

Intraoperative Determination of Muscle Vitality at the Level of Amputation in Patients with Diabetic Foot Syndrome

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Abstract The work analyzes the results of a comprehensive examination and treatment of 137 patients with diabetic foot syndrome who were hospitalized in the department of purulent surgery of the Bukhara Regional Multidisciplinary Medical Center in the period from 2020 to 2023. In order to assess the viability of muscle tissue at the level of amputation, the use of a rapid method of intraoperative macroscopic histochemical determination of the activity of muscle tissue dehydrogenases was proposed. Based on the study, we can conclude that there is a decrease in the activity of metabolic processes in muscle tissue during chronic ischemia in patients with diabetic foot syndrome, which is indicated by a slowdown in the formation of formazans during an intraoperative histochemical study for more than 5 minutes. It can be assumed that dystrophic, necrobiotic and inflammatory changes in the conditions of diabetic angiopathy predetermine disruption of regeneration processes and in some cases lead to the development of massive muscle necrosis in the postoperative period. The identified indicators of correlation between the results of intraoperative histochemical examination and histological signs of impaired muscle tissue viability confirm the objective nature of the intraoperative rapid test.

Keywords Diabetic angiopathy of the lower extremities, Lactate dehydrogenase, Intraoperative histochemical study

1. Introduction

One of the most common endocrine diseases is diabetes mellitus: on average, 4-5% of the world's population suffers from it, in Russia - up to 6%, in the USA - up to 20%. More than 70% of diabetic patients develop diabetic foot syndrome. In 1987, at the Geneva International Conference on Diabetes Mellitus, "diabetic foot" was identified as an independent complication along with diabetic nephro-, ophthalmo-, neuro- and angiopathy. Much has been achieved in the field of treatment of diabetic foot lesions, but as regards the use of vasoactive drugs, there is not yet a sufficient number of studies justifying the expediency of their widespread use in practice. Nowadays there are no drugs that significantly and permanently improve the state of microcirculation and the number of patients undergoing high amputations of the lower extremities due to diabetic lesions continues to increase steadily. Trental, traditionally used in such practice, can cause some undesirable consequences ("stealing

syndrome") and carries a potential risk of developing hemorrhages on the fundus in patients with diabetic retinopathy. Currently used methods of surgical and conservative treatment of such complications of diabetes mellitus as diabetic foot with necrotic lesion of the lower extremities at various levels do not always allow to achieve stabilization of the process, are quite expensive and often difficult to perform. The drugs used for topical treatment mainly have a narrowly targeted effect: antimicrobial, dehydrating, non-political, improving the regulatory process. However, they do not provide a comprehensive effect on the wound process as a whole.

The increase in the incidence of diabetes mellitus predicted by WHO experts worldwide makes it urgent to search for new, effective and cost-effective methods of treating patients with diabetic foot syndrome. In recent years, the method of ozone therapy has been successfully used in various fields of medicine. Ozone has a positive effect on carbohydrate and lipid metabolism, improves the oxygen transport function of blood, eliminates microcirculatory disorders, including due to its positive effect on the rheological properties of blood, and also actively affects the

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processes of lipid peroxidation and the antioxidant protection system, optimizes reparative processes in wounds. Recently, methods of topical application of ozone and ozone-saturated sodium chloride saline solution for active drainage of wounds, in which only low concentrations of ozone were used, have become increasingly important in the local treatment of patients with diabetic feet. The intraosseous route of drug administration has long been recognized as one of the most effective. However, there are isolated publications on the intraosseous administration of ozonated saline solution. Currently, almost the entire frequency range of electromagnetic oscillations is used in the diagnosis, treatment and prevention of various diseases. Simultaneous exposure to magnetic, infrared and laser radiation as therapeutic factors improves reparative processes in wound tissues and increases the effectiveness of treatment by about 1.5 times. At present, technologies for the integrated use of ozone, both local and intraosseous in the form of ozonized solutions, magneto-infrared laser radiation, with combined topical application of complex ozone therapy and magneto-infrared laser radiation in various phases of the wound process in patients with purulent necrotic form of diabetic foot syndrome have not been fully developed.

Thus, there is every reason to turn to the scientific study of this issue.

2. The Purpose of the Study

To determine intraoperatively the viability of muscles at the amputation level in patients with diabetic foot syndrome.

3. Materials and Methods of Research

The paper analyzes the results of a comprehensive examination and treatment of 137 patients with diabetic foot syndrome (DFS) who were hospitalized in the department of purulent surgery of the Bukhara Regional Multidisciplinary Medical Center in the period from 2020 to 2023.

Patients underwent ultrasound Dopplerography and color duplex vascular mapping of the lower extremities, X-ray of the foot. The state of carbohydrate metabolism was monitored in the laboratory. Wound healing was assessed according to laser Doppler flowmetry.

In order to assess the viability of muscle tissue at the amputation level, we proposed the use of an express method of intraoperative macroscopic histochemical determination of the activity of dehydrogenases of muscle tissue.

In the operating room, according to the standard procedure described above, the limb was amputated at the initially selected, as high as possible, level. After crossing the muscle arrays with a scalpel, muscle biopsy was performed at the expected amputation level, the thickness of the sections was about 3-6 mm.

During amputation at the hip level, the external broad and semimembranous muscles were examined, during surgery below the knee joint - the calf, flounder and anterior tibial muscles. After manufacturing a suitable biopsy with a cross-section of muscle fibers, it was incubated in a mixture of 0.02% solutions of nitrosine tetrazolium and 1M lactate solution at a temperature of 43°C. At this stage, the temperature regime was strictly observed. The acidity required for the reaction (pH=7.2-7.4) was achieved by using a phosphate buffer. Accordingly, the time for which the first and second stages of the reaction were manifested was taken into account. V1 corresponded to the time of the first reaction, V2 – the time of the end of the reaction. The initial changes began to occur in the first or second minute in the form of staining of individual fibers, then in the period from 3 to 10 minutes, depending on the degree of ischemia. This process can take up to 10 minutes. The course of the reaction was recorded in photographs. The initial stage of the reaction is presented – the formation of formazane in individual muscle fibers in the second minute of the study (Fig. 1).

The final stage of the reaction is presented – total staining of biopsies at the last minute of the study. The result is interpreted as the presence of signs of viability of the corresponding muscles. All changes were recorded in a specially designed patient record card (Fig. 2).



Figure 1. The type of muscle biopsies in the course of IHCE (initial stage): 1. m. gastrocnemius; 2. m. tibialis anterior



Figure 2. The type of muscle biopsies in the course of IHCE (the final stage): 1. m. gastrocnemius; 2. m. tibialis anterior

To confirm the reliability of the applied technique, biopsied muscle sections similar to those used for IHCI were sent for histological examination to the pathology department of the multidisciplinary Regional Clinical Hospital of Bukhara.

In our case, the method of making "frozen" sections was used, while the blocks of tissues are quickly frozen, which makes it possible to quickly produce thin sections with subsequent microscopic examination. For this purpose, a microtome equipped with a freezing table is used, adapted for making sections of frozen tissues.

Preparation of slices from frozen tissues

1. After the frozen tissue block has reached the cryostat temperature, sections 5-8 microns thick are prepared simultaneously from the same block.
2. With a cooled dental needle, two or three sections are transferred to the surface of each slide and left to dry at room temperature for 2-3 minutes. If the quality of the frozen samples was not good enough or they were fixed before microtomy, then the resulting sections should be placed on slides coated with PLL (L-lysine).
3. Then the slices were fixed for 10 minutes in acetone, then 10 minutes in ethanol, and after that they were quickly washed in acetone.
4. The fixed sections were left to dry, then applied with the backs of the slides to each other, wrapped in a tight-fitting polyethylene film or aluminum foil and stored at minus 20°C, placed in a sealed plastic bag with a desiccant (silica gel).
5. The sections were stained with hematoxylin and eosin in a standard battery.
6. The preparations were enclosed in Canadian balsam or polystyrene.

Histological signs of ischemia also include circulatory disorders at the level of the microcirculatory bed, which is manifested primarily in the uneven blood filling of capillaries.

The histological examination data were recorded in a specially designed patient record card and tables.

Early complications were recorded in the postoperative

period. The frequency and nature of complications were compared with the results of the express method of histochemical macroscopic determination of lactate dehydrogenase activity in muscle tissue, as well as with the results of histological examination of the corresponding muscles.

We selected the following formalized signs as histological criteria for impaired viability of muscle tissue:

- Leucocyte infiltration-the presence of neutrophilic granulocytes in muscle tissue outside the vascular bed;
- Karyopycnosis – shrinking of the cell nucleus of myocytes as the initial stage of necrobiotic changes;
- Karyolysis is the process of complete destruction of the cell nucleus during dystrophic changes in it. Cytolysis of myocytes is the destruction of a cell determined by light microscopy;
- Violation of the transverse striation of muscle fibres.

We considered changes in the nucleus and cytoplasm of cells to be microscopic signs of necrosis (Fig. 3-6). The nuclei successively undergo wrinkling (karyopycnosis), disintegration into lumps (karyorexis) and lysis (karyolysis).

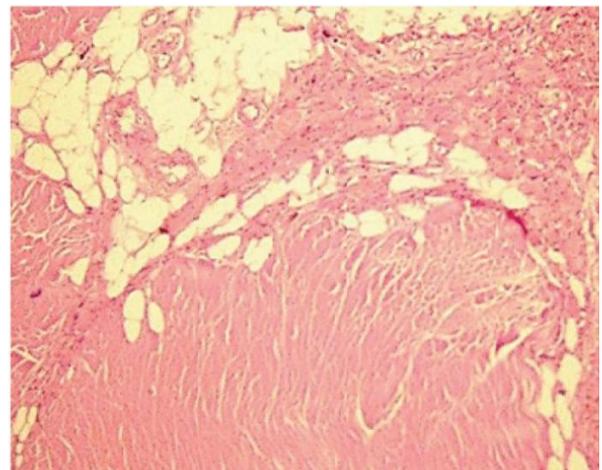


Figure 3. Karyorexis (karyolysis)

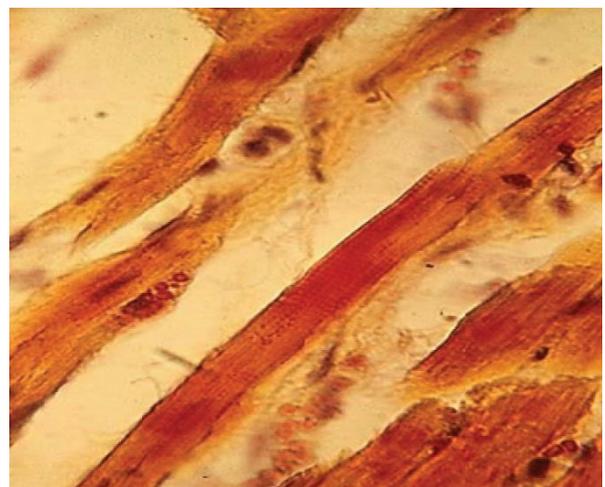


Figure 4. Contractural damage

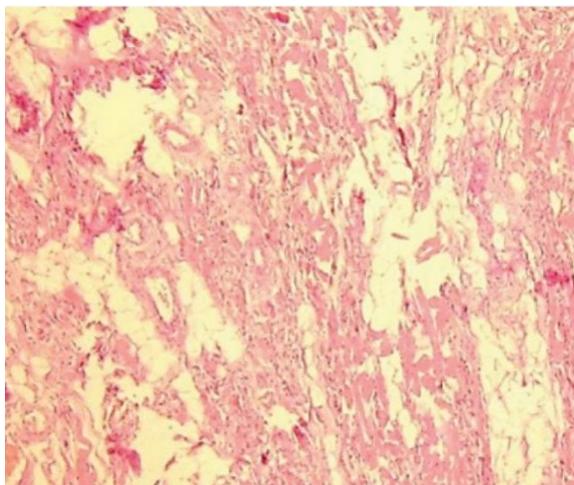


Figure 5. Necrosis and edema



Figure 6. Normal

4. The Results of the Study

Intraoperative histochemical examination of the activity of lactate dehydrogenase in muscle tissue was used by us as an objective criterion of tissue viability for the final determination of the level of limb amputation and the method of stump formation in 137 patients with SDS.

When the lower limb was truncated, the tissue condition was determined using both traditional tissue viability criteria and an intraoperative macroscopic histochemical rapid test. Based on this assessment, in 28 (20.4%) of the observed cases, a decision was made on the possibility of preserving the ankle joint, in 69 (50.4%) amputation was performed at the knee level, in 7 (5.1%) exarticulation was performed at the knee level and in 33 (24.1%) amputation at the hip level is complete.

The informativeness of intraoperative methods for assessing tissue viability was assessed by the clinical result - the presence of necrosis of the stump muscles in the early postoperative period, leading to impaired healing of the surgical wound. It should be emphasized that in this study we did not take into account early postoperative

complications unrelated to ischemic necrosis of the stump muscles, such as postoperative hematomas, marginal necrosis of the skin, superficial suppuration of the postoperative stump wound, unrelated to muscle necrosis, etc.

The healing of the postoperative wound of the stump by the type of primary tension, as we believed, indicated the absence of significant necrosis of the stump muscles.

We studied the results of the assessment of the intraoperative condition of 206 muscles.

With the development of postoperative necrosis, in most cases, during ligation, it was not possible to determine the anatomical affiliation of the altered stump muscle. Therefore, when establishing the fact of postoperative necrosis, we limited ourselves to stating the localization of necrotic changes in the anterior or posterior muscle groups.

As an intraoperative criterion for the viability of muscle tissue in patients with DFS, we used the method of rapid assessment of the activity of lactate dehydrogenase of muscle tissue by reaction with nitrosine tetrazolium developed at the Department of Faculty and Hospital Surgery of the Bukhara State Medical Institute.

To determine the effectiveness of this technique, we studied its informative value for predicting the development of early local postoperative complications, in particular postoperative necrosis of the stump muscles.

According to our data, the total staining time of a fragment of a viable muscle was < 5 minutes. Slower formation of formazanes indicated a marked decrease in redox processes in muscle tissue, which led to impaired regeneration processes and caused the development of clinically significant necrosis of the stump muscles.

In this study, the informativeness of intraoperative histochemical examination (IHC) was studied and compared with traditional criteria for intraoperative assessment of the viability of muscle tissue.

The dependence of the clinical outcome of the operation on the intraoperatively determined activity of lactate dehydrogenase of muscle tissue is presented in Table 1.

As can be seen from the data in Table 1, in the studied group of patients, a slowdown in the rate of total staining of a muscle biopsy for more than 5 minutes demonstrated a significantly positive result in 23 observed cases, a significantly negative result in 233 cases. A false positive result was noted for 16 muscles, a false negative result for 2 muscles.

Based on the data obtained, the indicators of the informative value of this diagnostic test were calculated: sensitivity - 94.7%, specificity - 94.1%, overall accuracy - 94.2%.

In our observations, a significantly higher accuracy of IGHI was noted in comparison with subjective criteria for assessing the viability of muscle tissue due to higher sensitivity and significantly higher specificity. In other words, if with reduced blood flow and muscle contractility, in most cases it was possible to count on an uncomplicated course of the postoperative period, then a slowdown in the formation of pigment in a muscle biopsy over 5 minutes with a high degree of reliability was a predictor of clinically significant myonecrosis of the stump. At the same time, the

high reaction rate indicated sufficient activity of aerobic metabolism processes in the muscle and, consequently, made it possible to consider the choice of amputation level adequate.

Thus, based on the presented data, it can be concluded that the intraoperative histochemical express method for determining the activity of lactate dehydrogenase of muscle tissue at the amputation level in patients with DFS (diabetic foot syndrome) is a highly informative method for assessing tissue viability and can be used as an objective criterion for choosing the optimal amputation level.

The range of preliminary selection of the possible amputation level was determined taking into account the spread of the zone of necrotic and inflammatory changes in soft tissues, the level of occlusion of the main arteries according to ultrasound, as well as determining the patency of the dorsal artery of the foot according to duplex scanning, radiopaque angiography or MRI angiography. Based on this set of diagnostic criteria, the most distal level of amputation was determined. Finally, the issue of the level of amputation and the method of stump formation was resolved after performing a tissue revision at this level. The viability of muscle tissue was assessed by such traditional viability criteria as contractility, bleeding, edema, as well as by the results of intraoperative macroscopic histochemical express study of lactate hydrogenase activity of various muscles.

Depending on the results obtained, a decision was made on the possibility of performing amputation at this, the most difficult, level, as well as on the possibility of simultaneous stump formation and the method of surgical closure of the wound. We used the following surgical tactics (Table 2).

Amputation below the knee joint in patients with gangrene of the distal extremities on the background of occlusion of the femoral-popliteal segment was possible in cases when blood supply to the shin tissues occurred due to collateral blood flow. The main ways of collateral blood supply to the muscles of the upper 1/3 of the lower leg in these cases is a network of arteries that encircle the knee joint, which in turn receive blood from the deep artery system of the thigh. However, it is extremely difficult to assess the adequacy of this blood supply pathway in the preoperative period.

Therefore, we considered it possible to make an attempt to preserve the knee joint in patients with occlusion of the femoral-popliteal segment arteries while maintaining blood flow through the deep artery of the thigh (according to duplex scanning, radiopaque or MRI angiography) and the absence of a necrosis zone above the average 1/3 of the lower leg (Fig. 7, 8).

Based on the conducted research, it can be concluded that a decrease in the activity of metabolic processes in muscle tissue in chronic ischemia in patients with DFS, an indicator of which is a slowdown in the reaction of formazane formation during IGHE for more than 5 minutes, finds its reflection in structural changes in muscle tissue determined by histological examination. It can be assumed that dystrophic, necrobiotic and inflammatory changes in conditions of chronic ischemia precede the disruption of regeneration processes and in some cases lead to the development of massive muscle necrosis in the postoperative period. The revealed correlation indicators of IHCE results and with histological signs of impaired viability of muscle tissue confirm the objective nature of the intraoperative rapid test.

Table 1. The ratio of the results of intraoperative macroscopic histochemical examination (IHC) and the clinical result of amputation

Sign	Result	Clinical result				Total	
		Healing without complications		Muscle necrosis		abs.	%
		abs.	%	abs.	%		
The time of total staining of the muscle biopsy	< 5 minute	233	93,6	2	8,0	235	85,8
	> 5 minute	16	6,4	23	92,0	39	14,2
	Total	249	100,0	25	100,0	274	100,0

Table 2. Objectification of the choice of the level of truncation and the method of stump formation using intraoperative histochemical examination

Surgical tactics	Number of patients	%
Exarticulation at the level of the metatarsal bones	11	8,0
Amputation of the foot by Sharpe	17	12,4
Amputation of the lower leg in the lower third	12	8,7
Amputation of the lower leg in the middle third	27	19,7
Amputation of the lower leg in the upper third	30	21,9
Exarticulation at the knee joint level	7	5,1
Amputation of the hip in the lower third	17	12,4
Hip amputation in the middle third	10	7,3
Hip amputation in the upper third	6	4,4
Total:	137	100,0



Figure 7. Type of limb of patient K. Diabetic foot syndrome, extensive trophic ulcer of the right shin. IHCE: m. gastrocnemius – 4,5 min, m. soleus – 6,5 min., m. tibialis ant. – 5 min



Figure 8. A day after amputation of the right shin, according to the Budget, at the level of the upper 1/3 of the shin. There are no signs of ischemia or inflammation. Wound healing by primary tension, the long-term result was followed for 14 months

5. Conclusions

The intraoperative histochemical express method for determining the activity of lactic acid dehydrogenase of muscle tissue at the amputation level in patients with diabetic foot syndrome is a highly informative method for assessing tissue viability, demonstrating sensitivity of 94.7% and specificity of 94.1%. The revealed correlation indicators of the results of IHC and with histological signs of impaired viability of muscle tissue confirm the objective nature of the intraoperative express study of the activity of lactate dehydrogenase of muscle tissue.

An intraoperative rapid study of the activity of lactate dehydrogenase of muscle tissue at the amputation level (IHCE) is an informative objective criterion for the final choice of the amputation level and the method of stump formation in patients with DFS. The use of IHCE made it

possible to perform amputation at a more distal level in 29.1% of patients and reduce the number of early postoperative complications due to necrosis of the stump muscles.

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