

# Association between small fiber neuropathy and higher skin accumulation of advanced glycation end products in patients with type 1 diabetes

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## KEY WORDS

advanced glycation end products, diabetic peripheral neuropathy, intraepidermal nerve fiber density, small fiber neuropathy, type 1 diabetes

## ABSTRACT

**INTRODUCTION** Advanced glycation end products (AGEs) play a crucial role in the pathogenesis of diabetic peripheral neuropathy (DPN).

**OBJECTIVES** The aim of the study was to assess the skin accumulation of AGEs in patients with long-lasting type 1 diabetes in relation to the presence of DPN.

**PATIENTS AND METHODS** We evaluated 178 patients with type 1 diabetes (99 men; age, 43 years [interquartile range [IQR], 34–54 years]; disease duration, 25 years [IQR, 18–31 years]). DPN was diagnosed if 2 or more of the following 5 abnormalities were present: symptoms of neuropathy, lack of ankle reflexes, and impaired sensation of touch, temperature, and/or vibration. PGP 9.5-immunoreactive nerve fibers were counted to assess intraepidermal nerve fiber density (IENFD) in skin biopsy. The accumulation of AGEs in the skin was assessed on the basis of skin autofluorescence (AF).

**RESULTS** Patients with DPN (45%), compared with those without neuropathy, had higher skin AF (2.6 AU [IQR, 2.3–3.1 AU] vs 2.1 AU [IQR, 1.8–2.5 AU];  $P < 0.001$ ) and lower IENFD (10 fibers/mm [IQR, 7–14 fibers/mm] vs 12 fibers/mm [IQR, 8–16 fibers/mm];  $P = 0.005$ ). We found a positive correlation between skin AF and patients' age ( $R_s = 0.44$ ;  $P < 0.001$ ), diabetes duration ( $R_s = 0.32$ ;  $P < 0.001$ ), and a negative correlation between skin AF and the estimated glomerular filtration rate ( $R_s = -0.26$ ,  $P < 0.001$ ) and IENFD ( $R_s = -0.22$ ;  $P = 0.004$ ). In a multiple linear regression analysis, skin AF was independently associated with age ( $\beta = 0.45$ ;  $P < 0.001$ ), glycosylated hemoglobin level ( $\beta = 0.19$ ;  $P = 0.007$ ), and IENFD ( $\beta = -0.14$ ;  $P = 0.04$ ) ( $R^2 = 0.27$ ;  $P < 0.001$ ). In multivariate logistic regression, the presence of DPN was independently associated with skin AF (odds ratio, 4.16; 95% confidence interval, 1.88–9.20;  $P < 0.001$ ).

**CONCLUSIONS** The presence of DPN, and particularly small fiber neuropathy, is associated with a higher accumulation of AGEs in the skin of patients with type 1 diabetes.

**INTRODUCTION** Diabetic neuropathy is one of the most common complications of diabetes. During its course, both small and large nerve fibers are damaged. Small fiber neuropathy (SFN) may occur early in diabetes and often is not revealed in physical examination or in electrophysiological

studies.<sup>1</sup> Skin biopsy with the assessment of intraepidermal nerve fiber density (IENFD) has been approved as a reliable technique and gold standard for confirming the clinical diagnosis of SFN.<sup>2,3</sup> The cut-off values of IENFD for the diagnosis of diabetic neuropathy have not yet been

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established. Nebuchennykh et al<sup>4</sup> reported different methods used to determine the reference values of IENFD in patients with different causes of polyneuropathy. The authors used Z-scores, fifth percentile, and receiver-operating characteristic (ROC) analysis. The cut-off point established in the ROC analysis for patients with SFN was 10.3 fibers/mm, with a sensitivity of 0.78 and specificity of 0.64. The value of 8.8 fibers/mm or less was postulated by Vlckova-Moravcova et al<sup>5</sup> as sensitive and specific enough. There is good intra- and interobserver variability for the assessment of IENF density, and the result might indicate the severity of neuropathy. For clinical practice, it is important that IENFD is decreased in diabetic patients with painful rather than with painless neuropathy.<sup>6</sup>

The formation of advanced glycation end products (AGEs) plays a crucial role in the pathogenesis of late diabetic neurovascular complications, including diabetic peripheral neuropathy (DPN). AGEs cause changes of long-lived proteins leading to modification of the protein matrix and increased stiffness of blood vessels. Via specific receptors, AGEs activate different cells, such as endothelial cells, monocytes, macrophages, and mesangial cells. Stimulation of these cells leads to the release of proinflammatory cytokines, production of toxic oxygen species, and the activation of transcription factors.<sup>7</sup> The accompanying oxidative stress and inflammation cause a cascade of phenomena leading to functional and structural changes in the cells and tissues, in particular in the structure of the vascular wall.<sup>8</sup>

There are several methods to assess AGEs. One of them is the measurement of skin autofluorescence (AF). It is well proven that this easy and noninvasive method reflects the accumulation of AGEs in the skin and provides insight into long and very long-term glycemic exposure.<sup>9</sup> The measurement of skin AF has been validated for the assessment of AGEs in skin biopsies.<sup>10</sup> Furthermore, we have previously shown that skin AF is increased in diabetic patients as compared with healthy controls.<sup>11</sup> The usefulness of skin AF to detect late diabetic complications has been investigated in several studies.<sup>12-14</sup> However, the association between AF and SFN is uncertain and has not yet been described.

The aim of the study was to assess the accumulation of AGEs in patients with long-lasting type 1 diabetes in relation to the presence of DPN, including SFN.

**PATIENTS AND METHODS** We evaluated 178 patients with type 1 diabetes (99 men), remaining under the care of the Department of Internal Medicine and Diabetology, Poznan University of Medical Science, Poznań, Poland, between the years 2013 and 2015. The median age of the patients was 43 years (interquartile range [IQR], 34–55 years) and the median disease duration was 25 years (IQR, 18–31 years). The characteristics of

the study population are shown in **TABLE 1**. All patients gave written informed consent to participate in the study, which was approved by the local Bioethics Committee of the Poznan University of Medical Science. The group was divided into 2 subgroups depending on the presence or absence of DPN, which was diagnosed using standard methods based on the Toronto definition of probable DPN.<sup>1</sup>

**Data collection** All patients underwent a physical examination, including anthropometric measurements and blood pressure recording. Blood pressure was measured twice by the Korotkov method in the sitting position, after 10 minutes in repose, using a mercury manometer. Arterial hypertension was diagnosed if the mean systolic blood pressure was more than 140 mmHg and diastolic blood pressure was more than 90 mmHg, or the patient had arterial hypertension diagnosed previously and had received appropriate treatment. The body mass index (BMI) was calculated from the following formula: BMI = weight (kg)/squared height (m<sup>2</sup>).

Blood samples were collected in a fasting state. Plasma glucose and serum concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and creatinine were measured using standard methods. Hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) was assessed by high-performance liquid chromatography with the Variant Haemoglobin A1c Program (Bio-Rad Laboratories, Hercules, California, United States). The serum C-reactive protein level was measured using the high-sensitive method, and the estimated glomerular filtration rate (eGFR) was calculated by the Modification of Diet in Renal Disease study equation.

**Skin autofluorescence** Skin autofluorescence 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. The tool has an excitation light source and optical spectrometer. At the beginning, the skin is affected by ultraviolet light of 300- to 420-nm spectrum, and, subsequently, the light emitted by the skin is assessed by spectrometer in a specific range (300–600 nm). Skin autofluorescence 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 site of the forearm, about 5 cm distal to antecubital space. The skin in all participants was free of tattoos or skin lesions.

For each patient, the AF measurement was done 3 times and the score was the arithmetic mean from those measurements. All the evaluations were performed in a fasting state, at room temperature.

**TABLE 1** Clinical characteristic of the study group and the subgroups with and without diabetic peripheral neuropathy

Characteristic	Diabetes (n = 178)	With DPN (n = 80)	Without DPN (n = 98)	P value <sup>a</sup>
women/men, n	79/99	34/46	45/53	NS
age, y	43 (34–55)	53 (43–58.5)	38 (31–44)	<0.001
diabetes duration, n	25 (18–31)	29 (21–33.5)	21 (17–29)	<0.001
smoking, n (%)	53 (30)	19 (24)	34 (35)	NS
history of hypertension, n (%)	88 (49)	55 (69)	33 (34)	<0.001
BMI, kg/m <sup>2</sup>	25 (23–29)	25 (24–30)	25 (22–29)	NS
VPT, V	19 (14–30)	32 (26–37)	16 (13–19)	<0.001
HbA <sub>1c</sub> , %	8.0 (7.3–9.2)	8.0 (7.3–9.1)	8.0 (7.3–9.2)	NS
HbA <sub>1c</sub> , mmol/mol	64 (56–77)	64 (56–76)	64 (56–77)	NS
TG, mmol/l	1.1 (0.8–1.5)	1.1 (0.9–1.6)	1.1 (0.8–1.4)	NS
LDL-C, mmol/l	2.9 (2.2–3.6)	2.8 (2.2–3.8)	2.9 (2.2–3.6)	NS
HDL-C, mmol/l	1.6 (1.4–2)	1.6 (1.4–2.0)	1.7 (1.4–2.0)	NS
hsCRP, mg/l	1.5 (0.7–2.9)	1.5 (0.8–3.1)	1.4 (0.7–2.6)	NS
eGFR (MDRD), ml/min/1.73 m <sup>2</sup>	89 (77–102)	82 (71–96)	95 (82–106)	<0.001
AF, AU	2.3 (2.0–2.7)	2.6 (2.3–3.1)	2.1 (1.8–2.5)	<0.001
IENFD fibers/mm	12 (8–15)	10 (7–14)	12 (8–16)	0.003

**a** comparison of patients with diabetes and DPN vs those without DPN (diagnosed using standard methods).

Data are presented as median values (interquartile ranges) or number (percentage) of patients. The Mann–Whitney test was used for continuous variables and the  $\chi^2$  test for categorical variables.

Abbreviations: AF, skin autofluorescence; BMI, body mass index, DPN, diabetic peripheral neuropathy; eGFR, estimated glomerular filtration rate; HbA<sub>1c</sub>, hemoglobin A<sub>1c</sub>; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; IENFD, intraepidermal nerve fiber density; LDL-C, low-density lipoprotein cholesterol; NS, nonsignificant; TG, triglycerides; VPT, vibration perception threshold

**Diagnosis of diabetic peripheral neuropathy** The assessment of DPN included examination of the pressure sensation (10-g monofilament perception), vibration perception (128-Hz tuning fork), temperature perception (TipTherm), and ankle reflex tests. DPN was diagnosed in patients when 2 or more of the following 5 abnormalities were detected: the presence of typical symptoms of neuropathy, the absence of ankle tendon reflexes, and/or abnormal scores for pressure, temperature, and/or vibration sensation. Additionally, we assessed the vibration perception threshold (VPT) with a neurothesiometer on a big toe as a mean value of the left and right feet.

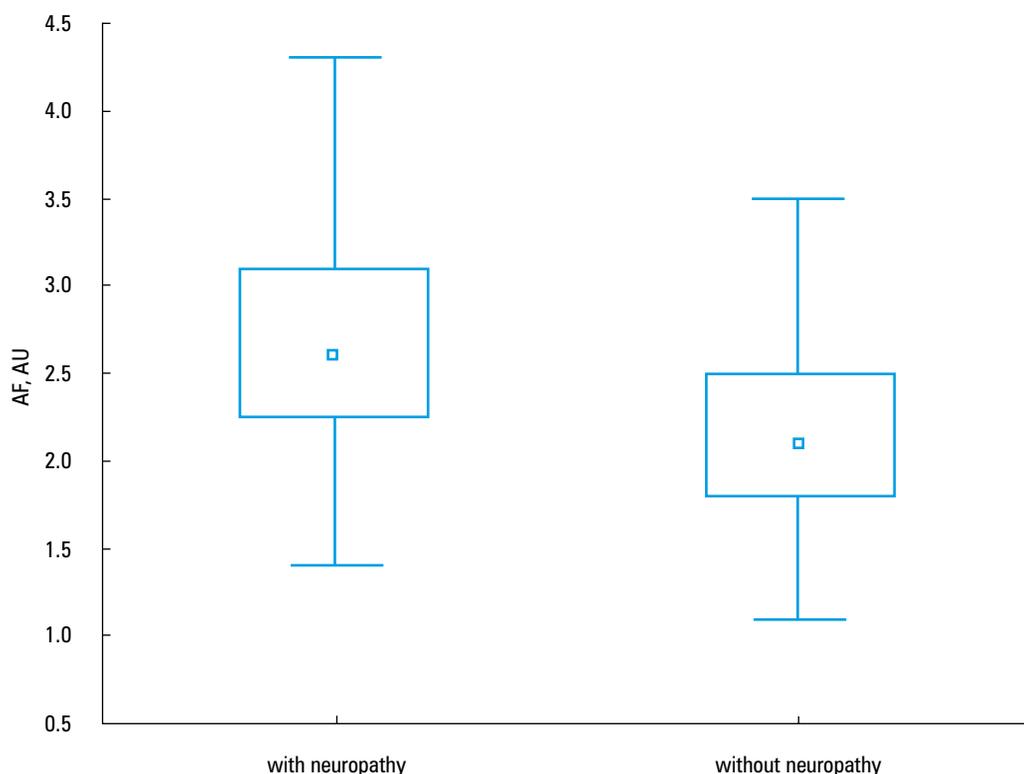
**Immunohistochemistry** A skin biopsy 10 cm above the lateral malleolus was performed in each patient. It was done using sterile, disposable 3 millimeter biopsy punches with a plunger (Integra York PA, York, Pennsylvania, United States) under local anesthesia with lidocaine (2%). Skin specimens were immediately fixed in Bouin solution containing picric and acetic acids as well as formaldehyde. After 24 hours, they were dehydrated, transferred into xylene, and finally embedded in paraffin. The paraffin blocks were subsequently cut into 50- $\mu$ m histological slides using fully automated rotary microtome (Leica 2255, KAWA.SKA Ltd., Poland). Three sections per study participant were subjected to further immunohistochemical analysis. Tissue specimens were

incubated overnight with monoclonal mouse antihuman PGP9.5 antibodies (1:1200, sc-390 883, Santa Cruz Biotechnology Inc., Heidelberg, Germany) and subsequently stained with biotinylated secondary goat antimouse antibodies (1:300) augmented by tyramine (Dako, Catalyzed Signal Amplification System, K1500, Gdynia, Poland). Finally, the sections were counterstained with eosin to improve localization of epidermis basement membrane and contrast the presence of nerve fibers. Negative controls consisted of replacing the anti-PGP9.5 antibody with phosphate-buffered saline.

The number of nerve fibers passing the basement membrane were estimated on a 1-mm length of the epidermis, using a Nikon Eclipse Ni-E motorized microscope and 5-megapixel color digital camera head (DS-Fi1c) cooperating with image analysis software (NIS Elements Documentation, KAWA.SKA Ltd., Poland). IENFD was estimated for individual study participants on 3 different sections and calculated as an average.

**Statistical analysis** Data for continuous variables were shown as mean values and standard deviations for normally distributed data or median and interquartile ranges (IQR) when the data were skewed, and data for categorical data were shown as number and percentage of patients. The normality of data distribution was tested using the Shapiro–Wilk test. The Mann–Whitney test

**FIGURE 1** Skin autofluorescence in type 1 diabetes patients with and without diabetic peripheral neuropathy



was used to compare patients' data with and without DPN. Correlations between variables were assessed by the Spearman's correlation coefficient. A multiple linear regression analysis was used to test different predictive models of skin autofluorescence. We used multivariate logistic regression to determine the independent relationship of variables with the presence of DPN. We included factors potentially associated with the presence of neuropathy. A multivariate logistic regression analysis included sex, age, diabetes duration, smoking, and skin AF. Statistical analyses were performed using Statistica version 8.0 (StatSoft Inc., Tulsa, Oklahoma, United States).

**RESULTS** We found DPN in 45% of patients with type 1 diabetes. Patients with DPN, as compared with those without DPN, were older (53 years [IQR, 43–58 years] vs 38 years [IQR, 31–44 years];  $P < 0.001$ ), had longer disease duration (29 years [IQR, 21–33 years] vs 21 years [IQR, 17–29 years];  $P < 0.001$ ), and more often had hypertension (69% vs 34%;  $P < 0.001$ ). They also had higher VPT (32 V [IQR, 26–37 V] vs 16 V [IQR, 13–19 V];  $P < 0.001$ ) and lower eGFR (82 ml/min/1.73 m<sup>2</sup> [IQR, 71–96 ml/min/1.73 m<sup>2</sup>] vs 95 ml/min/1.73 m<sup>2</sup> [IQR, 82–106 ml/min/1.73 m<sup>2</sup>];  $P = 0.002$ ) (TABLE 1). Patients with DPN had significantly lower IENFD (10 fibers/mm [IQR, 7–14 fibers/mm] vs 12 fibers/mm [IQR, 8–16 fibers/mm];  $P = 0.005$ ) and higher skin AF (2.6 AU [IQR, 2.3–3.1 AU] vs 2.1 AU [IQR, 1.8–2.5 AU];  $P < 0.001$ ) (FIGURE 1).

We observed a positive correlation between skin AF and patients' age ( $R_s = 0.44$ ,  $P < 0.001$ ), diabetes duration ( $R_s = 0.32$ ,  $P < 0.001$ ), and VPT ( $R_s = 0.46$ ,  $P < 0.001$ ), and a negative correlation between skin AF and eGFR ( $R_s = -0.26$ ,  $P < 0.001$ )

and IENFD ( $R_s = -0.22$ ,  $P = 0.004$ ). Additionally, IENFD correlated negatively with VPT ( $R_s = -0.19$ ,  $P = 0.02$ ).

Skin AF was independently associated with age ( $\beta = 0.45$ ,  $P < 0.001$ ), HbA<sub>1c</sub> level ( $\beta = 0.19$ ,  $P = 0.007$ ), and IENFD ( $\beta = -0.14$ ,  $P = 0.04$ );  $R^2 = 0.27$ ,  $P < 0.001$  in a multiple linear regression analysis (TABLE 2). In a multivariate logistic regression analysis, age (odds ratio [OR], 1.09; 95% confidence interval [CI], 1.05–1.14;  $P < 0.001$ ) and skin AF (OR, 4.16; 95% CI, 1.88–9.20;  $P < 0.001$ ) were independently associated with the presence of DPN after adjustment for sex, diabetes duration, and smoking (TABLE 3).

**DISCUSSION** The main finding of our study is a close relationship of accumulation of AGEs in the skin with DPN in type 1 diabetes patients. AGEs are formed and accumulated in the endoneurium, Schwann cells, extracellular matrix, and microvessels of peripheral nerve fibers. Glycation process and activation of inflammatory reaction results in the disruption of neuronal metabolism and axonal atrophy.<sup>15,16</sup> Sveen et al<sup>17</sup> described a negative correlation between SFN and serum levels of N-ε-(carboxymethyl)lysine (CML) in patients with type 1 diabetes lasting 40 years. In a multiple linear regression model, the authors showed that CML is independently associated with IENFD.

In our study, we used the AGE-Reader device to measure skin AF. This noninvasive assessment of tissue accumulation of AGEs is a reliable marker of long-lasting glycemic control and oxidative stress in diabetes. It was previously shown that skin AF measurement is useful in the detection of late diabetic complications.<sup>12</sup> We found that

**TABLE 2** Regression coefficients of multiple linear regression

Variable	Standardized coefficient	<i>P</i> value
sex	0.04	0.54
age	0.45	<0.001
duration of diabetes	0.08	0.4
smoking	0.14	0.06
HbA <sub>1c</sub>	0.19	0.007
IENFD	-0.14	0.04

Skin AF as dependent variable; sex, age, duration of diabetes, smoking, HbA<sub>1c</sub>, and IENFD as independent variables;  $R^2 = 0.27$ ,  $P < 0.001$

Abbreviations: HbA<sub>1c</sub>, hemoglobin A<sub>1c</sub>; others, see TABLE 1

**TABLE 3** Variables associated with the presence of diabetic peripheral neuropathy in multivariate logistic regression

Independent variable	DPN	
	<i>P</i> value	odds ratio (95% confidence interval)
male sex	0.93	0.96 (0.46–2.03)
age	<0.001	1.09 (1.05–1.14)
diabetes duration	0.51	0.98 (0.93–1.03)
smoking	0.70	0.84 (0.35–2.04)
skin AF	<0.001	4.16 (1.88–9.20)

Male sex, age, diabetes duration, smoking, and skin AF as independent variables

Abbreviations: see TABLE 1

the relationship between skin AF and neuropathy is independent of traditional risk factors for neuropathy such as age, sex, and smoking. Meerwaldt et al<sup>18</sup> reported an association between skin AF and clinical diabetic neuropathy, also using the AGE-Reader device. Skin AF correlated inversely with nerve conduction velocity and amplitude. However, the study group was not homogenous and included a small number of type 1 diabetes patients.

Similarly to our study, Conway et al,<sup>15</sup> in the Epidemiology of Diabetes Complications trial, showed higher skin intrinsic fluorescence (SIF) measured with a SCOUT DM device in 111 patients with type 1 diabetes and neuropathy. The authors emphasized that SIF was a better marker of neuropathy than cumulative glycemic exposure reflected by mean HbA<sub>1c</sub> levels. In our study, patients with neuropathy did not differ in terms of the HbA<sub>1c</sub> results as compared with patients without neuropathy. We also showed previously that skin AF is an independent predictor of diabetic microangiopathy.<sup>19</sup> As skin AF reflects the combined effect of chronic hyperglycemia and oxidative stress, it might have a larger significance for the risk of complications than HbA<sub>1c</sub>. Furthermore, it represents the effect of renal dysfunction, which was also confirmed in our study. We showed a negative correlation between skin AF and eGFR. AGEs are significantly elevated in renal disease, even in the absence of hyperglycemia. The accumulation of AGEs has been related to accelerated atherosclerosis. Meerwaldt

et al<sup>20</sup> indicated skin AF as a strong and independent predictor of total and cardiovascular mortality in patients on hemodialysis.

To our knowledge, we have shown for the first time that skin AF is correlated with IENFD. This relationship confirms that the accumulation of AGEs is related to SFN. Punch skin biopsy allows a direct assessment of pathological changes in C-nerve fibers. Credible and reproducible measurement techniques have been established for assessing IENFD.<sup>2,5,21</sup> Small unmyelinated fibers are the earliest nerve fibers that are exposed to damage in diabetes. It appears that large nerve fibers are subsequently destroyed. Therefore, an interesting result of our study is a strong correlation between skin AF and VPT. Martin et al<sup>22</sup> showed VPT as a sensitive measure of peripheral neuropathy in patients with type 1 diabetes participating in the DCCT/EDIC study. VPT may provide clinically important information about large nerve fiber dysfunction in diabetes. Abnormal VPT results have been shown to predict foot ulceration and amputation.<sup>23</sup>

The limitations of the skin AF assessment have been described previously.<sup>13,15,18,19</sup> Firstly, it is known that only some AGEs have fluorescent properties. Secondly, this technique does not allow a quantitative evaluation of the AGE accumulation. We cannot exclude the influence of diet on AGE concentrations in the skin. Moreover, prospective studies are required to show the potential role of skin AF in the prediction and monitoring of diabetic neuropathy. Finally, we are aware of the fact that the results of IENFD are relatively high in the study group. Our immunohistochemistry procedure was based entirely and precisely on the reference papers. The discrepancy between our results and those previously published might result from the inclusion of a quite well-controlled group of patients with type 1 diabetes in our study, as well as from the complexity of the testing methodology. To our knowledge, this is the first study in Poland that attempted to confirm the diagnostic usefulness of the calculation of IENFD in skin biopsy. The diagnosis of SFN based on the assessment of IENFD requires further research in the Polish setting.

In conclusion, the presence of DPN, and in particular SFN, is associated with a higher accumulation of AGEs in the skin of patients with type 1 diabetes.

**Contribution statement** AA designed the study, collected the data, and wrote the manuscript; AG recruited the patients, performed skin biopsy, and performed data analysis; MN performed immunohistochemical analysis and discussed the results; AU performed skin biopsy, collected the data, and discussed the results; AM performed immunohistochemical analysis; KK collected the data and performed immunohistochemical analysis; BW-W designed the study and discussed the results; DZ-Z designed the study and discussed

the results. All authors contributed to the interpretation of the data, reviewed the manuscript, and approved the final version of the manuscript before submission.

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# Związek między niższą gęstością śródskórkowych włókien nerwowych a zwiększonym gromadzeniem zaawansowanych produktów glikacji białek w skórze u osób z cukrzycą typu 1

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## SŁOWA KLUCZOWE

cukrzycowa neuropatia obwodowa, cukrzyca typu 1, neuropatia włókien cienkich, śródskórkowa gęstość włókien nerwowych, zaawansowane produkty glikacji białek

## STRESZCZENIE

**WPROWADZENIE** Zaawansowane produkty glikacji białek (*advanced glycation end products* – AGEs) odgrywają istotną rolę w patogenezie cukrzycowej polineuropatii obwodowej (*diabetic peripheral neuropathy* – DPN).

**CELE** Celem badania była ocena gromadzenia AGEs w skórze u pacjentów z długim wywiadem cukrzycy typu 1 w powiązaniu z obecnością DPN.

**PACJENCI I METODY** W badaniu uczestniczyło 178 osób z cukrzycą typu 1 (99 mężczyzn; wiek 43 lat [przedział międzykwartylowy: 34–54 lat]; czas trwania cukrzycy 25 lat [18–31 lat]). DPN rozpoznawano, jeśli wystąpiły 2 z 5 nieprawidłowości: objawy neuropatii, brak odruchów skokowych, zaburzenia czucia dotyku, temperatury i/lub wibracji. Śródskórkową gęstość włókien nerwowych (*intraepidermal nerve fiber density* – IENFD) obliczono na podstawie materiału z biopsji skóry, po barwieniu PGP 9.5. Gromadzenie AGEs w skórze oceniono na podstawie wskaźnika autofluorescencji skóry (AF).

**WYNIKI** Pacjenci z DPN (45%) w porównaniu z osobami bez neuropatii mieli wyższy wskaźnik AF (2,6 [2,3–3,1] AU vs 2,1 [1,8–2,5] AU;  $p < 0,001$ ) i niższą IENFD (10 [7–14]/mm vs 12/[8–16]/mm;  $p = 0,005$ ). Obserwowano dodatnią korelację pomiędzy AF a wiekiem pacjentów ( $R_s = 0,44$ ;  $p < 0,001$ ) i czasem trwania cukrzycy ( $R_s = 0,32$ ;  $p < 0,001$ ) oraz negatywną korelację pomiędzy AF a oszacowaną wielkością przesączania kłębuszkowego ( $R_s = -0,26$ ;  $p < 0,001$ ) i IENFD ( $R_s = -0,22$ ;  $p = 0,004$ ). W modelu wieloczynnikowej regresji liniowej wskaźnik AF był niezależnie związany z wiekiem ( $\beta = 0,45$ ;  $p < 0,001$ ), wartością hemoglobiny glikowanej ( $\beta = 0,19$ ;  $p = 0,007$ ) i IENFD ( $\beta = -0,14$ ;  $p = 0,04$ );  $R^2 = 0,27$ ,  $p < 0,001$ . W modelu wieloczynnikowej regresji logistycznej obecność DPN była niezależnie związana ze wskaźnikiem AF (OR 4,16; 95% CI: 1,88–9,20;  $p < 0,001$ ).

**WNIOSKI** Obecność DPN, a w szczególności neuropatii włókien cienkich, wiąże się ze zwiększonym gromadzeniem AGEs w skórze u osób z cukrzycą typu 1.

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