*Research Article (Araştırma Makalesi)*

# May Nesfatin-1 is a New Marker of Inflammation in Varicose Veins?

## *Nesfatin-1, Varisli Damarlarda İnflamasyonun Yeni Bir Belirteci Olabilir Mi?*

Mehmet KIRISCI[1], Sezen KOCAARSLAN[2]

[1] Kahramanmaras Sutcu Imam University, Faculty of Medicine, Department of Cardiovascular Surgery, Kahramanmaras, Türkiye
[2] Antalya Memorial Hospital, Medical Pathology, Antalya, Türkiye

**Özet**

**Amaç:** Varisli safen venlerinde bir anti-inflamatuar peptid olan nesfatin-1'in ekspresyonu, bu damarlarındaki inflamasyon sürecinin bir göstergesi olabilir. Bu amaçla, varisli venlerde nesfatin-1'in varlığını belirlemeyi hedefledik.

**Gereç ve yöntemler:** Bu çalışmada, varisli ven örnekleri 50 varis hastasından alındı. Kontrol grubu için, koroner bypass amacıyla alınan vena safena magna doku örnekleri 50 hastadan elde edildi. Nesfatin-1 antikoru ile immünohistokimyasal boyama yapıldı ve iki grup karşılaştırıldı.

**Bulgular:** Varisli ven örneklerinde nesfatin-1 immünohistokimyası yapıldığında, 38 (%76) hastada pozitif boyama saptandı, 12 (%24) hastada ise boyama gözlemlenmedi. Kontrol grubundan alınan safen ven dokusu örneklerinde ise 10 örnek (%20) nesfatin ile pozitif boyandı, 40 örnek (%80) ise boyanmadı. Varisli venlerde nesfatin immünohistokimyası ile yapılan boyama, kontrol grubundaki örneklerin boyama sonuçları ile istatistiksel olarak anlamlı bir fark gösterdi ($p<0,0001$).

**Sonuç:** Sağlıklı venlerle karşılaştırıldığında, varisli venlerin nesfatin-1 ile güçlü bir şekilde boyandığı gösterilmiştir. Nesfatin-1'in varisli venlerdeki ekspresyonu, inflamasyon sürecine bir yanıt olabilir ve varisli venlerin etiyopatogenezi açısından önemli bir rol oynayabilir. Nesfatin-1'in bir anti-inflamatuar peptid olarak varisli venlerdeki ifadesi, inflamasyon sürecine bir yanıt olabilir.

**Anahtar Kelimeler:** Nesfatin 1, Varisli ven, İnflamasyon, Koroner bypass

**Abstract**

**Objective:** Objective: The expression of nesfatin-1, an anti-inflammatory peptide, in varicose saphenous veins, may indicate the inflammation process in these veins. For this purpose, we aimed to determine the presence nesfatin-1 in varicose veins.

**Materials and methods:** In this study, varicose vein samples have been taken from 50 patients with varicose veins. For the control group, vena saphena magna tissue samples taken out for coronary bypass were obtained from 50 patients. Immunohistochemical staining was performed by staining tissues with nesfatin-1 antibody and two groups were compared.

**Results:** In the immunostaining of nesfatin-1 on varicose vein samples, 38 (76%) patients were determined to be positive, and no staining was observed in 12 (24%) patients. In saphenous tissue samples taken from the control group, 10 samples (20%) were stained positive with nesfatin immunostaining, while 40 samples (80%) were not stained. Staining of varicose veins with nesfatin immunostaining showed a statistically significant difference compared to the staining of samples taken from the control group ($p<0.0001$).

**Conclusion:** When compared to healthy veins, it was demonstrated that the varicose etiopathogenesis. It is concluded that the expression of nesfatin-1, which is an anti-inflammatory peptide, in varicose veins may be a response to the inflammatory process.

**Keywords:** Nesfatin 1, Varicose vein, Inflammation, Coronary bypass

**Correspondence:** Mehmet KIRISCI, Kahramanmaras Sutcu Imam University, Faculty of Medicine, Department of Cardiovascular Surgery, Kahramanmaras, Türkiye

**Phone:** +90 344 300 3378 **e-mail:** drkirisci@yahoo.com

**ORCID No (respectively):** 0000-0002-9618-4283, 0000-0002-3512-1588

**Submission date:** 31.07.2024

**Acceptance date:** 14.11.2024

**DOI:** 10.17517/ksutfd.1525932

## INTRODUCTION

Varicose veins (VV) are a common health problem in society. It is also a cosmetic problem. Patients with varicose veins have also accompanying symptoms such as pain, itching, swelling of feet, exhaustion, and pigmentation. Approximately one-third of women and men between ages 18-64 have VV (1). The etiology of VV is not exactly known (2). Heredity plays an important role in VV formation (3). Age, gender, pregnancy, obesity, smoking, and estrogen treatment have been shown to play a role in the formation of VV (4,5). The basic pathology in primary VV formation is venous wall degeneration and venous valve insufficiency (6). The flexibility and resistance of vein walls depend on collagen and elastic fibers. Morphological studies have shown changes in the VV wall in both collagen and elastic fibers (7,8). As a result of these inflammatory changes, decreased venous tone and therefore venous insufficiency are formed (9).

Nesfatin-1 was first defined in 2006 by Oh-I and colleagues (10). It is an anorexigenic molecule released by the hypothalamus (10). Nesfatin-1 is a peptide derived from nucleobindin 2, which is an energy homeostasis regulator and takes a role in suppressing appetite independent from the leptin pathway (11). The presence of nesfatin-1 has been shown in peripheral tissues. Nesfatin-1 is present in subcutaneous adipose tissue, gastric mucosa, pancreatic endocrine cells, testicles, skeletal muscles, pituitary gland, hepatic sympathetic nervous system, and sacral parasympathetic preganglionic neurons in the spinal cord (12-15). It has been demonstrated in studies that nesfatin-1 is also a molecule with anti-inflammatory and antiapoptotic efficacy (16). These findings indicate that nesfatin-1 may be a potential therapeutic agent for venous insufficiency. The purpose of this study is to investigate the staining of nesfatin-1, which may show the anti-inflammatory effect on varicose vein pathogenesis, in venous tissues.

## MATERIALS AND METHODS

This study has been performed in Kahramanmaras Sutcu Imam University Faculty of Medicine between the dates of October 2017 and June 2018. Varicose vena saphena magna (VSM) tissue samples were taken from 50 patients (27 females, 23 males; mean age 48 ± 12 years; range 26 to 75 years). As per the Clinical – Etiology – Anatomy – Pathophysiology (CEAP) classification, varicose veins between C2-C6 were collected during varicose vein operation. Healthy vein tissue samples were collected from 50 patients who were hospitalized for coronary bypass surgery and without venous dilatation and insufficiency with Doppler US. The control group was created from the tissue samples collected. (21 females, 29 males; mean age 63 ± 10 years; range 46 to 80 years). Varicose vein diagnosis was determined by measurements taken with Color Doppler US. Patients with a VSM diameter of 5.5 mm and above, and reflux flow above 2 seconds have been included in the study. The medications used continuously by patients and additional disorders have been recorded. Exclusion criteria included a history of deep vein thrombosis and thrombophlebitis.

### Immunohistochemical Staining

The venous tissue samples of the cases were fixed in 10% formaldehyde solution and embedded in paraffin blocks. Sections with a thickness of 4 μm were collected from all blocks. The tissue sections were deparaffinized in xylene and then rehydrated in ethanol solutions of decreasing concentrations (100%-95%-75%). They were irrigated in phosphate-buffered saline (PBS); then, they were incubated for 10 minutes in 3% hydrogen peroxide solution to allow the inhibition of endogenous peroxidase activity. The sections were boiled in 10 mmol/L of ethylenediaminetetraacetic acid buffer (pH 8.0) for antigen retrieval for five minutes at 850 watts and then for five minutes at 350 watts in a microwave. After that, the sections were treated with primary polyclonal rabbit antibody nucleobindin 2 Antibody (D-10): sc-376947 (Nesfatin) Santa Cruz, 1: 100 dilution) for 24 hours at 4 °C. All the sections were irrigated in PBS solution and then incubated for 60 minutes in horse radish peroxidase conjugate of goat anti-rabbit immunoglobulin G. Then, chromogen diaminobenzidine was applied and counterstaining was performed using Mayer's hematoxylin.

### Assessment of immunohistochemical expression

Two blinded pathologists evaluated and scored the specimens. In immunohistochemical staining, the cytoplasmic staining in endothelial and muscular cells of varicose veins was considered immunohistochemically positive. Immunohistochemical expression of nucleobindin 2 Antibody (D-10): Santa Cruz-376947 (Nesfatin) was assessed using a semi-quantitative scoring system for the presence of staining. Nucleobindin 2 Antibody (D-10): Santa Cruz-376947 (Nesfatin) immunostaining was negatively scored as 0 and positively scored as 1.

### Statistical Analysis

Statistical analysis was performed using the SPSS version 24.0 software (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to confirm the normality of the distribution of continuous variables. Continuous variables were expressed as mean ± SD or

median (min-max) in the presence of abnormal distribution, and categorical variables as percentages. Comparisons between the groups were made using the chi-square or Fisher's exact tests for categorical variables, independent samples t-test for normally distributed continuous variables, and Mann-Whitney U test when the distribution was skewed. A p-value of 0.05 was considered statistically significant.

## RESULTS

Demographics of patients with VV and control group patients are presented in **Table 1**. The mean age in the varicose vein group was 48± 12 and the mean age in the control group was 63±10. The number of patients with DM in the varicose vein group was 9 and it was 33 in the control group. Upon comparing the two groups, age hypertension (HT) and diabetes mellitus (DM) were observed to be significantly higher in the control group ($p<0.05$). We consider that the reason for this high level is that coronary bypass surgery is generally performed in a more advanced age group, and the combined prevalence of DM and coronary artery disease is higher (17).

It was observed in nesfatin-1 tissue immunostaining that 38 patients were stained positive in the varicose vein patient group, and 12 patients were not stained with nesfatin-1 immunostaining **(Figure 1a, 1b)**. In the control group, it was observed that the tissues of 10 patients were stained positive with nesfatin-1 immunostaining, and 40 patients were not stained **(Figure 2a, 2b)**. Nesfatin 1 was stained more potently in varicose vein tissues compared to healthy venous structures at a statistically significant level ($p<0.0001$).

## DISCUSSION

As far as we know, this study is the first study showing that varicose veins are stained with nesfatin immunostaining. A normal venous system depends on the integrity of the vein wall, valves, and venous blood flow hemodynamics. The pathophysiology of primary venous insufficiency is a complex entity including genetic and environmental factors, changes in venous endothelium, inflammatory biomolecules, dysfunctional valves, venous hypertension, and venous insufficiency clinical conditions. Inflammatory changes in the venous wall may be caused by various reasons such as mechanical pressure, venous hypertension, hypoxia, and venous blood stasis (18,19). The venous system in the lower extremities may evolve through the formation of varicose veins due to exposure to this type of stress. Of course, there may be differences between individuals depending on these environmental and genetic factors. In varicose vein pathogenesis, increased hydrostatic pressure causes the formation of inflammation and its increase on the vein wall and valves (20). This inflammatory response causes changes in leukocytes (particularly macrophage and monocytes), T lymphocytes, mast cells, inflammatory modulators, cytokine expression of chemokines, and many

**Table 1. Comparison of demographic characteristics and biochemical data between chronic venous insufficiency and control groups**

| | Control Group n=50 | Varicose Vein Group n=50 | p |
|---|---|---|---|
| **Age (years)** | 63±10 | 48±12 | <0.001 |
| **Male/Female (n)** | 29/21 | 23/27 | 0.230 |
| **Hypertension (%)** | 18(36%) | 8(16%) | 0.039 |
| **Smoke (%)** | 23(46%) | 22(44%) | 0.841 |
| **Diabetes Mellitus (%)** | 33(66%) | 9(18%) | <0.001 |
| **Nesfatin Staining(%)** | 10(20%) | 38(76%) | <0.0001 |
| **CEAP 0 (%)** | 50(100%) | 0 | <0.0001 |
| **CEAP 2 (%)** | 0 | 24(48%) | <0.0001 |
| **CEAP 3 (%)** | 0 | 18(36) | <0.001 |
| **CEAP 4 (%)** | 0 | 3(6%) | <0.001 |
| **CEAP 5(%)** | 0 | 1(%2) | <0.001 |
| **CEAP 6(%)** | 0 | 4(%8) | <0.001 |

**CEAP:** Clinical – Etiology – Anatomy – Pathophysiology classification

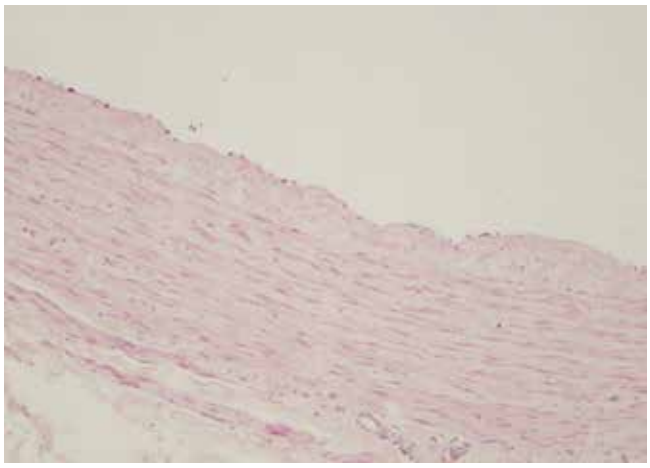

**Figure 1a** Histopathological appearance of normal saphenous vein. (Hematoxylin & eosin X100 objective.)

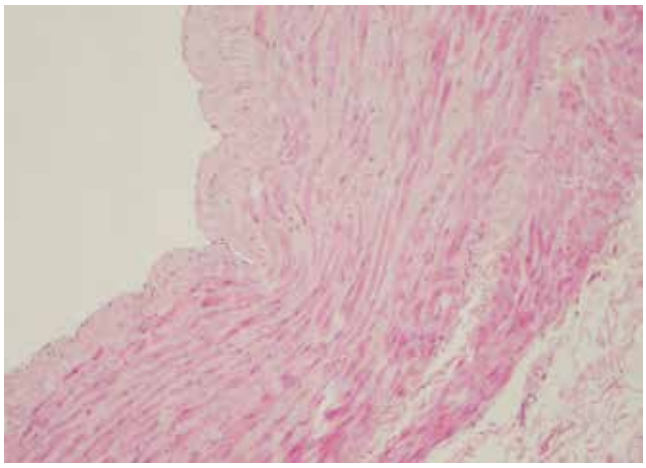

**Figure 1b** Histopathological appearance of varicose saphenous vein. (Hematoxylin & eosin X100 objective.)

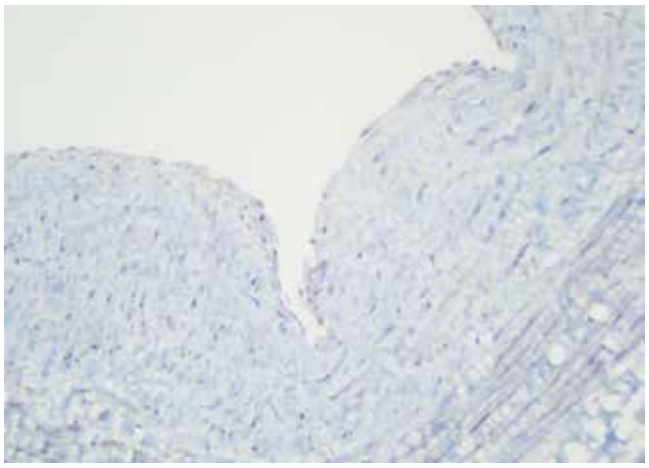

**Figure 2a** In the histopathological cross-section of normal saphenous vein, no staining was observed in the immunohistochemical study performed with nucleobindin 2 (Nesfatin). (Nucleobindin 2 antibody x200 Original magnification objective)

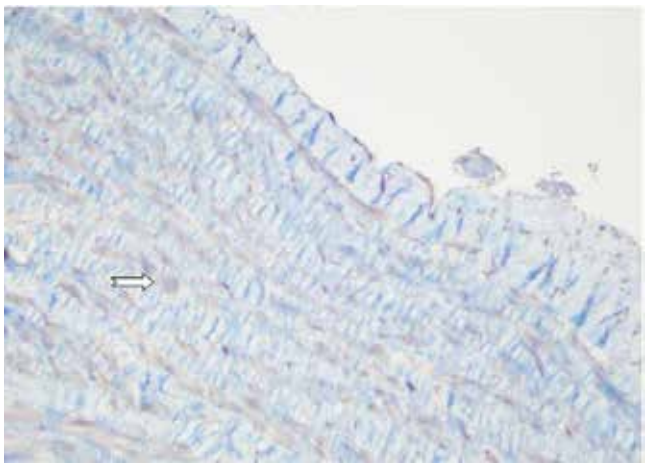

**Figure 2b** In the histopathological cross-section of varicose saphenous vein, positive cytoplasmic staining of vascular media smooth muscle cells in the immunohistochemical study was performed with nucleobindin 2 (Nesfatin). ( ⇨ ) has been observed. (Nucleobindin 2 antibody x200 Original magnification objective)

regulation pathways that drive inflammation in patients with venous insufficiency (21-24). In the study performed by Kocarslan and colleagues, it was shown that the varicose vein wall was stained with prolidase.

Staining of varicose vein wall by prolidase, which is an extracellular matrix metalloproteinase, has been evaluated as a response to inflammation (25). Similar to our study, Akar et al. examined and compared the prolidase enzyme activity, which is considered to be an indicator of oxidative stress, between the saphenous veins considered healthy during the coronary bypass and varicose saphenous veins. In their studies, they showed that Total Oxidant Status (TOS) and Oxidative Stress Index (OSI) values were higher in the varicose saphenous vein tissue than in the normal saphenous vein. This study shows that there is an inflammatory event in the varicose vein wall (26). Horecka et al. compared the healthy saphenous veins collected during the coronary bypass with varicose saphenous veins in terms of antioxidative mechanisms in their study. As a result of their studies, the increase in antioxidant enzymes measured in varicose saphenous vein wall was evaluated as an increase in antioxidant defense. The present study shows that when varicose saphenous veins and healthy saphenous veins are compared, varicose veins undergo a process with inflammation. In our study, staining of nesfatin-1 molecule with proinflammatory properties in the varicose saphenous vein wall shows that there is an inflammatory event in varicose veins (27).

Nesfatin-1 is known as an anorexigenic molecule that plays a role in suppressing appetite. In recently performed studies, a close relation was demonstrated between this peptide and diabetes (28), psychiatric disorders (29), neurogenic diseases (30), peripheral

arterial diseases (31), and acute myocardial infarction (32). Additionally, another remarkable feature is the demonstration of the anti-inflammatory and antiapoptotic efficacy of Nesfatin 1 in the study of Ozsavcı and colleagues evaluating the oxidative brain damage induced by subarachnoid hemorrhage (16,33). In a study conducted by Enfani et al.34, they investigated the protective effect of nesfatin-1 in cerebral tissue in the cerebral ischemia/reperfusion study in rats. In their study, they examined oxidative and antioxidative enzymes in serum between 2 groups as one group exposed to cerebral ischemia-reperfusion by injecting nesfatin-1 and the other group exposed to cerebral ischemia-reperfusion by not injecting nesfatin-1. In addition, cerebral tissue samples were collected and examined histopathologically. As a result of this study, when the two groups were compared, it was demonstrated that serum oxidative enzymes were increased in the non-nesfatin group while they were decreased in the nesfatin-1 group. In addition, the antioxidative enzyme activities in the nesfatin-1 group were significantly increased compared to the other group. As a result of histopathological examinations, apoptotic and necrotic cell death in the hippocampus CA1 region has been shown to decrease significantly in the group given nesfatin 1. As a result of these studies, they reported that the nesfatin-1 molecule has an antioxidative and anti-apoptotic activity as well as a cerebral protective effect (34). In our study, since varicose saphenous veins were stained with more nesfatin-1 compared to healthy saphenous veins, we think that the proximal activity of nesfatin-1 with its antioxidative and anti-inflammatory activity in the tissues tries to show the protective effect in the saphenous veins. In the study performed by Scotece and colleagues on human and murine chondrocytes, it was shown that chemotactic and proinflammatory mediators (IL 6 and TNF alpha) increased the expression of nesfatin-1 (35). In this study, we have demonstrated that varicose veins induced as a result of an inflammatory process are stained by nesfatin-1 immunostaining. The fact that nesfatin-1, the anti-inflammatory efficacy of which has been shown, has stained varicose veins in our study has been interpreted by the fact that nesfatin was accumulated in tissues to show its anti-inflammatory effect. In the study of Kuyumcu, the relationship between nesfatin-1 serum values in carotid artery stenosis was investigated. The study included three groups of 60 patients with carotid artery stenosis less than 60%, carotid artery stenosis more than 60%, and without atherosclerotic carotid artery stenosis according to computerized carotid artery angiography. All patients were evaluated for serum nesfatin-1 levels. Serum nesfatin-1 levels in the group without carotid artery stenosis were found statistically higher in groups with less than those with 60% carotid artery stenosis. Serum nesfatin-1 levels in the group with less than 60% carotid artery stenosis were found statistically higher than those with carotid artery stenosis more than 60%. It was found that there was a negative correlation between carotid artery stenosis and serum nesfatin-1 levels. According to these results, it was claimed that there was a close relationship between atherosclerotic carotid artery disease and nesfatin-1 levels in the inflammatory processes (36). In our study, the fact that nesfatin-1 was more stained in VV tissue showed that there was a close relationship of nesfatin-1 with anti-inflammatory activity in venous insufficiency resulting from an inflammation process. We think that it accumulated in the tissue to suppress the inflammatory process. This information is consistent with the anti-inflammatory efficacy of nesfatin in literature. In literature, most previous studies about nesfatin-1 have been conducted by analyzing serum levels of nesfatin and few studies investigated tissue levels of nesfatin-1 in rats. To the best of our knowledge, this is the first study analyzing tissue levels of nesfatin-1 in the human body. Zhang et al. studied the relationship between DM and nesfatin-1 and showed that serum levels of nesfatin-1 in newly diagnosed diabetic patients were significantly higher than serum levels of nesfatin-1 levels in both groups of patients with impaired and normal glucose tolerance (37). Li et al. showed that there was a positive correlation between age and serum levels of nesfatin-1 meaning that nesfatin-1 increased by aging (38). Gunes et al. showed that serum levels of nesfatin-1 in obese children with hypertension were higher than those of obese children without hypertension (39). In these three studies, serum levels of nesfatin-1 in the elderly or patients with DM or hypertension were seen to be higher than in the normal population. In our study, both the mean age of the control group and the number of diabetic and/or hypertensive subjects in the control group were higher than the study population meaning that the control group was supposed to have higher tissue levels of nesfatin 1. However, staining of wall of saphenous vein by nesfatin-1 stain was significantly more intense in patients with venous insufficiency as compared to the control group, which indicates higher tissue levels of nesfatin-1 in saphenous veins of the study group. This finding supports the idea suggesting that nesfatin-1 has accumulated in the walls of varicose veins to exert anti-inflammatory action.

In conclusion, new treatment methods may be developed to understand the pathophysiology of varicose vein formation and to prevent its formation. Although it is a neuropeptide assuming the roles of energy homeostasis and appetite regulation, in this study, we have

obtained results that suggest it is a molecule that may be used in the prevention and/or treatment of VV formation with its anti-inflammatory and antiapoptotic efficacy. At this point, further studies can be performed on a larger scale.

**Ethical Approval:** The study was approved by the Ethics Committee of the Kahramanmaras Sutcu Imam University Medical School Ethic Committee (date: 27.09.2017, protocol number 40, decision no 05) and International Declaration of Helsinki was followed in the study.

**Declaration of conflicting interests:** The author(s) declared no potential conflicts of interest concerning this article's research, authorship, and/or publication. Written informed consent was obtained from all patients for publication.

**Funding:** This work has not received any funding.

**Author Contribution Statement:** The authors declare they equally contributed to the manuscript.

## REFERENCES


1. Evans CJ, Fowkes FG, Ruckley CV, Lee AJ. Prevalence of varicose veins and chronic venous insufficiency in men and women in the general population: Edinburgh Vein Study. J Epidemiol Comm Health. 1999;53:149-53.
2. Chwała M, Szczeklik W, Szczeklik M, Aleksiejew-Kleszczynski T, Jagielska-Chwała M. Varicose veins of lower extremities, hemodynamics and treatment methods. Adv Clin Exp Med. 2015;24:5-14.
3. Krysa J, Jones GT, van Rij AM. Evidence for a genetic role in varicose veins and chronic venous insufficiency. Phlebology. 2012;27:329-35.
4. Scott TE, LaMorte WW, Gorin DR, Menzoian JO. Risk factors for chronic venous insufficiency: a dual case-control study. J Vasc Surg. 1995;22:622–8.
5. Jawien A. The influence of environmental factors in chronic venous insufficiency. Angiology. 2003;54: 19–31.
6. Myara I, Myara A, Mangeot M, Fabre M, Charpentier C, Lemonnier A. Plasma prolidase activity: a possible index of collagen catabolism in chronic liver disease. Clin Chem. 1984;30: 211-5.
7. Rose SS, Ahmed A. Some thoughts on the aetiology of varicose veins. J Cardiovasc Surg. 1986;27(5):534-43.
8. Birdina J, Pilmane M, Ligers A. The Morphofunctional Changes in the Wall of Varicose Veins. Ann Vasc Surg. 2017;42:274-84.
9. Vural M, Toy H, Camuzcuoglu H, Aksoy N. Comparison of prolidase enzyme activities of maternal serum and placental tissue in patients with early pregnancy failure. Arch Gynecol Obstet. 2011;283:953-8.
10. Oh-I S, Shimizu H, Satoh T, Okada S, Adachi S, Inoue K, et al. Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature. 2006;443(7112):709-12.
11. Yosten GL, Samson WK. Nesfatin-1 exerts cardiovascular actions in brain: possible interaction with the central melanocortin system. Am. J. Physiol. Regul. Interg. Comp. Physiol. 2009;297:330–6.
12. C Ayada, U Toru, Y Korkut. Nesfatin-1 and its effects on different systems. Hippokratia. 2015;19:4–10.
13. Ramanjaneya M, Chen J, Brown JE, Tripathi G, Hallschmid M, Patel S, et al. Identification of nesfatin-1 in human and murine adipose tissue: a novel depot-specific adipokine with increased levels in obesity. Endocrinology. 2010;151(7):3169-80.
14. Foo KS, Brauner H, Ostenson CG, Broberger C. Nucleobindin-2/ nesfatin in the endocrine pancreas: distribution and relationship to glycaemic state. J Endocrinol. 2010;204:255–63.
15. Osaki A, Shimizu H, Ishizuka N, Suzuki Y, Mori M, Inoue S. Enhanced expression of nesfatin/nucleobindin-2 in white adipose tissue of ventromedial hypothalamus-lesioned rats. Neurosci Lett. 2012;521:46–51.
16. CH Tang, X-J Fu, X-L Xu, X-J Wei, H-S Pan. The anti-inflammatory and antiapoptotic effects of nesfatin-1 in the traumatic rat brain, Peptides. 2012;36:39–45.
17. Low Wang CC, Hess CN, Hiatt WR, Goldfine AB. Clinical Update: Cardiovascular Disease in Diabetes Mellitus: Atherosclerotic Cardiovascular Disease and Heart Failure in Type 2 Diabetes Mellitus - Mechanisms, Management, and Clinical Considerations. Circulation. 2016;133:2459-502.
18. Naoum JJ, Hunter GC, Woodside KJ, Chen C. Current advances in the pathogenesis of, varicose veins. J Surg Res. 2007;141:311–6.
19. Michiels C, Bouaziz N, Remacle J. Role of the endothelium and blood stasis in the development of varicose veins. Int Angiol. 2002;21:18–25.
20. Eberhardt RT, Raffetto JD. Chronic venous insufficiency. Circulation. 2014;130:333-346
21. Raffetto JD, Khalil RA. Mechanisms of varicose vein formation: valve dysfunction and wall dilation. Phlebology. 2008;23:85-98.
22. Raffetto JD, Mannello F. Pathophysiology of chronic venous disease. Int Angiol. 2014;33: 212-21.
23. Ono T, Bergan JJ, Schmid-Schönbein GW, Takase S. Monocyte infiltration into venous valves. J Vasc Surg. 1998;27:158–66.
24. Mannello F, Ligi D, Raffetto JD. Glycosaminoglycan sulodexide modulates inflammatory pathways in chronic venous disease. Int Angiol. 2014;33:236-42.
25. Kocarslan A, Kocarslan S. What is the role of prolidase in pathogenesis of primary varicose veins? Turkish Journal of Thoracic And Cardiovascular Surgery. 2017;25(1):68-73.
26. Akar I, Ince I, Aslan C, Benli I, Demir O, Altindeger N, et al. Oxidative stress and prolidase enzyme activity in the pathogenesis of primary varicose veins. Vascular. 2018;26(3):315-21.
27. Horecka A, Biernacka J, Hordyjewska A, Dąbrowski W, Terlecki P, Zubilewicz T, et al. Antioxidative mechanism in the course of varicose veins. Phlebology. 2018;33(7):464-9.
28. Zuo H, Shi Z, Yuan B, Dai Y, Wu G, Hussain A. Association between serum leptin concentrations and insulin resistance: a population-based study from China. PLoS ONE. 2013;8:e54615.
29. Gunay H, Tutuncu R, Aydin S, Dag E, Abasli D. Decreased plasma nesfatin-1 levels in patients with generalized anxiety disorder. Psychoneuroendocrinology. 2012;37:1949–53.
30. Aydin S, Dag E, Ozkan Y, Erman F, Dagli AF, et al. Nesfatin-1 and ghrelin levels in serum and saliva of epileptic patients: hormonal changes can have a major effect on seizure disorders. Mol Cell Biochem. 2009;328(1-2):49-56.
31. Ding S, Qu W, Dang S, Xie X, Xu J, Wang Y, et al. Serum nesfatin-1 is reduced in type 2 diabetes mellitus patients with peripheral arterial disease. Med Sci Monit. 2015;21:987-91.
32. Dai H, Li X, He T, Wang Y, Wang Z, Wang S, et al. Decreased plasma nesfatin-1 levels in patients with acute myocardial infarction. Peptides. 2013;46:167-71.
33. Ozsavci D, Ersahin M, Sener A, Ozakpinar OB, Toklu HZ, Akakin D, et al. The novel function of nesfatin-1 as an anti-inflammatory and antiapoptotic peptide in subarachnoid hemorrhage-induced oxidative brain damage in rats. Neurosurgery. 2011;68(6):1699-708.

34. Erfani S, Moghimi A, Aboutaleb N, Khaksari M. Protective effects of Nesfatin-1 peptide on cerebral ischemia reperfusion injury via inhibition of neuronal cell death and enhancement of antioxidant defenses. Metab Brain Dis. 2019;34(1):79-85.
35. Scotece M, Conde J, Abella V, López V, Lago F, Pino J, et al. NUCB2/nesfatin-1: a new adipokine expressed in human and murine chondrocytes with pro-inflammatory properties, an in vitro study. J Orthop Res. 2014;32(5):653-60.
36. Kuyumcu A. The relationship between nesfatin-1 and carotid artery stenosis. Scand Cardiovasc J. 2018;52(6):328-34.
37. Zhang Z, Li L, Yang M, Liu H, Boden G, Yang G. Increased plasma levels of nesfatin-1 in patients with newly diagnosed type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes. 2012;120(2):91-5.
38. Li QC, Wang HY, Chen X, Guan HZ, Jiang ZY. Fasting plasma levels of nesfatin-1 in patients with type 1 and type 2 diabetes mellitus and the nutrient-related fluctuation of nesfatin-1 level in normal humans. Regul Pept. 2010;159(1-3):72-7.
39. Gunes H, Alkan BF, Gunes H, Temiz F. Can Nesfatin-1 Predict Hypertension in Obese Children. J Clin Res Pediatr Endocrinol. 2020;12(1):29-36.