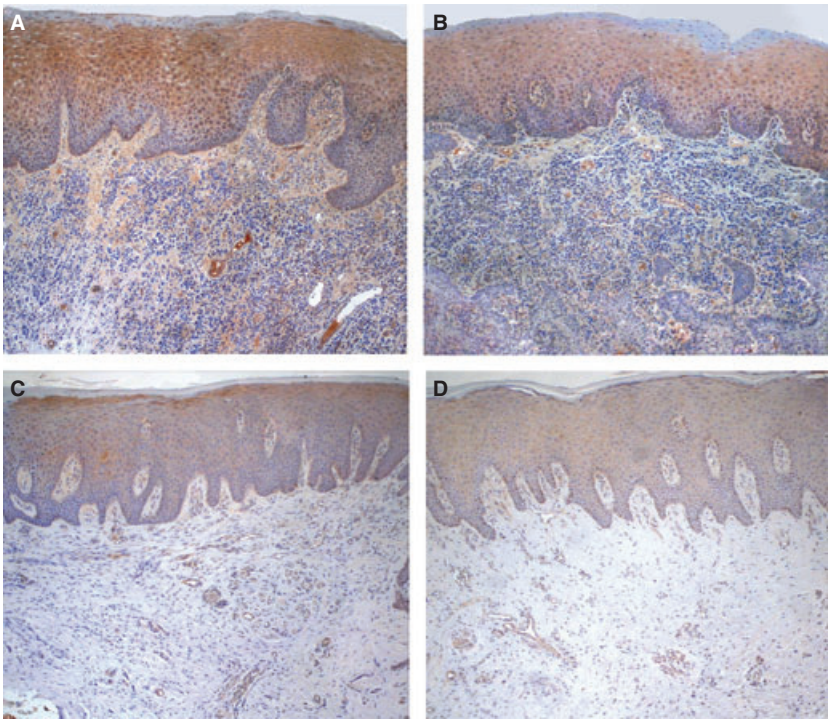

Fig. 2. Immunostaining of advanced glycosylation end-products (AGEs) on gingival tissue from: (A) periodontitis patients with type 1 diabetes mellitus (PD-DM1); (B) periodontitis patients with type 2 diabetes mellitus (PD-DM2); (C) periodontitis patients without diabetes mellitus (PD-S); and (D) systemically and periodontally healthy subjects (CT). Immunoperoxidase, original magnification: ×200.

make the groups comparable from a periodontal point of view and thus to eliminate the bias linked to the correlation between AGEs and periodontal deterioration (16).

In our study we unexpectedly found that subjects with type 1 DM showed both a higher immunostaining intensity of AGE and a higher percentage of AGE-positive cells in the epithelium, vessels and fibroblasts than did subjects with type 2 DM (Table 4). We found a possible explanation for this unexpected finding when analyzing the correlations between AGE levels and the clinical factors studied: both PD-DM1 and PD-DM2 subjects presented a positive and significant correlation between AGE levels and DM duration (Table 5); moreover, subjects affected by PD-DM1 had a significantly greater duration of DM than did PD-DM2 subjects (Table 2). Therefore, our hypothesis is that high gingival AGE levels in PD-DM1 subjects could specifically be a result of the long duration of DM. In support of our theory is the fact that the onset of AGE-mediated complications of DM, such as retinopathy, neuropathy or nephropathy, is determined by the long duration of hyperglycemia (25) and DM duration is a parameter that correlates with the presence and severity of these complications (25,26). Also important is the fact that glycemic control worsens with the progression of DM, thus stimulating an increase in the formation of AGEs (27–29). From a molecular point of view, long DM duration favors AGE accumulation in tissues because hyperglycemia-related AGE modification of proteins makes them more resistant to proteolysis, and therefore to turnover, (30,31), thus causing proteins containing various AGE adducts to accumulate rapidly after the onset of DM (31). Therefore, gingival AGEs could be the link in the well-known relationship between DM duration and severity of periodontitis (32,33). Interestingly, HbA1c did not correlate significantly with AGE values in any of the four study groups (Table 5); this finding may seem unusual given that the entity of glycemic variability affects the presence of AGEs in the various tissues (34). The explanation of this could lie in the fact that a single HbA1c gives us a picture of average blood glucose control for the past 2–3 mo, while glycemic control has an effect on AGE accumulation over a period of years (35).

We also evaluated the relationship of BMI, lipid profile and age with gingival AGE levels: we carried out these correlations in the light of the well-known effects of these parameters on AGE levels (36–38) and on the severity of periodontitis (39–41). However, our series did not present significant correlations of BMI, lipid profile and age with gingival AGE levels in any of the four study groups (Table 5). Regarding BMI and lipid profile, this finding could be justified by the fact that these two factors have an effect on the severity of periodontitis through mechanisms that are not related to the presence of AGEs. Where BMI is concerned, it is in fact known that the plasminogen activator inhibitor-1, which is strongly expressed in visceral fat, may also decrease blood flow in the periodontium of obese subjects and thus enhance the development of periodontal disease (42). Alternatively, it has been suggested that obese people may have different species of oral bacteria compared with normal-weight people, which might lead to periodontal disease (43); moreover, adipose tissue, especially visceral, can produce cytokines and prostaglandins, such as tumor necrosis factor alpha, for example, which may directly injure periodontal tissue (42). Regarding dyslipidemia, this metabolic alteration is able to influence the appearance and entity of periodontitis by stimulating cytokine hypersecretion on the part of polymorphonuclear leukocytes, which in adult periodontitis contributes to tissue destruction and pathologic wounding (39); in fact, Cutler *et al.* demonstrated that the addition of triglycerides resulted in a significant increase in the secretion of

Table 5. Spearman correlations between advanced glycosylation end-product (AGE) levels and clinical parameters potentially able to influence them

	CT		PD-S		PD-DM1				PD-DM2			
	AGE epithelium	AGE vessels	AGE epithelium	AGE vessels	AGE epithelium	AGE vessels	AGE fibroblasts	AGE gingival inflammatory cell infiltrates	AGE epithelium	AGE vessels	AGE fibroblasts	AGE gingival inflammatory cell infiltrates
Age	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
HbA1c	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
BMI	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Total cholesterol	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
HDL cholesterol	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Triglycerides	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
DM duration	–	–	–	–	*r*: 0.610 *p*: 0.012	*r*: 0.635 *p*: 0.008	*r*: 0.589 *p*: 0.016	NS	*r*: 0.672 *p*: 0.004	*r*: 0.652 *p*: 0.006	*r*: 0.572 *p*: 0.021	NS

BMI, body mass index; CT, periodontally healthy subjects; DM, diabetes mellitus; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; NS, not significant; PD-DM1, subjects affected by periodontitis and type 1 diabetes mellitus; PD-DM2, subjects affected by periodontitis and type 2 diabetes mellitus; PD-S, systemically healthy individuals affected by periodontitis.

interleukin-1beta by polymorphonuclear leukocytes that ranged from 7% to 150% over more than the one obtained with lipopolysaccharides alone (39). Furthermore, age did not correlate significantly with gingival AGE levels in any of the four groups; this finding was unexpected given that ample data have shown age to be one of the decisive factors in AGE accumulation in various organs (44–48); probably the correlation was not significant because the duration of DM modifies the natural temporal accumulation of AGEs in gingiva and has a predominant effect on the latter.

In conclusion, our study demonstrated that gingival AGEs are increased in both type 1 and type 2 DM-associated periodontitis; however, the clinical parameter determining their accumulation, and therefore their degree of influence on the development of DM-associated periodontitis, may be the duration of DM.

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