Haemoglobin of varicose vein, varicose vein with thrombophlebitis and in parietal thrombus of varicose vein

Guzowski A, Gacko M, Worowska A, Kowalewski R, Łapiński R, Ostapowicz R, Płoński A

Department of Vascular Surgery and Transplantology, Medical University of Bialystok, Poland

Abstract

Saphenous veins were taken for examination: unchanged, varicose with thrombophlebitis and varicose thrombus. The contents of haemoglobin and protein were determined in the homogenate of that material. Only small quantities of haemoglobin were found in walls of unchanged veins. Greater amounts of haemoglobin were observed in walls of varicose veins, especially in walls of varicose veins with thrombophlebitis. The varicose vein thrombus also contained marked quantities of haemoglobin.

Key words: varicose vein, varicose thrombus, haemoglobin.

Introduction

Chemical and enzymatic composition of the vein wall depends on the cellular composition, which changes in pathological conditions, such as varices and inflammatory processes [1, 2]. These changes are often accompanied by parietal thrombus [3, 4]. Erythrocytes dominate in vein thrombus. Haemoglobin, released during haemolysis of erythrocytes, may infiltrate into the vein wall. The aim of this report was to evaluate the contents of haemoglobin in unchanged veins, varicose veins and varicose veins with thrombophlebitis and in varicose vein thrombus.

ADDRESS FOR CORRESPONDENCE:
Marek Gacko
Department of Vascular Surgery and Transplantology
Medical University of Bialystok,
M. Skłodowskiej-Curie 24A, 15-276 Białystok, Poland.
tel. (85) 746 82 77; fax. (85) 746 88 96;
e-mail: mgacko@poczta.onet.pl

Material and methods

The following saphenous veins were taken for the study: unchanged macroscopically veins, varicose veins, varicose veins with thrombophlebitis and parietal thrombus, obtained during surgery from 8 patients. Retracted blood clots, obtained in vitro from blood samples, collected from the patients before the surgery, served as control material. Samples of unchanged and varicose vein walls, thrombus and blood clots were stored at the temperature of -75^{0}C. The samples were pulverized in nitrogen in a stainless steel pulverizer directly before determinations. Cooled 0.15 mol/l NaCI (1:9 v/v) was added to the pulverized material and extracted at the temperature of 2^{0}C for 30 minutes. Supernatant, obtained by centrifugation (2700 x g, 30 min, 2^{0}C), was used for the examinations. The contents of haemoglobin [5] and protein [6] were determined in the homogenates, and were converted to 1 g of wet tissue. Gell filtration of the homogenate proteins was carried out in a Sephadex G-75 column of 1 x 40 cm in size. Five (5) mg of the homogenate protein was placed in the column. A 0.15 mol/l NaCl solution was used for elution. One (1) ml fractions were collected in 10 minute intervals, using an automatic fraction collector. Haemoglobin was determined by the measure of absorbance at 625 nm [5]. Haemoglobin was identified by means of the calibration curve, expressing the dependence of elution volume on the logarithm of molecular weight. The results of the determination were analyzed by Student's 't' test. The values of $p<0.05$ were accepted as statistically significant.

Results

The content of haemoglobin in the homogenates of unchanged saphenous veins amounted to 4.0 mg/g of tissue, varicose vein - 6.8 mg/g of tissue and varicose vein with thrombophlebitis - 17.2 mg/g of tissue (Table 1). The content of protein in the vein homogenate amounted to: unchanged vein: 27.5 mg/g of tissue, varicose vein: 29.0 mg/g of tissue, varicose vein with thrombophlebitis: 34.5 mg/g

Figure 1. Gel filtration in Sephadex G-75 of saphanous vein homogenate haemoglobin:
o - unchanged vein, trangle - varicose vein, rectangle - varicose vein with thrombophlebitis

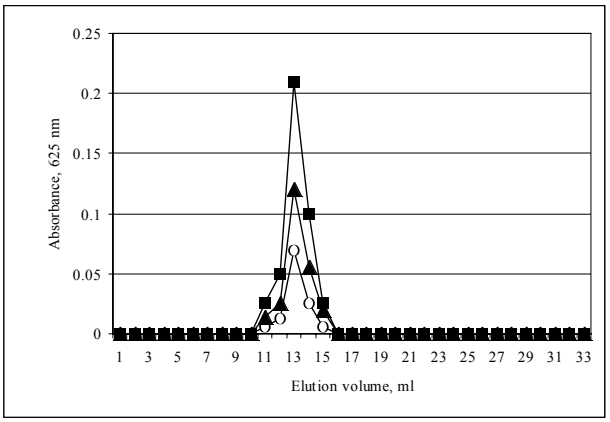
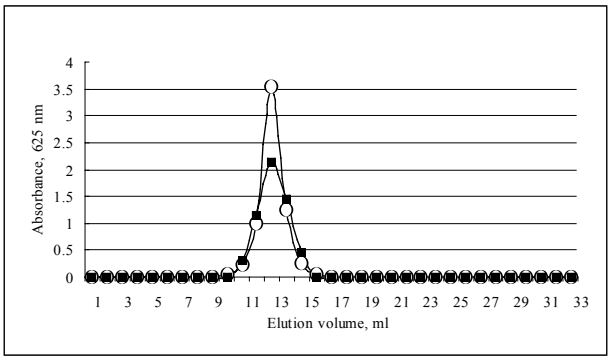


Figure 2. Gel filtration in Sephadex G-75 of homogenate haemoglobin: o - blood clot, rectangle - vein thrombus



of tissue. The content of haemoglobin in the vein thrombus homogenate amounted to 152.6 mg/g of tissue and in the blood clot homogenate amounts to 298.4 mg/g of tissue (Table 2). The content of protein in the vein thrombus homogenate amounted to 166.8 mg/g of tissue and in the blood clot homogenate, it amounted to 335.5 mg/g of tissue. Gel filtration in the Sephadex G-75 column showed that homogenates of the vein, varicose vein and varicose vein with thrombophlebitis contained haemoglobin (Fig.1). The homogenates of the vein thrombus and blood clot also contained haemoglobin (Fig. 2).

Discussion

In the physiological state, haemoglobin is found in erythrocytes of the circulating blood and it constitutes 90% of their dry mass. In pathological state, erythrocytes haemoglobin and free haemoglobin are also present in vein thrombus, in the wall of veins, changed in inflammatory processes, and in the blood extravasated into tissues and body cavities. Disturbances of oxidative-antioxidative processes are observed in walls of lower extremity varices [7, 8]. Haemoproteins, especially haemoglobin and its derivatives, may participate in these processes [9, 10]. The presence of haemoglobin, containing Fe2+ in a wall of varicose vein and, especially, in walls of veins, changed, due to thrombophlebitis, and in varicose thrombus should be taken into consideration, when estimating the systems, generating reactive forms of oxygen and, when estimating the concentrations of

Table 1. The contents of haemoglobin and protein in the saphenous vein wall: unchanged, varicose and varicose with thrombophlebitis.

Saphenous vein	Haemoglobin, mg/g of tissue	Protein, mg/g of tissue
Unchanged	4.0 ±0.2	27.5 ±2.2
Varicose	6.8 ±0.4*	29.0 ±3.0
Varicose with thrombophlebitis	17.2 ±1.2*	34.5 ±2.4*

* - statistically significant difference, p< 0.0001

Table 2. The contents of haemoproteins and protein in varicose thrombus and blood clot.

Examined material	Haemoglobin, mg/g of tissue	Protein, mg/g of tissue
Varicose thrombus	152.6 ±12,4	166.8 ±13.7
Bloot clot	298.4 ±23.8*	335.5 ±30.2*

* - statistically significant difference, p< 0.0001

antioxidants. Lower contents of haemoglobin in vein thrombus than those in blood clot, obtained in vitro, result in haemolysis of erythrocytes and in their release from the thrombus. The degree of haemoglobin loss may be a measure of thrombus duration. This time can also be determined by the content of von Willenbrand's factor, as well as by the contents of DNA and hydroxyproline.

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