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PECULIARITIES OF MICROCIRCULATORY DISTURBANCES IN PATIENTS WITH FOOT SORE COMPLICATIONS CAUSED BY DIABETES DEPENDING ON CLINICAL FORM

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Abstract

Totally 328 patients with complicated diabetic foot syndrome with abscess, phlegmon, purulent tenosynovitis, purulent arthritis and gangrene were examined and diagnosed in Therapeutic center of Zaporozhye, Ukraine, for the period 2015-2017. All patients were diagnosed with type 2 diabetes; its average duration was 12.5±1.8 years. Patients were divided into clinical forms – 118 patients with neuropathic, 74 with ischemic and 136 with mixed.

The study aimed to investigate the patients' blood flow in lower limbs by using the laser Doppler flowmetry method and to reveal specific microcirculatory disturbances for each clinical form.

The neuropathic form was noted with weakened arteriolar vascular tone and increased blood volume in the arterioles. A decrease in neurogenic and myogenic mechanisms of control was noted. Peripheral autosympathectomy results in the loss of neurogenic control and precapillary vasoconstriction, increased intracapillary pressure and increased blood flow through the arteriovenous shunts. The patients with ischemic form were noted with microcirculatory disorders; primarily, decrease in the volume of arterial influx and increase in the amplitudes of active and passive control factors were observed. Compensatory reaction in an attempt to improve microcirculation by increasing the respiratory amplitudes (passive factor). The patients with mixed form were noted with increase in the muscle tone of precapillary muscles and in the amplitude of the respiratory wave. Neurogenic and myogenic tone was increased due to the activity of the sympathetic component, which in its turn led to decrease in the diameter of the arterioles.

Keywords: *diabetic foot syndrome, microvascular disease, neuropathy, diagnostics.*

Introduction

Currently, diabetes mellitus is recognized by the WHO as an epidemic of non-infectious disease that is rapidly developing in both underdeveloped and highly developed countries [*Baltzis D et al., 2016*].

In European countries, including Ukraine, the prevalence of diabetes is from 3 to 10% among the general population, and among the elderly, up to 30%. Each year the number of patients with diabetes increases by 4-5%, every 12-15 years it doubles [*Mandrik O et al., 2013*].

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It was stated that diabetic neuro- and angiopathy in the late stages determine the prognosis of the patient's life, and the incidence of complications is caused by early and accelerated development of vessel atherosclerosis of large and medium caliber [*Klonizakis M et al., 2015*].

One of the diabetes complications is the diabetic foot syndrome (DFS) – a pathological condition of the feet of patients diagnosed with diabetes mellitus, which occurs due to lesions of peripheral nerves and vessels [*Kabbani M et al., 2013*].

In the development of DFS, great attention is paid to angiopathy, one of the reasons for which is the infringements of vessel patency of different diameters and the deterioration of the rheological properties of blood [*Epps J et al., 2016; Lowry D et*

al., 2017]. Ultimately, peripheral circulation and microcirculation suffer significantly [*Lewis J et al., 2017*]. The choice of the treatment method and the solution of surgical tactics of patients with DFS largely depend on the availability of information on the state of tissue blood flow and microcirculation [*Higashimori A et al., 2015; Körei A et al., 2016*].

There is an active search of non-invasive tests for verification of the triggering mechanisms of the disease [*Lal C et al., 2015; Bennett S et al., 2016*] at early stages of development of complications, possible options of drug therapy [*Kisch T et al., 2016*] and the need for invasive procedures [*Shapoval S et al., 2016*].

The aim of the present study was to investigate the blood flow of the lower limbs of patients with complicated DFS and to reveal specific microcirculatory disturbances for each clinical form using the laser Doppler flowmetry method.

Material and methods

A total of 328 patients with complicated DFS were treated in the city's purulent-septic center of Clinical Hospital No 3 in Zaporozhye, Ukraine, for the period 2015-2017.

The age of the examined patients ranged from 43 to 82 years and averaged 57.4±2.5 years. Men were 141 (43%), women – 187 (57%).

All patients had type 2 diabetes, with its mean duration 12.5±1.8 years. Depending on the severity of diabetes mellitus, the groups of patients were divided on average and severe course.

The concept of "complicated DFS" included purulent-necrotic lesion of the foot – abscess, phlegmon, purulent tendovaginitis, purulent arthritis and gangrene.

According to the classification of the International Working Group on Diabetic Foot Problems (Netherlands, 1991), patients were divided into clinical forms – 118 patients with neuropathic form of complicated DFS, 74 with ischemic and 136 with mixed.

The control group consisted of 32 healthy inhabitants of our region without diabetes, whose average age was 30.5±3.5 years, from which 14 (44%) men, 18 women (56%).

The microcirculation was studied by the laser Doppler flowmetry method with the help of the LAKK-02 analyzer (Lazma NPP, Russia). The blood flow of the foot was examined at resting state with the sensor placed on the back surface of the foot in the first intercellular space. The unit of measurement is the perfusion units (p.un).

The estimation and calculation of microcirculatory channel parameters was performed in two stages. At the first stage, the mean values of perfusion were calculated: Mav – the value of the average blood flow; σ is the average fluctuation of perfusion relative to the average blood flow; Kv (coefficient of variation) is the ratio of the quantities Mav and σ. At the second stage, the amplitude-frequency spectrum of perfusion was analyzed using the Wavelet transform algorithm.

The oscillatory process caused by active factors – endothelial (Ae), neurogenic (An), myogenic (Am) and passive – respiratory (Ar), cardiac (Ac) was recorded. Neurogenic and myogenic tonus, shunting index were calculated.

Methods of descriptive statistics were used for statistical analysis. Verification of the data for normal distribution was performed visually according to the histogram and using Kolmogorov-Smirnov test. Taking into consideration the normal distribution in the analyzed samples, we calculated the parameters of the parametric descriptive statistics in the format M±m (mean ± standard error of the mean value). The reliability of the differences was assessed depending on the analyzed data using the Student's parametric test. Differences were considered significant at $p<0.05$.

Results

For the neuropathic form of the complicated DFS constant component of the blood flow (Mav) was 5.75±0.22 p. un., which is significantly ($p<0.05$) higher than the control values by 111% (Table). This value characterizes the average perfusion, i.e. the change in the blood flow per time unit in the probed region and is proportional to the speed of movement of the erythrocytes. The increase in Mav indicates a decrease in arteriolar vascular tone and an increase in blood volume in the arterioles.

The variable component of the blood flow (σ) was 0.83±0.05 pF. units, which is 7% below the benchmark ($p>0.05$). This component is due to factors that influence the stability of the blood flow in the microcirculatory channel.

Table

Indices of microcirculation of patients with complicated DFS (M ± m)

Indices of microcirculation	Norm n = 32	Neuropathic form n = 118	Ischemic form n = 74	Mixed form n = 136
M_{av}, (*p. un.*)	2.7±0.24	5.75±0.22*	34.41±4.57*	9.28±0.58*
σ, (*p. un.*)	0.89±0.1	0.83±0.05	10.6±1.8*	1.4±0.19*
Kv, %	39.96±4.1	14.88±0.88*	27.4±3.81*	17.66±1.72*
An,(*p. un.*)	0.49±0.05	0.41±0.03*	3.05±0.47*	0.62±0.07*
Am, (*p. un.*)	0.41±0.05	0.34±0.02*	2.08±0.33*	0.51±0.05*
Ae, (*p. un.*)	0.51±0.07	0.37±0.03*	4.31±0.82*	0.65±0.14*
Ac, (*p. un.*)	0.12±0.01	0.17±0.02*	0.01±0.04*	0.11±0.01
Ar, (*p. un.*)	0.21±0.04	0.2±0.02	1.6±0.29*	0.25±0.29*
NT, (*p. un.*)	2.17±0.2	2.31±0.11	3.05±0.21*	2.6±0.17*
MT, (*p. un.*)	2.79±0.31	3.21±0.22*	3.91±0.38*	3.2±0.21*
SI, (*p. un.*)	1.4±0.05	3.66±2.18*	1.28±0.09	1.34±0.06

Note. * - the difference between the norm and the study group is statistically significant (p <0.05)., *NT – Neurogenic tonus, MT –myogenic tonus, SI – shunting index*

When analyzing the calculated parameters, we were guided by the ratio of the values of Mav and σ, i.e. by the coefficient of variation (Kv). The neuropathic form of the complicated DFS, Kv was 14.88±0.88%. A decrease in Kv below the normal values by 63% (p<0.05) indicated a decrease in activation of endothelial secretion and a worsening of neurogenic and myogenic mechanisms of control.

The magnitude of the oscillation amplitude of the microcirculation in specific frequency ranges assessed the state of functioning of certain perfusion control mechanisms.

The violation of the neurogenic regulation of the microcirculatory blood flow was indicated by a 16% decrease in the amplitude of the neurogenic tone (An) (p<0.05).

As a result of pathological processes, peripheral autosympathectomy developed, which lead to the loss of neurogenic control and increased blood flow through the arteriovenous shunts, thereby reducing the volume of nutritional capillary blood flow. Thus, the increase in basal blood flow (Mav) in the neuropathic form of DFS is caused by an increase in the volume of arterial blood supply due to the lack of sympathetic regulation of microcirculation and accelerated discharge of blood through arteriovenous shunts.

Myogenic regulation (Am) was localized in precapillary and sphincters. With the neuropathic form of complicated DFS, the amplitude of myogenic waves was reduced by 17% (p<0.05).

Endothelial oscillations (Ae) were caused by the functioning of the endothelium, and affected the transport function of the blood and promoted metabolic processes. With neuropathic DFS, the amplitude of endothelial oscillations corresponded to 0.37±0.03 p. un. and below the normal values by 27% (p<0.05), which indicated a decrease in endothelial dysfunction.

Passive factors (pulse and respiratory amplitudes) also affected the vascular wall with the help of blood flow oscillations.

An increase in the amplitude of the pulse wave (Ac) by 42% (p<0.05) was noted, which indicated an increase in the vascular tone and was possibly associated with mediacalcinosis.

The parameters of the respiratory wave (Ar) in the neuropathic form of complicated DFS have changed insignificantly, and the gradient of arteriovenous pressure approached the control values (p>0.05).

The nature of the neurogenic tone is associated with the activity of α-adrenoreceptors in the membrane of key, and partially with smooth connective muscle cells. With a neuropathic form of complicated DFS, the neurogenic tone index increases by 6% relative to the norm, but these changes are unreliable (p>0.05). Neurogenic tone can increase both due to the activity of sympathetic nerves-va-

soconstrictors, and against the background of denervation hypersensitivity of the vascular wall.

The recorded amplitudes of blood flow oscillations of endothelial, neurogenic and myogenic origins were associated with muscle tone. In the neuropathic form of DFS, myogenic tone was raised by 15% relative to the norm ($p<0.05$).

Using the data obtained in the calculation of neurogenic and myogenic tone, we can estimate the ratio of shunting and nutritional blood flow – the shunting index. With the neuropathic form of complicated DFS, there is a disruption in the regulation of arterioles and anastomoses (sympathetic adrenergic regulation mechanisms) on one hand and precapillary sphincters (absence of sympathetic innervation) on the other. An increase of 161% ($p<0.05$) was noted.

The ischemic form of complicated DFS shows a significant ($p<0.001$) increase in the constant component of the blood flow when compared to the norm – up to 34.41±4.57 p. un. Such an increase in Mav indicates the presence of blood stagnation in the arterioles and the venular link.

A significant ($p<0.001$) increase in the variable component of the microcirculation (σ), to 10.60±1.8 p. un. indicates an increase in the modulation of blood flow. This increase in σ may be due to both the more intensive functioning of active microcirculation control mechanisms, and as a result of an increase in cardiac and respiratory rhythms.

The ischemic form of complicated DFS, portrays Kv significantly below the norm by 32% ($p<0.05$). Despite a significant increase in Mav and σ, the Kv decreased below normal values, resulted by a disruption of the activation of endothelial secretion, neurogenic and myogenic control mechanisms.

The growth of amplitudes in the neurogenic range (An) in the ischemic form of complicated DFS is significantly higher than the norm by 522% ($p<0.001$), which indicates a disruption of control of the arteriolar tone. The pronounced activation of sympathetic vasomotor fibers leads to an increase in tone and an increase in the rigidity of the vascular wall.

The amplitude of myogenic fluctuations (Am) in the ischemic form of complicated DFS is 407% higher than the norm ($p<0.001$). The increase in myogenic fluctuations contributes to the reduction of peripheral resistance in the capillary network and is aimed to improve nutritional blood flow.

In the ischemic form of complicated DFS, the amplitude of endothelial oscillations (Ae) is significantly higher than the norm by 745% ($p<0.001$).

The amplitude of the pulse wave (Ac) in the ischemic form of complicated DFS was reliably reduced by 92% ($p<0.05$). This is due to a decrease in the elasticity of the vascular wall due to angiosclerosis on one side and a decrease in the volume of arterial blood flow into the microcirculatory bed on the other.

The amplitude of the respiratory wave (Ar) in the ischemic form of complicated DFS is 662% higher than the normal values ($p<0.001$). The increase is caused by a decrease in the pressure gradient at the arteriovenous anastomoses level. Reduction of blood flow to the microcirculatory bed is accompanied by a decrease in arteriovenous pressure, in response to this, the amplitudes of respiratory rhythms increase.

Neurogenic tone in the ischemic form of complicated DFS increases by an increase in the activity of sympathetic nerves of vasoconstrictors by 41% ($p<0.05$).

The increase in myogenic tonus by 40% ($p<0.05$) in the ischemic form of complicated DFS is caused by an atherosclerotic change in the vascular wall and a prolonged spasm aimed at maintaining the necessary pressure with a reduced volume of blood flow.

In the ischemic form of complicated DFS, the shunting index was reduced by 8% ($p>0.05$). This is resulted by a change in the regulation of arterioles and arteriolovenous anastomoses. Increased neurogenic and myogenic tone promotes redistribution of blood towards the nutritional blood flow.

In the mixed form of complicated DFS, the constant component of the blood flow (Mav) corresponds to 9.28±0.58 p. un., significantly ($p<0.001$) higher than the norm by 243%. The increase in Mav indicates an increase of blood volume in arterioles and venules.

The variable component of the blood flow (σ) is 1.4±0.19 p. un., which is higher than the norm by 57% ($p<0.05$). This increase indicates an increase in the modulation of blood flow and is caused by a more intensive functioning of the active microcirculation control mechanisms.

Kv in the mixed form of complicated DFS was reliably reduced below the norm by 55% ($p<0.05$),

which indicated a worsening of microcirculation.

Neurogenic vibration (An) with a mixed form of complicated DFS is significantly higher than norm values by 26% ($p<0.05$). An increase in the amplitude of neurogenic vibrations leads to an increase in the arteriolar tone.

The level of myogenic fluctuations (Am) in a mixed form of complicated DFS was higher than the norm by 24% ($p<0.05$). The increase in myogenic fluctuations contributed to the reduction of peripheral resistance in the capillary network and was aimed to improve nutritional blood flow.

The mixed form of complicated DFS performed the amplitude of endothelial oscillations (Ae) higher than the norm by 27% ($p<0.05$). The revealed disturbances of endothelial function were caused by a decrease in the response to NO or acceleration of its inactivation by free radicals.

The change in the amplitude of the pulse wave (Ac) in the mixed form of complicated DFS differed by 8% relative to the norm values and was unreliable ($p>0.05$).

The amplitude of the respiratory wave (Ar) with a mixed form of complicated DFS is significantly higher than the norm values by 19% ($p<0.05$).

The increase in the amplitude of the respiratory wave with an increase in the microcirculation index (Mav) indicates the appearance of stagnant phenomena in the venular part of the microcirculatory bed.

Neurogenic tone with a mixed form of complicated SDS increased by 20% ($p<0.05$) due to the activity of the sympathetic component.

The myogenic tone of precapillaries in a mixed form of complicated DFS is significantly increased by 15% ($p<0.05$). The detected increase in myogenic tone is caused by a decrease in the volume of blood entering the arterioles.

In the mixed form of complicated DFS, the shunting index is reduced, but there is no significant difference with the norm values ($p>0.05$). This is resulted by a change in the regulation of arterioles and arteriolovenous anastomoses. Increased neurogenic and myogenic tone promotes redistribution of blood towards the nutritional blood flow.

Discussion

With the neuropathic form of complicated DFS, there is an increase in the constant component of the blood flow (Mav), which indicates a weakening of the arteriolar vascular tone and an increase in blood volume in the arterioles. A decrease in Kv indicates a general deterioration in the state of microcirculation, which is associated with a decrease in activation of endothelial secretion, neurogenic and myogenic mechanisms of control. The violation of neurogenic regulation of microcirculatory blood flow is indicated by a decrease in neurogenic fluctuations, as well as an increase in neurogenic and myogenic tone. Peripheral autosympathoctomy results in the loss of neurogenic control and precapillary vasoconstriction, increased intracapillary pressure and increased blood flow through the arteriovenous shunts, thereby reducing the volume of nutritional capillary blood flow. The decrease in the amplitude of endothelial oscillations indicates a decrease in the endothelial function, a small increase in the amplitude of the pulse wave, and an increase in the vascular tone, which is apparently associated with mediacalcinosis.

Patients with ischemic form of complicated DFS are noted to have microcirculatory disorders associated, primarily, with a decrease in the volume of arterial inflow. A significant increase in Mav and σ, with a decrease in Kv, indicates violations of sympathetic regulation of microcirculation and arteriolovenous reactions. This is associated with a falsely high rate of microcirculation. In the ischemic form of complicated DFS, an increase in the amplitudes of active and passive control factors is observed. Compensatory reaction is an attempt to improve microcirculation by increasing the respiratory amplitudes (passive factor).

The mixed form of complicated DFS shows an increase in the parameters of perfusion of Mav and σ, which is due to the presence of stagnant phenomena in arterioles and venules. Decrease in Kv indicates a disruption of the microcirculation control mechanisms, resulting reduced endothelial secretion and activation of neurogenic and myogenic mechanisms of control. An increase in the amplitude of neurogenic fluctuations indicates an increase in the muscle tone of precapillaries, which regulates the flow of blood into the nutritional channel, and an in-

crease in the amplitude of the respiratory wave – the worsening of the blood outflow from the microcirculatory bed and is characterized by an increase in the volume of blood in the venular link. Neurogenic and myogenic tone is increased due to the activity of the sympathetic component, which in turn leads to a decrease in the diameter of the arterioles.

The study of microcirculation with the help of laser Doppler flowmetry made it possible to identify the most specific changes in blood flow parameters, to determine their numerical and border levels for each form of complicated DFS, which is undeniably important for predicting the course of the disease and the possibility of correcting the revealed disorders.

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