

The Determination of the Collagen and Elastin Amount in the Human Varicose Vein by the Computer Morphometric Method

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Abstract. The results of the works dealing with alterations of the connective tissue in varicose vein wall are not ambiguous, so the exact cause of the vein dilatation has still not been established. We were determining the collagen and elastin amounts in human varicose vein wall in comparison with non-dilated long saphenous vein by the light microscopy and computer morphometric method. We have found the lesser amount of collagen in varicose veins than in non-dilated veins, the amounts of the elastin in both the varicose and non-varicose veins were without the statistical significance.

Key words: Collagen — Elastin — Varicosis — Computer morphometry

Introduction

Chronic venous insufficiency of the lower extremities is a world-spread disease especially in its Western part. It affects about 30–40% of the human population (Durdík *et al* 1997). One of its main signs is varicosis – abnormal dilated, tortuous and elongated veins. The cause of the primary form of varicosis is still the subject of interest of several investigators in the world (Yamada *et al* 1996). Some hypothesis suppose and some investigators have noted the alterations of the connective tissue in venous wall to be responsible for the onset of the varicosis, but the exact cause of the vein dilatation has still not been established. The alterations of 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 the clear conclusion (Psaila *et al* 1989, de Carvalho *et al* 1990, Maurel *et al* 1990, Travers *et al* 1996, Venturi *et al* 1996). The aim of this study was to determine the collagen and elastin amounts in varicose vein wall by the computer morphometric method.

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Materials and Methods

6 varicose vein samples were collected from patients undergoing stripping surgery of the long saphenous vein, 6 control samples of the non-dilated long saphenous vein were taken from the pathologic material (with no previous history of varicosis). Samples were immediately fixed in 10% buffered formalin, processed, wax-embedded, sectioned and mounted on the glass microslides. Picroindigocarmin and Weigert (orcein) staining were used for differential staining of collagen and elastin (picroindigocarmin staining collagen deeply green and orcein in Weigert staining elastin black). For measuring 45 sections of each sample were used. Microslides were scanned with (black-white) CCD camera (Sony – Japan) connected with inverted microscope Olympus (Japan) IMT-4 with magnification 25×. Scanned microslides were analyzed with the morphometric programme CUE-2 (Galai – Israel) using PC 486 and magneto-optic recording apparatus Maxoptix TMT3-1300 (USA).

Results

At Picroindigocarmin staining we have observed the lesser occurrence of the collagen in a thin tunica intima of the varicose vein wall with relatively a lot of smooth muscle cells. The collagen was mainly concentrated in the subintimal region of the thick tunica media and the minimum of concentration was found in tunica adventitia. In the non-dilated samples of vein we have observed nearly equal arrangement of the collagen in all 3 layers of the vein wall. A little more was found in tunica adventitia with a less of muscle cells whereas in tunica intima and tunica media the fibers of collagen were intertwined with smooth muscle cells longitudinally and circularly arranged (Fig. 1).

At Weigert staining (orcein) we have observed the elastin occurrence in varicose vein samples in all 3 layers of the vein wall, a little more was found in tunica intima and with the rare accumulations in tunica media. In the non-dilated vein samples we observed the minimum of elastin in tunica media, a little more of it arranged in tunica intima and the maximum of it arranged in tunica adventitia (Fig. 2).

But the exact determination of the collagen and elastin contents for each vessel wall layer was not performed. For more exact distinguishing of the followed subjects – the

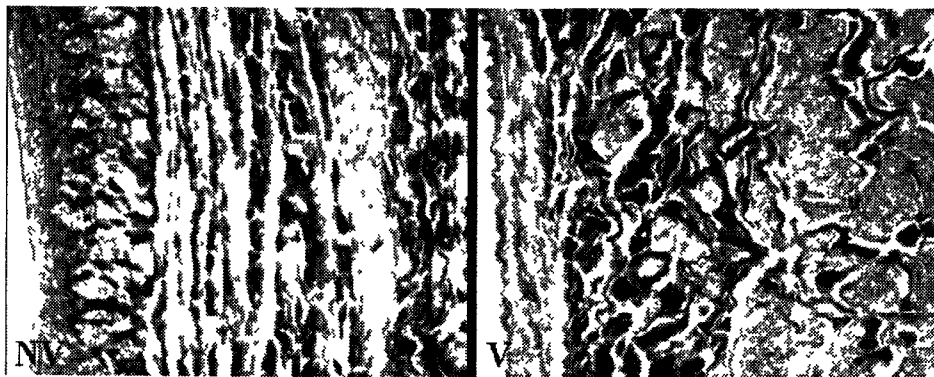


Figure 1. Collagen localisation (black colour) in human non-varicose (NV) and varicose (V) veins

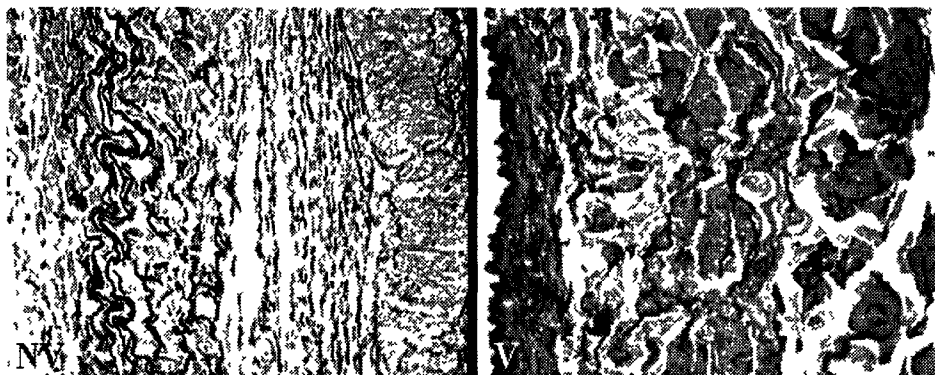


Figure 2. Elastin localisation (black colour) in human non-varicose (NV) and varicose (V) veins

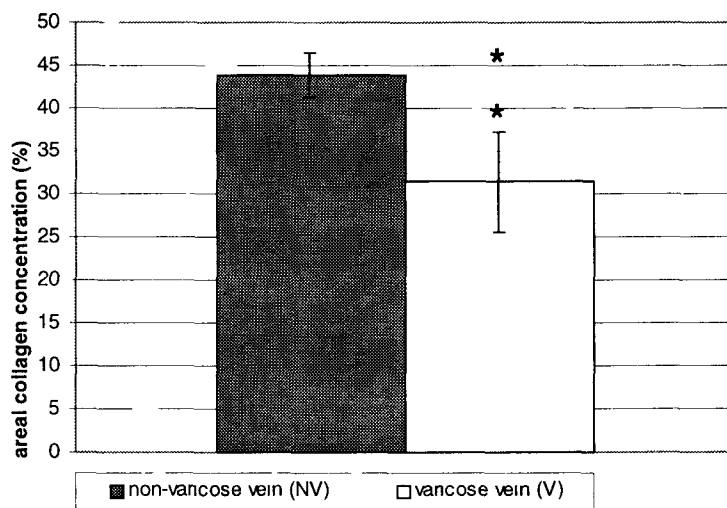


Figure 3. Comparison of the collagen content in varicose and non-varicose veins in the percentage of areal concentration (mm^2) ** $P < 0,01$

collagen and the elastin, we have equally divided the microscopic section of the vein into the relevant number of the square subareas which have been scanned separately. By the method of thresholding we determined the areal concentration of the collagen and elastin for the each microslide. By comparing the varicose and control samples (Fig. 3) we found that the varicose vein contains percentually lesser amount of the collagen (31.41 ± 5.82) than the control non-dilated vein (43.89 ± 2.64) ($P < 0.01$), the amounts of the elastin in the varicose and the non-varicose veins were nearly the same (with no statistical difference between them).

Discussion

The results of our work as for the collagen are in good correlation with the general opinion the varicose vein should contain the lesser amount of the elastin and collagen responsible for the elasticity, the firmness and the resistance of the vein wall against the hydrostatic pressure, than the non-varicose vein. Our results as for the elastin are not in a good correlation with the general opinion, but Travers *et al* (1996) found the same in his histological work (with the stereological analysis). The works of various investigators by using of various methods for the determining the collagen and the elastin amounts in the varicose vein wall have not led to the unambiguous conclusion, so the general opinion remains unconfirmed and unrefuted. For the future we are thinking about the use of the color CCD camera which enables the more precise – coloured distinguishing of the individual components of vein wall. It will be also interesting to visualize each collagen subtype with the immunofluorescent antibodies (Galbavý *et al* 1996, Waksman *et al* 1997).

Conclusion

The method of determining the concentration of the collagen and the elastin in the bioptic material processed with the classic histological procedures and analyzed by the computer morphometric method seems to be relatively easy, quick and precise in the case of using the experienced necessary staining technologies.

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