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STUDY OF ENDOTHELIAL INFLAMMATION IN PATIENTS WITH GOUT

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ABSTRACT

Background: Gout is a chronic inflammatory disease associated with hyperuricemia and systemic endothelial dysfunction. Recent studies have highlighted the role of endothelial and platelet activation in the development of vascular complications in gout.

Objective: To investigate endothelial inflammation in patients with gout by evaluating plasma P-selectin levels as a biomarker of endothelial and platelet activation.

Methods: A total of 65 patients with gout and 20 healthy controls were enrolled. Plasma P-selectin levels were measured using ELISA. Statistical analysis was performed using Student's t-test.

Results: Plasma P-selectin levels were significantly elevated in gout patients (mean 0.79 ± 0.63 ng/mL) compared to the control group (mean 0.08 ± 0.04 ng/mL, $p < 0.001$). Elevated levels were found in 58,4 % of patients, indicating widespread endothelial and/or platelet activation.

Conclusions: P-selectin may serve as a reliable biomarker for endothelial inflammation and thrombotic risk in gout. These findings support its potential role in disease monitoring and highlight the need for further research to explore its prognostic value.

Key words: Gout; Endothelial dysfunction; P-selectin; Hyperuricemia; Inflammation; Biomarkers; Thrombosis; ELISA.

INTRODUCTION

Gout is one of the most painful and acute conditions that affects mankind and one of the first defined diseases found in historical manuscripts. Over the centuries, gout has attracted the attention of many prominent physicians, including Egypt's Imhotep (27th century BCE) and Hippocrates (460-377 BCE) who spoke of the difference between gout and other arthritis and called it “the disease of the rich” due to the fact that it often occurs in individuals who consume a lot of alcohol and meat products [15]. Although gout is considered one of the oldest diseases, it has not lost its relevance nowadays and year by year new features of its course are defined and the rank of lesions expands day by day. Gout is an inflammatory disease of the joints, caused by the deposition of uric acid salts in the distal joints and peripheral tissues. It is promoted by an increase in the concentration of uric acid, which leads to the formation of uric acid salts and their deposition in different tissues of the body. Hyperuricemia has been shown in many studies to increase the risk of venous thrombosis, recurrent venous thromboembolism, pulmonary embolism, coronary artery atherothrombosis [11] or instability and mortality from cardiovascular diseases through endothelial dysfunction (inflammation of endothelial cells and proliferation of smooth muscle cells through ROS mechanisms) [13] and associated hypercoagulable mechanisms.

Endothelium is a thin layer of cells that covers blood vessels from the inside, and besides the barrier function, it performs many functions such as control of blood vessel tone, secretory, synthetic, metabolic, immune system and others [10]. As a result of many studies, it became known that the endothelium controls the blood clotting process, platelet adhesion and aggregation. Also, endothelium resists hemocoagulation and participates in fibrinolysis due to the thromboresistant surface of endothelial cells and tissue activator of plasminogen [1,3,10,14]. In addition, healthy endothelium plays an important role in monocyte adhesion, immunoregulation, and metabolism of circulating amines in the body [10]. One of the important functions of endothelial cells is to maintain a strong antithrombogenic activity [3,10,14]. Antithrombogenic activity includes antiaggregatory, anticoagulant and fibrinolytic activity. The antiaggregatory activity of the endothelium is due to the production of disaggregants such as prostacyclin and nitric oxide. Anticoagulant activity primarily consists of controlling the formation of thrombin, which performs various functions in hemostasis and coagulation. The endothelium is surrounded by a large number of glycosaminoglycans, which contribute to the activation of antithrombin III [10]. Antithrombin III performs 75%

of the anticoagulant function of blood plasma. More than 70% of thrombin interacts with antithrombin III and forms an inactive complex [3]. Antithrombin III deficiency indicates a high thrombogenic risk [3]. The fibrinolytic activity of the endothelium also plays an important role in the antithrombogenic activity of the blood vessel wall. Activation of fibrinolysis in the body occurs through extrinsic and intrinsic pathways [1]. Extrinsic activation of fibrinolysis occurs mainly due to tissue plasminogen activator, which is synthesized in the vascular endothelium. Disruption of the synthesis of tissue plasminogen activator, its decrease or disruption of its release from cells into the blood is one of the important factors in thrombus formation [1]. Procoagulant and proaggregant factors of the endothelium are also important in controlling the integrity of the vascular wall and the hemostasis system. These properties of the endothelium are achieved due to the von Willebrand factor, tissue thromboplastin, and other factors produced by it [3].

Endothelial dysfunction is often understood as an imbalance between endothelial-related factors [5]. Endothelial dysfunction develops in arterial hypertension [10], heart failure [4], and other pathologies. Many scientists have proposed that endothelial function indicators can be used as markers of arterial atherosclerosis, as well as gout [7,16]. A study by A.P. Rebrov et al. (2008) found that antithrombin III activity in gout patients before and after the test was slightly lower than in healthy subjects and patients with hypertension. It was found that the euglobulin-induced platelet aggregation time was prolonged in patients with gout. Also, the level of von Willebrand factor was significantly higher in the group of patients with gout. After treatment with allopurinol, these indicators were normalized in many patients. From the above, it can be seen that in patients with gout, the endothelium is damaged and endothelial dysfunction, that is, a decrease in the anticoagulant activity of the vascular wall, is noted [9]. In a scientific study by Kushnarenko N.N., endothelial dysfunction was detected in 64.6% of patients with gout. The functional state of the endothelium was explained by the activation of its ability to produce nitric oxide, an increase in the content of endothelin-1 in the blood serum, an increase in the activity of von Willebrand factor, a decrease in endothelium-dependent vasodilation, and thickening of the intima-media complex. Endothelial dysfunction was found to be associated with the course of gout, impaired structure and function of the coronary arteries [6]. Similar results were obtained by Magdeeva N.A et al, it was also noted in the study of, in addition to the above, the stiffness of the arteries increased and these changes varied depending on the amount of uric acid in the blood serum and the duration of the disease [8].

Materials and methods: The study involved 65 patients with gout and 20 healthy people. Laboratory tests were performed at the Tashkent Medical Academy's

Inter-Institutional Research Laboratory. For scientific research, blood was taken from the patient's vein in the amount of 3.0 ml. Each patient's blood was placed in a test tube with EDTA (ethylenediaminetetraacetate disodium salt) solution.

Immunoenzymatic studies were performed on a Mindray BA96A (China) spectrophotometer using Human Soluble P-Selectin (Hu sP-Sel/CD62) ELISA reagents. First, laboratory conditions were prepared, all reagents were prepared in advance to reach room temperature. Using a microtiter plate, each well was marked accordingly. Then, standard solutions were prepared, which were diluted to different concentrations and added to each well in an amount of 100µ. At the same time, serum samples were taken, each sample was separated in duplicate and added to the plate. The plate was placed in an incubator at 37°C for 2 hours. Then, excess liquid in the wells was removed. 100µ of biotin antibody was added to each well and covered with an adhesive strip. Then, it was placed in an incubator at 37 degrees for 1 hour. After incubation, it was brought to room temperature and the solution was gradually mixed until it became uniform in color. Then, the liquid in each well was removed and washed 3 times with a buffer wash solution (200 µl). After the last wash, the buffer solution residues in the wells were removed and the wells were dried thoroughly. Then, 200 µl of Horseradish peroxidase (HRP)-avidin solution was poured into each well, covered with a new strip and placed in an incubator at 37 degrees for 1 hour. Then, the wells were washed and dried 5 times. After washing and drying the wells, 90 µl of tetramethylbenzidine substrate was added to them and incubated for 15-30 minutes and stored in the dark. After incubation, 50 µl of stop solution was added to the wells and mixed thoroughly. At the final stage, the optical density of each well was read at a wavelength of 450 nm using a Mindray BA96A (China) spectrophotometer and the results were compared with a standard curve to accurately calculate the level of P-selectin. Using the results obtained, the concentration of P-selectin in the blood serum was estimated and biochemical changes in the hemostasis system of patients were analyzed.

Results

Plasma P-selectin levels were analyzed in 65 patients with gout and in 20 healthy people. According to the normative values (up to 0.30 ng/mL), the patients had significantly higher P-selectin levels compared to the control group.

In the patient group, the values ranged from 0.01 to 3.58 ng/mL. The mean value was 0.79 ± 0.63 ng/mL, while the control group ranged from 0.02 to 0.18 ng/mL, with a mean value of 0.08 ± 0.04 ng/mL.

In 38 of 65 patients (58,4%), P-selectin levels exceeded the upper limit of normal (0.30 ng/mL), whereas none of the controls demonstrated exceeding this threshold.

Statistical analysis using Student's t-criterion showed a significant difference between the mean values of the two groups ($p < 0.001$), indicating a significant increase in the level of P-selectin in patients compared to healthy individuals.

Discussion

The data obtained indicate a significant increase in the level of P-selectin in the examined patients compared to the control group. P-selectin is an adhesion molecule expressed by activated platelets and endothelial cells and plays an important role in the initiation of inflammatory and thrombotic processes.

The observed elevation may indicate platelet and/or endothelial activation in patients, which is consistent with the pathogenetic mechanisms of inflammation, vascular dysfunction and prothrombotic conditions. Particularly high levels of P-selectin (more than 1.0 ng/mL) were recorded in a number of patients, which may indicate a pronounced degree of pathologic process and potentially correlate with the severity of the clinical picture (this aspect requires further investigation).

Comparison with the control group confirms the diagnostic and prognostic significance of the P-selectin level, as well as its possible role in monitoring the course of the disease and assessing the risk of thrombosis. However, additional studies are needed to establish accurate risk thresholds and to assess the effect of therapy on the dynamics of this marker. So, the level of P-selectin in patients was significantly higher than in the control group ($p < 0.001$), which indicates its diagnostic significance in inflammatory and thrombotic conditions. In 58,4% of patients the concentration of P-selectin exceeded the upper limit of normal (0.30 ng/mL), while in the control group all values were within the reference range.

Conclusions. The obtained results confirm that P-selectin can serve as a biomarker of endothelial and platelet activation and be used as an auxiliary tool in clinical diagnostics and monitoring of diseases with inflammatory component. For a more accurate assessment of the prognostic value and dynamics of P-selectin depending on the clinical picture and therapy, further studies on an expanded sample with additional clinical and laboratory parameters are needed.

REFERENCES

1. Baluda V.P., Baluda M.V., Deyanov I.I., Tlepshukov I.K. Physiology of the hemostasis system. - Moscow: [Publisher not specified], 1995. - 243 c.

2. Barkagan, Z.S. Fundamentals of diagnostics of hemostasis disorders /Z. S. Barkagan, A.P. Momot. -M.: Newdiamed, 1999. - 217c

3. Bakhtiyarov, R.Z. Modern methods of endothelial function research / R.Z.Bakhtiyarov // Russian Cardiologic Journal. - 2004. -T. 2. -№46. -C. 76-79

4. Belenkov Y.N., Mareev V.Y., Aleev F.T. Endothelial dysfunction in heart failure: possibilities of therapy with IAPF // Cardiology. - 2001. - № 5. - C. 100-104..

5. Zateishchikova, A.A. Endothelial regulation of vascular tone: research methods and clinical significance / A.A. Zateishchikova. D.A. Zateishchikov// Cardiology. -1998. №9. -C -68-80.

6. Kushnarenko, Natalia Nikolaevna “Cardiovascular disorders in men with gout: clinical features, mechanisms of development, prediction” // Doctoral dissertation abstract.2012. Page 31

7. Lapshina N.A., Baranov A.A., Barsikova V.G. et al. Markers of vascular endothelial activation in gout // Therapeutic Archive. - 2005. - № 5. - C. 62-65.

8. Magdeeva Nadezhda Anatolievna. “Clinicodiagnostic significance of evdotelial dysfunction and artery hardness in podagroi patients in concentration with arterial hypertensisia” Ph.D. thesis abstract.2009. Page 21

9. Rebrov A.P. Features of endothelial dysfunction in patients with gout and its changes against the background of therapy / A.P. Rebrov, H.A. Magdeeva //.

10. Endothelial cells in physiology and in the pathophysiology of vascular disorders / Duglas B. Cines, Eleanor S. Pollak, Clayton A. Buck et al. // Blood. – 1998. – Vol. 91. –№10. –P. 3527- 3561.

11. Maruhashi, T., Hisatome, I., Kihara, Y. and Higashi, Y. (2018) Hyperuricemia and Endothelial Function: From Molecular Background to Clinical Perspectives. Atherosclerosis, 278, 226-231. https://doi.org/10.1016/j.atherosclerosis.2018.10.007

12. Nuki, G., and Simkin, P.A. 2006. A concise history of gout and hyperuricemia and their treatment. Arthritis Res. Ther. 8(Suppl. 1):S1.

13. Ozsu, S., Çoşar, A.M., Aksoy, H.B., et al. (2017) Prognostic Value of Uric Acid for Pulmonary Thromboembolism. Respiratory Care, 62, 1091-1096.https://doi.org/10.4187/respcare.05433

14. Sagripanti, A. Antithrombotic and prothrombotic activities of the vascular endothelium / A. Sagripanti, A. Carpi // Biomed. Pharmacother. – 2000. – Vol. 54. –№2. –P. 107-111

15. Zhang, W., Sun, K., Yang, Y., et al. (2009) Plasma Uric Acid and Hypertension in a Chinese Community: Prospective Study and Meta-Analysis. Clinical Chemistry, 55, 2026-2034. https://doi.org/10.1373/clinchem.2009.124891

16. Zoccali C., Maio R., Mallamaci F., et al. Uric acid and endothelial dysfunction in essential hypertension // Journal of the American Society of Nephrology. – 2006. – Vol. 17. – P. 1466–1471.