RESEARCH LETTER

Association of skin autofluorescence with periodontal inflammation in adults with type 1 diabetes

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Introduction The neurovascular complications of diabetes such as retinopathy, chronic kidney disease, or neuropathy are the most serious clinical manifestations of chronic exposure to hyperglycemia and major contributors to end-stage renal disease or blindness. Patients with diabetes are also at higher risk of developing periodontal disease.[1]

The accumulation of advanced glycation end products (AGEs) in local tissue is an important cause of low-grade inflammation in diabetes and is linked to late diabetic complications. The assessment of skin autofluorescence (AF) is a simple and noninvasive method that reflects the tissue accumulation of AGEs. The aim of the study was to assess skin AF to determine the association of AGE accumulation in the skin with periodontal inflammation in adults with type 1 diabetes.

Patients and methods The study was conducted in patients with type 1 diabetes referred to the outpatient clinic of the Department of Internal Medicine and Diabetology at the Poznan University of Medical Sciences in Poznań, Poland, in cooperation with the Department of Conservative Dentistry and Periodontology at the Poznan University of Medical Sciences. The study was approved by the local ethics committee (decision no., 1066/15) and conducted according to the guidelines of the Declaration of Helsinki on biomedical research involving human subjects. All participants provided written informed consent before enrollment to the study.

We evaluated 204 patients with type 1 diabetes (86 men), aged 29 years (interquartile range [IQR], 21–35 years). The median disease duration was 13 years (IQR, 9–19 years). The exclusion criteria were as follows: diabetic ketoacidosis at the time of enrollment, disease duration of less than 5 years, pregnancy, end-stage renal disease, liver cirrhosis, and malignancy (TABLE 1).

All patients underwent a complete physical examination with anthropometric measurements. We assessed the metabolic control of diabetes and the presence of chronic diabetic complications. A history of statin and angiotensin-converting enzyme inhibitor use was recorded as the drugs potentially affecting the gingival status. Blood samples were obtained after an overnight fast. Serum lipid and creatinine levels were measured with standard methods. Hemoglobin A_{1c} (HbA_{1c}) was measured using the high-performance liquid chromatography method according to the Diabetes Control and Complications Trial standard. As thyroid disorders are common comorbidities in type 1 diabetes and previous studies showed the possible impact of thyroid function on mucosal bleeding, we included serum thyrotropin in further analyses.[2] The thyrotropin concentration was assessed using electrochemiluminescence ECLIA Elecsys analyzers (Roche Diagnostics Ltd., Rotkreuz, Switzerland).

Evaluation of the periodontal status The clinical examination of the periodontal tissue was done by the same periodontist. The World Health Organization probe was used. The visual presence and severity of gingival inflammation were described by means of the gingival index (GI).[3] In the GI scale, code 0 means no visual bleeding and inflammation; code 1, mild; code 2, moderate; and code 3, severe inflammation. The activity of inflammation was assessed using the modified sulcus bleeding index (SBI).[4] In the SBI scale, severe gingivitis is recognized when SBI is between 1.0 and 0.5; moderate, between 0.5 and 0.2; mild, between 0.2 and 0.1; and no inflammation, when the SBI

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is below 0.1. The hygiene level was described by the approximal plaque index (API).[5] An API between 1.0 and 0.7 indicated bad oral hygiene; between 0.7 and 0.4, average hygiene; between 0.4 and 0.25, almost good hygiene; and below 0.25, good oral hygiene.

Skin autofluorescence Skin AF was evaluated using an AGE Reader device (Type 214D00 102, DiagnOptics, Groningen, the Netherlands). AGE Reader is a device for noninvasive assessment of the accumulation of AGEs with fluorescent properties in tissues. Skin AF is expressed as the ratio of the average intensity of light emitted in the wavelength range of 420 to 600 nm to the average light intensity in the wavelength range of 300 to 420 nm. AF was measured on the ventral side of the forearm, about 5 cm distal to the antecubital space. The mean AF values (displayed in arbitrary units) for the general population have been set for different populations and are strongly related to age and race. We therefore assumed the mean AF (1 SD) for healthy Caucasian population according to age as the reference value.[6] The study group was thus divided into 2 subgroups: with normal or increased skin AF.

Statistical analysis The results were presented as median (IQR, 25%–75%) for continuous variables or number (percentage) of patients for categorical variables. The relation between the SBI and selected parameters was assessed using the Spearman's rank correlation analysis, and the Mann–Whitney test was used to analyze the differences between the groups according to skin AF. The x^2 test was used to compare the frequencies. A P value of less than 0.05 was considered significant. Stepwise multivariate linear regression was used to assess factors related to the SBI. In the logistic regression analysis, we searched for predictors of elevated skin AF. Statistical analysis was performed using STATISTICA 10 software (StatSoft Inc., Tulsa, Oklahoma, United States).

Results Elevated skin AF was observed in 113 participants (55.4%). We found no significant correlations of SBI with HbA_{1c} or with skin AF. However, a comparison of the subgroup with normal and elevated skin AF showed higher SBI, higher HbA_{1c} levels, and younger mean age in the latter group. Data are presented in **TABLE 1**.

In a multiple regression analysis, elevated skin AF ($\beta = 0.15$, $P = 0.04$) and thyrotropin ($\beta = 0.23$, $P = 0.001$) were independently associated with SBI, after adjustment for age, sex, smoking, and HbA_{1c} ($R^2 = 0.13$, $P = 0.0004$). In the univariate logistic regression, SBI was associated with elevated skin AF (odds ratio [OR], 11.5; 95% confidence interval [CI], 2.0–66.7; $P = 0.006$). In the multivariate logistic regression, SBI remained significantly associated with skin AF after adjustment for age, sex, HbA_{1c}, thyrotropin, and cigarette smoking (OR, 7.4; 95% CI, 1.1–50.4, $P = 0.04$). There were no differences in oral hygiene assessed by the API between the subgroups, and the API did not correlate with the SBI either in the total study group ($Rs = 0.03$; $P = 0.6$) or in the subgroups with normal and increased skin AF ($Rs = 0.005$, $P = 0.9$ and $Rs = 0.08$, $P = 0.8$, respectively). Still, the subgroup with increased skin AF presented more inflammatory lesions located at the base of the gingival sulcus (higher SBI).

Discussion Participants in our study had good oral hygiene and no severe gingival inflammation. However, this observation is in contrast with previous studies.[7] One explanation is metabolic control of diabetes in our study group. Although the median HbA_{1c} level was above the therapeutic target, triglyceride and high-sensitivity C-reactive protein levels, which indicate decompensation of diabetes, remained within the reference range. Study participants were not obese, as indicated by their body mass index. This may be an important finding because obesity is associated with low-grade chronic inflammation.

The main finding was the relationship of skin AGE accumulation with gingival inflammation in type 1 diabetes. Chronic hyperglycemia leads to formation and accumulation of AGEs in local tissue, and this process is assumed to be one of the major pathogenic pathways in developing chronic complications of diabetes.[8] AGEs are resistant to enzymatic degradation and their accumulation triggers a range of cellular responses, such as osteoclast-induced bone resorption, vascular complications, and stimulation of the secretion of inflammatory cytokines, collagenase, and several growth factors. Many of the effects of AGEs are receptor-dependent and involve a multiligand member of the immunoglobulin superfamily of cell surface molecules. The best characterized of these is the receptor for advanced glycation end products (RAGEs), which appears to play a central role in oral infection, exaggerated inflammatory host responses, and destruction of the alveolar bone in diabetes.[9] Zizzi et al[10] showed the presence of AGE-positive cells in the endothelium, fibroblasts, vessels, and infiltrating inflammatory cells of the gingiva in patients with type 1 diabetes. The AGE–RAGE interactions lead to the release of reactive oxygen species by leukocytes. Leukocyte–endothelial cell interactions are also correlated with microvascular permeability. Sima et al,[11] using the mouse model of type 1 diabetes, described increased vascular permeability, leukocyte adhesion molecule expression, and leukocyte rolling in the gingiva. However, the assessment of the RAGE expression usually requires a tissue biopsy and is not widely available in clinical practice.

In our study, we used the AGE-Reader device to measure skin AF, a reliable marker of long-lasting glycemic control and oxidative stress in diabetes. This simple, noninvasive method of an indirect assessment of skin AGE accumulation is useful in the detection of late diabetic

TABLE 1 Characteristics of the study group and subgroups according to normal or elevated skin autofluorescence

Parameter	Whole study group n = 204	Normal skin AF n = 91	Elevated skin AF n = 113	*P* value
Age, y	29 (21–35)	32 (24–37)	25 (20–34)	0.001
Female sex, n (%)	118 (57.8)	51 (56)	67 (59.3)	0.6
Diabetes duration, y	13 (9–19)	15 (8–20)	13 (10–19)	0.7
Smoking, n (%)	48 (23.5)	22 (24.2)	26 (23)	0.8
BMI, kg/m^2	23.9 (21.8–26)	23.5 (21.3–26)	24 (22.5–26)	0.2
HbA_{1c}, %	8.1 (7.2–9.1)	7.6 (6.85–8.5)	8.5 (7.6–9.5)	0.00001
TC, mg/dl	180 (157–210)	178.5 (155–212)	184 (159–207)	0.7
LDL-C, mg/dl	92.8 (74.5–113)	91 (74–111)	94 (77–113)	0.6
HDL-C, mg/dl	62 (53–77)	63 (54.5–81.5)	61.5 (52.5–75.5)	0.3
TG, mg/dl	91 (65–125)	83 (60–117)	97.5 (70–141)	0.02
Hs-CRP, mg/dl	1.04 (0.54–2.43)	0.87 (0.51–1.55)	1.07 (0.58–2.92)	0.07
Thyrotropin, mU/l	1.67 (1.19–2.47)	1.66 (1.02–2.39)	1.67 (1.25–2.47)	0.4
Skin AF, AU	1.9 (1.6–2.1)	1.7 (1.2–2.3)	2.1 (1.8–2.3)	0.0000001
Retinopathy, n (%)	66 (32.4)	25 (27.5)	41 (36.3)	0.2
Neuropathy, n (%)	22 (10.8)	10 (11)	12 (10.1)	0.9
Chronic kidney disease, n (%)	19 (9.3)	6 (6.6)	13 (11.5)	0.2
ACEI use, n (%)	46 (22.5)	15 (16.5)	31 (27.4)	0.06
Statin use, n (%)	40 (19.6)	17 (18.7)	23 (20.3)	0.7
API	0.37 (0.23–0.57)	0.36 (0.24–0.58)	0.37 (0.21–0.56)	0.7
SBI	0.1 (0.03–0.21)	0.09 (0–0.19)	0.1 (0.03–0.32)	0.04
GI	0.88 (0.67–1.0)	0.83 (0.67–1)	0.92 (0.58–1)	0.9

Data are presented as median (interquartile range) unless stated otherwise. Statistical significance was assessed with the Mann–Whitney test or the χ^2 test.

Conversion factors to SI units are as follows: for TC, LDL-C, and HDL-C, 0.0259; for TG, 0.0114; and for HbA_{1c}, $(10.93 \times HbA_{1c}[\%]) - 23.5$.

Abbreviations: AF, autofluorescence; API, approximal plaque index; BMI, body mass index; GI, gingival index; HbA_{1c}, glycated hemoglobin A_{1c}; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; SBI, sulcus bleeding index; TC, total cholesterol; TG, triglycerides

complications: retinopathy, chronic kidney disease, and neuropathy.[12]

We showed that elevated skin AF was independently associated with a higher SBI, an indicator of inflammation in periodontal tissue. The differences in the SBI between subgroups with normal and elevated skin AF were subtle but significant, and therefore might be an indicator of some early microvascular changes in gingival tissues, perhaps appearing before any other indices of gingival abnormalities. Interestingly, there were no differences in oral hygiene assessed by the API between the compared subgroups. It may suggest that in type 1 diabetes gingival bleeding observed on clinical examination is not directly associated with plaque, but rather with changes in gingival blood vessels. Another explanation is that those patients might respond to plaque irritation by a more severe gingival inflammation developed earlier. In both cases, the inflammation could be modified by other factors, such as hormones. Although skin AGE accumulation is strongly associated with age in the general population, in our study the group with elevated skin AF was significantly younger than that with normal skin AF. The explanation may be worse metabolic control of diabetes in the former group.

Another finding in our study is the relationship of thyrotropin with gingival inflammation assessed by the SBI. Recent studies have shown the relationship between thyroid diseases and hemostasis, as well as an increased risk of mucosal bleeding in hypothyroidism.[2] Still, the underlying mechanisms and the clinical relevance of this association need to be clarified.

Conclusions To conclude, since the microvascular and macrovascular complications of diabetes may readily become irreversible, it is important for practitioners to detect these changes in patients at a stage where early interventions can minimize end-organ damage with simple diagnostic methods. To our knowledge, this is the first study indicating the relation of skin AF, a noninvasive method of assessing AGE accumulation in the skin, with early stages of gingival inflammation in adult patients with type 1 diabetes. More studies are needed to assess the relevance of skin AF in more advanced gingivitis in type 1 diabetes.

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