Intimal Changes in Varicose Veins: an Ultrastructural Study

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Abstract

In order to study the structural changes in the intimal layer of varicose veins, we prospectively collected a total of 23 vein specimens from both the normal proximal thigh long saphenous (LSV) in 3 young trauma patients and from the unstripped proximal LSV near the sapheno-femoral junction and the distal calf blowouts in 10 primary varicose veins patients. Paraffin sections stained with hematoxylin and eosin were examined under the light microscope while ultra-thin sections were examined under the transmission electron microscope (TEM). Compared with the normal control LSV, varicose vein sections showed increase in the diameter of the lumen, hypertrophy of the wall and elongation and invagination of the intima. Along these invaginations, endothelial cells were compressed, elongated and thinned out. The cells also showed progressive degeneration and were finally lost into the lumen, leaving only the basal lamina to form the luminal surface. This invited blood components like platelets and red blood cells to stick to the bare intima and to penetrate through the wall. This may form the basis of the clinical condition of superficial thrombophlebitis, which sometimes complicates cases of varicose veins. In conclusion, varicose veins showed increased diameter of the lumen and hypertrophy, elongation and invagination of the intima. There was marked degeneration of the endothelial cells and desquamation of the endothelial layer.

Key words: varicose veins, long saphenous vein, calf varicosities, transmission electron microscope (TEM), endothelium

Introduction

The exact mechanism by which primary varicosis occurs in the lower limbs in susceptible individuals has yet to be determined (Clarke et al., 1961). The valvular theory suggesting primary valvular incompetence as the most important factor for the development of varices (Moore, 1951) has been criticized in a number of morphological (Jurukova and Milenkov, 1982; Rose and Ahmed, 1986), functional (Psaila and Melhuish, 1989; Clarke et al., 1992), and biochemical studies (Svejcar et al., 1963; Prerovsky, 1981; Haardt, 1987; Psaila and Melhuish,
1989), which demonstrated that dilatation of the vein wall may occur before or even without valvular incompetence. Owing to the inadequacies in the explanation of the primary valve failure hypothesis, others have suggested a weak wall theory (Cockett and Jones, 1953). This theory states that if vein walls are easily distended under normal venous pressures, this will lead to dilatation and secondary valve failure (Miller, 1974). Heredity is an important factor as more than half of those affected have a positive family history, suggesting a congenital weakness of the vein wall (Lofgren, 1979). Hormonal factors can also increase vein distensibility during pregnancy and in those taking contraceptive pills, producing varicose veins (Goodrich and Wood, 1964). Others suggested a vein wall metabolic cause such as a decline in energy metabolism, which is associated with varicosis (Wood, 1968). The weak wall theory with secondary valvular incompetence has been, therefore, proposed as the most likely cause of primary varicose veins (Clarke et al., 1992).

Recent histological studies performed on varicose vein walls have lead to several hypotheses. Contradictory evidence exists on the connective tissue concentration of varicose veins. Several workers have found collagen decreases in varicose veins (Svejcar et al., 1963; Andreotti et al., 1978; Psafia and Melhuish, 1989). Andreotti et al. (1978) also found a significant decrease in elastin with an increase in total sugars and soluble non-scleroproteins. They proposed that a primary defect in the amount of collagen and elastin supporting an endothelium from the outside would lower resistance to venous pressure and presumably lead to dilatation. In contrast, Rose and Ahmad (1986) have shown that varicose vein walls have a higher than normal collagen content whereas Travers et al. (1992) found no difference in the ratio of collagen to total protein between control and varicose veins. Contradictory evidence also exists on the pathology of smooth muscle cells (SMCs) in varicose veins. Several studies have reported an increase in SMCs, or its activity (Prerovsky, 1981; Browse et al., 1988; Obitsu, 1990), whereas others reported reduced amount of SMCs due to replacement by connective tissue (Rose and Ahmed, 1986; Browse et al., 1988). In their earlier report, Khan et al. (2000) concluded that the endothelial lining was intact in varicose sections and that it was not significantly different from that seen in control veins.

In an attempt to contribute to the solution of this controversy, we studied the endothelial layer of the vein wall in patients who underwent surgery for varicose veins. We examined sections of the avulsed distal calf varicosities under the transmission electron microscope (TEM) with reference to the proximal thigh long saphenous vein in varicose veins patients and normal controls.

Material and Methods

A total of 23 vein specimens of the long saphenous vein (LSV) and the distal calf varicosities were collected from 13 patients at Asir Central Hospital (ACH), Abha, Saudi Arabia, during the period from February to September 1997. Three young trauma patients underwent repair of their arterial injures and 10 patients underwent surgery for their primary varicose veins. The patients were 7 males and 6 females with a mean age of 35 years ± SD 11.55 (13–51 years). The trauma patients had no clinical evidence of varicose veins. The varicose veins patients
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underwent both physical and continuous wave Doppler (CWD) examination to assess the extent of their varicosities and the presence of sapheno-femoral junction (SFJ), sapheno-popliteal junction (SPJ) and/or LSV incompetence. On Doppler examination, all varicose veins patients had sapheno-femoral junction (SFJ) incompetence, five had saphenopopliteal junction (SPJ) incompetence and nine patients had LSV incompetence. Based on these findings, varicose veins patients underwent SPJ and/or SFJ ligation, LSV stripping as appropriate, and multiple stab avulsions of the distal calf varicosities.

In the three trauma patients, specimens were collected from the normal proximal thigh LSV after it was harvested for use as an arterial graft, and were used as normal controls. In all the varicose veins patients, specimens were taken from both the proximal, unstripped long saphenous vein, distal to the tied SFJ and before insertion of the vein stripper, and also from the avulsed distal calf varicosities.

Specimens for light microscopy examination were collected in small test tubes containing 10% formalin and were sent for preparation of paraffin sections in the pathology laboratory. Paraffin sections were stained with hematoxylin and eosin (H&E). Specimens for electron microscopy examination were immediately put in small, labeled test tubes containing 2.5% glutaraldehyde solution and sent to the Electron Microscopy (EM) laboratory. Each specimen was then cut into 2–3 mm cubes and immediately fixed in 2.5% glutaraldehyde solution in 0.1 M sodium cacodylate buffer, pH 7.2, and kept at 4°C for two hours. Samples were later post-fixed in 1% osmium tetroxide in sodium cacodylate buffer, dehydrated in an ascending series of ethyl alcohol and embedded in Spurr’s resin. Semi-thin sections were stained with toluidine blue and examined under the light microscope. Ultra-thin sections stained with uranyl acetate and lead citrate were examined at 80 KV under the transmission electron microscope “TEM” (Jeol® 1200 EX, Japan).

Results

Compared with sections of the normal long saphenous vein (Fig. 1A), varicose vein sections stained with hematoxylin and eosin and examined under the light microscope, showed increase in the diameter of the lumen, generalized thickness of the wall and hypertrophy of the subendothelial tissues, which made the wall to protrude into the lumen. There was elongation of the intima, which was thrown into extensive folds and invaginations. There was discontinuity of the endothelial sheet and desquamation in many places (Fig. 1B).

Under the transmission electron microscope (TEM), the normal endothelium looked intact with regular arrangement of the endothelial cells, which laid flat on the sub-endothelial tissues (Fig. 2A). In varicose vein sections, there was wrinkling and invagination of the endothelial layer, which was more extensive in sections of the distal calf varicosities. Along the invaginations, crowded endothelial cells became vertically oriented, hypertrophied and elongated. These cells contained microfilaments in their cytoplasm, which may be related to their proposed contractility. Underneath these cells, ghost bodies, clear vacuoles and lipid droplets were seen (Fig. 2B). With further invagination, endothelial cells became compressed, thinned-out and elongated and some of them were lost into the lumen (Fig. 2C). Some of these
invaginations closed completely, entrapping blood components like erythrocytes (Fig. 2D). Along the invaginations, the thinned-out endothelial cells accumulated lipid droplets and started to peel off the intima (Fig. 3, A & B). Finally, they separated completely and fell into the lumen (Fig. 3C). The bare intima showed disorganized underlying internal elastic lamina, distorted and degenerated smooth muscle cells (SMCs) in addition to loss of the normal collagen/elastic lattice (Fig. 3D).

In the process of cellular degeneration, the endothelial cells swelled up and showed large vacuoles replacing most of the cytoplasm, which became compressed as a thin rim along the periphery of the cell (Fig. 4A). Some of these cells hypertrophied and contained damaged mitochondria and other cellular organelles (Fig. 4B). Other degenerating cells contained increased number of electron-dense granules (Fig. 4C). Especially in sections of the distal calf varicosities, break down of the intimal surface and loss of the endothelial cells exposed the basal lamina and the subintimal tissues to the lumen (Fig. 4D). This invited blood components, like platelets, erythrocytes and lipid droplets, to stick to the bare intima (Fig. 5, A & B). These were seen to migrate through the wall (Fig. 5C). Even with focal damage to the endothelial layer, lipid droplets migrated through and lay within the wall (Fig. 5D).

Although all the changes described were seen in all the sections of the distal calf blowouts, they were also observed to a lesser extent in sections of the proximal, clinically non-dilated long saphenous vein.

Fig. 1. Light micrographs of the intima in normal and varicose long saphenous vein. A. Normal intima of control long saphenous vein. Note the regular sheet-like intimal layer, and its normal thickness. Hematoxylin and eosin stain (× 1,030). B. Marked hypertrophy of the intima, which is thrown into irregular folds and clefts. Note the fraying of the endothelial layer off the intimal surface. Hematoxylin and eosin stain (× 1,030).
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Fig. 2. Electron micrographs of the intima in normal and varicose long saphenous vein. A. A normal long saphenous vein showing an endothelial cell (E) resting on the endothelial surface. Note the well-formed internal elastic lamina (e) between the tunica intima (I) and the tunica media (M) (× 6,200). B. Vertical orientation of a group of swollen endothelial cells (E). A swollen endothelial cell (asterisk), which split from the intima swim in a protein-like material (star) within the lumen (L). Ghost bodies (Gb) and clear vacuoles (V) are also seen underneath the endothelial cells (× 6,783). C. Wrinkling and invagination of the endothelial layer (E) in a distal calf varicosity showing atrophied (arrows) and pyknotic (double arrows) endothelial cells. L: lumen (× 3,455). D. An invaginated endothelial layer (E) enclosing an erythrocyte (R). L: Lumen (× 4,500).
Fig. 3. Cytological changes in the endothelium. A. In the proximal part of the long saphenous vein, the endothelial layer (arrow) with a pyknotic cell (E) starts to desquamate into the lumen (L) ($\times$ 7,059). B. A section of a distal calf varicosity showing a degenerated endothelial cell (E) with pyknotic nucleus (N) squeezed in the invaginated part of the endothelium (star). The cell is peeled off and rolled over into the invagination. Part of the endothelial layer is completely damaged (arrow). L: lumen ($\times$ 7,333). C. Complete disorganization of the intima (arrow) with loss of the degenerated endothelial cells (E) into the lumen (L) ($\times$ 4,230). D. Only the basal lamina (arrow) is left in the intima without any remaining endothelial cells. A large smooth muscle cell (SMC) is encroaching on the sub endothelial layer and is pressing on the intimal surface ($\times$ 9,429).
Fig. 4. Separation of the endothelial cells. A. Swollen endothelial cells (E) of the proximal long saphenous vein showing large vacuoles (V) and pyknotic nuclei (N). The cells prolong towards the lumen (L) (× 2,444). B. Balloon-shaped endothelial cell (E) in a distal calf varicosity, which prolong towards the lumen (L). The cell contains damaged mitochondria (m) and few vesicles (V). Fragmented elastic (arrows) and collagen (stars) fibers are also seen in sub endothelial layer (× 6,759). C. Damaged endothelial layer (arrow) of proximal long saphenous vein showing an endothelial cell (E), which contains increased number of electron-dense granules. Fragments of elastic fibers (double arrows) are lying on the bare intimal surface (× 13,333). D. An aggregation of blood platelets (P) is seen underneath a separated endothelial cell (E). Note the presence of erythrocytes (R) and elastic fibers (star) within the lumen (L) and in the subendothelial layer (× 4,357).
Fig. 5. Effect of loss of the endothelial cell barrier. A. Aggregated erythrocytes (R) are lying on the exposed subintimal layer (white arrow). Fragments of elastic fibers (black arrow) are approaching the luminal surface (× 2,500). B. Large number of erythrocytes (R) is being squeezed between collagen fibers (C) in the vein wall (× 4,375). C. Lipid droplets (Li) are invading the vein wall through a damaged part of the endothelium (E) (× 7,429). D. Increased number (and size) of lipid droplets (Li) is invading through the wall (× 4,792).
Discussion

The results of the present study show that there is increase in the diameter of the lumen, generalized thickness of the wall, hypertrophy of the intimal layer and elongation and invagination of the intimal surface. There was also degeneration and desquamation of the endothelial cells. Loss of the endothelial cell barrier exposed the subintimal tissues to the lumen with loss of the different wall components into the lumen and intramural migration of the blood components. These changes affected both the proximal long saphenous vein and the distal calf varicosities.

As proved by Sumner (1981), the lumen area of a vessel depends not only on the transmural pressure and tension generated in the vessel wall, but also on any increase in the wall area due to hypertrophy/hyperplasia of the constituent elements such as muscle, to counteract the distending force. And as previously noted by Travers et al. (1996), the increased lumen area found here suggests that this equilibrium has been disrupted in varicosis. Venturi et al. (1996) found a strong relationship between the reduced elastin content and the macroscopic finding of dilatation of the vein wall. Lengyel and Acsady (1990) also suggested that the relaxation of muscle cells together with their gradually increasing decay, as well as the decreased tensile strength and elasticity of the connective tissue fibers are direct reasons for the elongation and lateral blowouts of the veins.

Apart from Mashiah et al. (1991) who did not observe any intimal changes by scanning electron microscope, our finding of intimal hypertrophy confirms the results of previous investigators (Jurukova and Milenkov, 1982; Obitsu, 1990; Travers et al., 1996; Porto et al., 1998; Khan et al., 2000). The exact mechanism of intimal thickening in varicose veins is a matter of controversy and various explanations have been put forward by different investigators. According to Cheanvechai et al. (1975), uniform intimal fibroplasias, which is a loose proliferation of collagenous tissue confined strictly to the tunica intima, is the normal hemodynamic response of a vein to arterial pressure, as seen in patients of arteriovenous fistula. Brody et al. (1972) have reported this finding in animal experiments and believed that pressure and ischemia are responsible for intimal changes. Vlodaver and Edwards (1971) have also characterized intimal fibroplasias as a phenomenon responding to hemodynamic stress. It seems quite possible that the effect, which triggers the development of varicose veins disease, could be anything, which causes hypoxia (Lengyel and Acsady, 1990). Coleridge-Smith (1997) suggested an abnormality of oxygen delivery to the tissues as the cause of wall changes in varicose veins. Janssens et al. (1999) and Arnould et al. (1992; 1998) also recently suggested that the intimal changes are due to hypoxia of the endothelial cells leading to changes in the venous wall.

Although in their earlier report, Khan et al. (2000) could not find significant changes in the endothelial lining of varicose veins, in our present study we clearly demonstrated severe degeneration of the endothelial cells which peel off the intimal surface and desquamate into the lumen, with subsequent loss of the endothelial barrier and exposure of the subintima. According to Toda et al. (1987) and Munro et al. (1988), the endothelium is thought to cause the release of platelet-derived growth factor (PDGF) which results in the migration of smooth
muscle cells (SMCs) from the media to the intima in rats’ aortas. It is likely that similar cells in veins can do the same (Milroy et al., 1989). Furthermore, it is assumed that when SMCs come into close contact with blood-borne monocytes, their capacity for collagen phagocytosis, decomposition and disintegration is increased (Jurukova and Milenkov, 1982). Other investigators have suggested that after disruption of the endothelium, the SMCs are directly exposed to blood flow and their function might be modulated by changes in the local hemodynamic environment (Kohler et al., 1991; Yamamoto et al., 1954). As previously observed by Milroy et al. (1989), the frequent association of pathological changes in the muscle layer and in the intima suggests that both have a common cause. As we also demonstrated in the present study, loss of the endothelial barrier results in loss of wall components like elastin and collagen fibers into the lumen, with further distortion of the vein wall. It is possible that varicosis starts as an intimal disease and then progresses through the wall.

Although Cheauvechai et al. (1975) could not find any light microscopic evidence of intimal lipid, blood pigment or organizing thrombus, we have shown in this study that with focal damage to the endothelial layer or its complete loss, blood components like red blood cells (RBCs) and lipid droplets find their way through the wall. This might explain the process of clinical superficial thrombophlebitis, which sometimes complicates cases of varicose veins.

In conclusion, our study suggests that there are severe ultra structural changes, which affect the endothelial layer of varicose veins, probably initiated by hemodynamic stress and/or hypoxia. These changes affect not only the distal calf blowouts but also the proximal long saphenous vein. As proposed by Venturi et al. (1996), the rational surgical approach would therefore be to remove the whole pathological vein, i.e. stripping of the long saphenous vein, rather than removing the varices only as suggested by Mashiah et al. (1991).

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References


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