Morphopatological and histochemical highlights in normal and varicose vein wall

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Abstract: The nutrition of the venous wall appears to be an important factor in the vascular-fibrillar trophicity and in the dynamic of the extracellular matrix formation for the normal veins and, for the chronic venous ulcers of legs, on period of healing. Sequential biopsies were taken at various levels of venous wall of external and internal saphena in 16 cases presenting a chronic condition of legs venous system (35-58 years old patients, both sexes). 8 vein fragments with normal macroscopic appearance were also taken, in necropsy. These samples were analyzed using regular morphological methods and some histochemical reactions to reveal the glycogen, glycoproteins, and glycosaminoglycans substrates. There were used the Gomori silver impregnation and orcein to expose some specific substrates like reticulin or elastin. Other staining methods, like Gomori trichrome, were used to differentiate the specific structures of the vein wall, were used to differentiate the specific structures of the vein wall. A rich vascularization of normal and dilated vein wall could be remarked. Angiogenesis in vein wall and vasa vasorum changes as well as alcianophilic of vascular intima seem to be reactive and protective factors, depending on the applied therapeutic modalities. The veins are weak structures whose integrity depends on the thickness of the media and the support of neighboring structures.

INTRODUCTION

The emergence of varicosities it is supposed to be a consequence of defective development of venous wall and appears to be inherited. The proper nourishment of the venous walls appears to be an important factor in their trophicity. The incidence of varicose veins is about 1% of the adult population [5]. Both obesity and aging are involved as risk factors for emergence of defects in venous wall tissue support. The etiology and pathophysiology of varicose disease remains controversial. We emphasize the involvement of the following lesional links amid stasis hypoxia:

- Redistribution of microcirculation with arrangement in lobular aggregate of vessels in the superficial dermis;
- Areas of floride proliferation mimicking Kaposi's sarcoma (acro-angio-dermatitis), reticular dermis fibrosis and massive deposits of hemosiderin, degeneration of dermal fibroblasts (senescent fibroblasts converted by modulation into myofibroblasts having a role in periucleros tegument retraction) to extended tissue matrix lysis;

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Outbreaks of fat necrosis concomitant with a proliferative-alternative response of adjacent epidermal, hyper parakeratosis and orthokeratosis and pseudoepitheliomatous hyperplasia.

The presence of lytic degradative tisular factors (metalloproteinase) and activation of monocito-macrophagic system induced degradation of the basal membrane and chronic venous ulcers.

Morpho-histochemical current study aims to deepen the importance of veins wall nutrition in normal and dilated veins. It also envisages the re-active participation of supra-adjacent dermo-epidermal tissue, the possible influence on vessel permeability and vasoprotective or anticoagulant medication used in selected cases in the study.

MATERIALS AND METHODS

16 biopsies were taken sequentially from the varicose saphenous in areas of dilated vein (12 cases) and in the vicinity of shank chronic venous ulcer (4 cases); Saphenous fragments from normal vein were collected at necropsy (8 cases). Cases have included both sexes. The selected cases were with at least 5 years of disease duration and the type of treatment was also considered.

Patients in the study were selected from the inmates in Department of Surgery of Military Emergency Hospital and University Clinic of Dermatology in Timisoara.

Processing of samples was performed at the Department of Histology of Medicine and Pharmacy University in Timisoara by usual histological techniques: all samples were fixed in 10% formalin, buffered formalin, AFA fixator (Alcohol - Formaline - Acetic acid), paraffin inclusionated, sectioned at 5 μm, then colored by hematoxylin-eosin. For histochemical study, there were been applied reactions PAS (blue Alcian (BA)) and salivary amylase-PAS (Alcian blue used at pH 2.8 and pH 0.5); blue toluidine (BT) at pH 5 and pH 2.8; it was used Gomori silver impregnation for reticulin and it was also used trichrome Gomori reaction.

Orcein staining method was used to expose elastin.

RESULTS

In all examined cases, the wall of external and internal saphenous veins was well vascularized, containing arterioles, venules, capillaries in musculo-fiber media. Constant, parietal capillaries from vasa vasorum were not flattened. In varicose veins adventitia, in small and medium vessels, were frequently found leukocytic marginations and acidophilic deposits and PAS positive, weak metachromatic fibrin (Figure 1).

Fibrin sleeves were also observed in the wall vessels of varicose veins in which angiogenesis is very marked. For AT, were remarked weak metachromatic perivascular sheaths, at pH 5 and chromophobe tissue matrix of dilated veins sheaths, at pH 2.8. Orthochromatic of nuclei allows observing the phenomenon of leukocytes margination (mostly lymphocytes) in the parietal small and medium vessels up next to intima, for both pH used (Figure 2).
In all methods used in this study, the average media of varicose veins appears thickened through a rich smooth muscle proliferation (HE col., Gomori trichrome col.), with weak orceinic material fragmented through the wall layers (Orcein col.). There were observed fake elastic limitations, fragmented, disorganized (orcein, Gomori trichrome). In one case of all cases examined, using combined staining AA-PAS, which stands under anticoagulant therapy, an edemic intima and hyperplasia were observed, with fundamental alcianophilic substance (FS) and somewhere with deposits PAS positive as a rich interstitial material PAS positive all that pushing aside and cleaving muscle layers of the vascular media. Other intimal changes reported in our study are notches and extensive fibrosis, miointima hyperplasia of saphenous varicose veins.

Alcianophilic fibrillar material with reticular disposition is perivascular distinguished in the neo-capillaries that invade from adventitia media. Silver impregnation for reticulin (Figure 3) shows neo-formation basic membrane by budding vasa vasorum from adventitia to media to decline to extinction the reticulin network of media muscular interstice and vasa vasorum walls.

**Figure 3.** Reactive angiogenesis in variceal venous wall (silver impregnation, ×200)

The phenomenon seems to affect 2/3 of external walls of the dilated veins. In neighboring dermal and epidermal tissue and in the perivascular support appear lesions like: lipodermatosclerosis, fibrosis and perivascular fibrin deposits and interstitial fragmentation of elastic fibers in the deep of dermis, tinctorial variations of epidermal cells cytoplasm for which an accumulation of PAS positive material could be noted and cemented SF was PAS reactive. For silver impregnation, the dermal epidermal limit appears fibrillary fine, thinner than the normal appearance.

**DISCUSSIONS**

The changes of vein wall tissue architectural pattern take place simultaneously with the progression of venous distension in varicose vein disease. It is noticed an important reaction of vessels in vasa vasorum which angiomatosic proliferate, invading media, almost to intima, leading to muscular media hypertrophy [5]. Vascular proliferation can be reactive to miointima hyperplasia and to varicose wall media. Chronic shank venous ulcer it is possible to occur, in time.

Thus, as a reaction to increased venous pressure in a vein much thickened, on account of media and intima and hence to the shank edema, fibrin sleeves occur, those may inhibit the angiogenesis [4]. However, it is created a protection against increased venous additional pressure with a possible role in causing tisular ischemia [6][8].

Fibrin sleeves were found around the capillaries of the dermis in lipodermatosclerosis. Massive deposits of fibrin are involved in elastin fragmentation of the deep dermis and in elastic limitations of vascular walls. Vasa vasorum angiomatosic proliferate in invading varicose vein media. Since 1992, "leukocyte sleeve" was reported as a response to chronic stasis. White blood cells can accumulate in the lumen of capillary and venular, increasing perivascular resistance.

Activated endothelium becomes thrombogenic surface (via tissue factor secreted by endotheliocyte: factor Vlla, factor X-Xa). Simultaneously, activated endotheliocyte was involved in angiogenic peptide synthesis, stimulating neovascularization of dermis and ectasiated venous walls. Tissue anoxia and ulceration is installed.

Consistent with the cited literature, we found also an increased number of capillaries in the skin of patients with venous hypertension in the lower limbs.
Wall vessels are the only ones bringing oxygenated blood, draining interstitial fluid and extravasated colloidal material \([7][1][3]\). This explains the rapid drainage and metastasis of tumor cells via veins and lymphatics with rich vascularity, having intraparietal capillaries opened.

Adventitial enriched positive PAS material, periadventitial and intraparietal, is represented by fibrin, and also by a macromolecular glycoprotein substrate containing laminin, fibronectin, tenasin detected by immunocytochemical investigations by some cited authors \([2][5]\).

So we can correlate the "leukocytic margination" phenomenon in vasa vasorum and intraparietal vessels with depolymerization of SF tissue more evident in the 2/3 of external portion of the varicose venous wall.

The histochemical reactions used in our study, draw attention to the changes in the three fibrillar component. In varicose veins (dilated segments thereof) we found a quantitative increase of mature collagen fibers and reticulin, morpho-histochemical revealed, with a decrease and fragmentation of elastic fibers in the dilated walls. Together with the degradation of elastin, reticulin network growth can be an important factor in the parietal frailty in shank venous insufficiency evolution.

Marked alcianophilia of intima (AA pH 2.8) calls attention to presence at this level of complex carbohydrates with carboxyl groups and hydroxyl radicals in position of vic-glycol (sialic acid and hyaluronic acid) which act as intima hydrator, but may decrease macromolecular complexes stability of glycoproteins (with increase of sialic acid amount).

Sialic acid carboxyl groups and other substances not yet identified are responsible for basophilia and metacromazia of certain reticulin networks and basement membranes. The enriched PAS positive interstitial material in dilated vessels media is favorable to reticulin collagenization (gradual multiplication of preexistent collagen fibers in a glycoprotein matrix apparently unchanged).

Reticulin collagenization occurs when the hydroxyl group probably belonging to hydroxyproline and hydroxylysine achieve the appropriate concentration \([9]\).

In the cases we investigated, collagenization is conducted in a glycoprotein reticulin dominant matrix (in the external 2/3 dilated vein wall), progressively decreasing toward intima, simultaneously decreasing nourishment intake by vessels of the vein wall fibrosing.

**CONCLUSIONS**

Morpho-histochemical methods draw attention to the complex of walls reshuffles of venous leg during chronic venous insufficiency. There are being considered the three coats of veins in that appear to be successive lesional patterns: mio-intimal hyperplasia, medial muscle hypertrophy, reactive angiogenesis, lipodermatosclerosis, collagenization of medial muscle.

The chronic venous stasis can be quantified by morphological parameters: mio-intimal hyperplasia, medial muscle hypertrophy, reactive angiogenesis, lipodermatosclerosis, collagenization of medial muscle. The prolonged venous hypertension modifies both the local microcirculation conditions and selecting of fibril-forming cells, triggering the evolution of chronic venous ulcers.

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