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Histological Changes in Muscles During the Lower Extremities Thrombosis in Individuals with Gastrointestinal Tract Cancer

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ABSTRACT

Thrombosis is a common complication in cancer patients. In all cancer patients, changes occur that lead to arterial and venous thrombosis. It is not known for certain what exactly provokes the appearance of blood clots and causes disseminated intravascular coagulation (DIC). A morpho-histological study of the muscles of the lower extremities was carried out in two groups of people who suffered from cancer and had deep vein thrombosis of the lower extremities with subsequent migration of blood clots. In two groups of people who died due to the migration of blood clots from the veins of the lower extremities in the pulmonary artery, clear dystrophic changes in muscle fibers were found with narrowing of all arterioles and a strong expansion of the venous vessels. In all the venous vessels of the preparation, damage to the endothelium was found, which may indicate the presence of thrombosis of the venous vessels carrying blood from the muscles. People with cancer of the pancreas and/or colon have significant hemodynamic, hypertrophic, dystrophic, and atrophic changes in muscle fibers. There are signs of a systemic effect of the tumor on the vessels and hemodynamics in the lower extremities.

Key words: *Thrombosis, Embolism, Human, Oncology, Lower extremity, Muscles of the lower extremity*

INTRODUCTION

Cancer is one of the main causes of death in the world [1]. All cancer patients undergo changes that lead to arterial and venous thrombosis. It is not known for certain what exactly provokes the appearance of blood clots and causes the syndrome of disseminated intravascular coagulation (DIC). Venous thromboembolism is present in 15% of all cancer patients. Normally, venous outflow from the lower extremities becomes possible due to the pumping function of the heart and diaphragm [2]. Kovács [3-5] notes that constant contractions of the muscles of the lower extremities play an important role, due to which blood is transported through the veins between the valves [6, 7]. One of the reasons for the appearance of thrombosis is a change in the tone of the muscles of the lower extremities. Without active muscle contractions, blood flows through the valves and more slowly to the heart. As a result, there is an expansion of the areas between the valves, in which a lot of blood collects. At the same time, the speed of blood flow also decreases, areas appear in which blood may not move at all, in these areas, blood clots most often form. Conversely, patients who moved little during the first 3 days after starting cancer treatment, or after surgery, had a higher incidence of thrombosis than active patients. The problem of thrombosis of the lower

extremities in cancer patients is complex, and the pathogenesis occurs according to the mechanism of a “vicious circle” [8, 9]. It is impossible to establish which factor will act first - it is impossible after the almost simultaneous action of a large amount of chemical and biological substances on the blood coagulation function of people with cancer. Chemotherapy with cisplatin, L-asparaginase, and tamoxifen, as well as increased expression of tissue factor, sublamine, and an inhibitor of fibrinolysis by tumor cells increase the risk of venous thrombosis. Also, increase in the risk of thrombosis are microparticles of atypical cells, which have a size of 0.1 to 1 μm in diameter, tumor platelet agonists, pro-inflammatory cytokines, and adhesion molecules, as well as onco-associated fibroblasts, podoplanin, and cysteine protease. As a result, morphological changes occur that affect muscle tone and function [10]. Lack of oxygen in some areas of myofibrils leads to the launch of the arachidonic acid cascade, followed by the activation of phospholipase A2 and the release of a large amount of prostaglandins into the blood. The latter are platelet agonists and can activate neutrophils, which begin to adhere to the vascular endothelium of the lower extremities. In people who have problems with the muscles of the lower extremities, thrombosis is more common. An experimental decrease in blood flow through the deep veins of the lower extremities led to the appearance of blood clots. In addition, against the background of slowed blood flow, oxygen compression decreases, which in turn also affects the development of a thrombus [11]. Therefore, maintaining the functionality of muscle work can significantly reduce thrombosis in cancer patients. Hypoxia, which occurs as a result of disruption of the venous outflow, can contribute to thrombus formation. Under conditions of hypoxia, A2 begins to be released, which increases the level of prostaglandins, in turn, together with pro-inflammatory cytokines, trigger a cascade of reactions that lead to blood coagulation due to platelet activation. Aulin V [12, 13] is of the opinion that the formation of blood clots can occur not only in the deep or saphenous veins, but in the vessels that supply the muscles with blood. If such thrombosis occurs in a part of the vessels, then the muscle continues to perform its function, but due to the hypertrophy of a different number of fibers. In accordance with this, a decrease in muscle efficiency can lead to a slowdown in venous outflow and accumulation of blood in the intervalvular zones. Prolonged work of hypertrophied muscle fibers leads to the appearance of zones of atrophy, followed by dystrophy of the areas affected by hypoxia. Against the background of the appearance of venous stasis, atrophy of muscle fibers, and edema, there is still stagnation of lymph, which creates a slight compression on the muscle fibers, slowing down the outflow of venous blood. Considering that in the lower extremities, against the background of prolonged stagnation and disturbance of blood flow through the veins, aseptic inflammatory reactions in the muscles and subcutaneous tissue are often formed [14]. This, in turn, increases the level of pro-inflammatory cytokines, which are capable of activating the processes of thrombus formation. Despite the large number of thrombosis associated with damage to the vascular endothelium, few people take into account the importance of restructuring the muscles, which play one of the decisive roles in the formation of blood clots in the lower extremities. Morphological examination of the vessels of the lower extremities showed that remodeling and damage to the vascular wall in the complex lead to an indirect increase in the intima of the vessel, and changes in the structure of venous valves and other membranes, including leiomyocytes, occur. But the question of how much changes occur in the muscles of the lower extremities, and how this influences blood circulation in general, remains open [5].

The aims of the present study were as follows

- To study morphological changes in striated muscles and their vessels in patients with cancer of various localization, the course of which was complicated by deep vein thrombosis of the lower extremities.
- To check the patterns between changes in muscle fibers and thrombosis of the vessels supplying the muscles.
- To prove the importance of muscles in the development of deep vein thrombosis of the lower extremities in people with cancer of the pancreas and colon.

MATERIALS AND METHODS

A morpho-histological study of the muscles of the lower extremities was carried out in two groups of people who suffered from cancer and had deep vein thrombosis of the lower extremities with subsequent migration of blood clots. Group A consisted of 30 people who suffered from colon cancer and Group B consisted of 24 people who suffered from pancreatic cancer. Both groups were treated with both surgical and chemotherapy. The patients had different degrees of cancer, and different histological structures of the tumor. In addition, tumor sizes and clinical manifestations were also different. Blood biochemical parameters in patients did not differ significantly. The general urinalysis was also unremarkable. In all patients, material was taken from different topographic areas of the lower extremities. In most cases, this was the area of the calf muscles. For the manufacture of histological

micropreparations, classical methods of fixation, compaction and dehydration of tissue sections were used. For fixation, a 10% solution of neutral formalin was used. After that, the embedding was performed in paraffin, followed by the production of sections. The slides were stained with hematoxylin-eosin and trichrome according to the Malory method [7]. In addition, the Weigert [10] method was used. All actions were carried out according to the standard algorithm in compliance with all the rules and requirements in accordance with the author's methods. The imaging technique using deparaffized sections gives a clearer image of morphofunctional changes than the method using dyes. For this purpose, in our work, we used a trinocular with a polarizer and a SEOSCAN analyzer to study dewaxed sections. Submicroscopic studies were performed only on biopsy material. Biopsies were preliminarily fixed in a 2.5% solution of glutaraldehyde with an active reaction of the medium, pH 7.2-7.4, prepared in Millonig's phosphate buffer. Postfixation was carried out with 1% osmium tetroxide solution in Millonig's buffer for 60 minutes, after which the material was dehydrated in alcohols and acetone and embedded in epoxy resins according to the generally accepted technique. Ultrathin sections made on a UMPT-7 ultramicrotome were stained with a 1% aqueous solution of uranyl acetate, contrasted with lead citrate according to the Reynolds' method, and studied with a PEM-125K electron microscope. Semi-thin sections were stained with methylene blue. In parallel, the number of free circulating endothelial cells in citrated blood was determined in all patients according to the method of J. Hladovez. Then the preparations were dried from water using alcohol, acetone, and epoxy resin in accordance with the classical methods of dehydration of histological preparations. For ultrathin sections, UMPT-7 and Reynolds' staining were used. Microscopy was carried out using a PES-125hp electron microscope. All patients underwent a study of endotheliocytes by Hladovez's method. A SEOSCAN microscope was used for microscopic examination. The microscope image was displayed on a computer monitor using a VISION Color CCD Camera. In order for the computer to work in the DVR mode and record data from the camera, the Inter VideoWinDVR program was used.

RESULTS AND DISCUSSION

In two groups of people who died due to the migration of blood clots from the veins of the lower extremities in the pulmonary artery, clear dystrophic changes in muscle fibers were found with narrowing of all arterioles and a strong expansion of the venous vessels. Fiber striation was not clear, the entire cytoplasm was uniformly colored. Such changes are often observed with atrophy or dystrophy of part of the drug sites. Longitudinal sections showed the randomness of the placement of the fibers and the area of chaotic deformation behind the course of the fibers and their separation. A large number of fibers showed signs of hypertrophy against the background of atrophy of other fibers, which may indicate the compensatory function of the preserved muscle cells. During the study, in polarized light, areas of thinning of isotropic disks, and as a result, the convergence of anisotropic disks with each other were visualized. Large cells of anisotropy and fragmentation were observed. Studies using light well reflect the duration of the process and the presence of a prolonged inflammatory reaction, which led to partial dysfunction of the muscles of the lower extremities. In seven patients from group A and five from group B, areas of partial absence of luminescence of A-discs and manifestations of damage to muscle fibers and the beginning of their cytolysis were found. This picture indicates prolonged inflammation and damage of 1-3 degrees. In the future, this can lead to the appearance of zones of replacement of muscle fibers with connective tissue. Polarization microscopy, clearly pronounced contracture damage of 2-4 degrees [13]. Large zones of myocytolysis were found, followed by the breakdown of muscle fibers and the formation of inflammatory changes. 11 patients from group A and 8 from group B had grade 3-4 changes with no luminescence of A-discs. In these patients, the zones of myocytolysis were also more pronounced and the clinical manifestations of thrombosis were more pronounced than in other patients from these two groups. The stroma of the preparations showed signs of perivascular and interstitial edema with the involvement of collagen fibers in the process. All this can be regarded as a long-term process that led to irreversible changes in the vascular wall, an increase in its permeability, and destruction of the endothelial and subendothelial layers. The number of free circulating endothelial cells was also calculated, the level of which was $6.5 \pm 0.2 \times 10^4 / l$. This is important since it was investigated that in acute thrombosis, the level of free circulating endothelial cells was significantly higher than other groups ($p < 0.001$) and amounted to $9.4 \pm 0.5 \times 10^4 / l$.

Table 1. Comparison of muscle fiber injuries and the extent of these injuries in patients from different groups

Group	Percentage of patients with contraction changes in muscle fibers of 1-3 degrees	Percentage of patients with contraction changes in muscle fibers of 3-4 degrees	Percentage of patients with myocytolysis and muscle fiber loss followed by compensatory hypertrophy
A	23.3%	36.6%	19%
B	20.83%	33%	22.1%

Vascular disorders are clearly visible, which occur against the background of impaired blood flow in the vessels that feed the muscle fibers themselves. First of all, these are spasms of arterioles, expansion of venules, extensive edema of the intercellular fluid, and prevascular zones. In the study of the altered vessels, changes in the endotheliocytes that underwent desquamation were found. Similar manifestations occur in large venous vessels of the lower extremities, which indicates the cyclical nature of these impressions and the inclusion of the muscles of the lower extremities in this process. During electron microscopy, clear dystrophic and atrophic phenomena of all organelles were found [15]. It can be assumed that such changes are associated with a constant increase in the amount of intracellular fluid due to impaired vascular permeability of the lower extremities. Other organelles were deformed and had a significant number of cavities with smooth edges, but of different shapes. In mitochondria, zones of clearing and partial damage to the cristae were recorded, which significantly influenced the energy processes of myofibrils. The nuclei of the cells had an altered atypical shape and indistinct edges through the protrusion of the karyolem [6, 13, 14]. Primary and secondary lysosomes were found in the preparations, which were freely located in the cytoplasm and were as close as possible to the Golgi complex. In the center of the nucleoplasm, there is an electron-transparent karyoplasm. And given the fact that the muscles of the lower extremities play an important role in the transport of venous blood to the right heart, disruption of their work and morphological changes can be considered an important link in the pathogenesis of venous thrombosis in cancer patients with lesions of the colon or pancreas. It should be added that an important factor in the appearance of these changes is endotheliocytes, namely their dysfunction. Exposure of the basement membrane can be a trigger factor in the violation of hemodynamics and further development of dysfunction of the venous vessels [5].

CONCLUSION

Research data showed that these changes are indirectly associated with the destruction of endothelial cells and vascular membranes, leading to perivascular edema, changes in the structure of muscle fibers, and disruption of one of the mechanisms of venous outflow from the lower extremities [4, 16, 17]. Despite the experimental study carried out, it can be assumed that remodeling of muscle fibers with subsequent impairment of their function leads to a slowdown in venous outflow, vascular damage, and an increase in thrombus formation in the veins of the lower extremities in patients with cancer. Only the fact that the changes in the muscles are very similar indicates the general systemic effect of tumor growth on the microcirculation and veins of the lower extremities, confirming the data in **Table 1**.

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