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STUDY OF MATRIX METALLOPROTEINASE ACTIVITY IN PATIENTS WITH AUTOIMMUNE THYROIDITIS

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One of the most important pathogenetic mechanisms of autoimmune thyroiditis (AIT) is the violation of immunological tolerance and the development of the autoimmune process, the markers of which are various biologically active substances, in particular, matrix metalloproteinases (MMP) of the extracellular matrix (ECM). MMPs play a crucial role in the development of pathological processes in these diseases, contributing to matrix degradation due to imbalance between the activity of enzymes and their inhibitors. The aim of the work was to study the activity of key metalloproteinases and the level of α2-macroglobulin in patients with autoimmune thyroiditis. The diagnosis of AIT was established based on the study of data on anamnesis, thyroid status, the results of ultrasound of TG, and the presence of antibodies to the thyroid-stimulating hormone receptor (TSH) in blood plasma. Patients were enrolled in 2 groups: group 1 – 74 patients with a manifest form of the disease; group 2 – 96 patients with a subclinical form of the disease. The study of matrix metalloprotein activity in the examined patients showed a statistically significant (P = 0.015) increase in MMP-3 and MMP-7 activity in patients with AIT compared to the corresponding parameters in persons of the control group. Thus, levels of MMP-3 and 7 were in the group of patients, respectively 56 (51.0; 59.0) and 4.6 (4.3; 5.2) ng/ml, in control 23.0 (16.0; 26.0) and 3.6 (3.4; 4.1) ng/ml, respectively.

K e y w o r d s: autoimmune thyroiditis, matrix metalloproteinases, extracellular matrix, α2-macroglobulin.

Hashimoto's thyroiditis (HT), being the most common type of autoimmune thyroiditis (AIT), acts as the leading cause of hypothyroidism. To date, it has been found that the development of the disease is due to a combination of genetic factors and environmental factors [1,2]. One of the most important pathogenetic mechanisms of AIT is the violation of immunological tolerance and the development of the autoimmune process, the markers of which are various biologically active substances, in particular, matrix metalloproteinases (MMP) of the extracellular matrix (ECM) [3]. These enzymes are a group of 28 zinc-dependent endopeptidases involved in various links in the realization of the immune response - cell proliferation, migration and differentiation. MMPs are also involved in the mechanisms of angiogenesis and apoptosis [4].

Depending on the structural and functional features, as well as substrate specificity with respect to the components of ECM, MMPs are divided into several subfamilies. Initially, 4 subfamilies were described: collagenases (MMP-1, MMP-8, etc.), gelatinases/specific collagenases of collagen type IV (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-10) and matrilysins (MMP-7 and MMP-26) [4]. Currently, 6 large groups are isolated within the MMP family in accordance with structural features and types of substrates: 1) collagenases, 2) gelatinases, 3) stromelysins, 4) matrilysins, 5) membrane-type MMP, 6) all other MMPs [5,6].

Regulation of MMP activity is multilevel and is carried out by various mechanisms: at the level of expression of genes encoding these enzymes, by secretion and changes in MMP localization, due to activation of zymogens during cleavage of the prodomain, by suppressing activity by endogenous inhibitors (in particular, tissue MMP inhibitors), during enzyme degradation.

Regulation of MMP gene expression is realized at transcriptional and post-transcriptional levels, the first being due to the interaction of transcription factors with responsive elements in the promoter of MMP genes, as well as due to epigenetic mechanisms – by methylation of MMP gene promoters and due to changes in chromatin structure [5, 6]. The above mechanisms provide induction of gene

expression in response to various stimuli causing the development of various pathological processes in the body: inflammation, hypoxia, oxidative stress, hormonal shifts, changes in hemodynamics, changes szin cytokine activity, growth factors [7]. It has been shown that MMP expression level can increase in comparison with basal one by more than 100 times [8].

MMPs play a crucial role in the development of pathological processes in these diseases, contributing to matrix degradation due to imbalance between the activity of enzymes and their specific inhibitors. Tissue MMP inhibitors (TIMP) play an important role in maintaining a fine balance between the formation and utilization of ECM, so changes in their concentrations can serve as signs of dysfunction and a precursor to the development of pathological processes [8, 9].

To date, the clinical significance of MMP-2 and MMP-9 in neoplasms, autoimmune and chronic inflammatory diseases has been demonstrated [9], and it has been established that the increased activity of these enzymes is a marker of a number of oncological diseases (for example, multiple myeloma) [10, 11].

Destruction of TG cells in autoimmune pathology is due to the interaction of genetic and environmental factors. Dysfunction of immunoregulatory mechanisms and imbalance of cytokine synthesis are considered important trigger factors. In this regard, it is of interest to study the role of immunoregulatory proteins in the processes of inflammation, cell proliferation and apoptosis [12]. One such biologically active substance is potentially immunogenic alpha-2-macroglobulin (α2-MG). It was established that the concentration of complex α2-MG with IgG is increased in inflammatory processes and autoimmune diseases. It should also be noted that α2-MG can inhibit the activity of certain metalloproteinases, in particular MMP-3 [13]. In the literature, there is practically no data on the role of such proteins in the formation of autoimmune thyroopathies, in this regard, it seems interesting to study the level of α2-MG in AIT.

The available literature provides separate information on the role of MMP and α2-MG in autoimmune diseases of the thyroid gland (TG), however, these data are single and not systematized. There is no information on MMP levels and α2-MG concentration in the blood plasma of patients with AIT, there is no data on the association of their activity in the peripheral blood of patients with thyroiditis with changes in the function of TG and clinical manifestations of the disease.

The above confirms the relevance of the study of MMP activity level and α2-MG concentration with autoimmune thyroid diseases.

The aim of the work was to study the activity of key metalloproteinases and the level of α2-MG in patients with autoimmune thyroiditis.

Materials and Methods

In 2014-2020, a single-center open prospective non-randomized study was conducted, which conducted a complex examination of 170 patients with autoimmune thyroiditis, 64 men and 106 women, aged 18 to 64 years. The control group was 65 people without TG diseases and autoimmune pathology aged 20 to 65 years, of which 26 were men and 39 were women. The present study was approved by the university ethics committee (Ref.no: AMU/IEC/№12/ 07.02.2020)

The diagnosis of AIT was established based on the study of data on anamnesis, thyroid status, the results of ultrasound of TG, and the presence of antibodies to the thyroid-stimulating hormone receptor (TSH) in blood plasma.

Patients were enrolled in 2 groups: group 1 – 74 patients with a manifest form of the disease; group 2 – 96 patients with a subclinical form of the disease.

Diagnostics of the manifest form of AIT were carried out on the basis of the clinical picture of the disease: patient's complaints (increase in body weight, permanent feeling of cold, fatigue caused by metabolic disorders, yellowness of the skin, myxedema, shortness of breath, hearing impairment, voice changes, drowsiness, impaired cognitive processes, increased emotionality, pain behind the sternum and in the heart area, decreased heart rate, dyspepsia (tendency to constipation, diarrhea), loss of sensitivity of the limbs, thinning and loss of hair, violation or cessation of menstruation. From laboratory results, an increase in the level of thyroid-stimulating hormone (TSH) in peripheral blood, a decrease in the concentrations of the hormones triiodothyronine (T3) and thyroxine (T4), as well as the presence of elevated titers of antibodies to TSH were noted.

The diagnosis of the subclinical form of AT was made on the basis of an increase in TSH level in the background of normal levels of hormones T3 and T4 in blood plasma. This form of the disease was characterized, as a rule, by an erased clinical picture.

In patients of all groups, the activity of MMP-3 and 7 was determined by solid-phase enzyme immunoassay, "Biosource" kits (Belgium) were used to determine the level of MMP-3, and "Quantikine" reagents (USA) were used to evaluate the activity of MMP-7.

The level of α2-MG in patients with AIT was evaluated by an immunoturbometric method, the principle of which is based on the reaction between α2-MG and polyclonal antiserum in the presence of polyethylene glycol, using reagents from "Sentinel" (Italy).

Statistical analysis of the study results was performed using the Statsoft STATISTICA 10 software package. To represent quantitative parameters, medians, upper and lower quartiles were calculated. Inter-group quantitative comparisons were made using the Mann-Whitney rank non-parametric test, taking into account the difference in the distribution of the analyzed indicators from the normal one. Qualitative characteristics were evaluated as a percentage.

The differences were considered statistically significant when $P \leq 0.05$.

Results and Discussion

The study of matrix metalloprotein activity in the examined patients showed a statistically significant ($P = 0.015$) increase in MMP-3 and MMP-7 activity in patients with AIT compared to the corresponding parameters in persons of the control group. Thus, levels of MMP-3 and 7 were respectively 56 (51.0; 59.0) and 4.6 (4.3; 5.2) ng/ml, in the control group 23.0 (16.0; 26.0) and 3.6 (3.4; 4.1) ng/ml, respectively (Table).

A comparison of the study parameters in different clinical forms of AIT showed that patients with manifest form of AIT had a MMP-3 level of 59.0 (56.0; 65.0) ng/ml and was significantly higher ($P = 0.023$) than the corresponding index in patients with a subclinical form of the disease – 52.1 (48.0; 56.5) ng/ml.

At the same time, there were no statistically significant intergroup differences in the MMP-7 indicator in patients with different forms of AIT. At the same time, in both subclinical and manifest forms of the disease, activity levels MMP-3 and 7 were significantly higher than levels of similar indicators in the control group.

As can be seen from Table, in patients with AIT, the concentration of α2-MG was statistically significantly higher ($P = 0.008$) compared to the level in the control group, the values of the indicator were 2.6 (2.4; 2.9) and in the control group were 1.6 (1.4; 2.0) g/l.

α2-MG level in patients with manifest forms of AIT is significantly ($P = 0.031$) higher than in patients with the subclinical form of the disease, the values of α2-MG were 2.8 (2.5; 3.3) g/l and 2.5 (2.3; 2.9) g/l in manifest form and subclinical form of AIT, respectively. In both forms of the disease, concentrations of α2-MG were statistically significantly higher than control group results $P < 0.001$ for both comparisons).

The obtained results indicated that the degree of increase in MMP activity in the blood serum of patients with AIT can vary significantly. As can be seen from Fig. 1, MMP-3 activity at AIT is higher than in the control group by 143.5%, MMP-7 activity exceeds the corresponding level in the control group by 27.7%, and the α2-MG level is higher than in the control group results by 62.5%.

Differences in MMP-3 and α2-MG activity in groups of patients with different clinical forms of AIT were less pronounced. As can be seen from Fig. 2, median MMP-3 activity in patients with the manifest form of the disease was higher than in pa-

Matrix metalloproteinases (MMP) activity and α2-macroglobulin level in patients with autoimmune thyroiditis in AIT, Me (Q25; Q75)

Indicators	Control group ($n = 65$)	AIT ($n = 170$)	Subclinical form ($n = 96$)	Manifest form ($n = 74$)
MMP-3, ng/ml	23.0 (16.0; 26.0)	56.0* (51.0; 59.0)	52.1* (48.0; 56.5)	59.0*# (56.0; 65.0)
MMP-7, ng/ml	3.6 (3.4; 4.1)	4.6* (4,3; 5.2)	4.6* (4.2; 4.9)	4.7* (4.5; 5.4)
α2-macroglobulin, g/l	1.6 (1.4; 2.0)	2.6* (2.4; 2.9)	2.5* (2.3; 2.9)	2.8*# (2.5; 3.3)

Note *differences are statistically significant (at $P < 0.05$) when compared with the corresponding control group indicators; #differences are statistically significant (at $P < 0.05$) when compared with corresponding indicators of a group of patients with a subclinical form of AIT

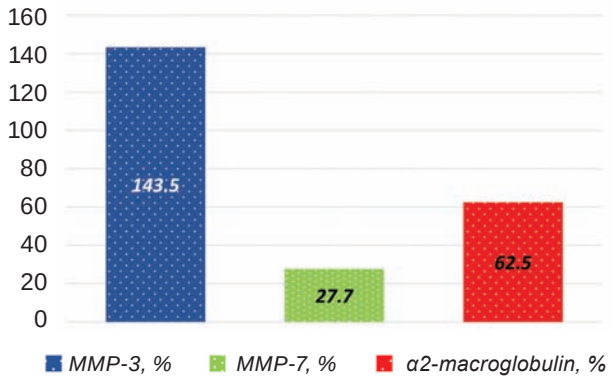

Fig. 1. The degree of increase in matrix metalloproteinases (MMP) and α2-macroglobulin level in patients with AIT compare to the corresponding values of the control group

tients with subclinical AIT by 13.2%, and the level of α2-MG in manifest form of AIT was 12% higher than in subclinical AIT.

The AIT multifactorial pathogenesis currently recognized by many authors demonstrates the need for an up-to-date algorithm for diagnosing immunological disorders and changes in the body's homeostasis, including various biochemical indicators [1, 2]. Recently, the role of matrix metalloproteinases has been actively studied in autoimmune diseases. MMPs are known to play an important role in the exchange of connective tissue proteins, in the processes of normal development and remodeling of the cell matrix, embryogenesis, tissue repair, neoangiogenesis, as well as in the processes of tumor transformation and metastasis [3, 4]. Studying the activity of this family of enzymes allows us to deepen ideas about the pathogenesis of AIT, as well as substantiate potential therapeutic targets for the development of promising therapies for the disease.

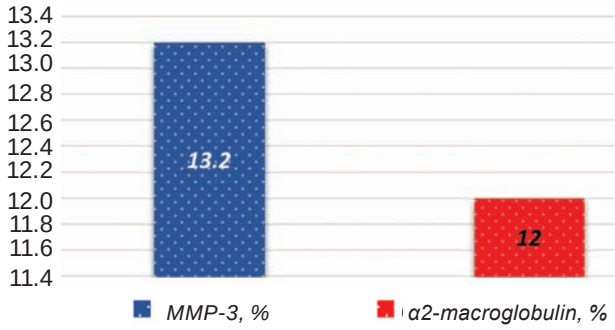

Fig. 2. The degree of increase in the studied parameters in patients with the manifest form of AIT relative to the corresponding levels in patients with the subclinical form of AIT

It is known that in the initial stage of development of Graves' disease (GD) inflammatory reaction prevails, which is replaced by intensive remodeling of connective tissue, accumulation of macromolecules by ECM and fibrosis [14, 15]. It is shown that GD therapy with glucocorticoids is accompanied by a decrease in blood plasma MMP-9 level, although the MMP-2 level does not change [16].

It has been demonstrated that MMP-9 concentrations in patients with ophthalmopathy in GD are significantly higher than in patients with GD without ophthalmopathy. It was also found that the concentration of MMP-9 decreases after the course of corticosteroid therapy, while the concentration of MMP-2 does not change [16].

Other authors have described an increase in TIMP-1 production by orbital fibroblasts obtained from ophthalmopathy patients at GD after their stimulation with interleukins-1β [17]. Other authors demonstrated that quercetin, a flavonoid phytoestrogen, suppresses inflammation in vitro and accumulation of extracellular matrix components in the medium with orbital fibroblasts after stimulation with proinflammatory cytokines also, the quercetin suppresses the synthesis of MMP-2 and MMP-9 fibroblasts, thereby preventing the development of chronic fibrosis in patients with GD [18].

At present, it has not been established which metalloproteinase, MMP-2 or MMP-9 determines the phenotype of the disease and the presence or absence of orbitopathy. The aim of the study by Kapelko-Słowik K. et al. (2018) was an assessment of serum concentrations of MMP-2 and MMP-9, as well as their inhibitors of TIMP-1 and TIMP-2 in patients with GD occurring with and without ophthalmopathy, in the active phase and after successful therapy. It was demonstrated that concentrations of all examined cytokines, as well as the ratio of MMP-9/TIMP-1, were significantly increased in all GD patients compared to the control [19]. Notably, MMP-9 concentrations were significantly higher in patients with ophthalmological manifestations of GD than in patients with GD without orbitopathy.

The above data may indicate that MMP-3 is a marker of disease activity and the process of thyroid tissue damage in AIT, and therefore may play a role in degenerative processes in other autoimmune diseases. In the body, there is a biological mechanism for limiting tissue proteolysis caused by active

MMPs. One of the substances inhibiting metalloproteinases may be macroglobulins, in particular α2-MG. So, in the study of Gorodetskaya I.V., Gusakova E.A. (2015), the authors determined the course features of anxiety stage in stress response (1 h after stress modeled by swimming rats in a cell for 1 h), with stimulation of trypsin-like activity (TpA) in the liver and in animal blood shown. It was found that experimental hypothyroidism in rats (25 mg/kg mercazolil, 20 days), causing a decrease in TpA, determines a more pronounced stimulation of proteolysis in the anxiety stage, prevents its leveling into the resistance stage, and in the depletion stage contributes to its excessive activation. It has been shown that the dependence of changes in the proteinase/inhibitor system under stress on the level of iodine-containing thyroid hormones in the blood is associated with their effect on the activity of endogenous proteinase inhibitors (a1-antitrypsin and a2-macroglobulin) and the permeability of lysosomal membranes [20].

The concentration of α2-MG usually depends on the MMP levels in tissues and extracellular fluid, thus limiting proteolytic activity in the focal cell space of the TG. High MMP-3 activity stimulates increased levels of α2-MG, as confirmed by the results of the present study. It was found that the concentration of this substance in patients with AIT is statistically significantly higher than the level of such in the control group. Also, the value of this indicator in patients with a manifest form of AIT was higher than that in patients with a subclinical form of the disease.

Conclusions. The results we obtained showed a significant increase in MMP-3 activity in patients with AIT compare to the control group. MMP-3 activity was significantly higher in patients with the manifest form of AIT than in patients with the subclinical form. Further research aimed at studying the trigger mechanisms of the autoimmune response and the role of individual signaling pathways in the pathogenesis of AIT, including the assessment of the activity of MMP and regulatory proteins, in particular α2-MG, allows identifying molecules that can be considered as markers and targets of therapeutic effects aimed at stopping autoimmune reactions.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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АКТИВНІСТЬ МАТРИКСНИХ МЕТАЛОПРОТЕЇНАЗ У ХВОРИХ НА АВТОІМУННИЙ ТИРЕОЇДИТ

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Одним із найважливіших патогенетичних механізмів автоімунного тиреоїдиту (AIT) є порушення імунологічної толерантності та розвиток автоімунного процесу, маркерами якого, зокрема, є матриксні металопротеїнази (ММП) позаклітинного матриксу. ММП грають вирішальну роль у розвитку автоімунного тиреоїдиту, а саме, сприяють деградації матриксу внаслідок дисбалансу між активністю ензимів та їхніх інгібіторів. Метою роботи було визначення активності ключових металопротеїназ та рівня макроглобуліну α2-МГ у плазмі крові хворих на автоімунний тиреоїдит. Діагноз AIT встановлювали на підставі даних анамнезу, тиреоїдного статусу, результатів УЗД ЩЗ, наявності антитіл до рецептора тиреотропного гормону (ТТГ) у плазмі крові. Пацієнтів було поділено на 2 групи: 1 – 74 пацієнти з маніфестною формою захворювання; 2 – 96 контрольних пацієнтів із субклінічною формою захворювання. Встановлено, що у пацієнтів з AIT рівень активності ММП-3 та 7 складав 56 (51,0; 59,0) та 4,6 (4,3; 5,2) нг/мл, відповідно, та у контрольній групі 23,0 (16,0; 26,0) та 3,6 (3,4; 4,1) нг/мл, відповідно. Таким чином, показано, що у плазмі крові пацієнтів із автоімунним тиреоїдитом спостерігалося достовірне підвищення активності матриксних металопротеїназ.

Ключові слова: матриксні металопротеїнази, автоімунний тиреоїдит, позаклітинний матрикс, α2-макроглобулін.

References

1. Hamous J, Dvořák J, Bušovský I, Šnajdr M. Hashimotos thyroiditis. *Rozhl Chir.* 2021; 100(3): 110-112.
2. Ralli M, Angeletti D, Fiore M, D'Aguanno V, Lambiase A, Artico M, de Vincentiis M, Greco A. Hashimoto's thyroiditis: An update on pathogenic mechanisms, diagnostic protocols, therapeutic strategies, and potential malignant transformation. *Autoimmun Rev.* 2020; 19(10): 102649.

3. Nagasaki T, Nagata Y, Wakita Y, Yamada S, Goto H, Imanishi Y, Onoda N, Emoto M, Inaba M. Association of heterogeneity of the thyroid gland with matrix metalloproteinase-3 in rheumatoid arthritis patients with Hashimoto's thyroiditis. *Minerva Endocrinol*. 2018; 43(4): 398-405.
4. Shadrina AS, Plieva YZ, Kushlinskiy DN, Morozov AA, Filipenko ML, Chang VL, Kushlinskii NE. Classification, regulation of activity, and genetic polymorphism of matrix metalloproteinases in health and disease. *Almanac Clin Med*. 2017; 45(4): 266-279. (In Russian).
5. Li J, Wang JM, Liu YH, Zhang Z, Han N, Wang JY, Xue SH, Wang P. Effect of microRNA-106b on the invasion and proliferation of trophoblasts through targeting MMP-2. *Zhonghua Fu Chan Ke Za Zhi*. 2017; 52(5): 327-332.
6. Yang L, Song X, Zhu J, Li M, Ji Y, Wu F, Chen Y, Cui X, Hu J, Wang L, Cao Y, Wei Y, Zhang W, Li F. Tumor suppressor microRNA-34a inhibits cell migration and invasion by targeting MMP-2/MMP-9/FNDC3B in esophageal squamous cell carcinoma. *Int J Oncol*. 2017; 51(1): 378-388.
7. Wortman BG, Bosse T, Nout RA, Lutgens LCHW, van der Steen-Banasik EM, Westerveld H, van den Berg H, Slot A, De Winter KAJ, Verhoeven-Adema KW, Smit VTHBM, Creutzberg CL, PORTEC Study Group. Molecular-integrated risk profile to determine adjuvant radiotherapy in endometrial cancer: Evaluation of the pilot phase of the PORTEC-4a trial. *Gynecol Oncol*. 2018; 151(1): 69-75.
8. Raeeszadeh-Sarmazdeh M, Do LD, Hritz BG. Metalloproteinases and Their Inhibitors: Potential for the Development of New Therapeutics. *Cells*. 2020; 9(5): 1313.
9. Young D, Das N, Anowai A, Dufour A. Matrix Metalloproteases as Influencers of the Cells' Social Media. *Int J Mol Sci*. 2019; 20(16): 3847.
10. Shay G, Tauro M, Loiodice F, Tortorella P, Sullivan DM, Hazlehurst LA, Lynch CC. Selective inhibition of matrix metalloproteinase-2 in the multiple myeloma-bone microenvironment. *Oncotarget*. 2017; 8(26): 41827-41840.
11. Sun W, Liu DB, Li WW, Zhang LL, Long GX, Wang JF, Mei Q, Hu GQ. Interleukin-6 promotes the migration and invasion of nasopharyngeal carcinoma cell lines and upregulates the expression of MMP-2 and MMP-9. *Int J Oncol*. 2014; 44(5): 1551-1560.
12. Singh D, Srivastava SK, Chaudhuri TK, Upadhyay G. Multifaceted role of matrix metalloproteinases (MMPs). *Front Mol Biosci*. 2015; 2: 19.
13. Rodríguez-Calvo R, Ferrán B, Alonso J, Martí-Pàmies I, Aguiló S, Calvayrac O, Rodríguez C, Martínez-González J. NR4A receptors up-regulate the antiproteinase alpha-2 macroglobulin (A2M) and modulate MMP-2 and MMP-9 in vascular smooth muscle cells. *Thromb Haemost*. 2015; 113(6): 1323-1334.
14. Tsai CC, Wu SB, Chang PC, Wei YH. Alteration of Connective Tissue Growth Factor (CTGF) Expression in Orbital Fibroblasts from Patients with Graves' Ophthalmopathy. *PLoS One*. 2015; 10(11): e0143514.
15. Slowik M, Urbaniak-Kujda D, Bohdanowicz-Pawlak A, Kapelko-Slowik K, Dybko J, Wolowiec D, Jazwiec B, Daroszewski J. CD8+CD28-lymphocytes in peripheral blood and serum concentrations of soluble interleukin 6 receptor are increased in patients with Graves' orbitopathy and correlate with disease activity. *Endocr Res*. 2012; 37(2): 89-95.
16. Beroun A, Mitra S, Michaluk P, Pijet B, Stefaniuk M, Kaczmarek L. MMPs in learning and memory and neuropsychiatric disorders. *Cell Mol Life Sci*. 2019; 76(16): 3207-3228.
17. Nawaz M, Shah N, Zanetti BR, Maugeri M, Silvestre RN, Fatima F, Neder L, Valadi H. Extracellular Vesicles and Matrix Remodeling Enzymes: The Emerging Roles in Extracellular Matrix Remodeling, Progression of Diseases and Tissue Repair. *Cells*. 2018; 7(10): 167.
18. Yoon JS, Chae MK, Lee SY, Lee EJ. Anti-inflammatory effect of quercetin in a whole orbital tissue culture of Graves' orbitopathy. *Br J Ophthalmol*. 2012; 96(8): 1117-1121.
19. Kapelko-Słowik K, Słowik M, Szaliński M, Dybko J, Wołowiec D, Prajs I, Bohdanowicz-Pawlak A, Biernat M, Urbaniak-Kujda D. Elevated serum concentrations of metalloproteinases (MMP-2, MMP-9) and their inhibitors (TIMP-1, TIMP-2) in patients with Graves' orbitopathy. *Adv Clin Exp Med*. 2018; 27(1): 99-103.
20. Gorodetskaya IV, Gusakova EA. Effect of the thyroid status on the proteinases/inhibitors system under stress. *Biomed Khim*. 2015; 61(3): 389-393. (In Russian).