REPUBLIC OF AZERBAIJAN

On the rights of the manuscript

ABSTRACT

of the dissertation presented for the degree of Doctor of Philosophy

ROLE OF ANTIMICROBIAL PEPTIDES AND CYTOKINES IN THE DIAGNOSIS OF RHEUMATOID ARTHRITIS

Speciality:	2406.02 – Biochemistry
Field of science:	Biology
Applicant:	**Asmaya Saftar Huseynova**

Baku – 2023

The dissertation was performed at the Educational-Clinical Biochemistry Laboratory and Department of Biochemistry of the Azerbaijan Medical University.

Scientific supervisor:	Doctor of Biological Sciences, Professor, **Arif Mustafa Efendiyev**
Official opponents:	Doctor of Biological Sciences, Associate Professor **Namig Oruj Gudratov**
	Doctor of Philosophy in Biology, Associate Professor **Farida Zohrab Mammadova**
	Doctor of Philosophy in Biology **Tarana Farahim Movsumova**

Dissertation Council FD 1.08 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at the Institute of Physiology named after Academician Abdulla Garayev of the Ministry of Science and Education of Azerbaijan Republic.

Chairman of the Dissertation Council:	Doctor of Biological Sciences, Professor **Ulduz Fayizi Hashimova**
Scientific secretary of the Dissertation Council:	Doctor of Philosophy in Biology, Associate Professor **Yegana Ogtay Bayramova**
Chairman of the scientific seminar:	Doctor of Biological Sciences, Professor **Adalat Nurulla Farajov**

GENERAL DESCRIPTION OF WORK

Actuality of the theme and the degree of research. Rheumatoid arthritis (RA) remains a prominent medical and social concern, attracting significant research attention over the years. This condition, comprising 10% of rheumatic diseases, is estimated to occur in 1% of the general population. The lack of timely diagnosis and appropriate therapy for RA can lead to the progressive destruction of joints, reducing patients' quality of life and increasing disability. Approximately 50% of patients experience disability within 5 years of RA onset, and up to 90% within 20 years[1,2].

The application of treatment methods in patients with rheumatoid arthritis is crucial in preserving their working capacity, reducing disability, increasing the quality of life, and significantly improving the disease prognosis. However, achieving maximum positive treatment outcomes requires early diagnosis of the patient, which remains an urgent challenge in medicine.

The modern laboratory diagnostics of RA is based on the determination of a wide range of biomarkers (autoantibodies, cytokines, acute-phase proteins of inflammation, markers of endothelial activation, lymphocyte subpopulations, bone and cartilage metabolism products, genetic markers, and etc.).

In recent years, there has been a particular focus on the study of cytokines, which play a pivotal role in the pathogenesis of rheumatoid arthritis (RA). The analysis of cytokine effects and their expression levels in various pathologies has proven valuable in the diagnosis, prophylaxis, therapy, and prognosis of the disease. The investigation of the role of cytokines in the chronic autoimmune inflammatory processes that are characteristic of RA is also an essential issue related

[1] Калюта, Т.Ю., Артанова, Е.Л., Кац, Я.А. Дебют ревматоидного артрита в старческом возрасте // Фундаментальные исследования, – 2012. 5, – p.36-43.
[2] Aletaha, D. Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative / D.Aletaha, T.Neogi, A.J.Silman [et al.] // Arthritis Rheum. – 2010. 62, – p.2569-81. doi: 10.1002/art.27584

to the development of a new generation of drugs, namely genetically modified biological preparations (GMBPs) for therapeutic purposes.

The study of certain representatives of antimicrobial peptides (AMPs) is of great interest in the realm of diagnostic and prognostic biomarkers for RA. AMPs are a heterogeneous group of molecules consisting of 12-15 amino acids, which participate in the development of innate and acquired immunity and possess various physical-chemical and biological properties. The general characteristic of AMPs is the ability to disrupt the cell membrane of prokaryotes, causing cell death[3].

Thus, the investigation of new biomarkers with high diagnostic sensitivity for RA is one of the critical issues in rheumatology. The complex and comparative determination of these biomarkers provides objective information about the nature of the resulting immunopathological disruptions, ensuring early diagnosis and adequate treatment methods.

The object of the research. Blood samples collected from patients diagnosed with rheumatoid arthritis were used in the study.

Aims of the study. The aim of the study was to investigate the involvement of certain AMPs and cytokines in the pathogenesis of RA and their potential diagnostic significance in the early identification of the disease.

Objectives of the study:

1. Evaluation of disease activity markers (CRP, RF, ESR) in the blood of healthy individuals and patients with RA included in the research cohort;

2. Investigation of calcium-phosphorus exchange indicators (calcium, phosphorus) and calcium-regulating hormones (parathyroid hormone, calcitonin, vitamin D) in the blood of healthy individuals and patients with RA included in the research cohort;

3. Evaluation of biochemical markers of bone metabolism (osteocalcin, osteopontin, alkaline phosphatase, free oxyproline) in the blood of healthy individuals and patients with RA included in the research cohort;

[3] Kopec-Medrek, M., Widuchowska, M., Kucharz E. Calprotectin in rheumatoid diseases: a review // Reumatologia, – 2016. 54(6), – p. 306-309

4. Investigation of cytokines (TNF-α, IL-2, IL-6, IL-8, IL-10) and AMPs (calprotectin, cathelicidin) in the blood of healthy individuals and patients with RA included in the research cohort, with the aim of determining their sensitivity and specificity to evaluate their diagnostic informativeness;

5. Assessment of correlation relationships between cytokines and AMPs with disease activity markers and bone metabolism biomarkers.

The research methods. In the research work, biochemical and immunoassay analysis methods were used.

The main provisions of the dissertation submitted for defense:

1. In patients with RA, hypovitaminosis D and hypocalcemia are observed, which leads to the development of secondary hyperparathyroidism. The vitamin D/parathormone metabolism and cytokine profile changes play a significant role in the disruption of bone metabolism and the development of osteoporosis in RA.

2. The impact of the serological variant of RA on the level of cytokines, AMPs, and bone metabolism indicators is investigated: more changes are revealed in seropositive RA compared to seronegative RA.

3. The involvement of pro-flogogen and anti-flogogen cytokines in the blood of RA patients increases compared to healthy individuals. These changes in the cytokine profile indicate their significant role in the pathogenetic mechanisms of the disease and condition the practical application in disease diagnosis.

4. The blood level of calprotectin increases in both patient groups regardless of the presence of RF compared to healthy individuals and correlates with disease activity markers. The obtained results indicate the possibility of using calprotectin in the evaluation of RA activity alongside CRP and other inflammation biomarkers.

Scientific novelty of the research: The levels of cytokines, AMPs, and bone metabolism markers in the blood serum were comprehensively studied during RA, and their interrelationships were revealed. The influence of different serological variants of the disease (seropositive and seronegative) on the levels of the investigated

biomarkers were examined. The role of cytokines and AMPs in the immunopathogenesis of RA was investigated, and their diagnostic significance was evaluated.

Practical significance of the research. The study of cytokines and AMPs yields significant scientific and practical importance in terms of obtaining information about the character of immunopathological disturbances, as well as the application of these indicators in the early diagnosis and monitoring of RA.

Approbation and application. The results of research work have been discussed at a series of international conferences. These include: International conference dedicated to the 85th anniversary of the birth of Prof. R.A. Asgarov (Baku, 2018); International scientific-practical conference "Actual Problems of Medicine" dedicated to the 100th anniversary of the Azerbaijan Democratic Republic (Baku, 2018); Scientific-practical conference dedicated to the 95th anniversary of Prof. T.A. Tagizade (Baku, 2018); International conference on "Modern molecular-biochemical markers in clinical and experimental medicine" (Czech Republic, 2019); 1st International conference on "Immunopathology Diseases" (Baku, 2019); 15th International European Conference on Mathematics, Engineering, Natural Medical Sciences (Adana, 2021).

Application of the obtained results. The results of the dissertation work are applied in the teaching process of the Department of Biological Chemistry at Azerbaijan Medical University.

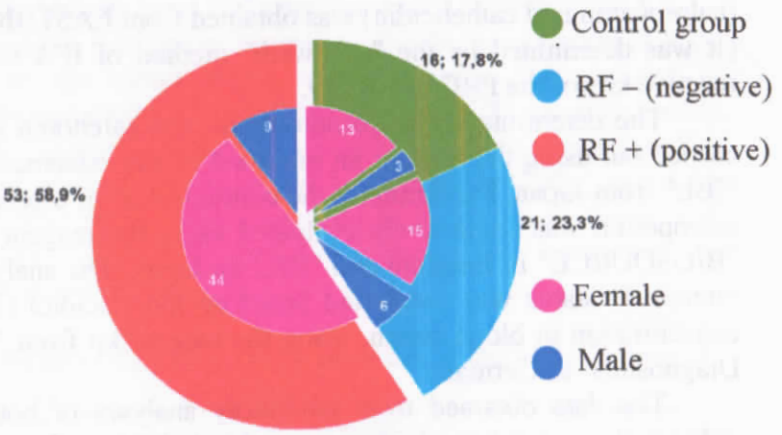
Name of the organization where the dissertation is performed. Educational-Clinical Biochemistry Laboratory of Department of Biological Chemistry, Azerbaijan Medical University.

Volume and structure of the dissertation. Dissertation written in Azerbaijani language and spans 138 pages (total 211943 characters) printed using a computer, "Table of Contents" (4253 characters), "Introduction" (11138 characters), "Literature review" (48854 characters), "Materials and methods" (13189 characters). , "Findings of personal research" (64144 marks), "Discussion of research results" (29180 marks), "Conclusions" (1758 marks), "Practical recommendations" (531 marks), "List of used literature" (38426 marks), " List of abbreviations and personal signs" (470 signs) consists

of sections. The dissertation work includes 23 tables and 18 pictures. The bibliography includes 227 sources, 11 of which are Azerbaijani, 1 Turkish, 72 Russian, and 143 works of other foreign scientists.

MATERIALS AND METHODS OF THE RESEARCH

74 individuals diagnosed with rheumatoid arthritis (RA) and ranging in age from 27 to 71 were assessed at the Teaching-Therapeutic Clinic and Clinical Biochemistry Laboratory of Azerbaijan Medical University. The average age of the RA patients was 49.1 years, with a predominance of women (59 women, 15 men) in the cohort. The control group comprised 16 individuals of similar age and in good health, including 3 men (18.7%) and 13 women (81.3%) (Figure 1).



Picture 1. Research groups

Based on the presence of rheumatoid factor (RF) in the blood, patients were categorized into seropositive and seronegative groups for RA diagnosis. The seronegative group comprised 21 patients, consisting of 6 males and 15 females (28.4%), while the seropositive group consisted of 53 patients (71.6%), including 9 males and 44 females. Laboratory investigations were carried out at the Clinical-Biochemical Laboratory of Azerbaijan Medical University.

The level of calcium and phosphorus in blood serum was

determined by the colorimetric method, and the activity of alkaline phosphatase was determined by the kinetic method using the reagent kit "Human" (Germany). The concentration of CRP and RF in the blood was determined by the immunoturbidimetric method with the help of the "Linear" (Spain) reagent kit. The level of free oxyproline in the blood was determined by the Berglan-Loxley, ECS-Panchenkov method.

The concentration of cytokines, AMPs, calcium-regulating hormones, markers of bone metabolism (osteocalcin, osteopontin) in serum was determined by enzyme immunoassay (IFA) on the STAT FAX 303 Plus (USA) analyzer.

During the study, the concentration of cytokines (TNF-α, IL-2, IL-6, IL-8, IL-10) in blood serum was obtained from the company "Vector-Best" (Russian Federation), and the level of AMPs (calprotectin and cathelicidin) was obtained from EASTBIOPHARM (It was determined by the "sandwich" method of IFA through the reactive kits of the PRC) company.

The determination of parathormone and calcitonin levels was carried out using the reagent kit provided by the esteemed company "IBL" from Japan. Furthermore, the concentration of osteocalcin and osteopontin was respectively evaluated using the reagent kits from "BIOSOURCE" in Belgium and "IBL" in Japan. The analysis of the vitamin D status was conducted based on the calcidiol (25(OH)D) concentration in blood serum, using the reagent kit from "Bioactiva Diagnostica" in Germany.

The data obtained from laboratory analyses of both healthy individuals and RA patients were subjected to mathematical and statistical analyses using a variation, discriminant, correlation and receiver operating characteristic (ROC) methods.

RESULTS AND DISCUSSION OF RESEARCH

Activity Markers of Rheumatoid Arthritis

Clinical observations indicate that RF-negative RA has a milder course and a more favorable prognosis in terms of joint damage and preservation of joint function. This necessitates the differentiation

between seronegative and seropositive clinical-immunological forms of the disease. During the study, patients were divided into seropositive and seronegative RA groups based on their RF levels in the blood.

It has been determined that the involvement of RF is 1.23 times higher in seronegative RA patients ($p<0.01$), while it is 7.24 times higher in seropositive patients ($p<0.001$) compared to control values. Comparative analysis between RA groups reveals that the rate of RF is significantly higher – 5.9 times in seropositive patients compared to seronegative patients ($p<0.001$) (Table 1).

Assessing the degree of inflammatory processes is an important aspect of RA treatment. CRP and ESR are inflammatory biomarkers that are more commonly used in clinical experience and scientific research. Both markers are included in various indices used to determine the activity level and effectiveness of treatment in RA.

In our study, it has been determined that the involvement of ESR and CRP in the blood serum of seronegative RA patients is significantly increased 3.08 times ($p<0.001$) and 6.25 times ($p<0.001$) respectively compared to the control group. In the seropositive RA group, these markers are increased 3.76 times ($p<0.001$) and 8.65 times ($p<0.001$) respectively compared to healthy individuals, and 22% ($p<0.01$) and 38% ($p<0.001$) respectively compared to seronegative patients (Table 1).

Table 1.

The levels of disease activity markers in the blood serum of RA patients.

Indicators	Groups		
	Control, n=16	RF-negative RA, n=21	RF-positive RA, n=53
RF, V/ml	7,63±0,47 (4,5-10,8)	9,36±0,28 (6,8-11,5) p<0,01	55,2±1,08 (38-83,6) p<0,001; p_1<0,001
ESR, mm/hour	8,17±0,68 (4-14)	25,1±1,59 (19-38) p<0,001	30,7±1,05 (23-46) p<0,001; p_1<0,001
CRP, V/ml	2,38±0,23 (2,0-5,7)	14,8±0,92 (6,7-22,8) p<0,001	20,5±0,64 (12,3-36,5) p<0,001; p_1<0,01

Note: p – compare to control group; p_1 – compare to RF-negative group;

The functions performed by CRP in the organism have not been fully elucidated, but it is speculated that this protein induces the classical pathway activation of the complement system and regulates the activity of phagocytes. In an environment where calcium ions are present, CRP interacts with microorganisms containing phosphatidylcholine in their membrane composition and certain cells released from damaged tissues. Formed complexes activate the complement system through the classical pathway, resulting in their opsonization and phagocytosis by the components of the complement system and microorganisms[4].

According to literary information, cytokines that provide an immune response direct and regulate the development of inflammatory reactions in conjunction with acute phase reactants: the CRP-IL-8 complex exhibits an anti-inflammatory effect, while the CRP-IL-4 complex exhibits a pro-inflammatory effect [5].

Indicators of calcium-phosphorus metabolism and status of hormones regulating this metabolism in Rheumatoid Arthritis

Despite significant advancements in the treatment of RA, patients' life expectancy is considerably reduced. This is attributed not only to the progression of the disease but also to the occurrence of complications such as cardiovascular diseases, amyloidosis, and secondary osteoporosis [6].

One of the serious complications considered is osteoporosis, which is estimated to occur 2-3 times more frequently in patients with RA compared to the general population. Local (periarticular) osteoporosis is considered an early diagnostic sign of RA and develops

[4] Аникин, С.Г., Беневоленская, Л.И., Александрова, Е.Н. Остеопороз и С-реактивный белок // Научно-практическая ревматология, – 2010. №1, – с. 46-50.

[5] Spasovski, D., Sotirova, T. C-reaktiv protein – the most useful marker in rheumatoid arthritis // Interdisciplinary J of Microinflammation, – 2014. – 1(2):e1000120.

[6] Sarkis, K.S., Salvador, M.B., Pinheiro, M.M. Association between osteoporosis and rheumatoid arthritis in women: a cross-sectional study // Sao Paulo Med J., – 2009. 127(4), – p.216-222.

before the appearance of cartilage erosions. In the later stages of the disease, diffuse osteoporosis is detected due to chronic inflammation and decreased physical activity. The progressive decrease in mineral density caused by cartilage damage creates medical and social problems leading to the development of osteoporotic fractures in RA patients[7].

In our research, the levels of calcium-phosphorus exchange indicators, hormones regulating this exchange (parathyroid hormone, calcitonin), vitamin D, and biochemical markers of bone metabolism (alkaline phosphatase, osteocalcin, osteopontin) were determined in the blood serum of patients with rheumatoid arthritis (RA) to assess their impact on the process of bone damage due to rheumatoid inflammation.

The study of calcium-phosphorus metabolism in RA patients showed that the level of calcium in the seronegative group decreased 19% ($p<0.001$) compared to the control group, and 24% in the seropositive group ($p<0.001$) (table 2).

Phosphorus concentration increases 53% ($p<0.001$) in the seronegative RA group and 56% ($p<0.001$) in the seropositive RA group compared to healthy individuals. There is no significant difference in the concentration of calcium and phosphorus between patient groups

It was discovered that the level of parathormone in the blood serum of RF-negative patients exhibited a significant increase of 46% ($p<0.001$) in comparison to the control group. The level of parathormone in RF-positive group increased 61% ($p<0.001$) compared to healthy individuals, and 11% compared to seronegative RA patients ($p<0.05$). Thus, hypocalcemia in RA patients increases the function of the parathyroid gland and causes secondary hyperparathyroidism.

[7] Островский, А.Б., Оттева, Э.Н., Тарнавская, Т.С. Остеопороз при ревматоидном артрите // Дальневосточный медицинский журнал. – 2012, № 4, – с. 142-144.

Table 2.

The levels of calcium-phosphorus metabolism indicators and calcium-regulating hormones in the blood serum of patients with RA

Indicators	Groups		
	Control, n=16	RF-negative RA, n=21	RF-positive RA, n=53
Calsium, mg%	9,2±0,25 (8,4-10,7)	7,74±0,11 (7,0-8,5) p<0,001	7,45±0,09 (6,8-8,7) p<0,001; p_1<0,05
Phosphorus, mmol/l	1,19±0,04 (0,68-1,56)	1,82±0,05 (1,16-1,98) p<0,001	1,86±0,02 (1,1-2,05) p<0,001; p_1<0,05
Parathormone, pg/ml	40,5±1,94 (26-63)	58,9±1,89 (33,7-75,4) p<0,001	65,3±1,52 (35-82) p<0,001; p_1<0,05
Calsitonin, pg/ml	3,31±0,24 (0,78-5,0)	5,08±0,31 (2,8-7,8) p<0,001	5,36±0,28 (3-8,4) p<0,001; p_1=0,499
Calsidiol, ng/ml	37,5±1,67 (30,3-48,7)	23,8±1,27 (12,3-34,8) p<0,001	20,4±0,95 (10,8-32) p<0,001; p_1<0,05

Note: p – compare to control group; p_1 – compare to RF-negative group;

In both RA groups, a statistically significant increase in the concentration of another calcium-regulating hormone, calcitonin, was observed compared to the control group. Therefore, the level of the hormone increased 53% in seronegative RA patients ($p<0.001$). The calcitonin content in seropositive RA patients differed 62% compared to healthy individuals ($p<0.001$). No statistically significant difference in the amount of calcitonin was found between the patient groups.

During the research, the status of vitamin D in patients was determined based on the concentration of calcidiol (25(OH)D). It should be noted that due to the long half-life of 25(OH)D (2-3 weeks), it is considered the best serological marker for evaluating the level of this vitamin. A level of vitamin D below 20 ng/mL is considered deficient, between 20-30 ng/mL is considered insufficient, and between 30-50 ng/mL is considered a normal value.

The serum level of vitamin D in patients with RA is lower compared to healthy individuals. In patients with seronegative RA,

this difference accounts for 57% ($p<0.001$). In this group, deficiency of vitamin D is observed in 12 out of 21 patients, while insufficiency is found in 4 patients. In patients with seropositive RA, a more profound hypovitaminosis is observed, and the average level of vitamin D is 84% ($p<0.001$) lower than that of the control group and 17% ($p<0.05$) lower than the other RA group. This group consists of 53 patients, among whom 17 individuals have vitamin D insufficiency and 28 individuals have vitamin D deficiency.

The effect of vitamin D on bone membrane inflammation is closely related to the activity of parathyroid hormone. In physiological doses, calcitriol cannot influence the transport of calcium and phosphorus without parathyroid hormone. The conversion of calcidiol to calcitriol occurs in the kidneys through the participation of mitochondrial 1-alpha-hydroxylase enzyme belonging to the cytochrome P-450 family. It has been discovered that calcitriol receptors are expressed on osteoblasts, and its effect on bone resorption is not direct but is mediated through osteoblasts, similar to parathyroid hormone [8].

After reaching a certain threshold, calcitriol attenuates the expression of the 1-α-hydroxylase gene through negative feedback, thereby reducing its own production. The effect of calcitriol inhibits parathyroid hormone secretion directly by increasing the production of fibroblast growth factor 23 (FGF23) in osteocytes, which in turn decreases parathyroid hormone secretion and indirectly by increasing the binding of calcium in the blood. This mechanism explains how secondary hyperparathyroidism can occur due to vitamin D deficiency[9].

Thus, in patients with RA, vitamin D deficiency and hypocalcemia are detected regardless of the presence of RF, which leads to the development of secondary hyperparathyroidism. During

[8] Gil, A., Plaza-Diaz, J., Mesa, M.D. Vitamin D: Classic and novel actions // Ann Nutr Metab., – 2018. 72(2), – p.87-95.

[9] Ермолаева, М.В. Остеотропные гормоны при ревматоидном артрите и остеоартрозе / М.В. Ермолаева, О.П. Сокрут, И.А. Гейко [и др.] // Проблемы остеологии. – 2016. 19(1), – p.8-14.

RA, alterations in the vitamin D/parathyroid hormone system play a significant role in the disruption of bone metabolism and the development of osteoporosis. The obtained results indicate the necessity of using vitamin D preparations in the complex treatment of the disease.

Evaluation of bone metabolism biomarkers during rheumatoid arthritis

The determination of bone metabolism markers has revealed that the levels of alkaline phosphatase and osteocalcin, which are markers of bone turnover, are increased 54% ($p<0.001$) and 22% ($p<0.05$) in the blood serum of seronegative rheumatoid arthritis (RA) patients compared to the control group. In the seropositive RA group, these indicators are increased 63% ($p<0.001$) and 50% ($p<0.001$) compared to healthy individuals, and they are increased 6% and 23% ($p<0.05$) compared to seronegative group (Table 3).

Table 3.

Bone metabolism biomarkers level in rheumatoid arthritis

Indicators	Groups		
	Control, n=16	RF-negative RA, n=21	RF-positive RA, n=53
Alkaline phosphatase U/l	198,4±16,17 (98-278)	306,4±18,53 (140-388) p<0,001	323,8±14,49 (145-408) p<0,001; p_1=0,465
Osteocalsin, pg/ml	18,3±1,17 (12,6-24,3)	22,3±1,48 (17,3-39) p<0,05	27,4±1,46 (18-45) p<0,001; p_1<0,05
Osteopontin, ng/ml	135,9±4,7 (112-184)	218,9±5,63 (187-285) p<0,001	256±6,75 (190-310) p<0,001; p_1<0,001
Free oxyprolin, mkg%	145,1±5,63 (106-187)	220±11,34 (123-294) p<0,001	224,9-12,23 (132-360) p<0,001; p_1=0,766

Note: p – compared to control group; p_1 - compare to RF-negative group

It is known that osteocalcin participates in bone mineralization and is considered one of the most informative indicators reflecting the rate of bone turnover. Through the involvement of vitamin K, its three glutamic acid residues undergo γ-carboxylation, acquiring the ability to bind calcium ions and incorporate them into the structure of hydroxyapatite crystals. According to conducted research, osteocalcin also plays a significant role in activating osteoclasts during bone resorption [10].

The determination of bone resorption markers, osteopontin and free oxyproline, in patients with RA has shown that their levels increase 61% ($p<0.001$) and 52% ($p<0.001$), respectively, compared to healthy individuals in seronegative patients. In seropositive patients, these parameters are elevated 88% ($p<0.001$) and 55% ($p<0.001$) compared to the control group results, and 17% ($p<0.001$) and 2% compared to seronegative patients, respectively.

Osteopontin is one of the key participants in various physiological and pathological processes in the organism. It has been determined that osteopontin creates conditions for the adhesion of hydroxyapatite crystals with cells. These molecules also facilitate the migration of macrophages and T lymphocytes to inflammatory foci in the immune system. The gene expression of osteopontin is regulated by various factors, including certain cytokines (such as IL-1 and TNF-α), which is why it is referred to as a link between the immune system and bone damage. Some researchers, who have observed a positive correlation between the levels of osteopontin in synovial fluid and CRP and ESR, consider it as a marker for local inflammatory processes [11,12].

---

[10] Панкратова, Ю.В., Пигарова, Е.А., Дзеранова, Л.К. Витамин К-зависимые белки: остеокальцин, матриксный Gla-белок и их внекостные эффекты // Ожирение и метаболизм. – 2013. т. 10, №2, – с.11-18.

[11] Clemente, N. Osteopontin bridging innate and adaptive immunity in autoimmune diseases / N.Clemente, D. Raineri, G. Cappellano [et al.] // J Immunol Res., – 2016. – p.1–15.

[12] Kahles, F., Findensen, H.M., Bruemmer, D. Osteopontin: a novel regulator at the cross roads of inflammation, obesity and diabetes // Molecular metabolism, – 2014. v.3, – p. 384-393.

Therefore, an increase in the levels of bone turnover markers (such as osteocalcin and alkaline phosphatase) and bone resorption markers (such as osteopontin and free oxyproline) is observed in both RA groups, which reflects an acceleration of bone metabolism in patients. The increased bone formation during RA is likely a compensatory response of the organism to the increased bone resorption. As a result of these changes, there is a decrease in mineral density of bone tissue and an increased risk of joint destruction and osteoporosis formation.

Cytokine profile in rheumatoid arthritis

It is known that an important component of RA pathogenesis is the imbalance of the cytokine system. During RA, the activation of T lymphocytes leads to the synthesis of a series of pro-inflammatory cytokines. The increase in these cytokines stimulates the activation of Th17 cells and the differentiation of B lymphocytes into mature plasma cells, which forms the basis of autoimmune responses. Pro-inflammatory cytokines induce the accumulation of leukocytes in the synovial cavity, bone destruction, and the formation of pannus. They also exert systemic effects by enhancing the synthesis of acute-phase proteins in hepatocytes [13] [14].

It was determined that the concentration of TNF-α in blood serum of seronegative RA patients increased 2.16 times ($p<0.05$) compared to the control group, and 2.52 times ($p<0.01$) in the seropositive group. No statistically significant difference in the value of this cytokine was detected during the comparative analysis between the RA groups (Table 4, Figure 2).

According to numerous studies, TNF-α has been identified as a crucial cytokine involved in the underlying mechanisms of RA. The pathogenic effects of cytokines in this condition encompass the induction of heightened expression of adhesion molecules, metalloproteinases, collagenases, chemokines, and prostaglandins.

[13] Rincon, M. Interleukin-6: from an inflammatory marker to a target for inflammatory diseases // Trends Immunol., – 2012. 33, – p. 571–7

[14] Yusof, M.Y., Emery, P. Targeting interleukin-6 in rheumatoid arthritis // Drugs, – 2013. 73, – p.341-346.

Furthermore, TNF-α stimulates the increased production of osteoprotegerin ligand (RANKL), which is responsible for the resorption of bone tissue. In synovial cell cultures, blockade of TNF-α has been observed to diminish the production of IL-1, IL-6 and IL-8[15].

Table 4.

Blood serum cytokines levels in rheumatoid arthritis

Indicators	Groups		
	Control group, n=16	RF-negative RA, n=21	RF-positive RA, n=53
TNF-α, pg/ml	0,75±0,28 (0,10-4,84)	1,62±0,32 (0,63-7,2) p<0,05	1,89±0,2 (0,72-9,4) p<0,01; p_1=0,471
IL-2, pg/ml	0,58-0,35) (0-5,8)	1,46±0,25 (0,84-6,1) p<0,05	1,97±0,18 (0,93-7,65) p<0,01; p_1=0,059
IL-6, pg/ml	2,73±0,19 (1,6-4,5)	3,6±0,27 (1,25-6,18) p<0,05	4,83±0,34 (2-12,2) p<0,001; p_1<0,01
IL-8, pg/ml	2,48±0,15 (1,3-3,64)	3,88±0,24 (2,62-7,24) p<0,001	4,15±0,3 (1,8-10,6) p<0,001; p_1=0,479
IL-10, pg/ml	5,69±0,43 (3,26-8,7)	8,48±0,57 (4,5-14,7) p<0,001	9,26±0,62 (2-19,8) p<0,001; p_1=0,354

Note: p – compare to control group; p_1 – compare to RF-negative group;

It was determined that the serum IL-2 level in the seronegative RA group increased 2.52 times ($p<0.05$), while in the seropositive group, it increased 3.4 times ($p<0.01$). There was no statistically significant difference found in the levels of this cytokine between the two RA groups.

In vivo studies have demonstrated elevated levels of IL-2 in autoimmune rheumatic diseases. Monoclonal antibodies targeting IL-2 receptors have been shown to impede the progression of experimental collagen-induced arthritis in animal models. These

[15] Gonzales-Alvaro, I. Baseline serum RANKL levels may serve to predict remission in rheumatoid arthritis patients treated with TNF antagonists / I.Gonzales-Alvaro, A.M. Ortiz, E.G. Tomero [et al.] Ann. Rheum. Dis., – 2007. 66(12), – p.1675-1678.

findings suggest a significant involvement of the IL-2-dependent T-cell immune response in the pathogenesis of inflammatory rheumatic diseases [16].

Our studies have shown that the concentration of proinflammatory cytokine IL-6 in patients with seronegative RA increased 32% ($p<0.05$) compared to healthy individuals. The same trend is observed in the seropositive group. The level of IL-6 in this group was 77% ($p<0.001$) higher than the control values, and 34% ($p<0.01$) higher than the value of the seronegative RA group.

The capacity of IL-6 to modulate the differentiation of B-lymphocytes into plasma cells assumes crucial pathogenetic significance, leading to excessive secretion of RF and gamma-globulins in RA. This cytokine establishes a connection between cellular and humoral immunity. It is widely acknowledged that impairments in cellular mechanisms play a pivotal role in joint damage, while disturbances in humoral immune responses are primarily implicated in extra-articular manifestations [17 18].

The direct involvement of IL-6 in joint destruction is not well-established; however, it is considered to be the sole cytokine that induces the synthesis of acute-phase reactants in hepatocytes. The results of clinical studies demonstrate close correlations between the plasma levels of acute-phase reactants and the rate of joint destruction [19, 20].

[16] Burska, A., Boissinot, M., Ponchel, F. Cytokines as Biomarkers in Rheumatoid Arthritis // Med. Inflamm., – 2014. – 545493. doi: 10.1155/2014/545493.

[17] Fonseca, J.E. Interleukin-6 as a key player in systemic inflammation and joint destruction / J.E. Fonseca, M.J. Santos, H. Canhao [et al.] // Autoimmun Rev., – 2009. v. 8, – p. 538-542.

[18] Rincon, M. Interleukin-6: from an inflammatory marker to a target for inflammatory diseases // Trends Immunol., – 2012. 33– p.571–7

[19] Аникин, С.Г., Беневоленская, Л.И., Александрова, Е.Н. Остеопороз и С-реактивный белок // Научно-практическая ревматология, – 2010. 1, – с.46-50.

[20] Трофименко, Н.А. Провоспалительные цитокины и реактанты острой фазы воспаления при заболеваниях суставов. / Дисс. … канд. мед. наук. – Новокузнецк, 2007, – 115 с.

It was determined that the blood serum level of IL-8 increased in both RA groups compared to the control group. The concentration of IL-8 in RF-negative RA and RF-positive was 57% ($p<0.001$) and 68% ($p<0.001$) higher than control group. No statistical difference was found in the value of IL-8 between RA groups.

According to modern concepts, IL-8 plays a central role as the mediator of the body's non-specific defense system, specifically related to neutrophils. Various factors such as trauma, hypoxia stimulate the production of IL-8 from different cells. In the case of RA, the concentration of IL-8 increases due to its hyperproduction by neutrophils. It is believed that the measurement of IL-8 levels provides more information than CRP in predicting the severity of the disease. Because IL-8 is secreted earlier than CRP, making it a valuable indicator for assessing disease progression.

It was found that the blood serum level of IL-10 increased in both RA groups compared to healthy individuals: 49% ($p<0.001$) in the RF-negative group, and 63% ($p<0.001$) in the seropositive group. Although the cytokine concentration was higher in the seropositive group than in the seronegative group, this difference was not statistically significant.

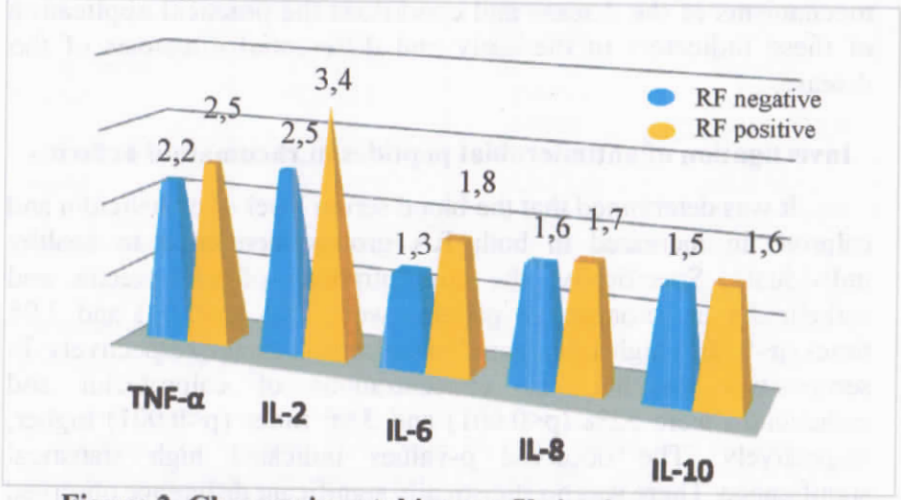


Figure 2. Changes in cytokine profile in rheumatoid arthritis patients compared to control group.

Macrophages, monocytes, CD5+ B lymphocytes, and CD4+ T lymphocytes synthesize IL-10, which, in in vitro studies, inhibits the expression of pro-inflammatory cytokines such as IL-1, IL-6, IL-8, and TNF-α, as well as matrix metalloproteinases, and the proliferation of T lymphocytes. In patients with RA, IL-10 inhibits the destruction of synovial cartilage by mononuclear cells. This cytokine is found in synovial fluid, but its presence is not sufficient to halt the inflammatory processes.

The serum levels of IL-2, IL-6, IL-8, IL-10 and TNF-α are increased in RA patients compared to healthy individuals. The cytokine profile is characterized by a higher concentration of cytokines in patients with seropositive RA than in patients with seronegative RA. In RA, the increase of phlogogenic cytokines leads to the activation of the endothelium, the accumulation of leukocytes in the joint cavity, the secretion of proteases and matrix metalloproteinases, and the development of local inflammatory reactions; induces pannus formation and bone destruction; participates in the emergence of autoimmune disorders and systemic manifestations. The activation of the cytokine system during RA proves that they play an important role in the pathogenetic mechanisms of the disease and conditions the practical application of these indicators in the early and differential diagnosis of the disease.

Investigation of antimicrobial peptides in rheumatoid arthritis

It was determined that the blood serum level of cathelicidin and calprotectin increased in both RA groups, compared to healthy individuals. Specifically, the concentrations of calprotectin and cathelicidin in seronegative patients were 35% ($p<0.01$) and 2.98 times ($p<0.001$) higher compared to the control group, respectively. In seropositive patients, the concentrations of calprotectin and cathelicidin were 52% ($p<0.001$) and 3.96 times ($p<0.001$) higher, respectively. The observed p-values indicated high statistical significance. There was no statistically significant difference observed in the levels of calprotectin between the different groups of RA patients. However, the concentration of cathelicidin was 33%

($p<0.001$) higher in the seropositive group when compared to the seronegative group (table 5, figure 3).

During inflammation, calprotectin is secreted from activated granulocytes and macrophages into the synovial membrane, as well as from granulocytes into the synovial fluid. Calprotectin emerges as the predominant protein in synovial fluid collected from inflamed joints[21].

It is important to note that inflammatory biomarkers play a crucial role in the diagnosis and monitoring of treatment for RA. One such biomarker is CRP, a well-established acute-phase protein that is commonly used as a laboratory marker for assessing inflammation and disease activity in RA. However, research has revealed that approximately 40% of RA patients exhibit CRP levels within the normal range. This necessitates the exploration of new indicators that can accurately determine the extent of disease activity. In this regard, investigating calprotectin as an inflammatory biomarker holds significant promise and generates considerable interest [22].

Table 5.

Blood serum level of AMPs in RA patients

Indicators	Groups		
	Control, n=16	RF-negative RA, n=21	RF-positive RA, n=53
Calprotectin, ng/ml	13,4±0,76 (7,5-18,6)	18,1±1,29 (7,4-34) p<0,01	20,4±1,42 (9,3-47,6) p<0,001; p_1=0,238
Cathelicidin, ng/ml	5,35±0,74 (0,12-9,6)	15,9±0,92 (11,0-27,6) p<0,001	21,2±0,68 (16,0-34,4) p<0,001; p_1<0,001

Note: p – compare to control group; p_1 – compare to RF-negative group;

Cathelicidin, also known as hCAP-18 (human cationic antimicrobial peptide) plays a significant role in inflammatory processes. This peptide is involved in regulating the production of

[21] Abildtrup, M., Kingsley, G.H., Scott DL. Calprotectin as a biomarker for rheumatoid arthritis: a systematic review // J Rheumatol., – 2015. 42, – p.760-770.

[22] Spasovski, D., Sotirova, T. C-reaktiv protein – the most useful marker in rheumatoid arthritis // Interdisciplinary J of Microinflammation, – 2014. 1(2), – e1000120.

reactive oxygen species (ROS) in neutrophils, as well as the secretion of TNF-α and IL-1β by these immune cells. Cathelicidin can bind to cathelicidin P2X7 and FPRL1 receptors, which helps inhibit the apoptotic activity of neutrophils, but it also activates a secondary form of apoptosis [23].

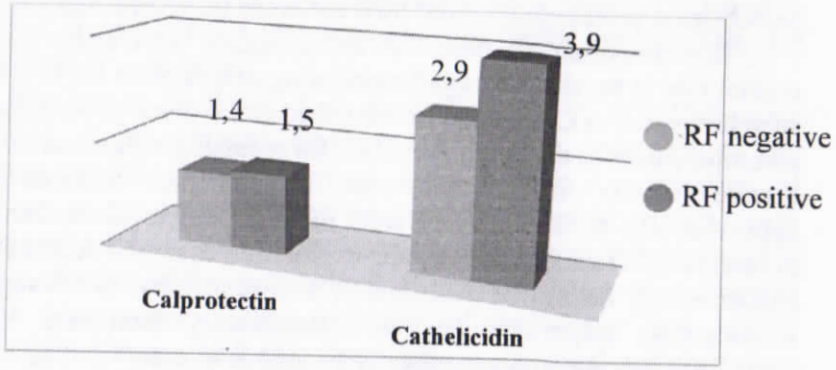


Figure 3. Alteration of AMPs in rheumatoid arthritis patients (compared to the control group).

In rheumatoid arthritis (RA), the increased level of AMPs in the blood occurs irrespective of the presence of rheumatoid factor (RF), in both patient groups, which is relatively higher compared to healthy individuals. This pathology necessitates further research to evaluate the diagnostic, prognostic, and therapeutic potential of AMPs.

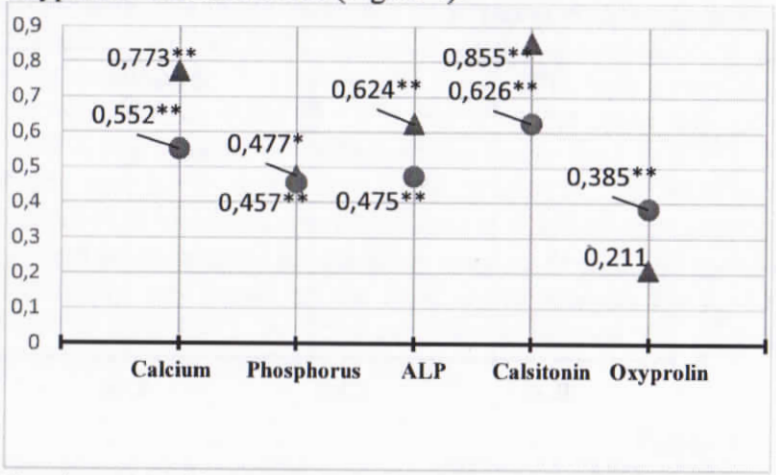
Evaluation of correlations between cytokine and AMPs and disease activity level and bone metabolism markers in RA patients

During the study, correlations of cytokines and AMPs with indicators of disease activity and bone metabolism biomarkers were evaluated, and a number of significant dependencies emerged.

[23] Пинегин, Б.В., Карсонова, М.И. Роль антимикробного пептида LL-37 в развитии аутоиунного процесса // Иммунология, – 2012. 5, – p. 276-280

Correlation between cytokines and markers of disease activity was found. Thus, a statistically reliable positive correlation of CRP with TNF-α and IL-6, considered the main proinflammatory cytokines, was observed in both groups of patients. In seropositive patients, a positive average correlation of EHS, another activity marker, with IL-2 (ρ=0.316, p=0.021) and IL-6 (ρ=0.352, p=0.010) was observed. These results indicate that cytokines play an important role in the pathogenetic mechanisms of RA.

Activation of the cytokine system affects bone remodeling processes as well as chronic inflammation. TNF-α, IL-6, and IL-8, which are potent proinflammatory cytokines, are known to be potent stimulators of bone resorption. Hypersecretion of these cytokines is accompanied by overexpression of RANKL, a potent inducer of bone tissue resorption. Among the cytokines in our studies, IL-8 was the one with the most correlations with bone metabolism biomarkers. Thus, the positive dependence of this cytokine with calcium, phosphorus, parthormone, calcitonin, osteocalcin, osteopontin, and free oxyproline was monitored (Figure 4).

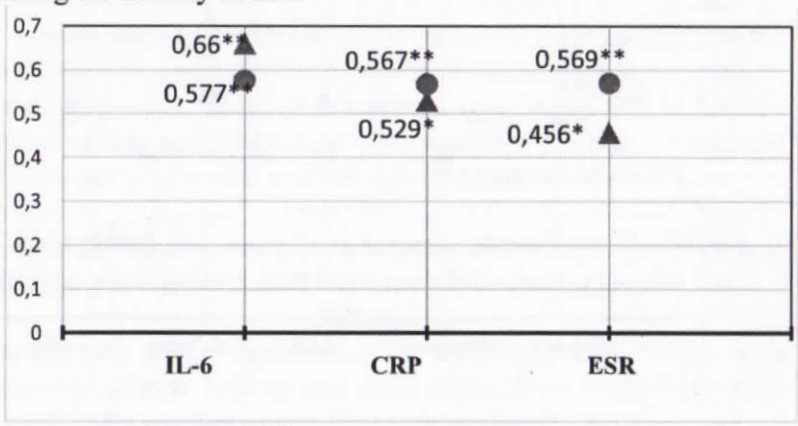


Note:* – p<0.05; ** – p<0.01.

Figure 4. Correlation of IL-8 with bone tissue metabolism indicators

In our study, we simultaneously evaluated the correlations between the concentration of antimicrobial peptides (AMPs) and other inflammatory biomarkers and cytokines. The findings revealed statistically significant positive correlations between the concentration of calprotectin and several markers in both the seronegative and seropositive RA groups. In the seronegative RA group, calprotectin concentration showed a significant positive correlation with the levels of CRP (ρ=0.529, p=0.014), TNF-α (ρ=0.666, p=0.001), and IL-6 (ρ=0.660, p=0.001). Similarly, in the seropositive RA group, calprotectin concentration exhibited a statistically significant positive correlation with the levels of CRP (ρ=0.567, p=0.000), ESR (ρ=0.569, p=0.000), and IL-6 (ρ=0.577, p=0.000) (Figure 5). However, no significant correlation was observed between cathelicidin, another type of AMP, and cytokines, bone tissue indicators, or activity level markers.

The obtained results demonstrate that it is essential the using calprotectin level with CRP and other inflammatory biomarkers for evaluating the activity of RA.



Note:* – p<0.05; ** – p<0.01.

Figure 5. The correlation between calprotectin and the activity level indicators of rheumatoid arthritis

It should be noted that during the study, negative correlations of vitamin D with cytokines such as TNF-α, IL-6 and IL-10 and CRP and calprotectin, which are considered inflammatory biomarkers, were determined in RA patients.

Calcitriol combines with vitamin D receptors located in the cells of the immune system, resulting in an anti-inflammatory effect and a reduction in the secretion of pro-inflammatory cytokines that accelerate bone resorption. It can be concluded that vitamin D deficiency in patients with rheumatoid arthritis (RA) both activates inflammatory processes and strengthens the negative effects of inflammation on bone tissue.

Evaluation of the sensitivity and specificity of antimicrobial peptides and cytokines in RA

During the research, the specificity and sensitivity of AMPs and cytokines, which have significant importance in the pathogenic mechanisms of RA have been determined through ROC analysis to investigate their role in the diagnosis and treatment of the disease.

According to the ROC curve analysis, the specificity area calculated based on IL-2 is 0.939±0.061 (p<0.001), with a confidence interval ranging from 0.868 to 0.979. For IL-6, the upper limit of the 95% confidence interval is 0.880, and the lower limit is 0.707. The specificity area for IL-6 is 0.804±0.056 (p<0.001). The ROC curve analysis for IL-8 yielded a specificity area of 0.800±0.052 (p<0.001), with a confidence interval for accuracy ranging from 0.703 to 0.877. The specificity area based on the ROC curve analysis for IL-10 is 0.793±0.056 (p<0.001). Its 95% confidence interval for accuracy varies between 0.694 and 0.871 (Table 6).

Table 6.

Sensitivity and specificity of cytokines and AMPs in RA

	TNF-α	IL-2	IL-6	IL-8	IL-10	Calprotectin	Cathelicidin
Se, %	87,8	100	63,5	68,9	87,8	59,5	79,7
Sp, %	87,5	93,8	87,5	87,5	62,5	81,3	100

The specificity of calprotectin is 0.743±0.063 ($p<0.001$), representing the range of specificity values. The 95% confidence interval for specificity ranges from 0.640 to 0.828. Similarly, for the ROC curve of catalysis, the specificity area is 0.960±0.021 ($p<0.001$). The 95% confidence interval for its specificity ranges from 0.896 to 0.990.

TNF-α, IL-2, IL-6, IL-8, IL-10, calprotectin and cathelicidin were assessed as laboratory tests exhibiting high specificity and sensitivity in RA, according to the results of ROC analysis.

RESULTS

1. Serum ESR and CRP rates increase correspondingly, 3.08 and 6.25 times respectively in seronegative RA patients group, and 3.76 and 8.65 times in the seropositive RA group, compared to the control group,

2. The levels of parathyroid hormone and calcitonin in the blood serum of RA patients increase compared to healthy individuals, while the level of vitamin D decreases. Vitamin D deficiency is detected in 12 out of 21 patients in the seronegative group, and its deficit is found in 4 patients. More severe hypovitaminosis is observed in patients with seropositive RA group, vitamin deficiency is found in 17 people, and its deficit is found in 28 people.

3. The serum levels of osteocalcin, osteopontin, and free oxiproline are higher in RA patients compared to healthy individuals, reflecting the acceleration of bone metabolism against the backdrop of exacerbation of rheumatoid inflammation and increased cytokine secretion.

4. In RA patients, the serum levels of pro-inflammatory cytokines – IL-2, IL-6, IL-8, and TNF-α, as well as anti-inflammatory cytokines –IL-10, increase significantly compared to healthy individuals. This increase is characterized by a more pronounced activation of the cytokine profile in the seropositive group compared to the seronegative group. Against the background of the activation of pro-inflammatory cytokines, the calprotectin and cathelicidin level increase in RA patients groups compared to the control group. Based

on ROC curves, TNF-α, IL-2, IL-6, IL-8, IL-10, calprotectin, and cathelicidin in RA patients have high specificity and sensitivity.

5. The serum calprotectin level in the seronegative RA group has a statistically significant positive correlation with CRP, TNF-α, and IL-6 and in the seropositive RA group with CRP and IL-6 serum level. The relationship of another AMP, cathelicidin, with cytokines, bone metabolism indicators, and markers of the degree of activity is not revealed. In RA patients, the correlation relationships of vitamin D with cytokines – TNF-α, IL-6, and IL-10, and inflammation biomarkers – CRP and calprotectin are negatively observed.

PRACTICAL RECOMMENDATIONS

1. In patients with rheumatoid arthritis (RA), regardless of seronegative or seropositive forms, vitamin D deficiency and hypocalcemia contribute to the development of secondary hyperparathyroidism. Therefore, the use of vitamin D supplements is considered essential in the comprehensive treatment of the disease.

2. Determining cytokines with high diagnostic informativeness in RA patients can assist in the application of new approaches in the diagnosis and treatment of the disease.

3. In RA patients it is recommended to assess the disease activity level not only by using C-reactive protein (CRP) and other inflammatory biomarkers but also by determining calprotectin levels.

LIST OF PUBLISHED SCIENTIFIC WORKS ON THE TOPIC OF THE DISSERTATION:

1. Məmmədli, Ə.S., Əfəndiyev, A.M., Kərimova, İ.A., Nuriyev, A.Ə. Revmatoid artriti olan xəstələrin qanında kalprotektin səviyyəsinin tədqiqi // Ə.e.x., prof. R.Ə.Əsgərovun anadan olmasının 85 illik yubileyinə həsr olunmuş beynəlxalq konfrans materiallarının toplusu, – Bakı, – 2018. – s.80-81.

2. Məmmədli, Ə.S., Əfəndiyev, A.M., Kərimova, İ.A Revmatoid artriti olan xəstələrdə sitokin sekresiyasının dəyərləndirilməsi // Azərbaycan Xalq Cumhur. yarad. 100 illiyinə həsr edilmiş"Təbabətin

aktual problemləri, 2018" beynəlxalq elmi-praktik kofransın materiallar, – Bakı, – 2018. – s.205

3. Гусейнова, А.С. Провоспалительные цитокины при ревматоидном артрите: взаимосвязь с дефицитом витамина D / А.С. Гусейнова, А.Г. Гаджиев, А.М. Эфендиев [и др.] // Theoret. Applied Science. – 2018. 11(67), – с.348-352

4. Hüseynova, Ə.S. Revmatoidli artrit zamanı D vitamini statusunun qiymətləndirilməsi / Ə.S. Hüseynova, İ.A. Kərimova, V.İ. Yaqubova [və b.] // Ə.e.x., t.e.d., prof. T.Ə. Tagızadənin 95 illik yubileyinə həsr edilmiş elmi-praktik konfransın materialları, – 2018, – Bakı, – s.121-123.

5. Hüseynova, Ə.S., Kərimova, İ.A., Rəhimova, R.R. Revmatoid artiti zamanı yaranan sümük metabolizmi pozulmalarının patogenezində D vitamini/parathormon sisteminin rolu // Azərbaycan Tibb Jurnalı, – 2019. №1, – s.36-39.

6. Hüseynova, Ə.S. Revmatoid artritinin aktivlik dərəcəsinin qiymətləndirilməsində kalprotektinin rolu // Azətbaycan Tibb Jurnalı, – 2019, №3, – s.19-23.

7. Huseynova, A.S. Cytocines and bone tissue biomarkers in patient with rheumatoid arthritis / A.S. Huseynova, M.R. Guliyev, V.İ. Yagubova [et al.] // The international conference "Modern molecular-biochemical markers in clinical and experimental medicine", Journal of the Czech Republic, – 2019, № 1, – c.17-18.

8. Гусейнова, А.С. Исследование некоторых лабораторных биомаркеров ревматоидного артрита // Биомедицина, – 2019. № 2, – с.13-17.

9. Гусейнова, А.С. Особенности изменения цитокинового профиля и кальцийрегулирующих гормонов при ревматоидном артрите // Клиническая лабораторная диагностика, – 2019. 64(11), – с.673-676.

10. Гусейнова, А.С. Комплексная оценка цитокинового профиля и метаболизма костной ткани у больных ревматоидным артритом / А.С. Гусейнова, А.Г. Гаджиев, А.М. Эфендиев [и др.] // Фундаментальные аспекты психического здоровья, – 2019, №3, – с. 4-8.

11. Гусейнова, А.С., Керимова, И.А., Ягубова, В.И. Оценка цитокинового профиля и статуса витамина D у больных ревматоидным артритом // Azərbaycan Allerqologiya və Klinik İmmunologiya Jurnalı (xüsusi buraxılış), – 2019. № 2, – s.39.

12. Hüseynova Ə.S. İkincili osteoporozların erkən diaqnostikasında D vitamini təyininin əhəmiyyəti / Ə.S. Hüseynova, A.Ə. Eyyubova, S.A. Bağırova [və b.] // İctimai sağlamlıq və səhiyyə, Bakı, – 2019, IV cild, – s.63-67.

13. Huseynova, A.S., Efendiyev, A.M., Yagubova, V.I. Evaluation of some endogenous antimicrobial peptides in rheumatoid arthritis // MAS 15th international European Conference on mathematics, engineering, natural and medical sciences, – Adana, –Turkey, september 7-8, 2021. – p.113

LIST OF ABBREVIATIONS

RA	– rheumatoid arthritis
AMP	– antimicrobial peptides
IL	– interleukins
RF	– rheumatoid factor
CRP	– C-reactive protein
ESR	– erythrocyte sedimentation rate
TNF-α	– tumor necrosis factor alpha
IFN	– interferon
Ig	– immunoglobulin
GEBP	– genetically engineered biological preparation
MHC	– major histocompatibility complex
HLA	- Human Leukocyte Antigens
DAS	- Disease Activity Score
ACCP	- anti-cyclic citrullinated peptide antibody
MMP	- Matrixmetalloproteinases
NF-kB	- nuclear factor kappa B

The defense of the dissertation will be held on «31» October 2023 at «11:00» at the meeting of the Dissertation Council FD 1.08 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at Institute of Physiology named after academician Abdulla Garayev.

Address: Sharifzada Street 78, Baku. AZ 1100

The dissertation is available at the Library of the Institute of Physiology named after academician Abdulla Garayev of the Ministry of Science and Education of Azerbaijan Republic.

Electronic versions of the abstract are posted on the official website (www.physiology.az) of the Institute of Physiology named after academician Abdulla Garayev.

Abstract was sent to the required addresses on «27» September 2023.

Signed for print: 18.09.2023

Paper format: 60x84 1/16

Volume: 37426 characters

Number of hard copies: 20