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Optical noninvasive diagnostics of dynamic changes in the level of blood microcirculation and oxidative metabolism using temperature tests

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ABSTRACT

The problem of diabetes mellitus (DM) has attracted scientists of different specialities, as its prevalence is increasing worldwide and assumes the character of a pandemic. The use of optical non-invasive diagnostic methods is a promising direction for the early determination of the presence and severity of diabetic complications. This work is aimed at assessing changes in the fluorescence intensity and the blood microcirculation level, evaluated in the skin of patients with DM with the use of local cold and heating tests. An experimental studies were carried out involving patients with type 2 DM and healthy controls. Significant differences were found in the changes in the level of microcirculation and oxidative metabolism under the influence of local temperature effects (local heating and cooling) among patients and control group. The results can be used in further development of an optical non-invasive diagnosis of diabetes complications.

Keywords: biomedical photonics, optical noninvasive diagnostics, laser Doppler flowmetry, fluorescence spectroscopy, diabetes mellitus, microcirculation, oxidative metabolism

1. INTRODUCTION

Light-based technologies present unique opportunities for the study and diagnosis of biological tissues. Modern advances in biophotonics allow to obtain information about the state of the body non-invasively and to diagnose various pathophysiological processes in the early stages of their development. Biophotonics methods are used in many areas of clinical practice, since the optical properties of biological tissues are subject to significant changes in the development of pathological conditions.[1]

Diagnosis of diabetes mellitus (DM) complications is one of the areas of medicine that particularly benefit from the implementation of optical non-invasive diagnostic methods. DM is a chronic disease, which in the absence of adequate therapy leads to disorders in many organs and systems of the body. Since the prevalence of diabetes is increasing every year and is already reaching alarmingly high values,[2] complications of this disease are considered among the most significant problems of the modern healthcare system. In diabetes, acute and chronic complications develop. Although acute complications are life-threatening conditions, such as hyperglycemia or ketoacidosis, the quality and duration of a patient's life is primarily determined by chronic complications.[3] In the absence of proper treatment, complications of diabetes can ultimately lead to the development of cardiovascular diseases, renal failure, the need for hemodialysis and kidney transplantation, amputation of the lower extremities.[4]

The development of diabetic complications is characterized primarily by vascular and metabolic disorders. In this aspect, microcirculation plays an important role in the development of DM complications, since changes

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in the microvascular wall, the hemodynamic regulatory mechanisms, and redistribution of blood flows lead to a decrease in capillary metabolism and thus to impaired tissue nutrition.[5] The microcirculatory function can be evaluated by means of various optical non-invasive methods, including laser Doppler flowmetry (LDF) and laser Doppler perfusion imaging, laser speckle contrast imaging, optical coherence tomography, photoacoustic tomography, etc.[6] LDF has a significant history of application in clinical practice and is often used to diagnose microcirculatory disorders in various socially significant diseases.[7,8] The principle of the method is based on the Doppler effect arising from the scattering of probe laser radiation from the surface of red blood cells moving in biological tissue.[9] LDF allows to evaluate the functional state of blood microcirculation and its pathological changes, as well as diagnose the mechanisms of regulation of hemomicrocirculation.[10]

Another optical non-invasive technology used in the clinic of diabetes to assess the severity of its complications is fluorescence spectroscopy (FS). Assessing the intensity of tissue fluorescence, one can judge its viability by evaluating mitochondrial function. As an assessment of mitochondrial function, the ratio of coenzymes NADH and FAD is used,[11] which can be calculated from the intensity of their fluorescence.[12] Significant contribution to the overall skin fluorescence is also made by the advanced glycation end-products (AGE), which are known to accumulate in tissues in diabetes. AGEs accumulation is considered a marker for the development and severity of diabetic complications.[13]

A multimodal approach implemented in the form of simultaneous measurement of several diagnostic parameters by optical non-invasive diagnostic methods can provide comprehensive information about the functional state of biological tissues.[14,15] This work is aimed at assessing changes in the fluorescence intensity and the level of blood microcirculation, evaluated in the skin of patients with DM with the use of local cold and heating tests.

2. MATERIALS AND METHODS

Experimental studies were conducted with the participation of 20 patients with type 2 DM and 20 healthy controls. Laser multifunctional complex "LAZMA-ST" (SPE "LAZMA", Russia) was used in the study. The complex includes LDF registration channels with single-mode near-infrared (1064 nm) laser and a fluorescence spectroscopy channel with UV (365 nm) and blue (450 nm) radiation sources. The device "LAZMA-TEST", designed for functional heating, was used to provide thermal effects. Patients rested supine with a probe located on the plantar surface of the big toe. The study included 3 consecutive stages: recording of the baseline signal for 8 minutes (BT), local cooling of tissue to 10 C and recording the signal for 1 minute and local heating to 35 C with recording for 4 minutes. A pair of fluorescence spectra was recorded during each stage of the experimental study. Fig. 1 presents a schematic description of the experimental research methodology. Empty gaps indicate stages of the experiment during which the LDF signal was not recorded.

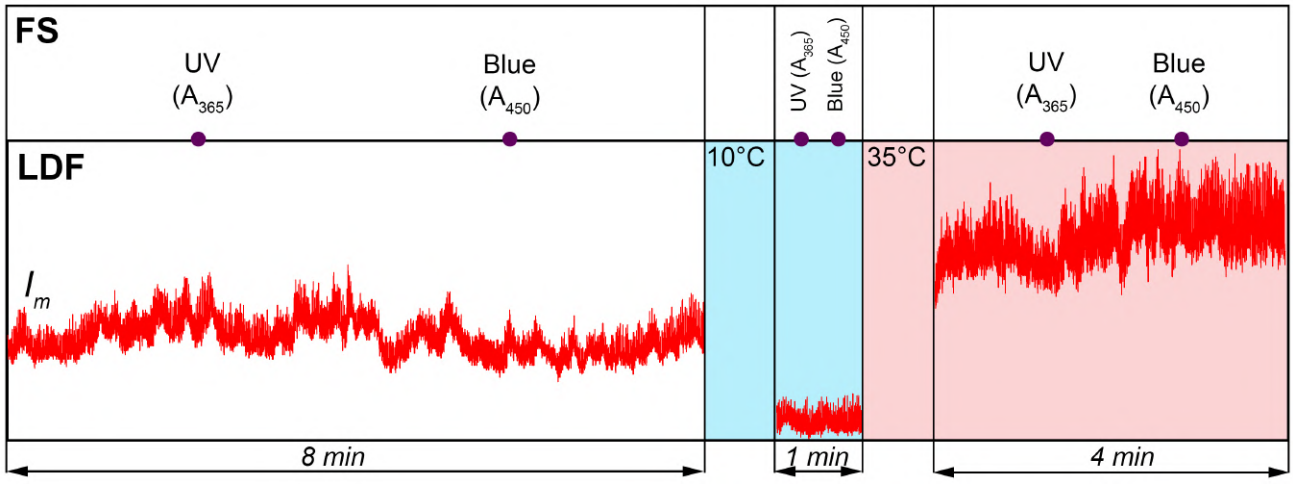

Figure 1. Schematic representation of experimental research protocol

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During the research, the microcirculation index (I_m) and the fluorescence amplitudes excited at wavelengths of 365 nm (A_{365}) and 450 nm (A_{450}) were recorded. To analyze the mechanisms of regulation of microcirculatory blood flow, the obtained LDF signals were subjected to wavelet analysis by means of the Morlet wavelet. The amplitudes of endothelial, neurogenic, myogenic, respiratory and cardiac oscillations were analyzed. Subsequently, the bypass index (BI) and nutritive blood flow (I_{mn}) were calculated according to the known formulas.[16,17]

The total value of the BI is obtained by adding BI_1 and BI_2, where BI_1 is the bypass index associated with differences in tone in the microvessels of the nutritive and non-nutritive blood flow pathways directly within the microcirculatory bed, and BI_2 is the bypass index associated with differences in perfusion of microvessels and larger vascular segments (arteries, venules and veins) in cases of arterial hyperemia or venous stagnation:

$$BI_1 = An/Am, \tag{1}$$

where An and Am - are the amplitudes of oscillations of neurogenic and myogenic frequency ranges respectively.

BI_2 is calculated according to the following equation:

$$BI_2 = Ac(r)/Am, \tag{2}$$

where $Ac(r)$ – the dominant amplitude of oscillations of the heart and respiratory rhythms. It is taken into account if it is greater than or equal to 1. By the calculation of BI, it is possible to assess the perfusion of nutritive and shunt pathways in microvascular networks. The value of nutritive perfusion I_{mn} is calculated by the expression:

$$I_{mn} = Im/(1 + BI). \tag{3}$$

For a comprehensive assessment of the relationship between the state of the microvasculature and oxidative metabolism in the tissue, the parameter oxidative metabolism index (OMI) was calculated using the following formula:

$$OMI = I_{mn}/(A_{365} + A_{450}), \tag{4}$$

The parameter allows the evaluation of the relationship between the flow of oxygenated blood into the capillary bed and its utilization in tissues.

3. RESULTS AND DISCUSSION

To assess the dynamic changes in the studied parameters when applying temperature tests, their relative increment was calculated in comparison with the data of the baseline recording. The calculation results for the microcirculation index and oxidative metabolism index are presented in the Fig. 2.

In the group of patients, increased perfusion and slightly higher feet temperature were observed in the baseline. When applying local tissue cooling, a significant decrease in microcirculation was observed in both groups. In the group of patients, a more marked decrease in the microcirculation index was observed, possibly due to the fact of higher initial values.

When applying local heating, the microcirculation index significantly increased both in the control group and among patients, exceeding the initial values. The increase in the parameter is explained by vasodilation caused by local heating of tissues.[18] However, it is worth noting the fact that among patients in 20% of cases there was no restoration of the initial level of the microcirculation index. In the control group under local heating, the index of microcirculation increased on average 2 times compared to the baseline. The increase in perfusion in patients at the third stage of the experimental study was significantly less pronounced. In diabetes depletion in the microvascular bed and decrease in the number of microvessels occurs. Thus, with local heating of tissues, larger vessels (arteries) predominantly react, while perfusion of microvessels does not change or decreases.

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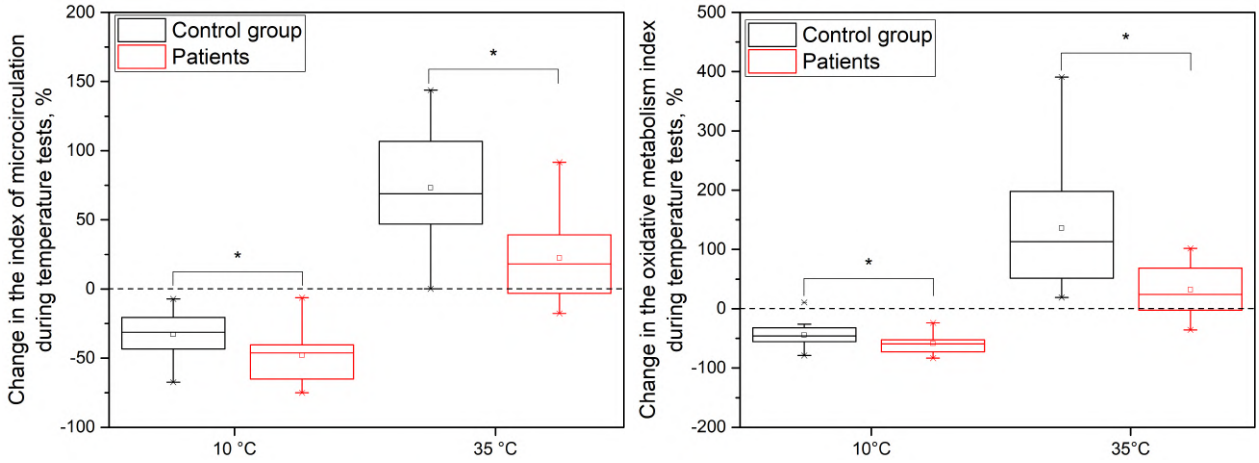

Figure 2. Relative increment of index of microcirculation (left panel) and oxidative metabolism index (right panel) when applying local temperature tests
* - The significance of the difference between the values was confirmed with $p < 0.05$ according to the Friedman-Anova test

OMI estimates showed similar results compared to perfusion. The parameter significantly decreased during local cooling stage with a more significant decrease in the patient group. The increase in the OMI during heating was significantly more pronounced in the control group compared with patients. Moreover, in the group of patients, there was a lack of growth or a decrease in this indicator in 30% of cases.

4. CONCLUSION

The study shows that the proposed research method in the form of applying local temperature effects while simultaneously recording laser Doppler flowmetry and fluorescence signals may have the potential to analyze the state of oxidative metabolism of biological tissues. It was shown that the increase in the microcirculation index and the oxidative metabolism index when using local heating of tissues is significantly less pronounced in patients with DM compared with a healthy controls.

The study provides preliminary results that need to be further studied with a larger sample size. The results can be used to further development of an optical non-invasive diagnosis of diabetes complications. Of potential interest is the observation of changes in the studied parameters in dynamics during inpatient treatment, as well as the evaluation of additional diagnostic parameters.

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