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Reduced microvascular reactivity in patients with diabetic neuropathy

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Abstract.
BACKGROUND: Neurogenic regulation is involved in the development of microcirculation response to local heating. We suggest that microvascular reactivity can be used to estimate the severity of diabetic polyneuropathy (DPN).
OBJECTIVE: To evaluate the prospects for using the parameters of skin microvascular reactivity to determine the severity of DPN.
METHODS: 26 patients with diabetes mellitus were included in the study (patients with retinopathy ($n = 15$), and without retinopathy ($n = 11$)). The severity of DPN was assessed using Michigan Neuropathy Screening Instrument (MNSI) and Norfolk QOL-DN (NQOLDN). Skin microcirculation was measured by laser Doppler flowmetry with local heating test.
RESULTS: There were revealed moderate negative correlations between microvascular reactivity and the severity of DPN (for MNSI (Rs = –0.430), for NQOLDN (Rs = –0.396)). In patients with retinopathy, correlations were stronger than in the general group (for MNSI (Rs = –0.770) and NQOLDN (Rs = –0.636)). No such correlations were found in patients without retinopathy.
CONCLUSIONS: Correlation of the microvascular reactivity and DPN was revealed in patients with registered structural disorders in microvessels (retinopathy). The lack of such correlation in patients without retinopathy may be explained by the intact compensatory mechanisms of microvessels without severe disorders.

Keywords: Diabetes mellitus, diabetic neuropathies, microcirculation, skin, laser-Doppler flowmetry, diabetic retinopathy

1. Background

Diabetic polyneuropathy (DPN) is a common chronic complication of diabetes mellitus (DM). It has been shown that sensory DPN occurs in at least 30% of patients with type 1 DM within 13-14 years from the onset of the disease and it is also diagnosed in 10–15% of people with the new onset type 2 DM [1]. Moreover, this complication develops in 42% of patients in 10 years [1]. DPN can be found in 10–30% of individuals with impaired glucose tolerance [2] or metabolic syndrome [3]. This complication can lead to problems in daily activities, disability, psychosocial disorders and the reduced quality of life [4]. All patients with DM should be screened for DPN since the diagnosis of type 2 diabetes, after 5 years from the onset of type 1 DM and further at least once a year [5]. This requires a careful medical history identification and the assessment of sensitivity: temperature, pain,

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vibration and light touch sensation [6]. Although these tests are adequate screening tools, they do not have sufficient sensitivity to provide an accurate evaluation of the dynamics of a patient's condition and may not be a good endpoint to assess the therapy in clinical trials [7]. However, they are used as a means of comparing the efficacy of treatment of DM and DPN. Skin biopsy and confocal corneal microscopy are also proposed to evaluate small nerve fiber lesions. Nerve conduction is recommended to assess the condition of large nerve fibers [7]. These methods are well researched, but they require expensive special equipment and qualification of specialists. Thus, the exploration of new ways to evaluate nerve fiber lesions remains actual.

It is known that diabetes mellitus leads not only to disorders of nerve fibers, but also microcirculation [8]. These changes may be pathogenetically related. Therefore, the literature shows that microvascular reactivity may reflect the severity of DPN [9]. According to some experts, noninvasive measurement of skin microvascular reactivity to various physical and chemical stimuli may be a prospective method of assessing the state of the peripheral nervous system, since neurogenic regulation is involved in the response of blood vessels to various exposures. For this purpose, the method of laser Doppler flowmetry (LDF) is widely used in scientific research [8, 10]. It is quantitative, objective and non-invasive. LDF is based on exposing an area of tissue to a monochromatic laser beam. The light is reflected by moving blood cells and laser frequency shift occurs. This change can be registered with special equipment. The final integral index is proportional to the number and velocity of blood cells, but does not allow the precise calculation of their specific values [11]. It is called "flux" or "flow", depending on the designation chosen by one or another scientific group. Therefore, the terms "flowmetry" and "fluxmetry" often have the same meaning [10, 12]. The laser Doppler signal is often associated with "microcirculation" [8, 13], although this assumption is not entirely correct, since vessels of a larger diameter than the microvascular bed will inevitably be involved in the measurement as well [11]. However, experts have not yet finally agreed on a definition of the term "microcirculation" and offer different interpretations [11]. In this paper, the name "Laser Doppler flowmetry", often applied by other authors [13–15], is used and the resulting integral LDF signal is also conventionally associated with terms such as "perfusion", "blood flow" and "microcirculation".

Ambiguous results were obtained in studies which included the application of pharmacological tests: it was shown that polyneuropathy in patients with DM is associated with a decrease in the microvascular bed reactivity [16–18] but also it has been observed that these disorders do not differ in patients with and without DPN and are not related to the severity of neuropathy [19]. It should be noted that most commonly, pharmacological tests are performed with the use of iontophoresis, which requires special equipment and operator qualification and therefore its widespread clinical use is unlikely.

A more convenient functional test is a thermal test and it is successfully used to assess the microvascular reactivity [13, 20, 21], but it is presented in few studies devoted to the relationship between microcirculation and neuropathy [15, 22]. It is known that during the heating of skin there occurs the initial vasodilatation response (local thermal hyperemia), which reaches a peak within a few minutes, decreases briefly and then increases again to the plateau, which may remain stable [23]. The amplitude of the initial peak is influenced by the axon reflex (when it is blocked by the application of anesthetics, the reaction to heating decreases) and endothelium (when NO synthase is inhibited, the reaction decreases as well) [23]. Thus, the contribution of nervous regulation to the development of thermal hyperemia suggests that its initial peak measured by LDF can be a marker of DPN severity. Kasalová Z. et al. found a reduced microcirculatory response to heat in participants with DPN only in the type 1 DM group in contrast to type 2 DM. However, the authors used vibration sensitivity to assess neuropathy, which is not a sufficiently accurate and objective method for detecting DPN [22]. Jan Y.K. et al. only suggested that thermal stimulus could be used to assess microvascular reactivity and the risk of diabetic ulcers as complications of DPN [15]. However, the authors did not standardize the technique for assessing nerve fiber condition.

In our work, we propose a convenient algorithm for the thermal test, which is supposed to be applicable in clinical practice to evaluate the severity of DPN.

2. Objectives

The aim of the study was to evaluate the prospects for using the parameters of skin microvascular reactivity to determine the severity of diabetic polyneuropathy.

3. Patients and methods

3.1. Study population

The participants ($n = 26$) were recruited from the endocrinology department of Moscow Regional Research and Clinical Institute ("MONIKI"): 8 males and 18 females. To be included in the study patients required a diagnosis of type 1 or 2 diabetes mellitus (15 and 11 people, respectively) and sensory/sensorimotor polyneuropathy confirmed by instrumental examination and neurologist consultation. Exclusion criteria were causes of peripheral neuropathy other than diabetes mellitus, malignant tumors, atrial fibrillation, acute illness, anemia (hemoglobin level is below 90 g/l, erythrocyte count is below $5.1*10^{12}$/l), platelet count above $400*10^{9}$/l, signs of inflammation in the complete blood count (leukocytosis, erythrocyte sedimentation rate > 15 mm/h), dermatitis at the measurement sites, peripheral artery or venous disease, lower limb edema, pregnancy. There were applied no exclusions in relation to current pharmacotherapy. All subjects were examined for diabetic microangiopathies (nephropathy, retinopathy). Therefore, the study participants were divided into 2 subgroups depending on the presence or absence of retinopathy as an indicator of severe structural disorders of the microvascular bed.

The informed consent was obtained from all the participants. The protocol of the study complies with the ethical principles of the Helsinki declaration (revision of 2013) and was approved by the Independent Ethics Committee at the Moscow Regional Research and Clinical Institute (Moscow, Russia) (Protocol No. 11 of 13 December, 2018).

3.2. Skin microcirculation measurement

Skin microcirculation was measured using laser Doppler flowmetry (LAKK-02 complex, SPE "LAZMA", Moscow, Russian Federation. (Fig. 1A)). Total skin blood flow was expressed in perfusion units according to the principles of laser-Doppler flowmetry. There was used a local heating test to assess the reactivity of skin microvascular bed. For this purpose, a titanium, temperature-controlled, square-shaped, custom-made probe with the side length of 20 mm was applied. It had four heating elements and a center hole for laser optic fiber sensor (Fig. 1B). The patient was in a sitting position, a probe was attached on dorsal surface of the left forearm at a distance of 4 cm from the wrist joint (Fig. 1C) and on the dorsum of the left foot between the first and second toes (Fig. 1D). Skin microcirculation measurements were accomplished after the participants were relaxing for 15 min in a sitting position. The temperature was set at 32°C for 2 minutes (baseline perfusion; BP) and then raised to 42°C at 0.6°C per second and maintained at this level for 5 minutes. An example of temperature and skin microcirculation curves is shown in Fig. 2.

The parameters used for the analysis of microvascular reactivity included baseline perfusion (BP) – average microcirculation during the rest, local thermal hyperemia (LTH) – average perfusion during the plateau after heating to 42°C, the tangent of the angle between the regression line (for the

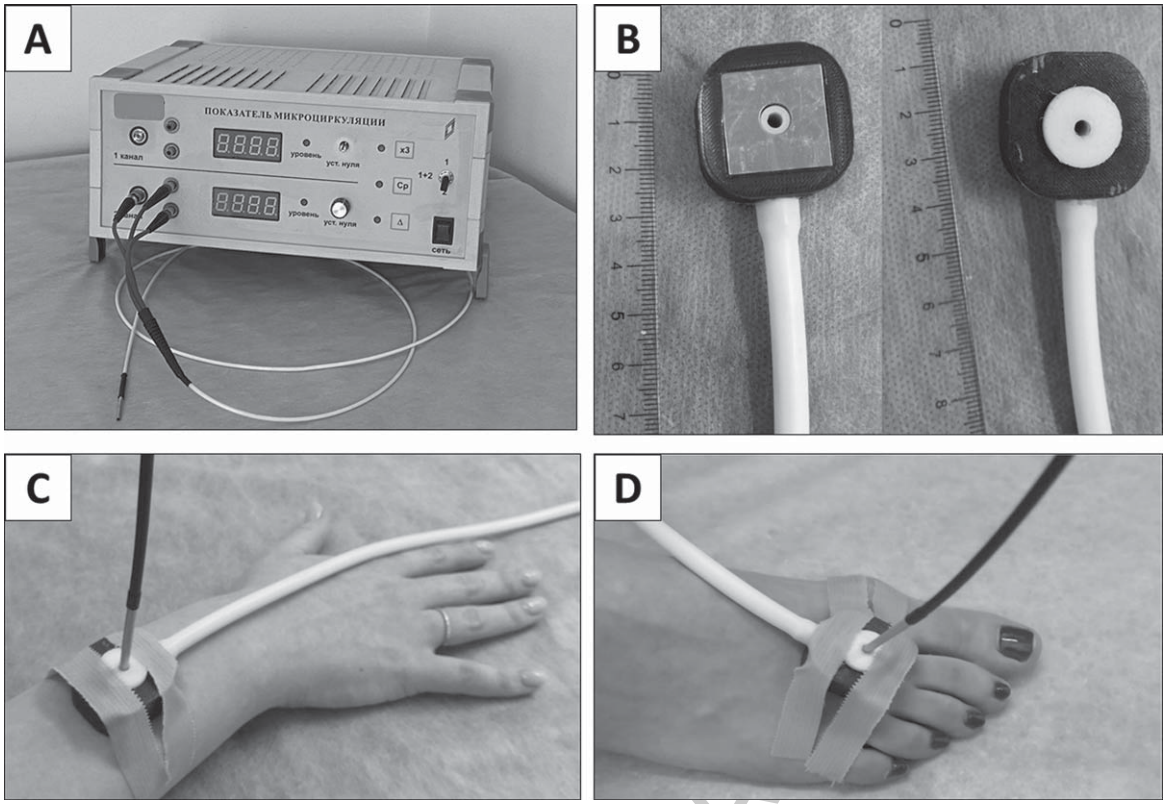

Fig. 1. A) LAKK-02 complex (SPE "LAZMA", Moscow, Russian Federation) is device for measuring of skin microcirculation by using laser Doppler flowmetry. B) Probe for carrying out local thermal hyperemia. C, D) The sites for measuring of skin microcirculation.

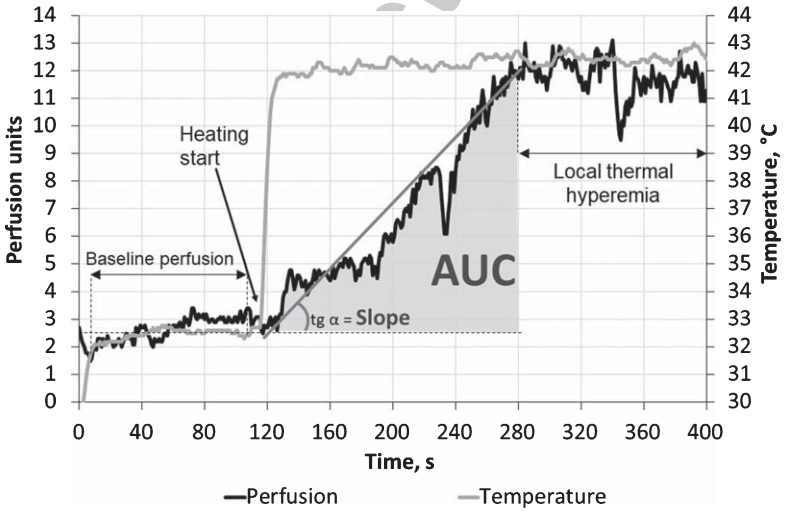

Fig. 2. The graphs of skin perfusion (microcirculation) on the forearm and temperature changes during heating test.

microcirculation curve) and the time axis within the first 120 seconds of heating multiplied by 10 (Slope-120), the area under the hyperemia curve after 120 seconds of heating (AUC-120), the relative increase of microcirculation – the difference (in percent) between the local thermal hyperemia and the baseline perfusion (LTH – BP(%)).

3.3. Assessment of diabetic polyneuropathy

The assessment of DPN was based on The Michigan Neuropathy Screening Instrument [24], which is most widely used in large cohort studies on types 1 and 2 DM [25, 26]. It includes participants' history (numbness, prickling, burning, sensitivity to touch, pain) and physical examination (appearance of feet, ulceration, ankle reflexes, vibration perception at great toe measured tuning fork 128 Hz, touch sensitivity measured Semmes-Weinstein monofilament). DPN was determined at 7 or more positive responses on the Part A ('History') or more 2 points on the Part B ('Physical assessment'). Symptoms and signs of DPN were also evaluated using the Norfolk Quality of Life-Diabetic Neuropathy (QOL-DN) [27]. This questionnaire allows to evaluate different aspects related to diabetic neuropathy since it is divided into five subscales: 1) symptoms; 2) signs of damage to small fibers; 3) signs of damage to large fibers; 4) symptoms associated with autonomic neuropathy; 5) activity of daily living. The maximum score of The Norfolk QOL-DN is 155.

Two scales were used to improve the accuracy of DPN diagnosis, as both have their advantages. MNSI is more objective and includes physical examination by a doctor. The Norfolk QOL-DN is subjective, but it allows to describe the symptoms and signs of DPN in detail and to characterize their severity.

3.4. Statistical analysis

The data were imported to Microsoft Excel 2016 (Microsoft, USA) to calculate BP, LTH, Slope-120, AUC-120, AUC-180, AUC-240, LTH – BP (%) and plots of blood perfusion units versus time were made for each participant and visually inspected for anomalous data. Afterwards the data were imported to the IBM SPSS Statistics v. 23 (IBM, USA) software for statistical analyses. The Mann-Whitney test was used to assess the differences in continuous variables between the studied groups. For the categorical data analysis there was applied the Fisher's exact. Bivariate correlations for continuous variables were verified using the Spearman correlation coefficient. P values < 0.05 (two-tailed) were considered statistically significant.

4. Results

The median score of MNSI was 9.5 (5; 13) and one of the Norfolk QOL-DN was 31.5 (19; 56) among the study participants. The maximum sum of the two parts of MNSI was 18.5, the minimum was 1.0. The maximum score of the Norfolk QOL-DN was 82.0, the minimum score was 1.0. The MNSI significantly correlated with the total score of the Norfolk QOL-DN ($Rs = 0.819$, $p < 0.001$).

The results of the correlations between the results of neuropathy severity estimation on two scales and parameters reflecting microvascular reactivity on the forearm and foot are shown in the Table 1. There were revealed moderate negative correlations between LTH – BP (%) on the foot and the results of MNSI ($Rs = –0.430$, $p = 0.028$) and The Norfolk QOL-DN ($Rs = –0.396$, $p = 0.045$). This result may demonstrate a decrease of the perfusion reaction on the lower limb with an increase in DPN severity. Therefore, the described approach using LDF and local heating up to 42 °C allows to reveal correlations between skin microvascular reactivity and DPN severity.

Table 1
Correlation coefficients (Spearman rank correlation) between skin microvascular reactivity and The Michigan Neuropathy Screening Instrument and Norfolk Quality of Life Questionnaire–Diabetic Neuropathy

	MNSI	NQOLDN
Forearm skin microcirculation		
BP	–0.137	–0.212
Slope-120	0.257	0.162
AUC-120	0.213	0.065
LTH	0.212	0.088
LTH – BP (%)	0.329	0.244
Foot skin microcirculation		
BP	0.175	0.191
Slope-120	–0.299	–0.251
AUC-120	–0.311	–0.272
LTH	–0.134	–0.056
LTH – BP (%)	**–0.430***	**–0.396***

*$p < 0.05$; **$p < 0.01$. BP: baseline perfusion (microcirculation during the rest); Slope-120: the tangent of the angle between the regression line (for the microcirculation curve) and the time axis within the first 120 seconds of heating multiplied by 10; AUC-120: the area under the hyperemia curve after 120 seconds; LTH: local thermal hyperemia (average perfusion during the plateau after heating to 42°C); LTH – BP (%): the relative difference (in percent) between local thermal hyperemia and baseline perfusion; MNSI: the total score of The Michigan Neuropathy Screening Instrument; NQOLDN: the total score of Norfolk Quality of Life Questionnaire–Diabetic Neuropathy.

It is known that reduced skin microvascular reactivity may be associated not only with neuropathy, but also with diabetic retinopathy. To exclude the influence of this factor on the estimated correlations, participants were divided into 2 subgroups. Table 2 demonstrates characteristics of these subgroups. As can be seen from this table, the subgroups were comparable in severity of diabetic neuropathy, age, diabetes duration, glycated hemoglobin level and body mass index.

There were calculated the correlations between the parameters of skin microvascular reactivity and the results of MNSI and The Norfolk QOL-DN scales in the subgroup of patients with diabetic retinopathy (Table 3). These significant correlations were found between DPN scores and several parameters of skin microcirculation: AUC-120, LTH – BP (%) ($p < 0.05$). The correlation between LTH – BP (%) and results of MNSI and The Norfolk QOL-DN is stronger in the subgroup of participants with retinopathy than in the total group (–0.738 vs –0.430 and –0.636 vs –0.396). The parameter "AUC-120" was also found to correlate significantly with these scores only in the subgroup of patients with retinopathy (–0.770 and –0.609 ($p < 0.05$)) in contrast to the total group (–0.311 and –0.272 ($p > 0.05$)). Significant correlations between the parameters of skin microvascular reactivity and the severity of DPN (results of MNSI and The Norfolk QOL-DN) were not identified in the subgroup of patients without diabetic retinopathy (Table 3).

Additionally, we compared reactivity of skin microvascular bed in two subgroups of patients: with diabetic retinopathy and without diabetic retinopathy (Table 4). Skin microcirculation parameters of reactivity on the forearm in patients with retinopathy are significantly lower ($p < 0.05$) than in participants without retinal damage. However, there were found no differences in perfusion on feet or DPN severity in these subgroups (Table 4).

Table 2
Baseline characteristics of the study participants. Description of the groups included to the study

	Patients without retinopathy	Patients with retinopathy	Total group	*P*-value
Number, *n*	15	11	26	-
Age, $M \pm SD$	52 ± 18	46 ± 13	49 ± 16	0.33
Male gender, *n*	5 (33.3%)	3 (27.3%)	8 (30.8%)	1
Diabetes duration, $M \pm SD$	12.9 ± 8.3	17.0 ± 8.8	14.6 ± 8.6	0.237
HbA1c (%), $M \pm SD$	8.51 ± 1.38	8.45 ± 1.18	8.49 ± 1.27	1
Body mass index (kg/m2), $M \pm SD$	28.05 ± 7.13	25.60 ± 6.86	27.01 ± 6.99	0.18
Nephropathy, *n*	8 (53.3%)	5 (45.5%)	13 (50.0%)	1
Arterial hypertension, *n*	10 (66.7%)	7 (63.6%)	17 (65.4%)	1
Chronic heart failure, *n*	3 (20.0%)	0 (0%)	3 (11.5%)	0.238
Coronary disease, *n*	3 (20.0%)	1 (9.1%)	4 (15.4%)	0.614
History of myocardial infarction, *n*	1 (6.7%)	1 (9.1%)	2 (7.7%)	1
MNSI, Me (LQ; UQ)	8 (4; 14)	10 (6; 12)	9,5 (5; 13)	0.610
NQOLDN, Me (LQ; UQ)	31 (19; 58)	32 (16; 56)	31,5 (19; 56)	1.000

Calculated parameters: Mean ± Standard Deviation: $M \pm SD$, Median and quartiles: Me (LQ; UQ), absolute and relative value: n (%). HbA1c: glycated hemoglobin level; MNSI: the total score of The Michigan Neuropathy Screening Instrument; NQOLDN: the total score of Norfolk Quality of Life Questionnaire–Diabetic Neuropathy.

Table 3
Correlation coefficients (Spearman rank correlation) between skin microvascular reactivity and results of neuropathy scales in patients with and without diabetic retinopathy

	Patients without diabetic retinopathy		Patients with diabetic retinopathy	
	MNSI	NQOLDN	MNSI	NQOLDN
Forearm microcirculation				
BP	–0.011	–0.179	–0.368	–0.164
Slope-120	0.297	0.270	0.075	0.073
AUC-120	0.358	0.198	0.023	–0.145
LTH	0.302	0.169	0.210	0.218
LTH – BP (%)	0.395	0.414	0.374	0.245
Foot microcirculation				
BP	0.149	0.131	0.269	0.309
Slope-120	–0.041	–0.079	–0.600	–0.365
AUC-120	0.090	0.080	**–0.770****	**–0.609***
LTH	0.172	0.093	–0.424	–0.164
LTH – BP (%)	–0.215	–0.216	**–0.738****	**–0.636***

*$p < 0.05$; **$p < 0.01$. BP: baseline perfusion (microcirculation during the rest); Slope-120: the tangent of the angle between the regression line (for the microcirculation curve) and the time axis within the first 120 seconds of heating multiplied by 10; AUC-120: the area under the hyperemia curve after 120 seconds; LTH: local thermal hyperemia (average perfusion during the plateau after heating to 42°C); LTH – BP (%): the relative difference (in percent) between local thermal hyperemia and baseline perfusion; MNSI: the total score of The Michigan Neuropathy Screening Instrument; NQOLDN: the total score of Norfolk Quality of Life Questionnaire–Diabetic Neuropathy.

Table 4
Microvascular reactivity in patients without retinopathy and with retinopathy

	Patients without retinopathy ($n = 15$) Me (LQ; UQ)	Patients with retinopathy ($n = 11$) Me (LQ; UQ)	*P* value
Forearm skin microcirculation			
BP	2.9 (1.91; 3.41)	2.59 (1.8; 2.84)	0.281
Slope-120	0.77 (0.29; 1.31)	0.1 (0.08; 0.41)	**0.011***
AUC-120	579.1 (201.2; 803.1)	216.8 (73; 376.6)	**0.009***
LTH	14.7 (9.5; 17.6)	7.4 (3.8; 11.8)	**0.015***
LTH – BP (%)	387.8 (200.3; 672.3)	160.4 (81.1; 329.9)	**0.038***
Foot skin microcirculation			
BP	2.17 (1.12; 2.86)	1.89 (1.3; 2.77)	0.959
Slope-120	0.16 (0.07; 0.26)	0.11 (0.03; 0.21)	0.574
AUC-120	142.6 (57.9; 331.8)	66.2 (46.8; 206.8)	0.198
LTH	5.3 (4.4; 9.5)	4.3 (2.6; 7.9)	0.217
LTH – BP (%)	145.2 (64.1; 368.4)	88.5 (9.3; 156.3)	0.164

*statistically significant difference ($p < 0.05$). BP: baseline perfusion (microcirculation during the rest; Slope-120: the tangent of the angle between the regression line (for the microcirculation curve) and the time axis within the first 120 seconds of heating multiplied by 10; AUC-120: the area under the hyperemia curve after 120 seconds); LTH: local thermal hyperemia (average perfusion during the plateau after heating to 42°C); LTH – BP (%): the relative difference (in percent) between local thermal hyperemia and baseline perfusion.

5. Discussion

In the present research, there were studied correlations between the reactivity of skin microvascular bed and the severity of DPN. Then, there were identified the key parameters reflecting the microvascular reactivity that were associated with the severity of neuropathy. LTH is used in studies of other authors most commonly [20, 21, 28]. It shows the level of perfusion after heating the skin to a certain temperature. In our study, LTH did not correlate with the severity of DPN. However, this parameter does not fully characterize the vasodilation features, because it does not reflect the relative increase of microcirculation compared to the initial baseline perfusion and does not describe the rate of vasodilation in response to the stimulus. We used other parameters besides LTH: Slope-120, AUC-120, LTH – BP(%). They characterize the rate of vasodilation and the increase in microcirculation relative to the baseline perfusion. Changes in these parameters are the additional signs of general microcirculation disorders, which allow to obtain a more complete description of the functional state of the microvascular bed. The decrease in them indicates a decline in the reactivity of the skin microvascular bed, and therefore the lesion of nerve fibers at DPN. It can be assumed that the lower the value of the analyzed parameters, more the severity of the DPN. Correlation analysis of DPN and skin microcirculation did not disprove the hypothesis that nerve fiber lesions affect the reactivity of skin blood flow, since there were identified moderate significant correlations between the questionnaire scores and LTH — BP(%) on the foot. The results obtained do not contradict other studies that show impaired skin perfusion in diabetic neuropathy [29, 30]. However, the revealed correlations are not strong and were observed only for the lower limb parameters, but not for the upper limb.

Due to the ambiguity of the results, we performed additional data analysis. The study participants were divided into 2 subgroups depending on the presence and absence of retinopathy as an indicator of registered structural microcirculation disorders. Patients with and without diabetic retinopathy did

not differ in the results of MNSI and The Norfolk QOL-DN, therefore they are comparable in DPN severity. At the same time the parameters of microvascular reactivity were worse in the subgroup of patients with retinopathy than in the subgroup of patients without retinopathy, which was expected and corresponds to the literature data [31, 32].

In the subgroup of patients with retinopathy correlations between skin microvascular reactivity and scores on scales of DPN assessment were revealed. However, no such correlations were found in a subgroup of participants without retinopathy.

It is possible that correlation between the DPN severity and skin microvascular reactivity was not detected in the subgroup without retinopathy due to the activity of compensatory mechanisms. Other authors have shown that damage to the peripheral nervous system begins at the earliest stages of the DM, including prediabetes [29]. Probably, this change can be compensated by the activity of local factors (endothelium, mast cells, etc.), due to which the reaction to heating is preserved. However, if the vessel wall is severely damaged, compensatory mechanisms stop working and the reactivity of skin microvascular bed decreases. This hypothesis can be indirectly confirmed by the result obtained by Sun P.-C. et al. who studied the frequency rhythms of microcirculation reflecting the functioning of certain regulatory mechanisms. It was found that endothelial activity was lower in clinical DPN patients than in patients without DPN and control subjects ($p < 0.05$), but in the subclinical DPN group there was a lower neurogenic activity and a higher myogenic activity than in patients without neuropathy ($p < 0.05$) [33]. The authors conclude that at the early stages of DPN nervous regulation of microcirculation is impaired, as evidenced by a decrease in the amplitude of neurogenic rhythm, but the myogenic regulation increases for compensation, which is expressed in an increase in myogenic rhythm. Thus, the relationship between the severity of DPN and the disturbance of skin microcirculation begins to be revealed when already affected vessels and endothelium cannot adequately compensate for the impaired nervous regulation.

B.E.K. Klein and et al. showed the relationship between microvascular and neuropathic complications of DM [34]. Proliferative retinopathy is associated with the presence of sensory DPN, signs of autonomic neuropathy (heart rate variability, standard deviation of RR intervals), but it is not proved that the nervous or vascular component is damaged first [34]. It is possible that autonomic neuropathy precedes or accelerates the development of diabetic retinopathy [35]. There is evidence that neural changes, such as retinal apoptosis, may antecede microvascular complications in humans [36]. However, retinal neurons are different from nerve fibers, which are affected by peripheral neuropathy, so it cannot be said that changes in nerve regulation lead to all microangiopathies in the body. It is possible that the heating test will allow detection of early signs of sensory DPH, since LTH depends on the axon reflex, but not on autonomous regulation, the disorder of which is supposed to promote retinopathy.

It should be noted that correlations between microvascular reactivity and DPN severity were detected only in the lower limb. This is probably due to the fact that vessels and nerves in the feet are affected earlier than in the hands [14]. In addition, there are many factors responsible for the skin vasodilatation, each of which can affect microcirculation and occurs differently in each patient.

6. Conclusion

This study shows that skin microvascular reactivity measured with LDF and thermal test is promising as a maker of DPN severity. It was found that statistically significant negative correlations between the microvascular reactivity in foot and the severity of neuropathy were revealed in total group and in patients with diabetic retinopathy. It can be assumed that a decrease in the reactivity of skin microvascular bed indicates an increase in the severity of neuropathy, but only in patients with registered structural disorders in microvessels. This is probably due to the early lesion of the nervous system, which is

compensated by the activity of local factors in patients without severe disorders in microvessels, so the local thermal hyperemia does not change. However, if the vascular wall is severely damaged, compensatory mechanisms stop working and the reaction of microcirculation to heating decreases. This fact limits the use of a heating test in clinical practice but reflects the prospects for its application in scientific research, including the study of the pathophysiology of the microvascular bed. Therefore, further research is necessary to develop a method of diagnosing the severity of nerve fiber damage by measuring the reactivity of skin microvascular bed and using it in the evaluation of the pharmacotherapy effectiveness.

7. Limitations

This work is a pilot study. The study design does not allow the effect of drug therapy on skin microcirculation to be assessed.

Conflicts of interest

The authors declare no conflict of interest.

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