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Thrombotic Impact on Muscle Tissue: Histological Insights in Gastrointestinal Cancer Cases

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Abstract

A frequent complication observed in cancer patients is thrombosis, which affects both arterial and venous circulation. However, the exact mechanisms of blood clot formation and the development of disseminated intravascular coagulation (DIC) remain unclear. This study performed a morpho-histological examination of the lower limb muscles in two groups of cancer patients who also experienced deep vein thrombosis leading to thrombus migration. In both groups, which succumbed to pulmonary embolism following clot migration from the lower limb veins, significant dystrophic changes in muscle fibers were identified. These changes were accompanied by the narrowing of the arteries and significant dilation of the venous vessels. In addition, endothelial damage was evident in all venous vessels examined, indicating the presence of thrombosis in the veins responsible for draining blood from the muscle tissue. Patients with pancreatic and/or colorectal cancer showed substantial hemodynamic, hypertrophic, dystrophic, and atrophic modifications in muscle fibers. The findings indicate a systemic impact of malignancies on vascular function and hemodynamics in the lower extremities.

Keywords: Muscles of the lower extremity, Embolism, Human, Oncology, Thrombosis, Lower extremity

Introduction

Cancer is a leading cause of mortality globally [1]. In individuals with cancer, various physiological changes occur that contribute to the development of both arterial and venous thrombosis. However, the precise triggers for blood clot formation and the onset of disseminated intravascular coagulation (DIC) remain poorly understood. Venous thromboembolism is observed in approximately 15% of cancer patients. Typically, venous blood return from the lower extremities is facilitated by the heart's pumping function and diaphragm movements [2]. Kovács emphasizes the significance of continuous muscle contractions in the lower limbs, which assist in blood flow through the veins, particularly between valve points [3-7]. A key factor in thrombosis formation is the alteration in the part of the lower limb muscles. Without sufficient muscle contractions, blood flow slows as it moves through the veins, causing blood to pool in the spaces between the valves. This slow-moving blood increases the likelihood of clot formation, especially in regions where blood flow may cease entirely. Furthermore, cancer patients who remain immobile during the first few days after the initiation of treatment

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or surgery tend to have a higher incidence of thrombosis compared to those who are more active. The thrombosis problem in the lower extremities of cancer patients is multifaceted, with the pathogenesis often following a "vicious circle" mechanism [8, 9]. It could be challenging to pinpoint which factor initiates the process, as cancer patients are exposed to a variety of biological and chemical substances that simultaneously affect the blood coagulation system. Chemotherapeutic agents such as cisplatin, L-asparaginase, and tamoxifen, along with elevated tissue factor expression, sublamine, and fibrinolysis inhibitors produced by tumor cells, all contribute to an increased risk of venous thrombosis. Additionally, microparticles from abnormal cells (ranging from 0.1-1 μm in size), tumor-derived platelet agonists, pro-inflammatory cytokines, adhesion molecules, as well as on-associated fibroblasts, podoplanin, and cysteine proteases, further elevate thrombosis risks. These factors together lead to morphological alterations that influence the function and tone of muscles [10].

Oxygen deficiency in specific regions of myofibrils triggers the arachidonic acid cascade, which activates phospholipase A2 and releases prostaglandins into the bloodstream. Prostaglandins act as platelet agonists and activate neutrophils, which begin to adhere to the vascular endothelium in the lower extremities. Individuals with muscle-related issues in their lower limbs are at a higher risk of thrombosis. Experimental reduction of blood flow in the deep veins of the lower extremities has been shown to result in blood clot formation. Furthermore, slower blood flow leads to decreased oxygen compression, further promoting thrombus development [11]. Therefore, preserving muscle function can be an important strategy to mitigate thrombosis in patients dealing with cancer. Hypoxia, caused by impaired venous drainage, contributes to thrombus formation. In hypoxic conditions, the release of A2 is triggered, elevating prostaglandin levels, which, along with pro-inflammatory cytokines, activate a chain reaction that promotes blood coagulation through platelet activation. According to Aulin *et al.,* blood clot formation can happen not only in the deep or saphenous veins but also in the vessels that provide blood to the muscles. If thrombosis affects a part of these vessels, muscle function can continue, but the muscle fibers undergo hypertrophy [12, 13]. This can reduce muscle efficiency, resulting in slower venous return and blood accumulation in valve intervals. Prolonged work of hypertrophied muscle fibers leads to muscle fiber atrophy, followed by dystrophy in hypoxic areas. In addition to venous stasis, muscle fiber atrophy, and edema, lymph stagnation further compresses the muscle fibers, impeding venous blood flow. In the lower extremities, prolonged venous stasis and disrupted blood flow often lead to aseptic inflammation in muscles and subcutaneous tissue [14]. This, in turn, raises levels of pro-inflammatory cytokines, which contribute to the activation of thrombus formation. While much attention has been given to thrombosis linked to vascular endothelial damage, the role of muscle remodeling in blood clot formation in the lower extremities has not been sufficiently emphasized. Morphological studies of the lower extremity vessels reveal that vascular wall remodeling and damage can indirectly increase the intima of the vessel, along with changes to venous valve structures and other components such as leiomyocytes. However, the extent of changes in the muscles of the lower extremities and their effect on overall blood circulation remains an unresolved issue [5, 15-17].

The objectives of this study were to investigate the morphological changes in striated muscles and their associated vessels in cancer patients with various types of cancer, particularly those complicated by deep vein thrombosis in the lower extremities. The study aimed to examine the relationship between alterations in muscle fibers and thrombosis in the vessels supplying these muscles. Additionally, the research sought to demonstrate the critical role of muscle changes in the development of deep vein thrombosis in the lower extremities of individuals with pancreatic and colon cancer.

Materials and Methods

The study involved a morpho-histological examination of the lower extremity muscles in 2 groups of cancer patients who had developed deep vein thrombosis with subsequent migration of blood clots. Group A included 30 individuals diagnosed with colon cancer, while group B comprised 24 individuals with pancreatic cancer. The two groups underwent a combination of surgical and chemotherapy treatments. The cancer severity, tumor histology, and clinical presentations varied among the patients. Tumor sizes and other clinical manifestations were diverse, though blood biochemical and urinalysis results showed no significant differences between the groups.

Tissue samples were obtained from various anatomical regions of the lower extremities, with the calf muscles being the most common site. Classical histological techniques were used to prepare tissue sections, which involved fixation in a 10% neutral formalin solution, followed by dehydration and embedding in paraffin. The sections were then stained with hematoxylin-eosin and trichrome using the Mallory method, along with Weigert's method. These steps were made according to standard protocols.

To obtain a more precise image of morphofunctional changes, deparaffinized sections were examined using a trinocular microscope equipped with a polarizer and an SEOSCAN analyzer. Submicroscopic analysis was performed exclusively on biopsy material, which was first fixed in a 2.5% glutaraldehyde solution with a pH of 7.2-7.4, prepared in Millonig's phosphate buffer.

Post-fixation involved treating the samples with a 1% osmium tetroxide solution in Millonig's buffer for 60 minutes. Afterward, the specimens were dehydrated using alcohols and acetone and embedded in epoxy resins following standard protocols. Ultrathin slices were obtained with a UMPT-7 ultramicrotome and stained with a 1% solution of uranyl acetate. The sections were further contrasted using lead citrate, keeping in mind what Reynolds procedure was, we analyzed with a PEM-125K electron microscope. For semi-thin slices, methylene blue was applied for staining.

The number of free endothelial cells circulating in the blood of all patients was measured using the J. Hladovez method. The preparations were dehydrated through a series of alcohol, acetone, and epoxy resin treatments following traditional histological dehydration steps. The ultrathin sections underwent UMPT-7 and Reynolds staining techniques and were examined under a PES-125hp electron microscope.

Endotheliocyte studies were conducted on all patients using Hladovez's method. The samples were analyzed using an SEOSCAN microscope, with images shown on a computer monitor via a VISION Color CCD Camera. The recorded data from the camera was processed using the Inter VideoWinDVR software for DVR operation.

Results and Discussion

In the two groups of individuals who died from the blood clots migration from the lower extremity veins to the pulmonary artery, notable dystrophic changes were observed in the muscle fibers. These included the narrowing of arterioles and significant dilation of venous vessels. The muscle fibers displayed a loss of striation, with the cytoplasm showing a uniform coloration. Such changes are typically seen in cases of atrophy or dystrophy in specific muscle regions. Longitudinal sections revealed a disordered arrangement of muscle fibers, with areas of chaotic deformation and fiber separation. Several fibers exhibited hypertrophy, alongside atrophy in others, suggesting a compensatory response from the surviving muscle cells.

Under polarized light, the thinning of isotropic disks was visible, leading to the convergence of anisotropic disks. This was accompanied by the appearance of large, fragmented anisotropic cells. The light microscope images highlighted the time of the pathological process and the presence of ongoing inflammation, which contributed to partial muscle dysfunction. In seven individuals from group A and five from group B, areas lacking luminescence in A-discs and signs of muscle fiber damage and cytolysis were evident. These observations pointed to chronic inflammation and damage at stages 1-3, which could potentially result in muscle fiber replacement by connective tissue. Polarization microscopy further revealed clear signs of contracture damage in grades 2-4.

Extensive areas of myocytolysis were identified, with muscle fibers breaking down and inflammatory changes developing. Among the patients, 11 from group A and eight from group B showed grade 3-4 changes, marked by absent luminescence of A-discs. These patients exhibited more pronounced myocytolysis zones, and their clinical symptoms of thrombosis were more severe compared to others in the groups. The stromal tissue of the specimens also exhibited signs of perivascular and interstitial edema, with collagen fibers involved in the process. This pattern suggested a prolonged pathological process that led to irreversible vascular changes, increased permeability, and damage to both the endothelial and subendothelial layers.

The study also measured the level of free circulating endothelial cells, which was found to be $6.5 \pm 0.2 \times 10^4$ /l. This finding is significant, as previous research has shown that during acute thrombosis, the level of circulating endothelial cells is notably higher ($P < 0.001$), reaching $9.4 \pm 0.5 \times 10^4$ /l.

A comparison of muscle fiber injuries and the extent of these injuries in patients from different groups is presented in **Table 1**.

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 abnormalities are evident, particularly in vessels that supply the muscle fibers, which occur as a result of compromised blood flow. These include arteriolar spasms, venule dilation, widespread intercellular fluid edema, and changes in the prevascular zones. Examination of the different vessels revealed desquamation of the endothelial cells. Similar findings were noted in the larger venous vessels of the lower extremities, suggesting a cyclical nature of the changes that involve the muscles in the lower limbs. Electron microscopy revealed distinct dystrophic and atrophic changes across all organelles. These alterations are likely linked to a continuous increase in intracellular fluid because of the compromised vascular permeability of the lower extremities.

Further, other organelles displayed deformation, with numerous cavities of varying shapes but smooth edges. In the mitochondria, it was observed that some areas were cleared and partially damaged to the cristae, which likely had a significant impact on the energy processes within the myofibrils. The cell nuclei exhibited an atypical shape with indistinct borders as the karyolem protruded. Primary and secondary lysosomes were present in the cytoplasm, located close to the Golgi complex. The nucleoplasm appeared electron-transparent at the center of the nucleus. Given the vital role of the lower extremity muscles in venous blood return to the right heart, the disruption of their function and the accompanying morphological changes are crucial to understanding the pathogenesis of venous thrombosis in cancer patients with colon or pancreatic involvement.

Additionally, a key factor contributing to these changes is the dysfunction of endothelial cells. Exposure of the basement membrane could serve as a triggering event, leading to impaired hemodynamics and the subsequent dysfunction of the venous vessels.

Conclusion

The findings of this study suggest that the observed changes are linked to the destruction of endothelial cells and vascular membranes, which contribute to perivascular edema, alterations in muscle fiber structure, and disruption of venous outflow mechanisms in the lower extremities. While the study was experimental, it is likely that remodeling of muscle fibers, followed by functional impairment, leads to a reduction in venous outflow, vascular damage, and an increased risk of thrombus formation in the lower extremity veins of cancer patients. The consistency of these changes across the subjects highlights the systemic impact of tumor growth on the microcirculation and venous function in the lower extremities, further supporting the data presented in **Table 1**.

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