ORIGINAL ARTICLES

DIAGNOSTIC CAPABILITIES OF DIFFERENT METHODS OF LASER DOPPLER FLOWMETRY SPECTRAL INDEXES ASSESSMENT IN PATIENTS WITH DIABETIC MICROANGIOPATHY

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Abstract

The article contains the results of a study of two different methods for calculating the spectral parameters of laser Doppler flowmetry in patients with diabetic microangiopathy caused by type 2 diabetes mellitus (main group) and those with excluded diabetes mellitus (control group). Spectral indices were calculated using either average or maximum amplitudes of the frequency ranges. When comparing the contribution of respiratory and pulse fluxmotions using average amplitudes, there were significant ($p < 0.05$) differences between the main and control groups. On the contrary, when using the maximum amplitudes, no significant differences were noted ($p > 0.05$). Also, significant correlations were found between the contributions of respiratory and pulse fluxmotions and the estimated glomerular filtration rate in the main group, using both calculation methods. These studies indicate the feasibility of using a technique based on the analysis of average amplitudes to increase the specificity of laser Doppler flowmetry as a method for diagnosing diabetic microangiopathy.

Key words: laser Doppler flowmetry, diabetes mellitus type 2, diabetic microangiopathy, spectral analysis, assessment of spectral indices.

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ДИАГНОСТИЧЕСКИЕ ВОЗМОЖНОСТИ РАЗЛИЧНЫХ МЕТОДИК ОЦЕНКИ СПЕКТРАЛЬНЫХ ПОКАЗАТЕЛЕЙ ЛАЗЕРНОЙ ДОППЛЕРОВСКОЙ ФЛОУМЕТРИИ У ПАЦИЕНТОВ С ДИАБЕТИЧЕСКОЙ МИКРОАНГИОПАТИЕЙ

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Резюме

В работе представлены результаты исследования двух различных методик расчёта спектральных показателей лазерной допплеровской флоуметрии у пациентов с диабетической микроангиопатией на фоне сахарного диабета 2 типа (основная группа) и лиц с достоверно исключённым сахарным диабетом (контрольная группа). Расчёт спектральных показателей выполнялся с использованием либо средних, либо максимальных амплитуд частотных диапазонов. При сравнении вклада дыхательных и пульсовых флаксмоций с использованием средних амплитуд были получены значимые ($p < 0{,}05$) различия между основной и контрольной группами, тогда как при использовании максимальных амплитуд значимых различий не отмечалось ($p > 0{,}05$). При проведении корреляционного анализа вклада дыхательных и пульсовых флаксмоций и расчётной скорости клубочковой фильтрации в основной группе были выявлены значимые корреляции при использовании обеих расчётных методик. Данные исследования свидетельствуют о целесообразности использования методики, основанной на анализе средних амплитуд, для повышения специфичности лазерной допплеровской флоуметрии как метода диагностики диабетической микроангиопатии.

Ключевые слова: лазерная допплеровская флоуметрия, сахарный диабет 2 типа, диабетическая микроангиопатия, спектральный анализ, расчёт спектральных показателей.

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Introduction

The relevance of the development of diagnostic methods for microvascular complications of diabetes mellitus is beyond any doubt. In 2019, according to the International Diabetes Federation, there were 463 million patients with diabetes in the world aged 20-79 years (9.3% of this age group), and by 2045 the projected incidence will be 700 million [1]. The total number of patients with diabetes in Russia as of 01.01.2019 was 4,584,575 people (3.12% of the population), of which patients with type 2 diabetes accounted for 92.4% (4.24 million). According to NATION national epidemiological study, the proportion of undetected cases of type 2 diabetes in Russia is on average 54% [2]. Cardiovascular diseases, resulting from or aggravated by vascular complications of diabetes, are the main cause of mortality in this group of patients [3, 4].

For the diagnosis of diabetic microangiopathy, intensive research is done into the possibilities of laser Doppler flowmetry (LDF) [5-8]. The wavelet analysis of the amplitude-frequency spectrum of the LDF signal allows us to identify groups of oscillations (harmonics) in certain frequency ranges, the amplitudes of which provide information about the function of local and systemic mechanisms of microcirculation modulation. In particular, with the help of LDF, it was shown that insulin therapy can affect the state of microcirculation even in the short term [9].

The large-scale application of LDF in the diagnosis of microcirculatory disorders is complicated not only by the relatively high cost of equipment, but also by the lack of a unified algorithmic approach to the interpretation of the data obtained. In this regard, there is an urgent need to develop new methods and numerical indices that characterize changes in the parameters of LDF in patients at different stages of diabetic microangiopathy.

The purpose of this work was a comparative analysis of the use of two different methods for calculating the spectral parameters of LDF in patients with diabetic microangiopathy.

Materials and methods

The study was conducted on the basis of St. Petersburg State Medical Institution «City Hospital of St. George the Great Martyr» (St. Petersburg, Russia) among patients receiving treatment on the basis of the Surgical Departments 2 and 4 (the main group) and Cardiology Department 1 (the control group). All patients signed a voluntary informed consent to participate in the study.

The main group included 40 patients. The criteria for inclusion in the main group were: the established diagnosis of type 2 diabetes mellitus, the presence of manifest signs of diabetic microangiopathy (diabetic foot syndrome) and age over 50 years. The following criteria were chosen as non–inclusion criteria: the presence of primary renal diseases in the anamnesis, oncological diseases, systemic connective tissue diseases, administration of nephrotoxic or immunosuppressive drugs, morbid obesity, the presence of varicose vein disease of the lower extremities and post–thrombophlebitic disease, the consequences of acute cerebral circulatory disorders in the form of spastic lower para- and tetraparesis.

For the control group, 30 patients over 50 years of age with reliably excluded diabetes mellitus were selected, in compliance with the above non-inclusion criteria. The summary characteristics of the groups of the surveyed patients are given in the table.

Laser Doppler flowmetry was performed with the diagnostic system «BIOPAC LDF 100C» (Biopac, USA) with a probing radiation wavelength of 830 nm. In each patient, a 10-minute registration of the LDF-gram was performed, in the supine position. The sensor was placed on the skin of the back of the foot in the distal part of the first metatarsal space.

To process the obtained LDF signals, a spectral wavelet analysis based on the Morlet wavelet was used. The following were taken as the boundaries of the corresponding frequency ranges: slow-wave flaxmotion (LF): 0.05–0.2 Hz, respiratory flaxmotion (HF): 0.2–0.4 Hz, pulse flaxmotion (CF): 0.8-1.6 Hz. The calculation of the indicators was based on the methodology proposed by V. I. Kozlov et al. [10].

The contribution of the corresponding frequency range (v: vLF, vHF, vCF) was determined as the percentage ratio of the square of the amplitude of this range (A) to the total power of the spectrum (M), which is the sum of the squares of the amplitudes over 3 ranges.

$$M = A^2LF + A^2HF + A^2CF$$
$$v = A^2 / M \times 100\%$$

Таблица
Характеристика обследованных пациентов
Table
Characteristics of investigated patients

Параметр Parameter	Основная группа Main group	Контрольная группа Control group
Количество обследованных Number of examined	n=40 (20 мужчин, 20 женщин) n=40 (20 male, 20 female)	n=30 (20 мужчин, 10 женщин) n=30 (20 male, 10 female)
Возраст, лет (среднее ± ст. отклонение) Age, years (mean ± st. deviation)	67,6 ± 6,7	65,2 ± 6,3
Продолжительность СД, лет Duration of diabetes, years	> 5	не применимо not applicable
Сахароснижающая терапия Hypoglycemic therapy		
Пероральные препараты Oral medications	n=37	не применимо not applicable
Инсулинотерапия Insulin therapy	n=5	
Форма синдрома диабетической стопы Diabetic foot syndrome form		
Нейропатическая Neuropathic	n=14	не применимо not applicable
Нейроишемическая Neuroischemic	n=26	
Гипертоническая болезнь Essential arterial hypertension	n=40	n=30

The calculation of these spectral indicators was performed with two different methods. In one case, the average amplitudes of the corresponding frequency ranges were used, in the other, the maximum ones.

One of the manifestations of diabetic microangiopathy is diabetic nephropathy, which leads to the development of chronic kidney disease with a progressive decrease in the filtration function of the kidneys. In this regard, it was of interest to evaluate the correlations of the spectral parameters of LDF with the calculated glomerular filtration rate (eGFR). To determine eGFR, the formula CKD–EPI was used, based on the concentration of creatinine in the blood serum.

Statistical processing was performed with the GraphPad Prism 8 software package. The intergroup differences were evaluated with the Mann-Whitney criterion. The Spearman correlation coefficient was used for the correlation analysis. The differences were considered statistically significant at $p < 0.05$.

Results and discussion

In both groups, the spectral parameters of the contribution of slow-wave (vLF), respiratory flaxmotions (vHF) and the contribution of pulse flaxmotions (vCF) were analyzed, obtained with the average or maximum amplitudes of the corresponding harmonic components.

When comparing the contribution of slow-wave flaxmotions in patients of the control and main groups, calculated with average and maximum amplitudes, no significant differences were found in either case ($p>0.05$). There were also no intergroup differences in the flaxmotion index ($p>0.05$), with both calculation methods.

The comparison of the contribution of respiratory flaxmotions with the use of average amplitudes showed that the indicator was significantly higher in the main group ($p<0.05$) (Fig. 1), with no significant differences observed with the use of maximum amplitudes ($p>0.05$) (Fig. 2).

On the contrary, the contribution of pulse flaxmotions with the use of average amplitudes was significantly higher in the control group ($p<0.05$) (Fig. 3), while no differences were detected with the use of maximum amplitudes ($p>0.05$) (Fig. 4).

The correlation analysis revealed significant correlations of the contribution of respiratory and pulse flaxmotions with the estimated glomerular filtration rate in the main group with both calculation methods ($p<0.05$). A negative correlation was observed for the contribution of respiratory flaxmotions (Fig. 5), while a positive correlation was observed for the contribution of pulse flaxmotions (Fig. 6). In the control group, there was no significant correlation between the LDF indicators and the calculated glomerular filtration rate ($p> 0.05$).

The obtained data can be interpreted as follows. According to our previous studies [11], a decrease in the contribution of slow-wave flaxmotion and the flaxmo-

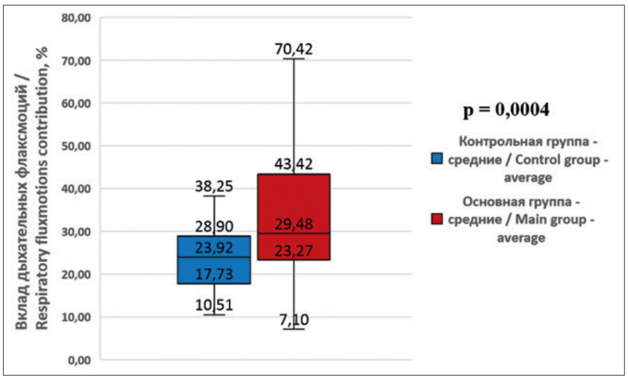

Рис. 1. Вклад дыхательных флаксмоций в основной и контрольной группах при использовании средних амплитуд
Fig. 1. The contribution of respiratory fluxmotions in the main and control groups when using average amplitudes

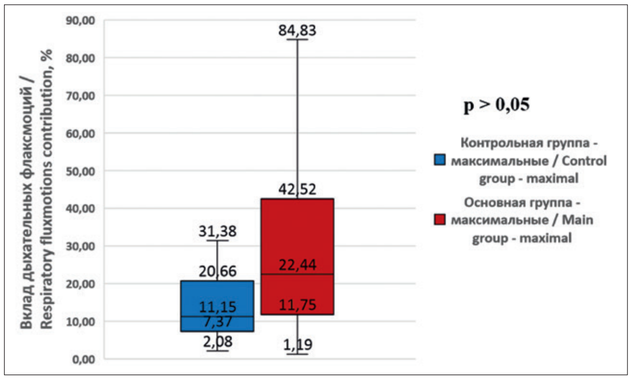

Рис. 2. Вклад дыхательных флаксмоций в основной и контрольной группах при использовании максимальных амплитуд
Fig. 2. The contribution of respiratory fluxmotions in the main and control groups when using maximum amplitudes

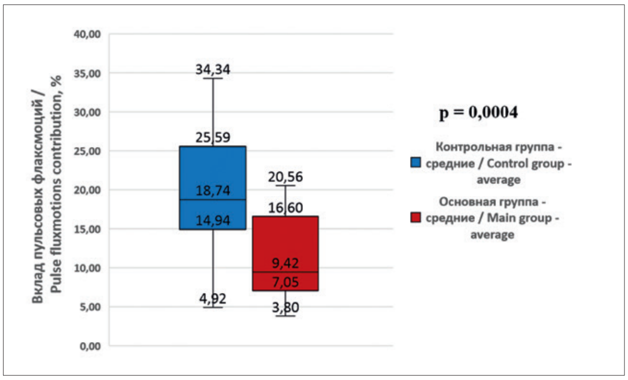

Рис. 3. Вклад пульсовых флаксмоций в основной и контрольной группах при использовании средних амплитуд
Fig. 3. The contribution of pulse fluxmotions in the main and control groups when using average amplitudes

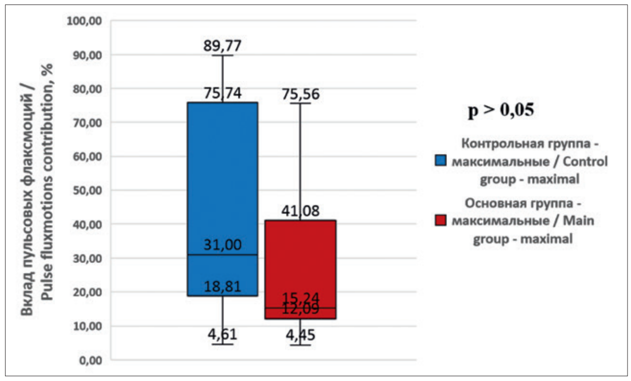

Рис. 4. Вклад пульсовых флаксмоций в основной и контрольной группах при использовании максимальных амплитуд
Fig. 4. The contribution of pulse fluxmotions in the main and control groups when using the maximum amplitudes

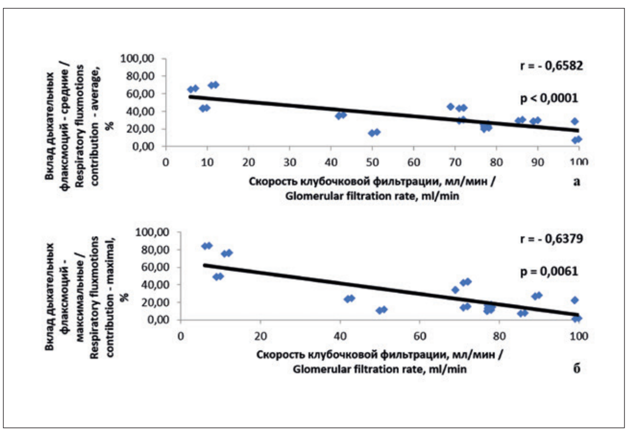

Рис. 5. Корреляции вклада дыхательных флаксмоций с расчётной скоростью клубочковой фильтрации при использовании средних (а) и максимальных (б) амплитуд
Fig. 5. Correlations of the contribution of respiratory fluxmotions with the estimated glomerular filtration rate using the mean (a) and maximum (b) amplitudes

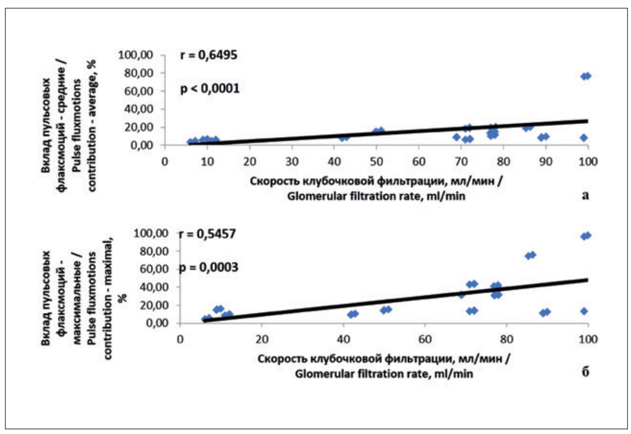

Рис. 6. Корреляции вклада пульсовых флаксмоций с расчётной скоростью клубочковой фильтрации при использовании средних (а) и максимальных (б) амплитуд
Fig. 6. Correlations of the contribution of pulse fluxmotions with the calculated glomerular filtration rate using the mean (a) and maximum (b) amplitudes

tion index was observed in various pathologies of microcirculation, including those not associated with diabetes mellitus. The reason for this lies in the integration of the circulatory, lymphatic and interstitial compartments of the internal environment of the body at the level of the microcirculatory bed. Changes in the microcirculatory blood flow can be caused by pathological processes both at the level of the microvessels themselves and by disorders of arterial inflow or venous outflow [13]. The balance of factors of modulation of microcirculatory blood flow in the case of a decrease in the role of local factors is shifted towards systemic factors, such as the suction effect of the chest and the propulsive activity of the left ventricle.

As it was shown earlier [11], it is the nature of the relationship between the systemic factors of microcirculation modulation, which is displayed by the indicators of the contribution of respiratory and pulse flaxmotions, that is the criterion for differentiating pathogenetic variants of microcirculatory dysfunction.

In the present study, the calculation using the maximum amplitudes of harmonics did not reveal significant differences in microcirculatory dysfunction in patients of the main and control groups, despite the fundamentally different mechanisms of its development. It was possible to differentiate between microcirculation insufficiency caused by diabetic microangiopathy and other causes only when using a calculation algorithm based on average amplitudes.

At the same time, the contribution of respiratory flaxmotions was significantly higher in patients of the main group (with diabetic microangiopathy), whereas in the control group the contribution of pulse flaxmotions was higher. This can be explained by the formation of persistent disorders of venous outflow with stasis phenomena [10, 12]. The reverse situation in patients with essential arterial hypertension and excluded diabetes mellitus may be due to remodeling of the arteriole wall with a decrease in the number of myocytes in the middle layer of the vessel wall (tunica media), a decrease in the compliance of the vascular wall and an increase in its rigidity.

It is worth paying attention to the fact that the patients of the main group also had essential arterial hypertension, and, therefore, the wall of the arterioles in them was also highly likely to undergo remodeling. Moreover, according to available data [13], changes in arterioles caused by diabetic microangiopathy are similar in their morphomechanical properties to those caused by primary arterial hypertension: in both cases, the phenomena of hyalinosis and sclerosis are noted, which often complicates morphological identification of the particular type of damage. Type 2 diabetes mellitus itself is a risk factor for the development of primary arterial hypertension, so in patients of the main group, remodeling of the walls of arterioles may have a dual pathogenesis (due to diabetic microangiopathy and essential arterial hypertension) [4]. From our point of view, the lower values of the contribution of pulse flaxmotions in the main group are due to the fact that the lesion of arterioles becomes somehow less important in connection with the lesion of the venous link, which causes an increase in the contribution of respiratory flaxmotions.

An additional factor in reducing the contribution of pulse flaxmotions in patients with type 2 diabetes is the atherosclerosis-type lesions of large arterial vessels. At the same time, the transmission of the pulse wave to the periphery becomes difficult or completely impossible. This explains the decrease in the contribution of pulse flaxmotions to the total power of the amplitude-frequency spectrum of the LDF signal in these patients when registering LDF in the basin of the above-mentioned arteries.

The revealed correlations in the main group can be explained in the framework of the pathogenesis of diabetic microangiopathy. As it was shown earlier [7, 11], in diabetic microangiopathy, there is a progressive dysfunction of local microcirculation mechanisms caused by a decrease in the number of pacemaker myocytes in the t. media of arterioles, the phenomena of neuropathy and endothelial dysfunction. At the same time, the role of systemic factors of microcirculatory blood flow modulation, such as the pulse wave and the suction effect of the chest, increases in the compensatory manner.

An increase in the contribution of respiratory flaxmotions is associated with an increase in the phenomena of venous stagnation [5]. Venous stasis with subsequent formation of microcirculatory stasis is a natural component of the progression of diabetic microangiopathy [13]. The increase in the contribution of cardiac flaxmotions, apparently, is a consequence of remodeling of the wall of blood vessels, primarily arterioles. It is known that in diabetic microangiopathy, there is a decrease in the number of smooth myocytes in the t. media of arterioles, sclerosis and hyalinosis [13, 14]. As a result, the rigidity of the arteriole wall increases, which makes it difficult to transmit the pulse wave to the periphery and reduces the amplitude of fluctuations in the microcirculatory blood flow under the influence of the pulse wave. Diabetic nephropathy is a particular variant of diabetic microangiopathy and at the same time a factor in its progression due to increased activation of local renin-angiotensin-aldosterone systems [14]. In this regard, it can be assumed that the severity of the above processes: remodeling of the arteriole wall and the progression of venous congestion, will correlate with the estimated glomerular filtration rate as an indicator of the progression of diabetic nephropathy, which is confirmed by the data obtained.

Conclusion

In connection with the obtained results, in our opinion, for the differential diagnosis of diabetic microangiopathy, it is advisable to use a technique that allows to obtain higher values of the contribution of respiratory flaxmotions and lower values of pulse flaxmotions. This requirement is largely met by the method using the average amplitudes of harmonic components. This feature can increase the specificity of laser Doppler flowmetry as a method for diagnosing diabetic microangiopathy.

The revealed correlations are: the negative contribution of respiratory flaxmotions and the positive contribution of pulse flaxmotions, indicate that as chronic kidney disease progress, the changes in the nature of microcirculation modulation described above are increasingly observed. Therefore, the data of laser Doppler flowmetry are consistent with the ideas about the pathogenesis of diabetic microangiopathy. Thus, laser Doppler flowmetry allows us to identify the progressive nature of diabetic microangiopathy.

Based on the above, it can be concluded that laser Doppler flowmetry can be considered as a potential method of dynamic monitoring of microcirculation, in particular, in patients with diabetic microangiopathy. By improving the methods of spectral analysis results processing, it is possible to significantly expand the diagnostic potential of the method and its value for practical medicine.

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