CHAPTER 17

CHRONIC VENOUS INSUFFICIENCY AND BIOCHEMICAL PERSPECTIVE ON PHARMACOLOGICAL THERAPY

Nurkay KATRANCIOĞLU[1]
Önder OTLU[2]
Tuğba Raika KIRAN[3]
Ercan KARABULUT[4]

CHRONIC VENOUS INSUFFICIENCY

Chronic venous insufficiency (CVI) and varicose veins are important causes of morbidity and appear as common chronic health and socioeconomic problems. Venous insufficiency (VI) and varicose veins affect approximately 40% of the population (1). It has been reported that leg complaints compatible with venous disease symptoms such as heaviness and swelling are present in 49.1% of the male population and 62.1% of the female population (2,3). The Millennium Research Group estimates that by the end of 2021, CVI treatments will double from 2011 levels (4).

In venous circulation, the leg veins work in the opposite direction of gravity as they send blood to the lungs. The transport of blood to the lungs occurs when the veins are compressed as a result of the contraction of our leg muscles and by the musculovenous pump and when the muscles relax, the venous valves in the leg veins prevent the return of blood. Valves open as blood goes up and close when blood returns. In this way, they ensure that venous blood flows in one direction to the lungs (5). When one or more of the components in this system fail, clinical venous insufficiency with a wide range of symptoms occurs (6).

In CVI, dysfunction occurs due to congenital or acquired factors in the valves

[1] Prof. Dr. Malatya Turgut Ozal University, Faculty of Medicine, Department of Cardiovascular Surgery nurkay.katrancioglu@ozal.edu.tr

[2] Assistant Prof. Malatya Turgut Ozal University, Faculty of Medicine, Department of Biochemistry onder.otlu@ozal.edu.tr

[3] Associate Prof. Malatya Turgut Ozal University, Faculty of Medicine, Department of Biochemistry raika.kiran@ozal.edu.tr

[4] Associate Prof. Ankara Yıldırım Beyazıt University, Faculty of Medicine, Department of Pharmacology ekarabulut@ybu.edu.tr

in the lower extremity veins that prevent the backflow of blood with the effect of gravity. Varicose veins occur due to insufficiency of the valves of the deep, superficial, and/or perforating veins. This insufficiency causes backflow of blood and an increase in venous pressure, causing problems with the enlarged, elongated or tortuous subcutaneous veins of the lower legs (7).

Anatomical, ultrasound, and plethysmographic studies have determined that valvular regurgitation is widely distributed throughout the leg in affected individuals. Insufficiency is most commonly seen in the great saphenous vein below the knee. It has been shown that increased venous pressures are associated with increased disease severity (8). Impairments in valve function cause hydrostatic pressure to be reflected in the venous segments and backflow of blood. With an increase in venous pressure, the veins expand and the valves diverge. As a result, valve insufficiency and reflux become evident. Proximal valvular regurgitation progresses distally and may involve long segments. Venous hypertension can cause protein-rich fluid and blood cells to migrate through the capillary walls into the intercellular space. Thus, soft tissue edema occurs in the early period. In the long term, the risk of skin thickening, hyperpigmentation and ulceration increases (9,10).

Risk factors for varicose veins include family history, age, gender, occupation or previous history of deep vein thrombosis, sedentary life, sitting work, hypertension, long-term use of birth control pills, smoking, obesity, pregnancy, and chronic constipation etc. (11). Women are more likely to have varicose veins than men, and their severity increases in successive pregnancies. Estrogen and progesterone affect the formation of varicose veins in different ways. Estrogen increases venous capacitance by changing blood volume, intra-abdominal pressure. Progesterone on the other hand causes venous relaxation and body weight is known as another risk factor in the formation of varicose veins. There are data showing that the incidence of varicose veins is higher in overweight or obese women (12).

The clinical manifestations of chronic venous insufficiency can range from mild symptoms such as swelling, edema, pigmentation to more serious symptoms such as induration and ulceration. The most common early symptoms of CVI include the appearance of varicose veins and lengthening of the saphenous veins, and leg distention (13).

The treatment of varicose veins can be listed as conservative treatment, compression treatment, pharmacological treatment and surgical treatment (14).

Conservative treatment: Conservative treatment aims to reduce or control symptoms. Basic life style changes such as increasing daily walking, being careful

not to stand for a long time, leg elevation, weight loss constitute the first stage of conservative treatment. It is necessary to avoid wearing tight clothing and smoking, which may cause an increase in pressure in the proximal region. Since the sudden increase in temperature causes vascular enlargement, effects such as hot bath and sauna should be avoided (15).

Compression Therapy: Elastic compression stockings are recommended in the treatment of CVI to support veins with reduced vascular wall resistance, increase venous hemodynamics, reduce edema, and relieve symptoms (16).

Surgical Treatment: Surgical techniques are classified in two groups as Ablative Surgery and Conservative Surgery. Ablative surgery methods; includes saphenous scraping, simple crossectomy, and phlebectomy. Conservative surgery technique is aimed to continue saphenous vein drainage and to prevent reflux. Safenofemoral external valvuloplasty or CHIVA (Conservatrice Hemodynamique de Insuffisance Venieuse en Ambulatorie) method is applied (17).

PATHOPHYSIOLOGY OF CHRONIC VENOUS DISEASE

The venous wall be formed of the endothelial lining and this lining encircled by an extracellular matrix (ECM) formed of collagen, elastin, smooth muscle cell (SMC) and proteoglycans. Structural and functional unity of this type of capacitance is preserved by a susceptible balance of protein synthesis and degradation between the vessels and the extra-cellular matrix. Scientific reports showed that there is a significant correlation between decreased elastin content and enlargement of the venous wall (18). Similarly, Aunapuu et al. found an increase in the connective tissue of varicose veins and a deterioration in the elastic network around the SMC bundles. In the results of the study, an increase in laminin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) was found in the elderly and varicose group. In addition, it was found that women have higher expression levels of ICAM-1 and VCAM-1 (19). SMC and fibroblast culture from varicose patients showed significantly less collagen III and fibronectin and regular collagen V levels compared to normal controls (18).

Venous hypertension and shear stress play crucial role in the development of inflammation in both the vessel wall and surrounding tissue (20). In general, releasing of the signals for suppressing inflammation and reactive free radical production is activated by laminar shear stress. It is recognized that low shear stress, tempestuous flow of blood and stasis lead to inflammation and the thrombotic mediators relase from vessels because of inflammation.

IL-6 is an inflammatory cytokine. In case of trauma or other tissue damage

that leads to inflammation it is secreted by T cells and macrophages secreted IL-6 in order to induce the immune response. IL-6 also supports the arrengement of metabolic, regenerative and neural processes. In a study, a significantly increased IL-6 level was found in varicose veins (21). This data shows that inflammatory response elevated in the damaged blood vessels. According to the findings of Yasim et al., there were no significant differences in systemic IL-6 levels between the group has varicose veins and the group of normal vessels (22).

According to Virchow's triad, endothelial dysfunction and damage to the venous wall represent one of the fundamental mechanisms not only in the development of deep vein thrombosis, but also in the development of varicose veins and complications. Endothelial damage is expected in varicose veins. Trauma and shear stress are the most common major cause of endothelial damage. In a study by Poredos et al., von Willebrand factor (vWF) levels formed by endothelial cells were measured in order to examine endothelial activity and/or damage. As a result, they reported that vWF levels increased significantly in varicose veins compared to systemic blood (23).

Yasim et al. measured the plasma levels of procoagulant and endothelial markers in volunteers who has varicose veins and healthy people. According to their results there where not a significant difference between two groups in plasma levels of protein C, fibrinogen, tPA, IL-6 and NO. However, they reported that some parameters such as protein S, vWf, Vascular endothelial growth factor (VEGF) and IL-12 are statistically higher in the varicose vein group than the control group (24).

Major factors known to play an critical role in CVI are hypoxia, hemosiderin deposition, fibrinogen/fibrinolysis, and endothelial/glycocalyx dysfunction. Inflammation is at the center of the damage process, and leukocytes secrete intensely cytokines (23). Mansilha et. al. compared the blood sample drawn from the varicose area with the venous blood sample from arm of the same person. They reported that IL-6, fibrinogen and hemoglobin levels were higher in the varicose area compared to arm blood. With this data, it was confirmed that some inflammatory processes are activated in patients with varicose veins and this activation increases the concentration of IL-6 and fibrinogen (25).

The balance between matrix metalloproteinase (MMP) enzymes and their endogenous tissue inhibitors is very important for regulating the homeostasis of the extra cellular matrix. MMPs belong to a family of proteases known as metzincins and are zinc-dependent endopeptidases. These enzymes can degrade most components of the ECM. Humans are known to have 23 MMPs, and vascular tis-

sues can express 14 of these MMPs. Although the structures of MMPs are similar, they can effect differently to homeostasis of the extra-cellular matrix. Gene transcription, protein translation and pro-MMP activation are the control points for the regulation of MMPs. Plasma proteins and tissue inhibitors of metalloprotein (TIMPs) are also act as inhibitors of MMPs. There is scientific evidence that varicose veins have instability between MMP and TIMP (26, 27). Varicose veins also have some other disfunctions such as loss of the collagen-elastin network, changes in collagen type I to III ratio and high activity of matrix-metalloproteinases (28, 29). It has been shown that activation of EP (EP1-EP4) receptors in endothelial cells triggers proinflammatory responses, MMP-9 expression and angiogenesis (26). In vascular smooth muscle cells, the prostacyclin receptor usually associates with the G-protein subunit Gαs, which is linked to a signaling cascade that promotes relaxation and a decrease in vascular tone (30).

PHARMACOLOGICAL THERAPY

Venoactive drugs and diuretic drugs are generally used as pharmacological treatment in the treatment of varicose veins. There is no consensus on the optimal treatment yet. Venoactive drugs are drugs that interfere with the pathophysiological mechanism of CVI and are used to reduce the symptoms of varicose veins, especially pain and edema. Venoactive drugs used in the treatment of varicose veins play an active role in reducing edema formation and hemorheological healing by regulating blood flow, reducing capillary filtration and regulating lymph circulation. These drugs usually contain active metabolites of herbal origin obtained by extraction methods. For this reason, the pharmacological activities of venoactive drugs are high and the use of these drugs is quite safe. Some natural and synthetic venoactive drugs are listed below.

Natural:

- Benzopines: Coumarin, flavonoids, hydroxyrutosides etc.,
- Saponosides: Horse chestnut extras,
- Micronized purified flavonoid fraction (MPFF)
- Plant extracts; Anthocyans, oligomers, ginko biloba.

Synthetic:

- Adenosinephosphate,
- Benzarone,
- Calcium dobesilate (31,32).

In 2005, experts came together and exchanged scientific views on randomized clinical and meta-analysis studies in the literature to establish standards in the treatment of chronic venous insufficiency. Following this meeting, an internation-

al consensus was reached in 2008, giving recommendations on the evidence levels of venotonic drugs at Grade A, B and C levels that will guide chronic venous insufficiency (33).

Apart from these studies, researches on drugs used in pharmacological treatment are still continuing today. MPFF, for example Daflon®, contains large amount of diosmin and less other active flavonoids such as hesperidin, diosmetin, linarin, and isorhoifolin. Daflon® is also prescribed very often and it is one of the well-researched venoactive drugs. MPFF's major effects have been investigated by both clinical and non-clinical researches. Improvements in venous tone and contractility, microcirculation and venous ulcer healing have been reported. Also researchers have showed decreases in edema, inflammation, leukocyte adhesion and inflammatory mediator production (34,35). Coleridge Smith et al. studied the effects of 500 mg Daflon® on the surface expression of leukocyte adhesion molecules in chronic venous disease. Administiration of Daflon® for 60 days have been chanced the CD11b and CD62L expression levels in neutrophils and monocytes respectively. These results suggest that Daflon® can be inhibit of leukocyte adhesion and activation (36, 37). The anti-hypertensive effects of diosmin have been demonstrated in a rat model, probably due to its activity in the elimination of superoxide anions (38). Diosmin, a pharmacological vasoactive agent, has the ability to inhibit inflammatory pathways by increasing anti-angiogenic factors and decreasing the level of pro-angiogenic factors in plasma (39,40). It has been shown that MPFF has the ability to effectively reduce endothelin 1 and TNF-α levels in patients with CVD (41). Another form of venoactive bioflavonoids is Rutosides. Anti-inflammatory properties of rutosides have been showed and rutosides can heal cronic venous disease signs and symptoms. In vitro experiments have shown rutoside's potential on the inhibition of the inflammation-related gene expression such as TNF-α, IL-1, and IL-6 (42). Another research using peripheral blood neutrophils has shown that Rutin, another flavonid, can reduce nitric oxide, and TNF-α production and can also suppress myeloperoxidase activity (43). One of the most prescripted drug for treatment of cronic venous disease is Calcium dobesilate and its antithrombotic properties have been shown. It has been reported that calcium dobesilate regulates vascular homeostasis by raising the nitric oxide-synthase activity (44, 45). Sulodexide is a highly purified glycosaminoglycan mixture (46) and it can significantly reduc the secretion of pro – and complexed MMPs from leukocytes and monocytes (47,48).

Loss of endothelial glycocalyx and degradation of some proteolytic enzymes due to increased shear stress or hydrostatic pressure in CVI reduce the barrier function and facilitate the extravasation of reactive "leukocytes". The highly neg-

atively charged, unbranched polysaccharide family of GAGs can modulate the involvement of leukocytes and neutrophils, a vital process in inflammation, by mediating chemokine mobilization (49).

Glycosaminoglycans physiologically function as a protective layer and an important barrier for all endothelial cells. In addition, commercially available GAG-based drugs can limit the deleterious inflammatory and proteolytic cascade and enhance anti-inflammatory pathways and tissue remodeling through modulation of a wide variety of biocompounds with opposing biological activities (50,51).

CONCLUSION

There is no consensus yet on the gold standard pharmacological treatment applied in the treatment of chronic venous insufficiency. However, venoactive drugs, which have become widespread recently, are drugs that interfere with the pathophysiological mechanism of chronic venous insufficiency and are used to reduce the symptoms of varicose veins, especially pain and edema. We think that serious steps will be taken in the permanent treatment of CVI as the pathophysiology of chronic venous insufficiency and the mechanisms of action of venoactive drugs are clarified.

REFERENCES

1. Beebe-Dimmer, J.L., Pfeifer, J.R., Engle, J.S. The epidemiology of chronic venous insufficiency and varicose veins. *Annals of Epidemiology*, 2005; *15 (3)*, 175-184.
2. Rabe, E., Pannier, F. (2017). Epidemiology of chronic venous disorders. Peter Gloviczki (Ed.), *Handbook of Venous and Lymphatic Disorders* in (p. 121-128). USA: CRC Press.
3. Robertson, L., Evans, C., Fowkes, F.G. Epidemiology of chronic venous disease. *Phlebology,* 2008; *23 (3)*, 103-111.
4. Grand Wiew Research (2019). *Varicose Veins Treatment Devices Market Size, Share & Trends Analysis Report By Type (Endovenous Ablation, Sclerotherapy, Surgical Ligation & Stripping), By Region, And Segment Forecasts, 2019 – 2025.* (Accessed at 22/06/2021, https://www.grandviewresearch.com/industry-analysis/varicose-veins-treatment-devices-market)
5. Yılmaz, S. Venöz Yetmezlik ve Varis Tedavisinde Güncel Yaklaşımlar. *Klinik Gelişim*, *2010*; 23, 57-62.
6. Nguyen, T.H. Evaluation of venousinsufficiency. *Semin Cutan Med Surg*., 2005; *24*, 162-174
7. Joseph N.B.A., FaizanThouseef, M., Devi, M.U. A multicenter review of epidemiology and management of varicose veins for national guidance. *Annals of Medicine and Surgery*, 2016; *8*, 21–27.
8. Hanrahan, L.M., Kechejian, G.J., Cordts, P.R. Patterns of venous insufficiency in patients with varicose veins. *Archives of Surgery*, 1991; *126 (6)*, 687-690.
9. Meissner, M.H., Gloviczki, P., Bergan, J. Primary chronic venous disorders. *Journal of Vascular Surgery*, 2007; *46 (Suppl)*, 54-67.
10. Beebe-Dimmer, J.L., Pfeifer, J.R., Engle, J.S. The epidemiology of chronic venous insufficiency and varicose veins. *Annals of Epidemiology*, 2005; *15 (3)*, 175-184.
11. Scherger, J. Varicose veins. Vice President for Primary Care. 2012;1-67.
12. Ascher, E., Jacob, T., Hingorani, A. Expression of molecular mediators of apoptosis and their

role in the pathogenesis of lower-extremity varicose veins. *Journal of vasculer Surgery*, 2001; *33 (5)*, 1080-1086.
13. Naoum, J.J., Hunter, G.C., Woodside, K.J. Current Advances in the Pathogenesis of Varicose Veins. *Journal of Surgical Research*, 2007; *141 (2)*, 311–316.
14. Sayın, A., Dayıoğlu, E. Kronik venöz yetersizlikte klinik ve CEAP Sınıflaması. *İ.Ü. Cerrahpaşa Tıp Fakültesi Sürekli Tıp Eğitimi Etkinlikleri Sempozyum Dizisi*, 2007; *56*, 39-46.
15. Köksal, C., Alsalehi, S., Kocamaz, Ö. Treatment of Chronic Venous Insufficiency (Kronik venöz yetmezlik tedavisi). *Kosuyolu Heart J.,* 2010; *13 (2)*, 28-33.
16. Beale, R.J., Gough, M.J. Treatment options for primary varicose veins--a review. *Eur J Vasc Endovasc Surg.* 2005; *30 (1)*, 83-95.
17. Bozkurt, K., Demirkılıç, U., Topcuoğlu, Ş. (2008). *Türk Kalp Damar Cerrahisi Derneği Periferik Arter ve Ven Hastalıkları Tedavi Klavuzu*. Ankara: Öncü Basımevi.
18. Venturi, M., Bonavina, L., Annoni, F. Biochemical assay of collagen and elastin in the normal and varicose vein wall. *Journal of Surgical Research*, 1996; *60 (1)*, 245-248.
19. Aunapuu, M., Arend, A. Histopathological changes and expression of adhesion molecules and laminin in varicose veins. *Vasa*, 2005; *34 (3)*, 170-175.
20. Kockx, M.M., Knaapen, M.W., Bortier, H.E. Vascular remodeling in varicose veins. *Angiology*, 1998; *49 (11)*, 871-877.
21. Sorescu, G.P., Song, H., Tressel, S.L. Bone morphogenic protein 4 produced in endothelial cells by oscillatory shear stress induces monocyte adhesion by stimulating reactive oxygen species production from a nox1 based NADPH oxidase. *Circulation Research*, 2004; *95 (8)*, 773-779.
22. Scheller, J., Chalaris, A., Schmidt-Arras, D. The pro and anti-inflammatory properties of thecytokine interleukin-6. *Biochimica et Biophysica Acta (BBA) Molecular Cell Research*, 2011; *1813 (5)*, 878-888.
23. Yasim, A., Kilinc, M.¸ Aral, M. Serum concentration of procoagulant, endothelial and oxidative stress markers in early primary varicose veins. *Phlebology*, 2008; *23 (1)*, 15–20.
24. Poredos, P., Spirkoska, A., Rucigaj, T. Do Blood Constituents in Varicose Veins Differ From the Systemic Blood Constituents? *European Journal of Vascular and Endovascular Surgery*, 2015; *50 (2)*, 250-256.
25. Castro-Ferreira, R., Cardoso, R., Leite-Moreira, A. The role of endothelial dysfunction and inflammation in chronic venous disease. *Annals of Vascular Surgery*, 2018; *46,* 380–393.
26. Lim, C.S., Davies, A.H. Pathogenesis of primary varicose veins. *British Journal of Surgery,* 2009; *96 (11),* 1231–1242.
27. Lim, C.S., Shalhoub, J., Gohel, M.S. Matrix metalloproteinases in vascular disease – a potential therapeutic target? *Current Vascular Pharmacology*, 2010; *8 (1)*,75-85.
28. Tiwary, S.K., Kumar, A., Mishra, S.P. Study of association of varicose veins and inflammation by inflammatory markers. *Phlebology*, 2020; *35 (9),* 679–685.
29. Somers, P., Knaapen, M. The histopathology of varicose vein disease. *Angiology*, 2006; *57*, 546-555.
30. Foudi, N., Gomez, I., Benyahia, C. Prostaglandin E2 receptor subtypes in human blood and vascular cells. *European Journal of Pharmacology*, 2012; *695 (1-3)*,1-6.
31. Damnjanovic, I., Stefanovic, N., Zlatkovic-Guberinic, S. Self Medication Practices among the patients with Chronic Venous Disease. *Farmacia*, 2020; *68 (2)*, 225-231
32. Belczak, S.Q., Sincos, I.R., Campos, W. Veno-active drugs for chronic venous disease: A randomized, double-blinf, placebo-controlled parallel-design trial. *Phlebology*, 2014; *29 (7)*, 454-460.
33. Ramelet, A.A., Boisseau, M.R., Allegra, C. Veno-active drugs in the management of chronic venous disease. An international consensus statement: current medical position, prospective views and final resolution. *Clin Hemorheol Microcirc*., 2005; *33 (4)*, 309-319.
34. Katsenis, K. Micronized purified flavonoid fraction (MPFF): A review of its pharmacological effects, therapeutic efficacy and benefits in the management of chronic venous insufficiency. *Current Vascular Pharmacology*, 2005; *3 (1)*, 1-9.

35. Bush, R., Comerota, A., Meissner, M. Recommendations for the medical management of chronic venous disease: The role of Micronized Purified Flavanoid Fraction (MPFF). *Phlebology,* 2017; *32(1_suppl)*, 3-19
36. Shoab, S.S., Porter, J.B., Scurr, J.H. Effect of oral micronized purified flavonoid fraction treatment on leukocyte adhesion molecule expression in patients with chronic venous disease: A pilot study. *Journal of Vascular Surgery*, 2000; *31 (3)*, 456-461.
37. Shoab, S.S., Scurr, J.H., Coleridge Smith, P.D. Plasma VGEF during oral therapy in patients with chronic venous disease with purified micronized fraction. A pilot study. *European Journal of Vascular and Endovascular Surgery*, 1999; *18 (4)*, 334-348.
38. Zheng, Y., Zhang, R., Shi, W. Metabolism and pharmacological activities of the natural health-benefiting compound diosmin. *Food & Function*, 2020; *11 (10)*, 8472-8492.
39. Feldo, M., Wójciak-Kosior, M., Sowa, I. Effect of Diosmin Administration in Patients with Chronic Venous Disorders on Selected Factors Affecting Angiogenesis. *Molecules*, 2019; *24 (18)*, 3316-3327.
40. Smetanina, M.A., Shevela, A.I., Gavrilov, K.A. The genetic constituent of varicose vein pathogenesis as a key for future treatment option development. *Vessel Plus*, 2021; *5 (19)*, 1-13.
41. Pietrzycka, A., Kózka, M., Urbanek, T. Effect of Micronized Purified Flavonoid Fraction Therapy on Endothelin-1 and TNF-α Levels in Relation to Antioxidant Enzyme Balance in the Peripheral Blood of Women with Varicose Veins. *Current Vascular Pharmacology*, 2015; *13 (6)*, 801-808.
42. Kauss, T., Moynet, D., Rambert, J. Rutoside decreases human macrophage-derived inflammatory mediators and improves clinical signs in adjuvant-induced arthritis. *Arthritis Research & Therapy*, 2008; *10 (1)*, 1-9.
43. Nikfarjam, B.A., Adineh, M., Hajiali, F. Treatment with Rutin—A Therapeutic Strategy for Neutrophil-Mediated Inflammatory and Autoimmune Diseases—Anti-inflammatory Effects of Rutin on Neutrophils. *Journal of Pharmacopuncture*, 2017; *20 (1)*, 52–56.
44. Benarroch, I.S., Brodsky, M., Rubinstein, A. Treatment of blood hyperviscosity with calcium dobesilate in patients with diabetic retinopathy. *Ophthalmic Research*, 1985; *17 (3)*, 131–138.
45. Suschek, C., Kolb, H., Kolb-Bachofen, V. Dobesilate enhances endothelial nitricoxide synthase-activity in macro – and microvascular endothelial cells. *British Journal of Pharmacology*, 1997; *122 (7)*, 1502–1508.
46. Coccheri, S., Mannello, F. Development and use of sulodexide in vascular diseases: Implications for treatment. *Drug Design, Development and Therapy*, 2014; *24 (8)*, 49–65.
47. Mannello, F., Medda, V., Ligi, D. Glycosaminoglycan sulodexide inhibition of MMP-9 gelatinase secretion and activity: Possible pharmacological role against collagen degradation in vascular chronic diseases. *Current Vascular Pharmacology*, 2013; *11 (3)*, 354–365.
48. Ligi, D., Mosti, G., Croce, L. Chronic venous disease—Part II: Proteolytic biomarkers in wound healing. *Biochimica et Biophysica Acta (BBA) – Molecular Basis of Disease*, 2016; *1862 (10)*, 1900-1908.
49. Midgett, C., Stitham, J., Martin, K. Prostacyclin receptor regulation – – from transcription to trafficking. *Current Molecular Medicine*, 2011; *11: (7)*, 517-528.
50. Alphonsus, C.S., Rodseth, R.N. The endothelial glycocalyx: A review of the vascular barrier. *Anaesthesia*, 2014; *69 (7)*, 777–784.
51. Gschwandtner, M., Strutzmann, E., Teixeira, M.M. Glycosaminoglycans are important mediators of neutrophilic inflammation in vivo. *Cytokine*, 2017; *91*, 65–73.