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Application of optical non-invasive methods to diagnose the state of the lower limb tissues in patients with diabetes mellitus

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Abstract. The paper shows the possibility of assessing the functional state of microcirculatory-tissue systems of patients with diabetes mellitus by laser Doppler flowmetry (LDF), diffuse reflectance spectroscopy (DRS) and fluorescence spectroscopy (FS) methods. A review of the existing non-invasive optical technologies used to assess the state of microcirculation and oxygen metabolism in tissues of patients with diabetes is conducted. A series of experimental studies involving 76 patients with diabetes and 46 healthy volunteers was carried out. A wavelet analysis of LDF-grams was used to evaluate the adaptive changes of microcirculation during the temperature tests. The obtained data revealed that the proposed methodology in the form of combined use of several diagnostic technologies (LDF, FS and DRS) allows us to detect the presence or absence of trophic disorders and to evaluate adaptation processes during thermal tests.

1. Introduction

One of the acute problems of modern medicine is the diagnosis of diabetes mellitus (DM) complications. Diagnosis of diabetic microangiopathy and neuropathy is relevant in view of the still unanswered questions about their role in the pathogenesis of diabetic foot syndrome.

It is known that during diabetes, vascular walls and nerve endings suffer damage due to metabolic disorders, lack of oxygen in the tissues and an excess of toxic products of carbohydrate metabolism. As a result, the direct control of these deviations to assess the functional state of patients with DM tissues is the most logical.

Currently, a variety of optical non-invasive methods is used for the study of the microcirculatory-tissue system state.

1.1 Spectrophotometric techniques

The diffuse reflectance spectroscopy (DRS) method has been applied in the assessment of tissue saturation. This method allows to noninvasive continuous measure of changes in the concentration of oxygenated haemoglobin (cHbO₂) and deoxygenated haemoglobin (cHb). The level of oxygen saturation of tissues (including disorders in diabetes) is judged by the view of the diffuse reflection spectrum based on different mathematical models or empirical relationships derived for communication the reflection coefficient with a concentration of specified chromophores. As valued



characteristics of the skin diffuse reflection spectrum, the slope of the spectrum and the area under the spectrum curve are used most often. One of the diabetes complications is the development of vascular occlusive lesions that may lead to the development of tissue ischaemia. At the same time, a high level of oxyhaemoglobin in the blood can be observed. A number of scientific papers are dedicated to the research status of tissue metabolism in diabetes by diffuse reflectance spectroscopy method. Sujatha *et al* in their work demonstrated a statistically significant decrease in the total haemoglobin concentration in diabetic subjects compared to a control group without diabetes using the diffuse reflectance spectroscopy method [1]. There are study approaches where the relationship of diffuse reflectance coefficients at different wavelengths is selected as a diagnostic parameter. These wavelengths are most often isosbestic points of oxy and deoxyhaemoglobin. The DRS method can be used to study the processes occurring immediately in places of severe metabolic disorders such as tumours or diabetic trophic ulcers.

To assess the state of blood flow and detect possible vascular disorders when applying the method of diffuse reflectance spectroscopy, various stress tests are also used, including venous occlusion and exercises [2, 3]. Diffuse reflectance spectroscopy also is used for assessment of foot ulceration and wound healing processes [1].

Another spectrophotometric technology to evaluate the delivery and tissue oxygen consumption is a hyperspectral imaging technology. The use of hyperspectral technology allows detection of systemic and local microcirculatory disorders in diabetes mellitus [4]. In a number of papers, hyperspectral imaging technology is considered as a tool to noninvasively monitor and predict ulcer formation and healing on the diabetic foot [5]. The paper Nauvong *et al* shows the possibility of using hyperspectral imaging technology to assess cutaneous Hb oxygen saturation under wound healing.

1.2 Optical coherence tomography

As one of the methods to identify possible violations in the state of peripheral blood vessels, an assessment of retinal vessels is applied because they are the first target in diabetes damages. Changes in retinal vascular state reflect a range of pathophysiologic responses to hyperglycaemia, hypertension, inflammation, hypoxia, and endothelial dysfunction, and can predict different diabetes microvascular complications. Disorders of retinal blood vessels may be a marker of the development of neuropathy at an early stage [6]. To assess the state of retinal vessels various methods are used, including Doppler Fourier domain optical coherence tomography. The high-speed Fourier domain OCT enables obtaining of blood velocity and vessel volumes that allows measurements of pulsatile blood flow. Optical coherence tomography also allows measurement of retinal thickness as a marker of disease progression [7].

1.3 Fluorescence techniques

Fluorescence techniques are widely used in the study of disorders associated with the development of diabetes. The fluorescence spectroscopy (FS) method allows recording the fluorescence spectra of endogenous and exogenous skin fluorophores.

An important parameter is the tissue viability is the mitochondrial function. Using indicators of respiratory chain activity, it is possible to predict the death of the cells, to diagnose the state of tissue ischemia, or even to talk about their cancerous activity [8]. One of the assessments of mitochondrial function is the ratio of coenzymes NADH (the reduced form) and FAD (oxidized form). At hyperglycaemic conditions, changes occur in the mitochondrial respiratory chain, resulting in an overall imbalance of NADH/FAD.

Another violation that occurs during prolonged hyperglycaemia and leads to oxidative stress - excessive production of advanced glycation end products (AGE). The accumulation of sugars in the body leads to the glycation of proteins and thus to increase the production of AGEs, which initiate the expression of collagen genes and other proteins of the capillary membrane and skin and lead to the formation of adhesions between the collagen fibres. In diabetic disorders, AGEs accumulate in large amounts in the extracellular matrix. The presence of glycated proteins in the bloodstream may affect

many tissues and cells. For example, at glycation of collagen and elastin, fibrosis and atherosclerosis may develop, leading to violations of blood vessel elasticity. A number of papers is devoted to research of AGEs accumulation in tissue by the fluorescence spectroscopy method [9]. Currently to record changes in diabetes related to the accumulation of AGEs various AGE-readers are used [10].

Nowadays one of the promising directions for an effortless and automatic assessment of larger tissue areas in DM is the use of fluorescence imaging technology. This technology allows to evaluate the redox ratio, which characterizes the ratio of coenzyme NADH/FAD in tissues [11]. Furthermore, the use of endogenous fluorescence imaging enables a clear visualization and discrimination of distinct wound regions. AGEs accumulation measurement technology by fluorescence imaging methods are also being developed [12].

1.4 Laser Doppler techniques

For the diagnosis of peripheral blood flow state, laser Doppler flowmetry (LDF) is widely used. This method is highly sensitive and allows measurement of blood flow and detecting of microcirculatory disorders in diagnostic of different microvascular disorders [13]. LDF has frequently been used to measure blood flow in the limbs of diabetic patients. The oscillatory process recorded by this method is a superposition of fluctuations caused by active and passive factors. To diagnose the state of the microcirculation system it is important to determine the contribution of various components of the rhythmic oscillations. One of the most important properties of the microvasculature, according to many researchers, is the reactive hyperaemia that occurs in response to the increased needs of the tissues for oxygen and nutrients. In this regard, different functional stress tests are used for a better assessment of the regulatory mechanisms state. To assess the state of microcirculation in diabetes, orthostatic tests [14], pharmacological tests [15], physical exercises [16] are widely adopted. Local heating test allows to evaluate the response of local regulatory mechanisms to the tissue heating [17]. Numerous studies use LDF for elucidating NO synthase mechanisms for quantification of abnormalities in the endothelium-dependent NO vasodilation and other microvascular research issues.

Similar data can be provided by laser Doppler imaging technique. This technique assesses blood perfusion over large areas and find widespread application in wound healing assessment [18].

1.5 Combined technologies

For a comprehensive diagnosis of the microcirculatory-tissue systems state currently it is promising to apply several diagnostic technologies simultaneously in one diagnostic volume. To assess the state of blood flow and detect possible vascular disorders different combinations of methods are applied. For instance, simultaneous measurements of the intensities of fluorescence and Rayleigh components allow decreasing the age dependency of the separation between diabetic subjects and control group, as well as increasing the overall magnitude of the separation [19]. Also the DRS method can be used for the correction of the data obtained during fluorescence studies [20]. Another example of combined technologies is a lightguide tissue spectrophotometry (O2C). It is a combination of LDF and tissue spectrometry. This method allows measuring both relative blood flow by the laser Doppler technique and haemoglobin oxygenation and haemoglobin amount in tissue by spectrometric techniques [21].

2. Experimental method and equipment

The aim of this work was to evaluate the possibilities of simultaneous application of LDF and the FS methods to identify trophic disorders at earlier stages in the skin of feet of patients with diabetes mellitus. To achieve this goal, the experimental studies were conducted. Local heating probe was chosen as the provocative exposure. Experimental studies included the registration of parameters during 4 of the successive stages. The first stage included registration of a base test of LDF-gram for a 4 minute-period. The second and third stages included registration of a local cold test ($t=25\text{ }^{\circ}\text{C}$) and a local heating test ($t=35\text{ }^{\circ}\text{C}$) for 4 minutes each. The fourth stage included registration of a local heating

test ($t=42\text{ }^{\circ}\text{C}$) for 10 minutes. A pair of fluorescence spectra at the excitation source with wavelengths of 365 nm and 450 nm were recorded in the first examination stage to avoid the photobleaching effect.

One study on each foot was 22 min, and indices of both feet recorded successively. Simultaneously with the continuous recording of perfusion a pair of fluorescence spectra under excitation of UV (365 nm) and blue (450 nm) light were recorded at each stage. The light guide probe was brought into contact with the dorsal surface of the foot on a point located on a plateau between the 1st and 2nd metatarsals. All studies were performed in the supine position. After carrying out experiments, LDF-grams of each phase of the study were subjected to adaptive wavelet analysis by LDF 3.0.2.384 software. Experimental studies were carried out by LDF and FS methods using the multifunctional laser noninvasive diagnostic system «LAZMA MC». The «LAZMA-TEST» complex was used to provide thermal effects. Measurements were carried out on 76 patients diagnosed with diabetes and 46 apparently healthy volunteers of approximately the same age. Figure 1 presents schematic views of location of the optical sensor on patient's foot (a) and the equipment used in the experimental studies (b, c).

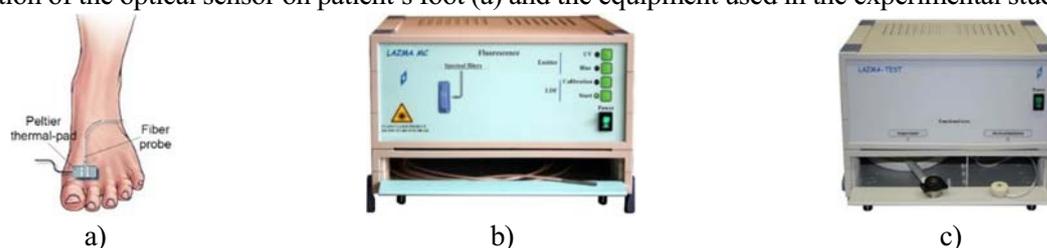


Figure 1 - Location of the optical sensor on foot (a) and the appearance of the “LAZMA MC” complex (b) and the “LAZMA-TEST” device (c)

Registration of the blood microcirculation index (I_m) and the I_{365}^F and I_{450}^F fluorescence amplitudes were performed. Before the beginning of each study at the specified point registration of the spectra of skin diffuse reflection was carried out by a compact spectrometer “FLAME” (Ocean Optics, USA). In addition, for patients with ulcers, spectra were recorded directly at ulcers and at one centimetre away from the ulcers (at the intact region).

During the study, the oscillation amplitude values for the 5 main ranges were determined: endothelial (A_e), neurogenic (A_n), myogenic (A_m), respiratory (A_r) and cardiac (A_c). These values were normalized to the standard deviation (σ) and the average value of the index of microcirculation (I_m) were also determined.

3. Results and discussion

The research process revealed that the fluorescence intensity was higher in patients (2.7 ± 0.8 AU, 1.8 ± 0.7 AU) at both excitation wavelengths in comparison to the control group (2.1 ± 0.8 AU, 1.2 ± 0.4 AU). This fact can be explained by the accumulation of advanced glycation end products and formation of glycated proteins, including collagen, which is the largest contributor to the fluorescence spectrum at these wavelengths, in the body of patients with diabetes. At the same time microcirculation index in the group of patients reduced, which is especially pronounced during the local heating tests.

The results obtained in patients and in the control group were compared. Significant differences in some of the above parameters were identified and statistically proved (by One-way ANOVA, $p < 0.05$) for the group of patients relative to the control group.

According to the data of DRS measured, spectra curves of the skin diffuse reflection were analysed. To study the ulcerative processes ratio of the diffuse reflection coefficient, the absorption wavelength of oxyhaemoglobin (540 and 578 nm) was calculated. Erythema coefficient was calculated and analysed according to the method in which area under the curve of the skin optical density in the spectral range of 510-610 nm is estimated.

Table 1 shows the numerical indicators of the study results.

Table 1. The results of 3rd and 4th stages of experimental studies.

Parameters	3 stage		4 stage	
	Control group	Patients	Control group	Patients
I _m , PU	8,68±3,29	6,70±2,65*	20,09±4,46	11,56±3,67*
A _e , PU	0,58±0,31	0,40±0,24*	0,6±0,39	0,42±0,23*
A _n , PU	0,60±0,32	0,38±0,17*	0,5±0,48	0,35±0,17*
A _m , PU	0,49±0,24	0,29±0,17*	0,46±0,36	0,25±0,15*
A _r , PU	0,22±0,15	0,21±0,11	0,33±0,16	0,28±0,17*
A _c , PU	0,36±0,23	0,53±0,25*	1,19±0,48	0,89±0,42*

* - statistical significance of the differences of the indicator values of patients in relation to healthy volunteers with $p < 0,05$

The patients have reduced value of nutritive blood flow during local heating (2.42 ± 1.67 PU) in comparison to the control group (4.91 ± 2.85 PU), which indicates redistribution of blood flow in the direction of the shunt component and reducing of capillary perfusion. Reduced values of endothelial oscillations indicate the weakening of the endothelium-dependent vasodilation associated with the secretion of nitric oxide (NO) by vascular endothelium [17].

The lack of growth of the neurogenic oscillation amplitudes in patients shows disorders in the work of sensory peptidergic nerve fibres responsible for the initial stage of vasodilation during local heating.

During the research, the highest blood circulation observed was also revealed in patients with focal disorders. Erythema index for patients without ulcers was higher than that for the control group (17.1 ± 11.7 vs 13.3 ± 8.6), which may indicate the presence of disorders in the peripheral circulation.

4. Conclusions

The proposed methodology allows us not only to detect the presence or absence of trophic disorders, but also to determine possible causes by evaluating adaptation processes during thermal tests and by comparing them with the results obtained in the control group. The use of wavelet analysis for assessing the regulatory mechanisms of peripheral blood flow during heating tests gives the opportunity to explore changes in autoregulation of vascular tone, as well as the regulation of the value of shunt blood flow by sympathetic fibres, which gives an indirect idea of the nervous innervation of blood flow and can indicate the presence of neuropathies.

The combined use of several optics diagnostic technologies allows for a comprehensive approach to the problem of assessing the state of microcirculatory-tissue systems, as well as to identify and predict the development of trophic disorders and diabetic foot syndrome at an earlier stage.

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