GEORGIAN MEDICAL NEWS


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Abstract.
The aim of the study: To investigate the hemicrocirculatory channel (HCMC) in chronic critical lower extremity ischemia (CHLIC) in patients with diabetes mellitus (DM). To develop a method for calculating the indicator of degree of diabetic microangiopathy (PSDMAP).

Material and methods: Skin and muscle samples of 63 patients with type 2 diabetes mellitus with CKD and skin and muscle samples of 30 patients without systemic diseases. Histological and immunohistochemical studies were conducted to detect type IV collagen, CD31, and smooth muscle action. We measured the diameters of blood vessels of the skin and muscles, the density of capillaries per unit area for the calculation of PSDMAP.

Results and conclusions: Characteristic changes were revealed in the morphometric parameters of the HCMC of the skin in patients with type II diabetes mellitus with CKD. A method for calculating PSDMAP was developed. It was revealed a correlation between the type of diabetic microangiopathy and the probability of thrombosis after hybrid arterial reconstruction, and an algorithm for the examination of patients in the postoperative period was developed.

Key words. Diabetes, diabetic microangiopathy, hemicrocirculatory channel, ischemia, hybrid arterial reconstruction.

Introduction.
In recent decades, the incidence of DM has reached the proportions of a non-infectious pandemic. The prevalence of diabetes doubles every 10-15 years. Diabetic microangiopathy (DMAP) occurs in more than half of patients [1]. This complication of diabetes is associated with a high risk of development and rapid progression of vascular pathology [2].

The group of patients with ischemic form of the diabetic foot syndrome in combination with occlusion of the superficial femoral artery (SFA) and stenotic-occlusive lesion (SOU) of the arteries of the popliteal-shin-foot segment requires particularly complex tactical and technical solutions as standard methods of arterial reconstruction are not always effective [3].

The pathogenetic role of morphological changes of the HCMC in the development of such a complication of diabetes as the diabetic foot syndrome is indisputable. There is no doubt that vascular disorders like this can lead to irreversible changes in the tissues of the foot [4].

The level of their vascularization is of great importance for tissue regeneration. Modern immunohistochemical methods allow detecting a wide range of cell types and extracellular components with high reliability. Knowledge of immunohistochemical characteristics of various tissue components is necessary for using these methods in the diagnosis of a range of diseases [5].

The aim of the study. To analyze the results of the research of HCMC in patients with diabetes mellitus. To develop a method for calculating PSDMAP based on the determination of the quantitative characteristics of the lesions of the HCMC of the skin in patients with diabetes mellitus and CKD.

Materials and methods.
For immunohistochemical and histological studies, 487 micropreparations were prepared and examined from 178 paraffin blocks of skin, subcutaneous tissue, and muscles of 63 patients (research group). All patients had type II diabetes with CKD on the background of SOU of the arteries of the femoral and popliteal segments in combination with ulcerative-necrotic lesions of the foot. All patients received treatment at the Center for Vascular Surgery of the "FEOFANIYA" Clinical Hospital of the State Administration of Affairs during 2013 - 2017. There were 38 (60.3%) men and 25 (39.7%) women aged 59 to 84 years. The average age of the patients was (67.3 ± 5.2) years.

For comparison, it was selected skin and muscle samples of 30 patients (control group) who did not have systemic diseases.

The material was obtained from patients of the experimental group during hybrid arterial reconstructions in order to restore arterial blood circulation in the lower limb, which consisted in an open surgical intervention on PSA in the form of shunting or endarterectomy and endovascular intervention on the arteries of the popliteal-shin segment, and from patients of the control group under during general surgical and orthopedic operations in compliance with the rules of ethics and deontology without moral and legal violations in accordance with the provisions of the Convention of the Council of Europe “On the Protection of Human Rights and Dignity in the Aspect of Biomedicine” (1997).

The obtained material was fixed in a 10% solution of neutral formalin. After fixation, the selected tissue sections were sealed in paraffin according to the generally accepted scheme. Paraffin sections 5 μm thick were made using a Leica 2025 microtome. Some of sections were stained with hematoxylin and eosin, van Gieson's picrofuchsin. To assess the condition of the basement membranes and metabolic disorders in the tissues, the McManus Schiff-iodic acid (SHIK) method with amylase control was used. Some sections were stained with hematoxylin and eosin, van Gieson's picrofuchsin. To assess the condition of the basement membranes and metabolic disorders in the tissues, the McManus Schiff-iodic acid (SHIK) method with amylase control was used. We conducted immunohistochemical studies using antigens: VEGF – growth factor of endothelial vessels. CD31 – endothelial factor (endothelial cell marker). Smooth muscle action is a marker of a benign vascular process, also reveals cells with partial smooth muscle differentiation. Type IV collagen is a marker of the vascular process and is found in basement membranes.

Immunohistochemical reactions were performed according to standard protocols using monoclonal mouse and rabbit antibodies.
Results and Discussion.

In the skin of the patients of the experimental group, thickening of the basement membrane with increased expression of type IV collagen was observed, which led to the narrowing of the vessels of the HCMC of varying degrees, which progressed. In addition, there was loosening of the basal membrane of the vessel wall of the HCMC of the papillary skin layer (Fig. 1), which impaired their permeability.

When studying the density and distribution of blood vessels of the HCMC of the skin after immunohistochemical staining with anti-CD31 antibodies in patients of the control group, uniform expression of the antigen in the endothelium of the vessels was detected, which indicated a normal physiological state. However, after the same staining of skin and muscle preparations of patients with DM, a high intensity and focal character of CD31 expression was revealed (Fig. 2).

Counting the number of capillaries of the papillary layer of the dermis showed that patients with the 1st degree of DMAP, their density per 1 mm2 was the lowest and gradually increased, the maximum - with the 3rd degree of DMAP (Table 1).

Angiogenesis processes were changing in the skin of patients with diabetes. The formation of new blood vessels in DM was enhanced with increased expression of VEGF receptors in the skin of patients with DMAP, correlating with the severity of disorders in the MHCR. Thus, in the vessels of the skin of patients with II and III degrees of DMAP, the immunopositive reaction was observed, whereas in the vessels of the skin of patients of the control group, a weakly expressed reaction of blood vessels was clearly expressed. Given the relative simplicity of skin biopsy, its use for determining DMAP PS is promising.

Based on the results of the study, we developed a method for determining PSDMAP (Table 2).

An example of PSDMAP calculation: the average diameter of an arteriole is 15.28 μm - 2 points, the average diameter of a venule is 27.32 μm - 1 point, the average diameter of a capillary is 4.96 μm - 3 points, the average number of capillaries per 1 mm2 is 162.45 - 3 points. Sum of points: 1+2+3+3=9. PSDMAP = 2.25 (9/4).

It is clear that PSDMAP can range from 0 to 3. DMAP was considered as mild when PSDMAP was in the range of 0-0.9, moderate when PSDMAP was in the range of 1-1.9, and severe when PSDMAP was in the range 2-3.

Out of 63 patients whose skin and muscle preparations were examined, 8 of them (12.7%) had PSDMAP scores in the range of 0-0.9, which indicated mild DMAP, 11 of them (17.5%) had PSDMAP scores in the range of 1-1.9, which indicated the average severity of DMAP, 44 of them (69.8%) had PSDMAP scores in the range of 2-3, which indicated severe DMAP.

After hybrid arterial reconstruction, all patients received antplatelet therapy with clopidogrel 75 mg per os once a day during the observation period. Among 8 patients with mild DMAP during the observation period, there were no thromboses in the zone of arterial reconstruction (AR). Out of 11 patients with an average degree of severity of DMAP during the observation period, thrombosis of the ZAR was recorded in 1 (9.1%), out of 44 patients with severe DMAP - in 12 (27.3%). This highlights the important role of DMAP in the creation of peripheral vascular resistance and the need to take into account the severity of DMAP to determine the examination algorithm with the possible performance of preventive operations before the occurrence of thrombosis of the ZAR, the nature and intensity of conservative treatment after arterial reconstructions in patients with DM with CKD.

In parallel with the above study, we studied the results of hybrid reconstructive surgical interventions on arteries in 75 patients with type 2 diabetes with CKD on the background of SOU of the arteries of the infragluteal segment in combination with ulcerative-necrotic lesions of the foot.

During the observation period, which lasted for 12 months, 11 (14.7%) patients developed thrombosis of the ZAR. In order to

Table 1. Changes in vascular parameters of the HCMC of the papillary layer of the dermis.

<table>
<thead>
<tr>
<th>Blood-bearing vessels</th>
<th>Groups of patients without diabetes (control)</th>
<th>with Sugar Dabet with DMAP (experimental)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I degree</td>
<td>II degree</td>
</tr>
<tr>
<td>Diameter, mкm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>arterioles</td>
<td>26,63 ± 1,69</td>
<td>24,02 ± 2,7*</td>
</tr>
<tr>
<td>venules</td>
<td>38,98 ± 2,15</td>
<td>33,26 ± 3,07</td>
</tr>
<tr>
<td>capillaries</td>
<td>8,19 ± 0,69</td>
<td>7,58 ± 1,1*</td>
</tr>
<tr>
<td>Capillary density per 1 mm2</td>
<td>98,5 ± 3,4</td>
<td>112,1 ± 5,6</td>
</tr>
</tbody>
</table>

Note. * p<0.05 – (statistical significance of indicators in comparison with the corresponding indicators of patients who did not have diabetes).

Table 2. PSDMAP calculation method.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>The number of points depending on the diameter of the lumen of the vessels and the number of capillaries per 1 mm2 of the HMC of the skin in patients with DMAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter, mкm</td>
<td></td>
</tr>
<tr>
<td>arterioles</td>
<td>≥26 25-18 17-12 ≤11</td>
</tr>
<tr>
<td>venule</td>
<td>≥39 38-25 24-14 ≤14</td>
</tr>
<tr>
<td>capillaries</td>
<td>≥8,2 8,2-6,5 6,4-5,5 ≤5,4</td>
</tr>
<tr>
<td>The number of capillaries per 1 mm2</td>
<td>≤98 99-128 129-157 ≥157</td>
</tr>
</tbody>
</table>
Table 3. Risk factors for the development of thrombosis of ZAR.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>The degree of peripheral resistance (according to R. B. Rutherford and co-authors, 1997)</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Smoking</td>
<td>Didn’t smoke</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>&lt; 30</td>
</tr>
<tr>
<td>Myocardial infarction and/or history of stroke</td>
<td>There was no</td>
</tr>
<tr>
<td>Sex</td>
<td>Women</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Compensation</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>None</td>
</tr>
<tr>
<td>Severity of DMAP</td>
<td>Light</td>
</tr>
<tr>
<td>Thrombosis of the ZAR in the early postoperative period</td>
<td>Was not.</td>
</tr>
<tr>
<td>Reconstructive surgery was performed</td>
<td></td>
</tr>
<tr>
<td>Amputation in anamnesis</td>
<td>Was not.</td>
</tr>
</tbody>
</table>

Figure 1. Photomicrograph. Loosening and thickening of the basal membrane of vessels of the HCMC of the papillary skin layer. Staining with antibodies to type IV collagen. Coll. 400.

Figure 2. Photomicrograph. Skin of a patient with diabetes. Focal expression in the vessels of the dermis. Immunohistochemical staining with antibodies to CD31. Coll. 400.
improve the results of treatment, we proposed an algorithm for the postoperative examination of patients taking into account the presence of risk factors for the recurrence of SOU and thrombosis of the ZAR and in the inflow and outflow tracts.

In our opinion, the development of thrombosis of the ZAR is most likely in patients with risk factors listed in the table. To determine the degree of severity of DMAP, we considered the number of points, the tactics of examining patients in the postoperative period were determined:

- if the sum of points is less than 10, determination of the regional systolic pressure index (RSPI) and control ultrasound dopplerography (UZDH) are indicated after 3, 6, 12 months, then 2 times a year.
- with a score of 10-16, IRST determinations and control ultrasound every 3 months are indicated.
- with a total score of 17-22, IRST determinations and control ultrasound are indicated every 3 months + after 6 months control angiography, then - once a year control angiography.

Timely examination of operated patients and, if necessary, the implementation of interventions (usually balloon angioplasty) prevent the occurrence of complications such as SOU in the ZAR and the inflow and outflow pathways and, as a result, ZAR thrombosis. The most accessible and safe method of diagnosis is ultrasound, which allows to determine the peak systolic blood flow velocity, detect stenosis of the ZAR, as well as the inflow and outflow arteries, but the most informative method of diagnosing the state of the arterial bed in operated patients was considered to be X-ray contrast angiography, which allows to establish indications for performing a preventive operation.

Conclusion.

1. The totality of the obtained data on qualitative and quantitative changes in the HMC of the skin made it possible to develop criteria for the calculation of PSDMAP.
2. A correlation was found between the degree of DMAP and the probability of thrombosis after hybrid arterial reconstruction.
3. Determining the range of severity of DMAP can be used when creating an examination algorithm to establish indications for preventive operations that prevent thrombosis of the ZAR, and the nature of conservative treatment after arterial reconstructions in patients with DM with CKD.

REFERENCES