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Surface Morphology of the Endothelium in Varicosis of the Great Saphenous Vein — Scanning- and Transmission Electron Microscopy Study

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The results obtained in these parallel SEM and TEM studies display the significant informativeness of this approach in the investigations on venous endothelium and its changes during varicosis. It was established that in varicose veins great alterations are observed in the spatial structural organization of the endothelium including in the area of the "valve complex". With the development of varicosis, the lumen relief of the endothelium becomes more complex. The considerably uneven relief of this surface exerts an increased local resistance to venous blood circulation. The availability of deep crypts accompanied by the shortening of the valve cusp with an unevenness of its thickness and with a thickening of its free border lead to changes in its biomechanical properties. The altered valve cusp cannot perform the fine movements with which it reacts in normal conditions to the changes in the venous circulation in order to prevent venous reflux.

Key words: vein, valves, varicosis, SEM, TEM.

Introduction

In recent years a lot of dependence has been established accounting for the changes in the structure of the endothelium of the venous blood vessels during the development of the varicose process in the lower limb veins [1-5, 7-10, 12-14, 16, 17]. Among them most noteworthy are the established alterations in the venous valve endothelium structure — taking place during the varicose process before the clinical picture of venous valve insufficiency has developed [7-9].

The investigation of these changes with various methods — histological, immunocytochemical and transmission electron microscopy brought about to the elucidation of certain aspects of the etiology and pathogenesis of the varicosis [1, 2, 5, 7-9, 12-13]. Nevertheless, the parallel studies with scanning (SEM) and transmission electron

microscopy (TEM) [1,10] allowing for the performance of an integral investigation of the form, size and relief of the endothelial all lumen surfaces and the fine ultrastructural alterations in it represent a very limited number and stand out as unique. This fact drew our attention to a more detailed comparative study on the changes taking place in the endothelium of the great (long) saphenous vein in the process of development of varicosis in it by the help of SEM and TEM.

Material and Methods

Selection of the operative material

The study was carried out after all the established in the Medical University of Varna requirements for ethical attitude towards the patient and the research material were strictly observed. Portions of the great saphenous vein wall have been taken from twenty patients in its operative removal due to its varicose dilatation. As controls have been used parts of great saphenous vein wall removed from five patients in whom the great saphenous vein was used for autologous arterial by-pass graft as well as from one amputated due to obliterating atherosclerosis lower limb. In all patients under study no anamnestic or clinical data for convalescent thrombophlebitis were found.

Methods

Macroscopic study, classification and sectioning of the material

The material for SEM and TEM was taken at times of operation in the operative hall. The extirpated segments of the great saphenous vein were opened very carefully along the blood flow direction, then washed with saline, fixed as it is further on described and inspected by the help of a stereomicroscope so that their lumen relief could be investigated and the vein valves located. The parts of the found vein valves were designated after the classification of B u t t e r w o r t h et al. [2] for the so-called "valve complex". The degree of atrophy of the found valves was registered after the Vankov's classification [18] according to their cusp type as:

1. Partially emaciated — valves with a part of their cusps preserved
2. Totally emaciated ones — valves displaying what has remained of them as small bands along the vein wall, named "valve agger".

Representative portions of valves and such located outside the valves were carefully removed for further SEM and TEM studies.

Scanning electron microscopy

The removed vein parts were fixed in 4 per cent glutaraldehyde in phosphate buffer, pH 7.4, post-fixed in 1 per cent OsO_4, dehydrated through an ascending ethanol series, soaked in hexamethyldisilazane for 5 min and then dried in the air. The were coated with gold-palladium and were studied on a JEOL JSM 35-CF scanning electron microscope (Soquelec LTD, Montréal, Québec, Canada) at a 15-25 kV accelerating voltage.

Transmission electron microscopy

The removed venous parts were fixed in 3 per cent or 4 per cent solution of glutaraldehyde in 0.1M phosphate buffer, post-fixed in OsO_4, dehydrated through the ascend-

ing ethanol series and embedded in Durcupan. Semi-thin sections of 1μm thickness and ultra-thin sections were prepared. The ultra-thin sections were studied on a transmission electron microscope JEM 7A and Opton.

Results

I. Scanning electron microscopy

1. Venous wall outside the valve area and mouths of tributaries

Along the lumen surfaces of the endothelial cells, vesicles and thread-like outgrowth projections of various shape and size have been observed. On the surface of the larger protrusions, imaginations of the cytolemma of various diameters are found. In some parts of the lumen surface of the vein wall the endothelial cells encircle deep pits and tunnels. Their walls are of a complex relief since the endothelial cells surrounding them display two types of projections — flat ones and single or grouped vesiculous projections.

2. Valves with two shortened partially emaciated cusps.

A. Vein wall in the valve sinus area

The endothelial cells on the surface of the valve sinus of the vein wall are arranged as roof tiles. Single short cytoplasmic projections are formed on the lumenal surface of the cytolemma.

Fig. 1. Lumenal surface of valve cusp endothelial cells from a valve with two partially emaciated cusps SEM × 2000

B. Valve cusp

The lumenal surface of the valve cusp in its major part is uneven because of the present of a multitude of vesiculous and thread-like cytolemmal projections (Fig. 1).

It is noteworthy that upon comparison of the lumenal with the sinus surface the latter is much more uneven — deep crypts are formed on it, penetrating to different depths into the stroma of the valve cusp and the deepest crypts reaching the elastic membrane of the cusp. The endothelial cells covering this surface descend into the depths of the crypts. The endothelial cells on the sinus surface outside the crypt area display a great number of cytolemmal invaginations and projections.

The endothelial surface of the thickened free border of the valve cusp is uneven. Cytoplasmic thread-like projections of various lengths and thickness are observed on it. Single invaginations of the cytolemma of varying shape and size are also observed (Fig. 1).

3. Valves with two totally emaciated cusps.

A. Vein wall in the valve sinus area.

Along the valve sinus-to-valve agger transmission in its lowest portions, the lumenal surface of the endothelial cells is uneven with a large number of cytoplasmic projections of varying height and thickness being observed on it. The cytoplasmic projec-

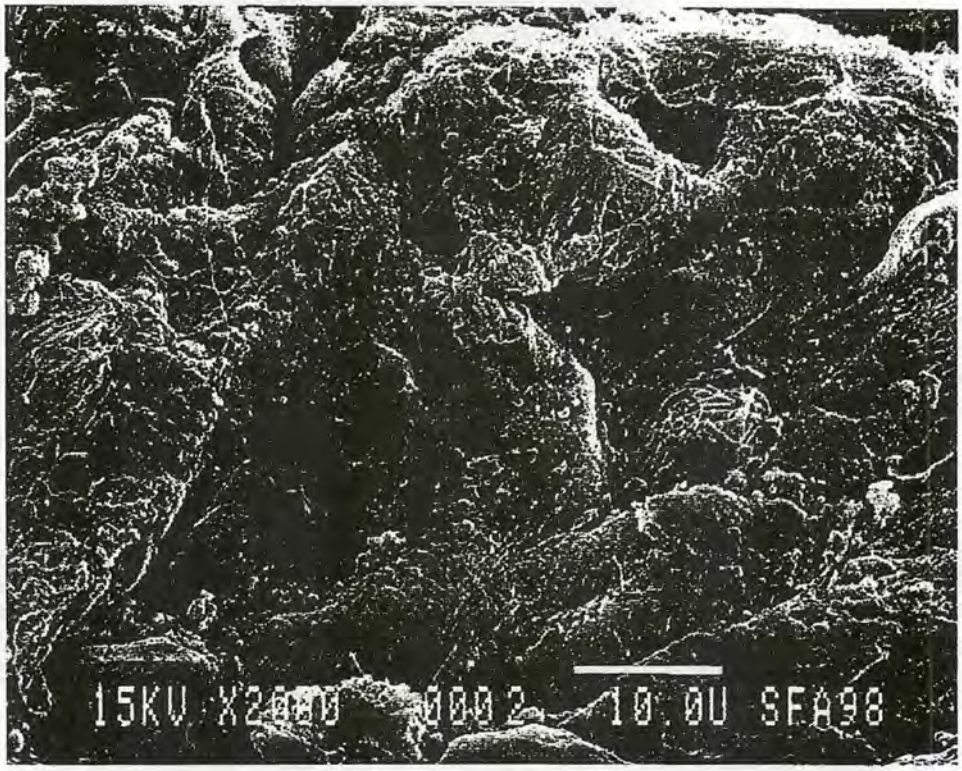

Fig. 2. Lumenal surface of a valve agger endothelial cells from a valve with two totally emaciated cusps. SEM × 2000

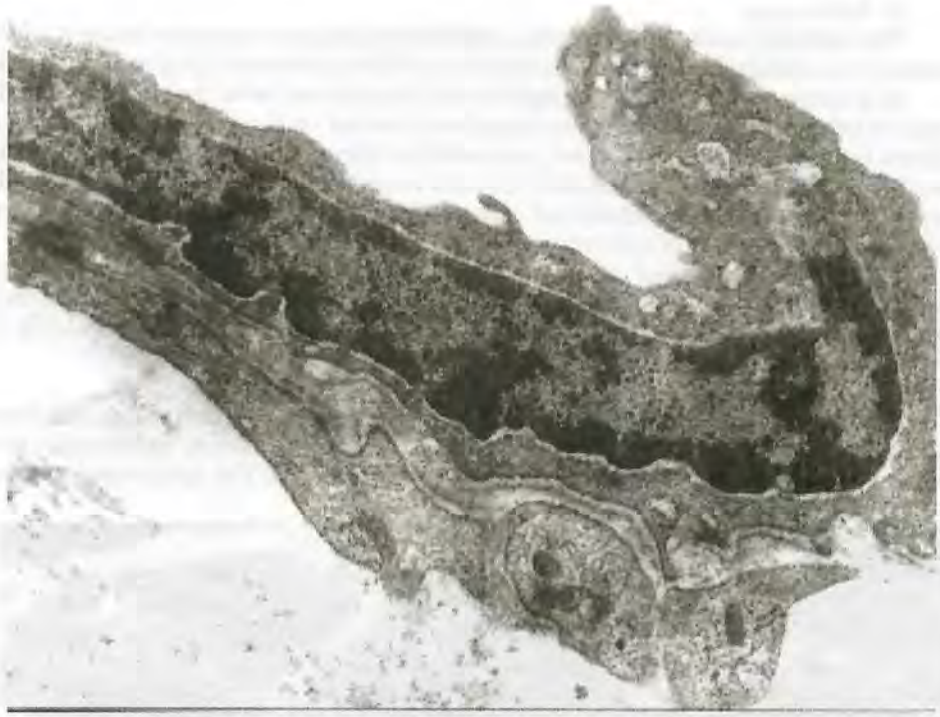

Fig. 3. Endothelial and subendothelial cells of a valve cusp from a valve with two partially emaciated cusps. Nuclear portion. TEM × 10 000

tions display a wide, almost spherical top part, which is gradually narrowing towards its basis. Except the projections thus described other lower and elongated folds of the endothelial cell cytolemma are also observed. On the lumenal surface of certain endothelial cells vault-like cytolemmal formation are observed creating the impression of an ongoing channel formation.

The latter are observed on the sinus surface being most pronounced in proximity to the valve agger.

B. Valve agger

The lumenal surface of the valve agger is strongly undulating covered both by endothelial cells following the complicated wave-like relief and grouped roof like arranged endothelial cells. Amid the endothelial cells, pits of a varying shape and orientation to the valve agger are enclosed. They penetrate to differing depths being in capacity to form tunnels. The endothelial cell cytolemma forms lumenal projections of various shapes — spherical ones, such resembling vesicles, others — thread-like of different lengths as well as flattened ones of irregular shape (Fig. 2).

II. Transmission electron microscopy

1. Valve cusp

Upon TEM studies of the endothelial cell lumen surface, short lumen projections and invaginations were established. The invaginations penetrate to depths of different size both in the nuclear area of the cell and its perinuclear zones. The invaginations of the cytolemma in the nuclear area of the cell can alter to a different degree the shape of the nucleus, in some it follows the relief of the lumen surface (Fig. 3).

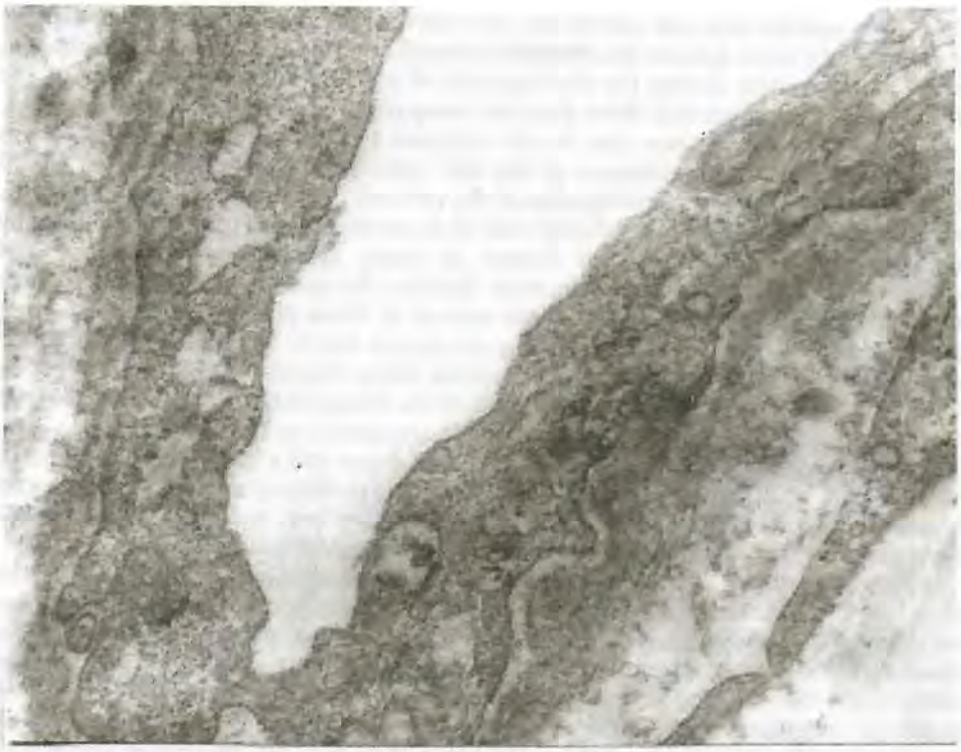

Fig. 4. Endothelial cell of a valve cusp from a valve with two partially emaciated cusps. TEM × 20 000

The parts of the endothelial cells covering the pits' bottoms on the lumenal surface of the vein valve cusps are strongly attenuated (Fig. 4). The projections found on their surface are of various lengths and thicknesses jut out into different depths in these pits. It is noteworthy that alongside the pit mouth periphery asymmetrically localized projections are observed which cover parts of the pit like a "shade". In the thin parts of the endothelial cell the cell organelles are rather scanty — cytofilaments and vesicles are observed. The basement membrane is preserved. In the regions in which the subendothelial space is very thin the subendothelial cells are in close proximity to the bottom of the pits formed by the endothelial cells. In this areas the amount of the collagen fibrils between the endothelial and subendothelial cells is strongly reduced. The portions of the subendothelial cells situated in close proximity to the endothelial cells forming the pits display a number of pinocytosis vesicles, mitochondria, granular endoplasmic reticulum, cytofilaments and microtubules.

Discussion

The results obtained from the SEM study in the present investigation render an in-detail spatial notion of the general state of the endothelium covering the vein wall in the development of a varicosis process. Especially interest provoking is the comparison of the data about the changes of the endothelium of the so-called "valve complex"

of the veins and the vein wall outside the vein valve areas. These data broaden, supplement and make more precise the available scientific literature data about the changes taking place in them during the development of primary varicosis. An asset of the present study is the fact that these data are compared with parallel TEM studies.

The obtained data show that in vein varicosis significant changes take place in the spatial structural organization of the wall endothelium including the so-called "valve complex". With the development of the varicose process, certain complications in the lumenal relief of the endothelial cells (6,9) are observed. These are displayed by the formation of numerous and diverse in shape cytolemmal projections and cytolemmal invaginations of various sizes. Besides, the endothelial cells take part in the enclosures of deep pits. Around the mouth of these pits, projections covering asymmetrically like a "tent" a part of the pit mouth can be found. This leads to the conclusion that the latter could be adaptations whose functional role is the creation of conditions for a more continuous contact of the blood with the endothelium of the vein wall. In support of this suggestion come the results from TEM which show a considerable slimming of the endothelium in the pit areas and a sparse development of the intracellular organelles in such parts. Amidst the thinned endothelial cells and the underlying subendothelial cells of the vein valves the base membrane may be missing, a reduction of the collagen fibrils among them is established.

The presence of deep crypts accompanied by shortening of the length of the valve cusp with a roughness in its thickness and by a thickening of its free border described by us in previous works change its biomechanical properties [8, 9, 11]. The valve cusp thus altered cannot perform the fine movements by which under normal conditions it reacts to the changes in the vein blood flow to prevent venous reflux. A significant roughness of the surfaces of the partially emaciated valve cusp as well as the considerable projections on the valve agger surface of the emaciated valves and on the surface of the vein wall in the vicinity of the valve sinus exert an increased local resistance to the venous blood flow. In the areas of the vein walls outside the valves and tributaries the uneven lumenal surface, the lumenal projections and invaginations on the surface of the endothelial cells most probably also exercises a certain influence on some parameters of the blood flow in the vein.

Conclusion

The results obtained in this TEM and SEM parallel study show a considerable informativeness of this approach in the investigation of the vein endothelium. In the varicose veins are established significant rough areas on the surface of the partially emaciated valve cusps and valve aggers, which together with the other morphological alterations of the valve cusps change the biomechanics and function of the vein valve complex.

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