

MORPHOLOGICAL STUDY

Comparison of collagen subtype I and III presence in varicose and non-varicose vein walls

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Abstract: *Study objectives:* The connective tissue alterations in varicose vein wall are supposed to be one of the main causes of primary varicose vein (main sign of human lower limbs chronic venous insufficiency).

Methods: 5 varicose vein samples from 5 patients undergoing stripping surgery of long saphenous vein were compared with 5 control samples of healthy (non-dilated) long saphenous veins from necroptic material (with no history of varicosis). They were fixed in a Baker solution, processed by use of light microscopic method, cut to ultra-thin sections (4–5 µm) and stained with PicroSirius Red for collagen. Sections were scanned with light microscope (Leica, Germany) and camera Canon S50 (Germany) and analysed by morphometric programme Image J v.1.38g (National Institute of Health, USA).

Results: In the group of healthy (non-dilated) veins the mean collagen I/III ratio value was 31.40 and in the group of varicose veins the mean collagen I/III ratio was 12.35; the difference is statistically significant: healthy veins contain significantly more of collagen subtype I and varicose veins contain significantly more of collagen subtype III in their walls.

Conclusion: The statistically significant difference in the collagen I/III ratio between the groups of healthy (non-dilated) and varicose (dilated) vein walls is worthy of further following (Tab. 2, Fig. 7, Ref. 12). Full Text (Free, PDF) www.bmj.sk.

Key words: varicose veins, collagen subtype I and III, collagen subtype ratio I/III.

Chronic venous disease is a relatively frequent vascular disease of the lower limbs affecting mainly the persons of productive age. After statistical data from literature the incidence of this disease in the developed countries of Europe and USA is about 40–60 % in females and 15–30 % in males (1). Beside the postthrombotic syndrome and crural ulcer, varicose veins represent only one of the symptoms of the chronic venous disease. It is characterized by abnormal dilatation, tortuous course and elongation of veins (2). According to its etiology we distinguish its primary form (cause unknown) and secondary form (occurring usually as a consequence of the surviving deep lower limb phlebothrombosis). As the exact cause of the primary form has not been yet revealed, the therapy of this form still resides mainly in reducing the symptoms. The cause of the primary form of varicosis remains to be the subject of interest of several investigators in the world. There is etiopathogenetic association between a) weakness of venous wall associated with alterations in the connective tissue and smooth muscle cells (3, 4, 5), b) altered

Tab. 1. Fibrillar collagens and their distribution in various tissues.

Type	Chains	Distribution in tissues
I	alfa1(I), alfa2(I)	Skin, bone, tendon, dentin
III	alfa1(III)	skin, vessels
V	alfa1(V), alfa2(V), alfa3(V)	Hamster lung cells culture, fetal Membranes, skin, bone, placenta, synovial membranes
II	alfa1(II)	hyaline cartilage, vitreous humor
XI	alfa1(XI), alfa2(XI), alfa3(XI)	Hyaline cartilage

function of venous endothelium, c) venous valve damage, d) alterations in microcirculation and venous wall nourishment (6, 7).

Some hypotheses in accord with some investigators suggest the alterations in the connective tissue of venous wall to be responsible for the onset of varicosis, yet the exact cause of venous dilatation has not been established. The alterations in the connective tissue in varicose vein wall – especially its two components: collagen and elastin, have been investigated by several workers and by various methods but they still have not come to a clear conclusion (3, 5, 8, 9, 10, 11, 12).

The extracellular matrix of connective tissues is composed of molecules belonging to four protein groups: collagens, proteoglycans, glycoproteins and elastin. Collagens are structural proteins with one or several triple-helix domains. This struc-

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ture results from the association of three polypeptidic chains presenting characteristic amino acid sequences. Every chain is built up at the level of triple-helix domains by the repetition of triplets (Gly-Xaa-Yaa), where X and Y are very often proline and hydroxyproline respectively. Because of the presence of cyclo amino acids, the chains take a left-handed helix structure of type poly-proline II. The association with two other chains leads to the formation of a right-handed super helix. Till nowadays 19 types of collagens have been described and new types are currently under characterization. All of these collagens have triple-helix domains, but in variable proportions. The structure and the function of the molecule in the extracellular matrix depend thus on the number, the size of these different helicoidal domains but also on the size of globular domains that separate them. Collagens can be divided into two sub-families: fibrillar and non-fibrillar collagens (8). Their compositions and tissue localizations are displayed in Tab I and Tab II (Lethias et al, 1996).

So far it has been documented that collagen in venous wall is present in subtypes I, III, IV, V, VI, XII, and XIV. It is supposed that collagen I provides traction firmness and collagen III is probably responsible for dilatibility and elasticity of the vascular wall (3, 9, 12). The aim of our work was to compare the presence of I and III collagen subtypes in the walls of human varicose and non-varicose (healthy, non-dilated) veins of lower limbs, while in both groups we compared the mutual ratios of these two collagen subtypes (I/III ratio).

Tab. 2. Non fibrillar collagens and their distribution in various tissues

Type	Chains	Distribution in tissues
IV	alfa1(IV),alfa2(IV) alfa3(IV),alfa4(IV),alfa5(IV) alfa6(IV)	basement membranes, glomerular basement membranes
VI	alfa1(VI),alfa2(VI),alfa1(VI)	vessels, skin, intervertebral discs
VII	alfa1(VII)	dermoepidermal junction
VIII	alfa1(VIII),alfa2(VIII)	Descemet's membrane, endothelial cells
IX	alfa1(IX),alfa2(IX),alfa3(IX)	hyaline cartilage, vitreous humor
X	alfa1(X)	growth plate
XII	alfa1(XII)	tendon, skin, periodontal ligaments
XIII	alfa1(XIII)	endothelial cells
XIV	alfa1(XIV)	skin, tendon
XV	alfa1(XV)	fibroblasts, smooth muscle cells
XVI	alfa1(XVI)	placenta, fibroblasts, smooth muscle cells
XVII	alfa1(XVII)	bullous pemphigoid antigen (BP180)
XVIII	alfa1(XVIII)	liver, kidney, placenta
XIX	alfa1(XIX)	?

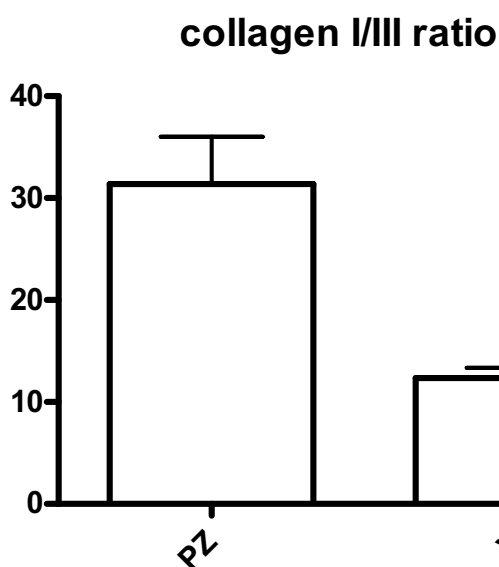


Table Analyzed		Data 1	
Column A	vs	PZ vs V	
Column B			
Unpaired t test			
P value		0.0010	
P value summary		**	
Are means signif. different? (P < 0.05)		Yes	
One- or two-tailed P value?		Two-tailed	
t, df		t=3.351 df=142	
How big is the difference?			
Mean ± SEM of column A		31.40 ± 4.615 N=86	
Mean ± SEM of column B		12.35 ± 0.9983 N=58	
Difference between means		19.05 ± 5.686	
95% confidence interval		7.908 to 30.20	
R squared		0.07328	
F test to compare variances			
F,DFn, Dfd		31.69, 85, 57	
P value		P<0.0001	
P value summary		***	
Are variances significantly different?		Yes	

	PZ	V
Number of values	86	58
25% Percentile	9.012	6.919
Median	10.61	10.96
75% Percentile	48.94	14.21
Mean	31.40	12.35
Std. Deviation	42.80	7.603
Std. Error	4.615	0.9983
Lower 95% CI of mean	22.22	10.35
Upper 95% CI of mean	40.57	14.34
Sum	2700	716.1

Fig. 1. Graph and table of results for the compared collagen I/III ratio of both sample groups.

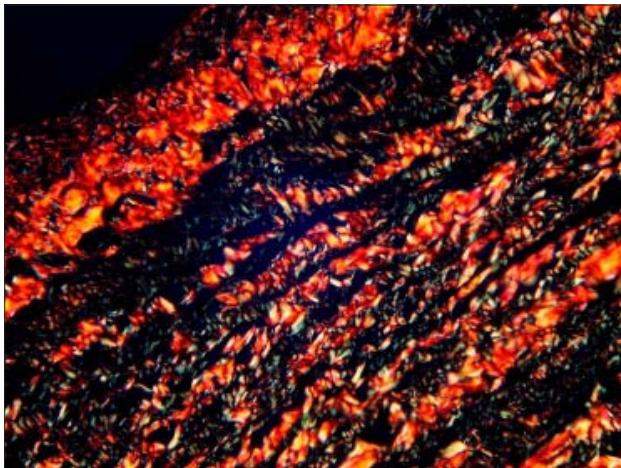


Fig. 2. Healthy (non-dilated) venous subarea, summary digital image.

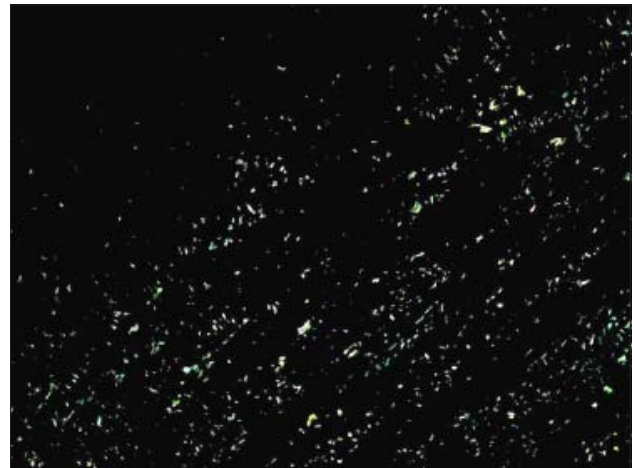


Fig. 4. Healthy (non-dilated) venous subarea, polarized light-digital subtraction, collagen 3 (green).

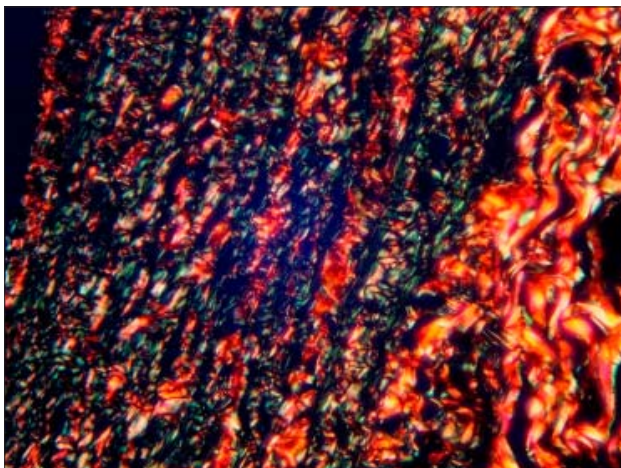


Fig. 3. Varicose (dilated) venous subarea, summary digital image.

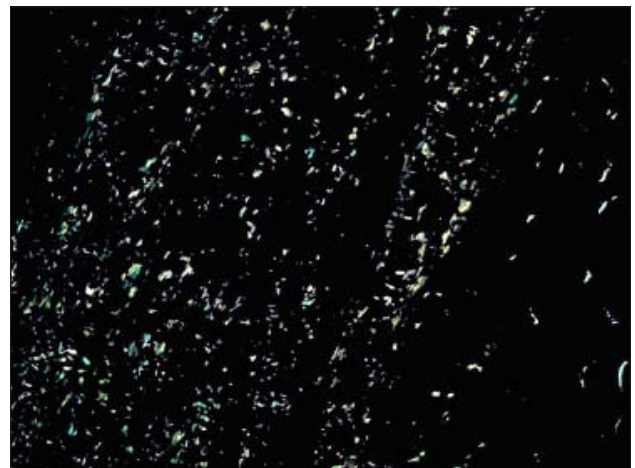


Fig. 5. Varicose (dilated) venous subarea, polarized light-digital subtraction, collagen 3 (green).

Material and methods

The followed group was composed of 5 varicose vein samples taken from the patients undergoing the stripping surgery of the long saphenous vein (5 males, age range 26–50 yrs, average age 40.8 yrs, median 43 yrs). They were compared with the control group composed of 5 samples of healthy (non-dilated) long saphenous vein taken from necroptic material (with no history of varicosis, 1 female, 4 males, age range 20–35 yrs, average age 25.6 yrs, median 24 yrs). The samples were fixed in Baker solution (24–48 hours) and further processed by the light microscopic method into paraffine tissue blocks, then cut to ultra-thin sections (4–5 μm), stained with PicroSirius Red for collagen presence. The sections were scanned in polarized light of the light microscope (Leica, Germany) and camera Canon S50 (Germany). In the polarized light the different collagen subtypes of fibrils show different colour according to their thickness: collagen I red to yellow, collagen III green. Each colour density was morpho-

metrically analysed with the morphometric software Image J v.1.38g (National Institute of Health, USA) and the results were processed with Excel (Microsoft, USA) (Fig. 1).

Results

In the group of healthy (non-dilated) human superficial lower limb veins the mean collagen I/III ratio value was 31.40 and in the group of varicose veins the mean collagen I/III ratio was 12.35, while $p < 0.0001$. The difference is statistically significant: healthy veins contain significantly more of collagen I subtype and varicose veins contain significantly more of collagen III subtype in their walls. Both group results and more statistical details are shown in Figure 1.

Discussion

In the group of healthy (non-dilated) human superficial veins



Fig. 6. Healthy (non-dilated) venous subarea, polarized light-digital subtraction, collagen 1 (red-yellow).

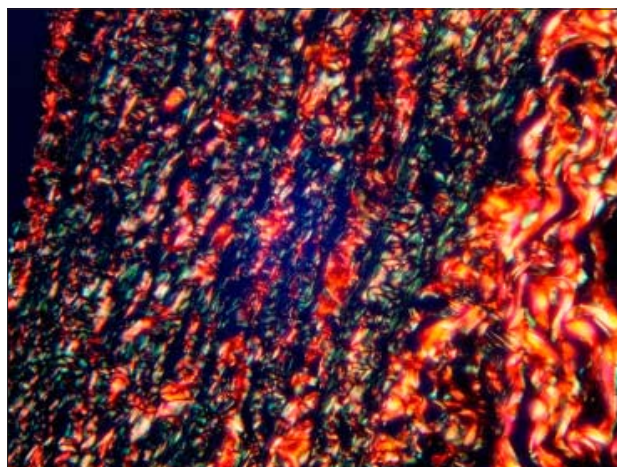


Fig. 7. Varicose (dilated) venous subarea, polarized light-digital subtraction, collagen 1 (red-yellow).

of the lower limbs the collagen I/III ratio was significantly higher than in the group of varicose (dilated) human superficial veins of lower limbs. This means that in the group of non-dilated veins prevailed the presence of collagen 1 and in the group of dilated (varicose) veins prevailed the presence of collagen 3. Distribution of both collagen subtypes was approximately similar. This means that we did not observe any noticeable differences as to the presence of collagen subtypes among individual vascular layers.

Conclusion

The results of our preliminary study helped to reveal the fact that varicose venous walls really show different collagen subtype patterns compared to the healthy venous wall as well as that the collagen subtype pattern (and contents) are worthy of further following. It will be also worthy of repeating this study in more numerous sample groups.

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