Role of saphenous vein wall in the pathogenesis of primary varicose veins

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Abstract

Varicose veins may be due to weakness of the vein wall as a result of structural problems. There are conflicting findings in the literature about these problems especially concerning collagen, elastin and smooth muscle cells content. The aim of this study was to look at the structural abnormalities of varicose veins (with and without valvular incompetence). Materials and methods: We studied 70 specimens of long saphenous veins from 35 patients (24 with varicose and 11 with normal veins). Two specimens were taken from each vein approximately 3–4 cm from the saphenofemoral junction. Vein specimens were processed for histological and electron microscopic studies. Both qualitative and quantitative analyses were performed to assess the degree of wall changes. Using the image analyzer, contents of collagen, elastin and smooth muscle cells, in addition to intimal and medial thickness, were measured. Results: Light microscopy revealed significant increase in intimal and medial thickness and collagen content of media and significant decrease in elastin content in varicose veins compared with normal veins. There was no statistical significant difference between varicose veins with and without saphenofemoral valve incompetence. Electron microscopy showed marked degenerative changes in intima and media of varicose veins. Conclusion: The findings in our study supported the theory of primary weakness of the vein wall as a cause of varicosity. This weakness is due to intimal changes, disturbance in the connective tissue components and smooth muscle cells.

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Keywords: Varicose veins; Collagen; Elastin; Smooth muscle cells

1. Introduction

Primary varicose vein disease is a widely prevalent condition. It affects about 10–40% of 30–70-year-old people [1]. Varicose veins were regarded as a secondary manifestation of valvular incompetence and exposure of the vein wall to pressures that it cannot withstand, i.e. the hydrostatic head of pressure from the right auricle in the upright position [2]. Recently, it has been reported that varicose veins can develop without valvular incompetence [3,4]. The theory of primary venous dilatation leading to secondary valvular incompetence has received more attention nowadays [5]. This venous dilatation may be due to weakness of the vein wall as a result of structural problems [6–11]. However, there are conflicting findings in the literature about these problems. Some authors demonstrated a reduction in collagen and elastin content in primary varicose veins compared to normal veins [8]. In contrast, some have found an increase in the collagen content without change [10] or reduction [7,9] in elastin content in segments of varicose veins compared to normal [8]. On the other hand, some have demonstrated reduction in elastin content with normal collagen content of varicose veins compared to normal [11]. There were also conflicting reports about the smooth muscle cells (SMCs) of the tunica media. While some reported muscle hypertrophy [12], others have shown muscle loss with replacement by fibrous tissue [10,13,14].

No available studies compared the structural problems of varicose veins in the case of the presence of valvular incompetence and in the case of the presence of normal valves. The aim of this work was to study the structural wall abnormalities of varicose veins (with and without valvular incompetence) to clarify the cause of varicosity.

2. Patients and methods

Seventy specimens of long saphenous veins (LSV) were obtained from 35 patients admitted to the Vascular Unit, King Fahd Hospital of the University, Saudi Arabia and Suez Canal University Hospital, Egypt. The study was agreed by the local Ethical Committee and informed consent was taken from each patient. All patients had proximal thigh LSV excised. The LSV specimens were divided into two groups: normal vein group and varicose vein group. Two specimens were taken from each vein, approximately...
3–4 cm from the saphenofemoral junction. The specimens were obtained from:

2.1. Normal vein group (11 patients)

These were eight males and three females with mean age (years) 30 ± 6.5. None of the patients had clinical evidence of chronic venous insufficiency in both lower limbs. The vein was required for repair of lower limb arterial injury in four patients, axillary artery injury in three patients, repair of artery in traumatic arteriovenous fistula in two patients and reversed vein femoro-popliteal above knee bypass in two patients with superficial femoral artery disease.

2.2. Varicose vein group (24 patients)

Those included 16 females and eight males with mean age (years) 31.6 ± 4.3. They had full history, preoperative physical examination and whole leg duplex mapping. All patients had a history of varicose veins for less than one year. This minimized the changes in the vein wall secondary to venous dilatation. All patients had primary class 2 (uncomplicated) chronic venous insufficiency affecting the LSV. The saphenofemoral valve (SFV) was competent in five and incompetent in 19 patients. All patients underwent high ligation of the saphenofemoral junction, excision of the specimens, stripping down to knee and multiple stab avulsions of distal calf varicosities.

All vein specimens were processed and prepared for microscopic examination.

2.3. Light microscope

Specimens for light microscopy were fixed in 10% neutral buffered formalin solution. They were then processed to prepare 5 μm thick paraffin sections. Sections were stained with Hematoxylin & Eosin (H&E), Masson’s trichrome and Verhoeff-Van Gieson.

Quantitative measurements were carried out using the image analyzer (Super eye-Heidi soft) to measure: (1) Intimal thickness. (2) Medial thickness; both intimal and medial thicknesses were measured in H&E stained sections. (3) Color area percentage of collagen fibers (blue colored) in intima. (4) Color area percentage of collagen fibers in media, (5) Color area percentage of smooth muscle (red colored). Both collagen and smooth muscles were measured in Masson’s trichrome stained sections. (6) Color area percentage of elastin (black colored) at the junction between intima and media (internal elastic lamina) was measured in Verhoeff’s stained sections. The image analyzer was calibrated for color and distance measurements before use.

3. Statistical analysis

Results have been summarized using descriptive statistics. These are presented as mean ± S.D. and compared using Student’s t-test. Significance was set at P < 0.05 for all comparisons. All statistical analyses were performed with the aid of SPSS-9 (Chicago, IL, USA) statistical analysis software.

3.1. Electron microscope

Specimens for electron microscopy were immediately immersed in 2.5% glutaraldehyde solution. Each specimen was trimmed, immediately fixed in glutaraldehyde solution in 0.1 M sodium cacodylate buffer, pH 7.2, and kept at 4 °C for 2 h. They were post-fixed in 1% osmium tetroxide in sodium cacodylate buffer then dehydrated in an ascending series of ethyl alcohol and embedded in Spurr’s resin. Semi-thin sections (1 μm) stained with toluidine blue were obtained for observation. Ultra-thin sections stained with uranyl acetate and lead citrate were examined at 80 kV under the transmission electron microscope ‘TEM’ (Jeol 100 CXII, Japan).

4. Results

4.1. Light microscopy

4.1.1. H&E stained sections

Normal vein group revealed that vein wall was composed of three tunicae. The first tunica from the luminal side was the intima which appeared very thin, consisting of endothelium and subendothelial connective tissue (Fig. 1a). The media was formed of longitudinally oriented smooth muscle cells next to the intima, in addition to a circular layer of smooth muscle cells. The muscles were separated by collagen fibers. The third tunica; the adventitia, consisted of bundles of collagen fibers, fibroblasts and capillaries. Clusters of longitudinally oriented smooth muscle fibers were also observed. In the varicose vein group, sections showed a high variability in the organization of the vessel wall. All sections showed dilatation of the lumen and most of them showed thickened wall. The mean thickness of intima was significantly increased compared to normal veins (Table 1). In many cases the intima appeared folded with an irregular surface. Discontinuity of the endothelium was also noticed.
Table 1

<table>
<thead>
<tr>
<th>Stain</th>
<th>Morphometric parameter</th>
<th>Vein wall</th>
<th>Normal vein</th>
<th>Varicose vein</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H&amp;E</td>
<td>Thickness (μm)±S.D.</td>
<td>Intima</td>
<td>60.07±1.12</td>
<td>100.25±2.14</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Thickness (μm)±S.D.</td>
<td>Media</td>
<td>360.54±4.56</td>
<td>410.61±3.82</td>
<td>0.025</td>
</tr>
<tr>
<td>Masson’s Trichrome</td>
<td>Mean color area percentage of collagen±S.D.</td>
<td>Intima</td>
<td>0.17±0.02</td>
<td>0.19±0.02</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Mean color area percentage of smooth muscle±S.D.</td>
<td>Media</td>
<td>0.19±0.04</td>
<td>0.26±0.06</td>
<td>0.003</td>
</tr>
<tr>
<td>Verhoeff</td>
<td>Mean color area percentage of elastic lamina±S.D.</td>
<td>Internal elastic lamina</td>
<td>0.02±0.009</td>
<td>0.01±0.007</td>
<td>0.04</td>
</tr>
</tbody>
</table>

in many places (Fig. 1b). The mean thickness of media was significantly increased compared to normal veins (Table 1).

4.1.2. Masson’s trichrome stained sections

Normal vein group revealed blue colored collagen fibers in the subendothelial layer of the intima, between SMCs of the media and in the adventitia (Fig. 2a). In the varicose vein group, sections revealed accumulation of collagen fibers in both intima and media (Fig. 2b). There was insignificant increase of the mean color area percentage of collagen in intima compared to normal vein while that in media was significantly increased compared to normal vein (Table 1). There was also insignificant increase of the mean color area percentage of muscles in media compared to normal veins (Table 1).

4.1.3. Verhoeff’s stained sections

Normal vein group showed elastic fibers between intima and media forming poorly developed internal elastic lamina. Elastic fibers were also seen between SMCs of the media (Fig. 3a). In the varicose vein group, sections revealed the presence of fewer elastic fibers in the area of internal elastic lamina, while there was an apparent increase between the smooth muscles of the media (Fig. 3b). The mean color area percentage of varicose vein elastin at the area of internal elastic lamina was significantly decreased compared to the normal vein group (Table 1).

There was no significant difference in morphometric parameters between varicose veins with and without SFV incompetence (Table 2).

4.2. Electron microscopy

Normal vein group: showed that the endothelial cells appeared flat with flattened nucleus (Fig. 4a). The SMCs of the media appeared fusiform in shape (Fig. 4b). Varicose vein group: the endothelial cells were abnormally shaped with irregular borders. They also contained many vacuoles in their cytoplasm and their nuclei showed chromatin margination (Fig. 5a). In some sections, complete disorganization of the intima was observed where the endothelial cells were fragmented into pieces with destruction of subendothelial tissue and desquamation of some cellular fragments into the lumen (Fig. 5b). SMCs of the media were abnormal in shape. They lost their regular borders and showed many plasmalemmal projections (Fig. 5b). Smooth muscles were distorted and markedly elongated giving the shape of fiber-like material in some areas. Abnormal vacuoles which sometimes contained residual material were noticed. Another finding concerning these

Fig. 2. (a) Section in normal LSV showing blue colored collagen fibers in the subendothelial layer of the intima (I), between smooth muscles of the media (M) and in the adventitia (Ad). (b) Section in varicose LSV showing increased blue colored collagen fibers in both intima (I) and media (M). (Masson’s trichrome × 100).

Fig. 3. (a) Section in normal LSV showing black colored elastic fibers forming poorly developed internal elastic lamina between intima and media (arrows). Elastic fibers are also present between smooth muscles of the media and adventitia. (b) Section in varicose LSV showing loss of internal elastic lamina. (Verhoeff × 100).
Table 2
Histological analysis of varicose vein with and without SFV incompetence

<table>
<thead>
<tr>
<th>Stain</th>
<th>Morphometric parameter</th>
<th>Vein wall</th>
<th>Varicose vein with SFV incompetence</th>
<th>Varicose vein without SFV incompetence</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H&amp;E</td>
<td>Thickness (μm)</td>
<td>Intima</td>
<td>102.08 ± 1.33</td>
<td>100.09 ± 2.50</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>420.33 ± 4.52</td>
<td>410.22 ± 4.20</td>
<td>0.81</td>
</tr>
<tr>
<td>Masson’s trichrome</td>
<td>Thickness (μm)</td>
<td>Media</td>
<td>0.19 ± 0.12</td>
<td>0.19 ± 0.22</td>
<td>0.72</td>
</tr>
<tr>
<td>Mean color area</td>
<td></td>
<td>Intima</td>
<td>0.26 ± 0.12</td>
<td>0.25 ± 0.40</td>
<td>0.66</td>
</tr>
<tr>
<td>percentage of collagen</td>
<td></td>
<td>Media</td>
<td>0.38 ± 0.11</td>
<td>0.39 ± 0.10</td>
<td>0.61</td>
</tr>
<tr>
<td>Verhoeff</td>
<td></td>
<td>Internal</td>
<td>0.01 ± 0.009</td>
<td>0.01 ± 0.007</td>
<td>0.84</td>
</tr>
<tr>
<td>Mean color area</td>
<td></td>
<td>Elastic lamina</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>percentage of elastin</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

muscle cells was that some of them were seen extending pseudopodia-like projections around other damaged SMCs (Fig. 6a). With further destruction of the SMCs, some of them were disintegrated into small fragments which were scattered in the extracellular matrix (Fig. 6b). In other areas, wide separation of SMCs by increased amounts of extracellular matrix and collagen fibers was noticed. Destruction of extracellular matrix was also evident in some sections.

5. Discussion

Vein wall distensibility is controlled by SMCs, collagen and elastin. Smooth muscles in the tunica media are responsible for wall tone, which is influenced by autonomic nerves and circulating stimulants. Passive tone is provided by collagen and elastin. Loss of tone in varicose veins could be due to defects in these wall components [10]. The present work confirmed the findings of previous studies [6–11] that there were significant changes in collagen, elastin and smooth muscle contents of the wall of varicose veins compared to the normal, even without saphenofemoral valve incompetence.

The present study agreed with most of the previous ones [10,16] that there was an increase in intimal thickness in varicose veins compared to normal. This increased thickness could be due to increase in collagen content of intima areas.

Fig. 4. (a) Electron micrograph of a section of normal LSV showing an intimal endothelial cell (E) which lays flat on the subendothelial connective tissue; L is the lumen. (×14000). (b) Electron micrograph of a section of normal LSV showing 2 adjacent SMCs of the media. They appear fusiform in shape. (×5000).

Fig. 5. (a) Electron micrograph of a section of varicose LSV showing abnormally shaped endothelial cells with irregular boundaries. The nucleus of one cell shows chromatin margination (arrow). The cells also contain many vacuoles (V). Note the abnormally shaped SMCs of the underlying media (×4000). (b) Electron micrograph of a section of varicose LSV showing fragmentation of an endothelial cell (arrows) with loss of some fragments into the lumen (curved arrow). Note that the subendothelial connective tissue (C) is also destroyed. (×6700).

Fig. 6. (a) Electron micrograph of a section of varicose LSV showing one of SMCs extending pseudopodia (arrows) around another muscle cell as if it is phagocytosing it. Note that the SMCs are abnormal in shape and contain vacuoles (V) (×8000). (b) Electron micrograph of a section of varicose LSV showing damage of SMC of the media which appears fragmented into pieces (arrows) that are scattered in the disintegrated extra-cellular matrix. Note that parts of the damaged cell give the shape of fiber like material. (×5000).
and migration of SMCs from media to intima [16]. Other intimal changes reported in the present and other studies [16] included folding, invagination, fragmentation and peeling of intimal endothelium with disintegration of subendothelial connective tissue and internal elastic lamina. The same authors referred intimal folding to the increase in the lumen diameter with increased surface area. Venous stasis was suggested to result in intimal hypoxia and consequently causes intimal changes [17]. Invasion of intima by small-sized newly formed SMCs as well as increased collagen content could be attributed to the degenerative changes caused by this venous stasis which stimulates these compensatory mechanisms.

Many studies [6,7,9,10,18,19] including the present one have shown that LSV contained a significantly higher amount of collagen in varicose veins compared to normal. This increase was more evident in tunica media causing separation of SMCs. The contradictions reported in the literature about the amount of collagen; whether it may be decreased [8] or not changed [11] could be attributed to the hypothesis that collagenosis is not the only factor for the development of varicose vein disease. Another explanation for these conflicting findings may be due to difference in collagen concentration in the vein wall but the absolute amount is increased in all [10].

In the current study, there was insignificant increase in muscle thickness in media of varicose veins when compared to normal. Conflicting findings were met in the literature: increase [12], no change [20,21] or decrease [10,13,14] in the density of SMCs in varicose veins. However, the overall amount of SMCs in varicose veins was reported to be high [10]. Therefore, the pathological abnormalities in varicose veins were not due to deficiency of smooth muscles, but could be referred to the inability of muscle cells to provide the necessary tone in the vessel wall leading to vein wall dilatation [6]. This muscle dysfunction [22,23] may be due to the break up of its regular arrangement by fibrous tissue [10,18] as effective contraction cannot occur when individual cells are not in direct communication with each other [6]. This has been shown also in the present study. Moreover, some studies [11,12], including ours, have shown ultra-structural changes in SMCs of varicose veins compared to normal veins. These changes included abnormality in shape, conversion into fiber-like material, degeneration, vacuolization and break up of SMCs. Vacuoles have been described to contain plasminogen activator receptors, which are involved in the regulation of pericellular proteolysis [24]. This proteolysis could affect the wall tone as it disconnects the contractile-elastin units between the smooth muscle and elastin fibers, resulting in decreased elastin near the border of the SMCs, as well as a disorganized elastin pattern which appeared even clumped in the adventitial layer. Another important finding, in this study, as well as others [12], was that the abnormally shaped SMCs (which contain vacuoles most probably lysosomal) would have the ability to form pseudopodia and phagocytose other SMCs. Moreover, SMCs were seen to convert into fiber-like material, in this study and a previous one [12], which could be sequelae of its degeneration. Because of these muscle abnormalities, some authors [25] considered that the primary defect might be in the SMCs of the vein wall and this defect could be genetically determined [10].

In addition to the above-mentioned changes concerning the wall components, the present study and others [7,18,25] have demonstrated that elastic fibers were significantly decreased in internal elastic lamina of varicose veins when compared to normal. Moreover, there was loss of normal elastin/collagen lattice network [18]. These morphological alterations of elastin may explain the functional finding of reduced strength [7] and elasticity of varicose veins [22].

The present study has shown that there was no significant difference in the vein wall structural changes between varicose veins with and without valve incompetence. This supports the theory of primary weakness in the vein wall leading to dilatation of the vein with resultant separation of valve cusps [5].

In conclusion, studying the histopathological changes of varicose veins in comparison to normal ones, revealed that varicose veins showed intimal changes, disturbance in connective tissue components and smooth muscles. These findings supported the theory of primary weakness of the vein wall as a cause of varicosity.

References


ICVTS on-line discussion A

Title: Valves and primary varicose veins

Author: Narcis Hudorovic, University Hospital Sestre Milosrdnice, Zgobr 10000, Croatia
doi:10.1510/icvts.2006.136937A
eComment: The authors stated that there are no available studies which compare the structural problems of varicose veins in the case of the presence of valvular incompetence and that they study findings of the histopathologic changes which support the theory of primary weakness of the vein wall as a cause of varicosity [1]. Studies on the pathophysiology of chronic venous insufficiency [CVI] should acknowledge that the valvular ‘chain’ is not limited to large veins, but extends down to the venular level where microscopic venous valves (MVVs) play an important role in venous hemodynamics. Role of MVVs was demonstrated in limbs afflicted with CVI by Photoplethysmography [2]. These findings evaluated the venous refilling time (VRT) in limbs with severe CVI [CEAP: C4–C6] and attributed to the increase in VRT exclusively to the transfer of MVV. The improvement of the local venous haemodynamics was also confirmed by the clinical results with no recurrent ulceration and no recurrent tissue lipodermatosclerosis up to 9 years. This data suggests that MVVs play a role in countering venous hypertension caused by valvular failure of larger veins. Therefore, MVV incompetence could explain the occurrence of skin changes associated with CVI in limbs with competence of proximal valves and a short VRT and in those without any relevant venous disease. Clinicians consider the venous bed as ‘valvless’ from the venular level up to 2 mm large veins.

The above mentioned statements demonstrate that MVVs are present in vascular territories with unfavourable venous haemodynamics to play a functional role which must be still comprehensively evaluated. The available evidence suggests that MVV incompetence could explain clinical syndromes characterized by signs and symptoms of CVI in limbs with competent venous valves in large veins. Investigation of the pathophysiology of CVI must take into account the possible haemodynamic role of a valvular ‘chain’ extending down to the venular level. In the future, the functional role of MVV in CVI will be investigated by techniques such as capillaroscopy, high frequency ultrasound probes, Laser Doppler and micro fibre angioscopy. The huge body of knowledge available concerning MVVs urges us to study further the combined histopathological changes of varicose vein walls and valves.

References
