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Original Article

The relationship between leukocyte activation and apoptosis in venous insufficiency etiopathogenesis

Varis etiyopatogenezinde lökosit aktivasyonu ile apopitozisin ilişkisi

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Abstract

Aim: Varicose veins are the most common disorders of the lower extremities. The purpose of this study is to investigate the effect of leukocyte activation in the vein wall and apoptosis in the etiopathogenesis of varicose veins.

Material and Methods: Forty-six patients with varicose veins were included in the study. High ligation with stripping with or without additional individual ligation and excision was performed for symptoms, complications or cosmetic needs. ELISA was used to measure the serum concentration of caspase-8 and caspase-9.

Results: There was a statistically significant difference between the cephalic vein and saphenous vein groups ($p=0.04$ and $p<0.0001$ respectively).

Conclusion: As a conclusion, the complex pathophysiology underlying varicose veins has yet to be fully defined. It is suggested that both leukocyte activation and dysregulation of apoptosis are associated with susceptibility for varicose vein formation however this argument needs further research.

Keywords: apoptosis; venous insufficiency; leukocyte activation; caspase

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Öz

Amaç: Variköz venler alt ekstremitelerin en sık görülen rahatsızlıklarıdır. Bu çalışmanın amacı, lökosit aktivasyonunun ven duvarındaki apoptozis ve variköz venlerin patogenezindeki etkisini araştırmaktır.

Gereç ve Yöntemler: Çalışmaya variköz venleriolan 46 hasta dahil edildi. Hastalara semptomları, komplikasyonları veya kozmetik sebeplerle strippingle yüksek ligasyon, ve eksizyon yapıldı. Serum kaspaz-8 ve kaspaz-9 ölçümüleri ELİSA kullanılarak yapıldı.

Bulgular: Sağlıklı sefalik ven ile variköz safen ven gruplarının sonuçları arasında istatistiksel olarak anlamlı bir fark tespit edildi ($p=0.04$ and $p<0.0001$).

Sonuç: Variköz ven oluşumunun altındaki karmaşık patofizyoloji henüz yeterince açıklanamamıştır. Hem lökosit aktivasyonunun hemde apopitozisdeki bozulmanın variköz ven oluşumuna katkısı olduğunu düşünmekteyiz.

Anahtar kelimeler: apoptozis; venöz yetmezlik; lökosit aktivasyonu; kaspaz

Introduction

Varicose veins are the most common disorders of the lower extremities. It affects nearly half of female and one-quarter of the male population in general [1]. Veins have different wall structure than arteries, and they work in lower pressure environment and function against gravity as a result of their structural valves and musculovenous pumps [3]. The known major risk factors of varicose veins are increased age, prolonged standing, pregnancy, ligamentous laxity, obesity, leg trauma and previous venous thromboses [4-7]. It is suggested that venous thrombosis increases the inflammatory process in the vein wall and thus results in fibrosis and destruction of venous valves leading to chronic venous insufficiency[8,9].

Family history and genetic predisposition are other important risk factors of varicose vein formation however there is still ongoing debate about the genetic factors that cause varicose veins and also chronic venous insufficiency [10].

Vein wall has three layers which are; tunica intimae, tunica media and tunica adventitia. In the inner layer of tunica media, the smooth muscle cells are arranged longitudinally whereas they are arranged circularly in the outer layer. Usually these smooth muscle cells in the tunica media are located regularly and side-by-side, however, in the varicose veins this usual manner of smooth muscle cells are destructed, and collagen deposits are seen. Although the exact mechanism of this destruction is unknown, failure or down-regulation of programmed cell death, apoptosis, is suggested to be one of the essential factors [11, 12].

More recently, the role of inflammation and leukocyte activation resulting in localised endothelial activation/ dysfunction is suggested to be an essential factor in the aetiology of varicose veins [13]. Shear stress reduction due to venous reflux and consequent venous hypertension triggers activation of leukocytes and endothelial cells. These cells express chemokines, cytokines, growth factors, proteases, that further all result in inflammation [14].

The purpose of this study is to investigate the effect of leukocyte activation in the vein wall and apoptosis in the etiopathogenesis of varicose veins.

Material and Methods

Patients:

Forty-six patients with varicose veins were included in the study. The study protocol and informed consent were approved by the Gazi University Ethics Committee (Approval number 2009/0.9-2.6), and all patients gave informed consent. The patients were classified according to clinical, etiological, anatomical and pathophysiological (CEAP) classifications (20) (Table 1). High ligation with stripping with or without additional individual ligation and excision was performed for symptoms, complications or cosmetic needs. After written informed consent was given, 10 ml of blood was drawn from the greater saphenous vein (GSV), and at the same time, 10 ml of blood was pulled from the cephalic vein of upper extremity for the control group in the same patient. The blood samples were put into EDTA and serum separation tubes for analyses.

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Table 1. Clinical, etiological, anatomical and pathophysiological (CEAP) classification (20).			
Clinical classification (C0-C6)	**Etiologic classification**	**Anatomic classification**	**Pathophysiologic classification**
C0: No visible or palpable signs of venous disease	Ec: Congenital	As: Superficial veins	Pr: Reflux
C1: Telangiectasies or reticular veins	Ep: Primary	Ap: Perforator veins	Po: Obstruction
C2: Varicose veins	Es: Secondary (post-thrombotic)	Ad: Deep veins	Pr,o: Reflux and obstruction
C3: Edema	En: No venous cause identified	An: No venous location identified	Pn: No venous pathophysiology identifiable
C4a: Pigmentation or eczema			
C4b: Lipodermatosclerosis or atrophie blanche			
C5: Healed venous ulcer			
C6: Active venous ulcer			

Biochemical and haematological analyses:

Samples used for serum collection were allowed to clot at room temperature before centrifugation, and the supernatants were stored at –80°C until further use. ELISA was used to measure the serum concentration of caspase-8 and caspase-9 (Bender MedSystems GmbH, Austria). The samples in the EDTA tubes were sent for complete blood count and measured by routine laboratory methods.

Statistical analysis: All statistics were performed using SPSS version 12.0 for Windows (SPSS Inc. Chicago, IL, USA). Continuous variables were expressed as the mean±SD and were compared by unpaired Student's t-test or Mann Whitney U-test. All the data for the normality of distribution using the Kolmogorov-Smirnov test were analysed. Statistical significance was assumed if the p-value was less than 0.05.

Results

Demographic Parameters:

Forty-six patients enrolled in this study. The demographic data is shown in table 2. Venous pressures of lower and upper extremities were measured in both upright and recumbent positions. Mean lower extremity venous pressures were 79.17±5.28 and 39.5±6.36 mm Hg respectively and mean upper extremity venous pressures were 64.13±4.29 and 28.33±5.22 respectively in upright and supine positions.

Biochemical and haematological results:

Caspase 8 was measured 0.47±0.04 ng/ml in cephalic vein group and 0.49±0.02 ng/ml in saphenous vein group. Caspase 9 was measured 1.40±0.22 ng/ml in cephalic vein group and 1.58±0.23 ng/ml in saphenous vein group. There was a statistically significant difference between the cephalic vein and saphenous vein groups (p=0.04 and p<0.0001 respectively) as shown in table 3.

Table 2: Demographic properties of the study group	
Clinical characteristic	**Study Group (n=46)**
Mean age ± SD, (years)	47.63±8.12
Mean body mass index ±SD, kg/m2	27.25±2.67
Female gender, n	21
CEAP stage	
CEAP 3, n (%)	14 (30.4)
CEAP 4a, n (%)	13 (28.3)
CEAP 4b, n (%)	12 (26.1)
CEAP 5, n (%)	4 (8,7)
CEAP 6, n (%)	3 (6.5)
CEAP: Clinical, Etiological, Anatomical and Pathophysiological classification.	

Table 3: Caspase values in study groups			
Apoptotic enzymes	**Cephalic vein group (n=46)**	**Saphenous vein group (n=46)**	**P**
Caspase 8 (mean±SD), ng/ml	0.47±0.04	0.49±0.02	0.040*
Caspase 9 (mean±SD), ng/ml	1.40±0.22	1.58±0.23	<0.0001*
*p value <0.05, statistically significant			

The mean values of haemoglobin, hematocrit, red blood cells, MVC, MCH, MCHC, RDW data are shown in Table 4. There was no statistically significant difference between the cephalic and saphenous groups. Platelet, MPW, Pct, PDW values are also evaluated in table 5. The mean values were found similar in cephalic vein and saphenous vein groups. The mean values of leukocytes, lymphocyte, monocytes, neutrophil, eosinophils and basophils are shown in Table 6. There was a statistically significant difference between the mean values of leukocyte, lymphocyte (%), monocyte, neutrophil, neutrophil (%) and eosinophil (%) when compared between cephalic and saphenous vein groups (p <0.05).

Table 4. The mean values of hemoglobin, hematocrit, red blood cells, MVC, MCH, MCHC, RDW in study groups

	Cephalic vein group (n=46)	Saphenous vein group (n=46)	P
Red blood cells (106/µl)	4.06±0.47	4.09±0.51	0.786
Hemoglobin (g/dl)	12.26±1.48	12.61±1.52	0.266
Hematocrit (%)	36.30±4.12	37.14±4.10	0.334
MVC (fL)	86.50±3.49	86.82±3.35	0.660
MCH (pg)	29.30±1.63	29.50±1.47	0.547
MCHC (g/dl)	33.80±1.38	34.09±1.20	0.287
RDW (%)	13.26±1.12	13.48±1.19	0.366

Table 5: The mean values of platelet, MPW, PCT ve PCT in study groups

	Cephalic vein group (n=46)	Saphenous vein group (n=46)	P
Platelets (103/µl)	197.09±43.09	208.11±48.14	0.250
MPW (fL)	8.76±1.24	9.05±1.39	0.290
Pct (%)	0.17±0.06	0.18±0.07	0.534
PDW (%)	16.01±0.93	16.34±0.90	0.088

Table 6: The mean values of Leukocytes, Lymphocytes, Monocytes, Neutrophils, Eosinophils and Basophilic cells in the study groups

	Cephalic vein group (n=46)	Saphenous vein group (n=46)	P
White Blood Cells (103/µl)	7.70±1.76	6.67±1.93	0.009*
Lymphocytes (%)	28.52±7.47	32.90±6.42	0.003*
Lymphocytes ((103/µl)	1.69±0.52	1.80±0.58	0.363
Monocytes (%)	7.83±2.29	7.38±1.94	0.305
Monocytes(103/µl)	0.58±0.22	0.49±0.18	0.030*
Neutrophils (%)	61.73±7.85	57.44±6.40	0.005*
Neutrophils (103/µl)	4.16±1.85	3.40±1.53	0.035*
Eosinophils (%)	1.49±0.85	1.89±1.02	0.047*
Eosinophils (103/µl)	0.11±0.08	0.14±0.12	0.135
Basophils (%)	0.54±0.34	0.52±0.31	0.726
Basophils (103/µl)	0.03±0.06	0.03±0.10	0.896

*p value <0.05, statistically significant

Discussion

Varicose veins, although mostly considered a cosmetic problem, usually result in a poor quality of life and even severe morbidity due to ulcers of the lower extremity as a result of chronic venous insufficiency. There are many theories in the literature regarding the pathophysiology and aetiology of varicose veins however there is no consensus on the issue.

Our study showed that apoptotic dysregulation was present in patients with varicose veins. The serum levels of caspase-8 and caspase-9 that were withdrawn from the GSV were higher than the cephalic vein. This result suggests that there is an increase in apoptotic activity.

Apoptosis, programmed cell death, is a biological process necessary for the continuity and homeostasis of the organism. Dysregulated apoptosis is suggested to be an essential factor in the pathogenesis of cancer, ischemic heart disease, autoimmune diseases, inflammation and stroke [15, 16]. It is reported that increased apoptotic activity was present in the endarterectomy and atherectomy specimens from renal, coronary and carotid arteries [17]. In contrast, this increased activity was not present in the varicose veins in some studies and additionally it was reported that apoptosis was downregulated in these cohorts [11,18,19].

Caspases are a family of cysteine proteases, which are involved in the extrinsic phase of apoptosis [20]. In the present study, we observed a significant difference in serum levels of both caspase-8 and caspase-9 in the GSV than the cephalic vein of the same patient as a control. We demonstrated that the serum levels of both caspase-8 and caspase-9 were increased in the saphenous veins of the patients meaning that the apoptosis was up-regulated. This result seems to contradict with the literature as in the research most of the studies reported a decreased apoptosis in varicose veins. However, in all these studies, the authors dealt with the tissue expressions of the apoptotic proteins, not the serum levels. We think that this is the limitation of our research that we did not study the tissue expression of apoptosis.

One of the many theories about the pathophysiology of venous insufficiency is the leukocyte entrapment theory and local hypoxia [21]. According to the entrapment theory, inflammation is activated in the vessels due to venous hypertension and reflux. Inflammation results in the activation of leukocytes, and they release cytokines such as transforming growth factor [TGF]-b1, interleukin-1 and tumour necrosis factor- α, as well as oxygen free radicals. It is also reported that the expression of matrix metalloproteinases is increased which are neutral endopeptidases that catalyse the degradation of the proteins of the extracellular matrix (ECM) [22]. Due to his entrapment of leukocytes in the vein wall and valves, the number of circulating neutrophils decreases in the serum. In our study, the numbers of leukocytes were significantly reduced in the serum drawn from the GSV.

Local hypoxia due to venous hypertension, dilatation of vein and blood pooling is another problem in varicose veins. It is demonstrated that there is upregulation of hypoxia-

inducible factors and their target genes in a varicose vein [23]. Dysregulation of hypoxia-inducible factor pathways may also affect apoptosis in the cell wall and abnormality of the ECM [19, 24]. The cytokines released from the leukocytes are suggested to be related with the dysregulation of the apoptosis.

As a conclusion, the complex pathophysiology underlying varicose veins has yet to be fully defined. It is suggested that both leukocyte activation and dysregulation of apoptosis are associated with susceptibility for varicose vein formation however this argument needs further research.

Declaration of conflict of interest

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