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РЕВМАТОИДНЫЙ АРТРИТ: КЛИНИКО-ЛАБОРАТОРНЫЕ И УЛЬТРАЗВУКОВЫЕ ПАРАЛЛЕЛИ

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Rheumatoid Arthritis: Clinical-Laboratory and Ultrasound Parallels

Резюме

Цель. Установить взаимосвязь показателей сывороточного адипонектина и лептина у больных ревматоидным артритом с клиническими данными, серологическими показателями, активностью заболевания, результатами ультразвукового исследования опорно-двигательного аппарата и рентгенологическим поражением суставов. **Материалы и методы.** В статье представлена сравнительная характеристика уровней адипокинов среди 64 женщин с диагнозом ревматоидный артрит (I группа) и 30 здоровых женщин (II группа). У больных ревматоидным артритом выявлена зависимость уровней адипокинов от клинико-лабораторных, ультразвуковых и рентгенологических изменений. **Результаты.** Концентрация адипонектина была значительно выше у больных ревматоидным артритом по сравнению с группой здоровых (p <0,0001) и имела достоверные корреляционные связи с рентгенологическими изменениями в суставах (r=0,4; p <0,001) и длительностью приема метотрексата (r=0,4; p <0,001) и глюкокортикостероидов (r=0,3; p <0,05). Уровень лептина у больных ревматоидным артритом и контрольной группы был примерно одинаковым. Однако, были отмечены положительные взаимосвязи между уровнем лептина и числом болезненных суставов (r=0,5; p <0,0001), уровнями С-реактивного белка (r=0,3; p <0,05) и интерлейкина-17 (r=0,3; p <0,05), индексом Disease Activity Score 28 (r=0,4; p <0,001), а также усилением кровотока при допплерографии (r=0,4; p <0,001). **Заключение.** Таким образом, у больных ревматоидным артритом отмечается значительное повышение уровня адипонектина по сравнению с группой здоровых, что связано с выраженными деструктивными изменениями в суставах и длительностью приема метотрексата и глюкокортикостероидов. Однако, положительная взаимосвязь между показателями активности заболевания и наличием допплеровского сигнала отмечается только у лептина.

Ключевые слова: *адипонектин; лептин; ультразвуковое исследование суставов*

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Abstract

Purpose. To establish the relationship of serum adiponectin and leptin with clinical data, serological parameters, disease activity, results of ultrasound examination of the musculoskeletal system and X-ray damage of joints in rheumatoid arthritis patients. **Materials and methods.** The article presents a comparative characteristic of adipokine levels among 64 women diagnosed with rheumatoid arthritis (group I) and 30 healthy women (group II). The dependence of adipokine levels on clinical, laboratory, ultrasound and radiological changes was revealed in patients with rheumatoid arthritis. **Results.** The concentration adiponectin level was significantly higher in rheumatoid arthritis patients compared to the control group (p <0.0001) and had significant correlations with radiological changes in the joints (r=0.40; p <0.001) and the intake duration of methotrexate (r=0.4; p <0.001) and glucocorticosteroids (r=0.3; p <0.05). The level of leptin in the blood serum of women with rheumatoid arthritis and healthy individuals was approximately the same. However, there were positive correlations between the level of leptin and of the tender joint count (r=0.5; p <0.0001), the levels of C-reactive protein (r=0.3; p <0.05) and interleukin-17 (r=0.3; p <0.05), the index Disease Activity Score 28 (r=0.4; p <0.001) and increased blood flow during Doppler imaging (r=0.4; p <0.001). **Conclusion.** Thus, patients with rheumatoid arthritis have a significant increase in the level of adiponectin compared to the health group, which is associated with pronounced destructive changes in the joints and the intake duration of methotrexate and glucocorticosteroids. However, a positive relationship between the indicators of disease activity and the presence of a Doppler signal is observed only in leptin.

Key words: *adiponectin; leptin; ultrasound examination of joints*

Anti-CCP — anti-cyclic citrulinated peptide antibodies, GCS — glucocorticosteroids, IL-17 –interleukin-17, BMI — body mass index, WJ — wrist joint, MTX — methotrexate, NSAIDs — non-steroidal anti-inflammatory drugs, RA — rheumatoid arthritis , CRP — C-reactive protein, US — ultrasound examination, TJC — tender joint count, SJC — swollen joint count, TNF-α — tumor necrosis factor-α, PD — power Doppler, DAS28 — Disease Activity Score 28

Introduction

Rheumatoid arthritis (RA) is one of the most common severe immune-mediated inflammatory diseases in humans. It is characterized by chronic erosive arthritis and systemic damage to internal organs, which leads to early disability and shorter life expectancy. This, in turn, determines the high social significance of the disease [1]. The pathogenetic mechanisms of RA are still being studied. A number of studies have shown that adipose tissue is actively involved in the development of systemic inflammation in RA, producing pro-inflammatory cytokines and adipose tissue hormones (adipokines), which can independently modulate inflammatory and destructive processes in joints [2, 3].

However, data on the pro- and anti-inflammatory role of adipokines in the pathogenesis of RA remain poorly understood, which makes this study relevant.

Objective

To establish the relationship between serum adiponectin and leptin in patients with RA with clinical data, serological parameters, disease activity, results of ultrasound examination (US) of the musculoskeletal system and radiological damage to the joints.

Materials and Methods

Clinical, laboratory and ultrasound examinations of 64 women with RA confirmed according to the classification criteria of RA (American College of Rheumatology / European League Against Rheumatism, 2010) [1] were performed in the Orenburg State Medical University Hospital from September 2018 to September 2020. The average age of patients with RA was 46.1 ± 7.1 years; the average duration of the disease was 8.3 ± 5.8 years. Most of the examined patients 52 (81.3%) were seropositive for rheumatoid factor (RF).

Inclusion criteria: the patient's age over 18 years.

The exclusion criteria were hand injury and surgery during the last six months, another concomitant rheumatic disease, cancer, pregnancy and lactation, acute or exacerbation of chronic infections at the time of examination, and diabetes mellitus.

The study was approved by the local ethics committee of the Federal State Budgetary Educational Institution of Higher Education of the Orenburg State Medical University (Protocol No. 208 of September 28, 2018). All patients signed Informed Consent.

Patients with RA underwent a physical examination, which included estimation of the tender joint count (TJC) (mean value 10.0 ± 7.4), swollen joint count (SJC)

(mean value 4.4 ± 2.8), duration of morning stiffness (mean value 100.8 ± 54.4 min), a general assessment of the intensity of pain using a 100-mm visual-analogue scale [1] (mean value 46.2 ± 23.5). Disease activity was assessed by the DAS28 (Disease Activity Score 28) [1] (mean value 4.4 ± 1.7): remission, low, medium and high disease activity were observed in 11 (17.2%), 9 (14.0%), 32 (50.0%) and 12 (18.8%) patients, respectively. The X-ray stage was determined according to the modified Steinbrocker classification [1]: I, II, III and IV radiological stages of the disease were detected in 6 (9.4%), 37 (57.8%), 17 (26.6%) and 4 (6.3%) patients, respectively. The functional status of the musculoskeletal system was evaluated according to the clinical classification criteria of RA (2007) [1]: Functional classes I, II, III, and IV were determined in 6 (9.4%), 36 (56.3%), 20 (31.3%), and 2 (3.1%) patients, respectively.

The control group consisted of 30 women without inflammatory joint diseases, similar in gender, age and body mass index (BMI) to patients with RA.

The BMI index was calculated using the formula: $ИМТ = \frac{weight\ (kg)}{height\ (m)^2}$ [4]. The mean BMI in patients with RA was 26.3 ± 6.2 kg/m², in healthy individuals — 24.6 ± 5.0 (p > 0.05).

The following laboratory tests were performed in all study subjects: serum RF, anti-cyclic citrullinated peptide antibodies (anti-CCP), and C-reactive protein (CRP).

The concentrations of tumor necrosis factor-α (TNF-α), interleukin-17 (IL-17), adiponectin and leptin were determined by ELISA on a Bio-Rad Model 680 microplate photometer using commercial Bender MedSystems kits (Austria — USA), Mediagnost (Germany) and Diagnostics Biochem Canada Inc. (Canada), respectively.

We performed ultrasound examination to determine the inflammatory and destructive changes in 30 joints in each patient with RA (wrist (Wrist joint), from the first to fifth metacarpophalangeal joints, from the second to fifth proximal and distal interphalangeal joints and the first interphalangeal joint on the palmar and dorsal sides of both hands) using the Philips EPIQ 7 device with a multi-frequency linear transducer with a frequency of 4–18 MHz and power Doppler (PD). US exam included the detection of joint effusion, synovial hypertrophy and angiogenesis (the presence of a vascular signal), erosive changes in the hand joints, as well as the assessment of periarticular tissues to detect tenosynovitis on the palmar and dorsal hand sides. We examined 1920 joints in patients with RA.

In our work, we used the term "synovitis", which combines the concepts of "joint effusion" and "hypertrophy of the synovial membrane".

At the time of examination, 29 (45.3%) patients with RA received treatment with disease-modifying anti-rheumatic drugs (methotrexate (MTX) in combination with folic acid, hydroxychloroquine, leflunamide); seven patients (10.9%) received glucocorticosteroids (GCS) orally; 12 patients (18.8%) received a combination of disease-modifying antirheumatic drugs and GCS. Nine (14.1%) patients with RA received non-steroidal anti-inflammatory drugs (NSAIDs).

STATISTICA 12.0 software was used for statistical analysis. Qualitative results are given as the absolute number and percentage of the total number. Mann—Whitney U-test was used to assess the significance of differences between values. To identify the relationships between the variables, the Spearman pair correlation coefficient was calculated. Values were considered significant at p ≤ 0.05. To determine the agreement between the results of the two examination methods, the Cohen's kappa coefficient (κ) was used, the values of which at κ < 0.2 reflect poor, 0.2–0.40 — mediocre, 0.41–0.60 — moderate, 0.61–0.80 — good and > 0.81 excellent agreement [5].

Results

Table 1 presents a comparative assessment of the results of physical and ultrasound examination of patients with RA.

B-mode ultrasound revealed inflammation in the form of synovitis and tenosynovitis in 965 (50.3%) joints in patients with RA, while clinical manifestations of arthritis (TJC and SJC) were noted in 755 (39.3%) (Table 1). No ultrasound signs of inflammation were detected in 210 (10.9%) joints in RA during physical

Table 1. *Comparative assessment of clinical symptoms of arthritis and US signs of inflammation in WJ and small joints of the hands by B-mode US in RA, n (%)*

	RA (n=1920)			
	Clinical manifestations of inflammation in the joint			
US-signs	**TJC and SJC**	**TJC**	**SJC**	**No TJC and SJC**
Synovitis and tenosynovitis	96/5,0	11/0,6	4/0,2	13/0,7
Only synovitis	154/8,0	58/3,0	96/5,0	167/8,7
Only tenosynovitis	123/6,4	186/9,7	27/1,4	30/1,6
Absence of US-signs	6/0,3	58/3,0	4/0,2	887/46,2

Table 2 *Comparative assessment of clinical symptoms of arthritis and signs of hypervascularization of synovial stratum in the WJ and small joints of the hands detected with using PD US in RA, n (%)*

US-signs	RA (n=1920)			
	Clinical manifestations of inflammation in the joint			
	TJC and SJC	TJC	SJC	No TJC and SJC
Signal present	146/7,6	96/5,0	68/3,5	90/4,7
Signal absent	248/12,9	215/11,2	58/3,0	995/51,8

Table 3. *Adipocytokine concentrations in the blood serum of RA patients and the control group*

Adipocytokine	RA patients (n=64)	Control group (n=30)	p
Adiponectin (ng/ml), M±σ	40,9±13,6	22,8±11,3	p <0,0001
Leptin (ng/ml), M±σ	18,1±14,0	16,6±11,4	nr

Note: nr — not reliable

examination (Table 1). At the same time, swelling and/or pain in 68 (3.5%) joints in patients with RA noted during physical examination were not confirmed with US (Table 1).

When comparing the data of physical examination and US, the agreement was observed in 85.5% of cases (κ = 0.7) in patients with RA.

The Doppler signal was detected in 400 (20.8%) joints in RA, of which clinical signs of arthritis were found in 310 (16.2%) joints (Table 2). The Doppler signal was not detected in 1,516 (78.9%) joints in RA, of which swelling and/or tenderness were noted in 521 (27.1%) joints, respectively (Table 2).

The agreement between the results of the physical examination and US in the PD mode was 68.3% (κ = 0.3) in RA.

When determining the relationship between ultrasound and clinical/laboratory data, correlations between US signs of synovitis were detected in B-mode and PD (r = 0.4; p < 0.001) and SJC (r = 0.5; p < 0.0001). Significant correlations were determined between the presence of the Doppler signal and CRP (r = 0.5; p <0.0001) and anti-CCP (r = 0.3; p < 0.05) levels, as well as higher DAS28-CRP (r = 0.5; p <0.0001).

The serum level of adiponectin in patients with RA was significantly higher compared with the control group (p <0.0001). The serum leptin level in women with RA and healthy individuals was approximately the same (Table 3).

In RA, there was a negative correlation between the concentrations of adiponectin and leptin (r = −0.3; p < 0.05). The level of serum adiponectin negatively correlated with the level of CRP (r = −0.3; p < 0.05). In this case, significant correlations between the X-ray stage of RA and adiponectin level were found (r = 0.4; p <0.001). Additional correlations were observed between adiponectin level and duration of MTX treatment (r = 0.4; p < 0.001) and GCS (r = 0.4; p < 0.001) (Table 4).

We noted a positive correlation between the level of leptin and BMI (r = 0.6; p <0.0001). In addition, significant correlations were found between the level of serum leptin and the duration of morning joint stiffness (r = 0.3; p < 0.05), TJC (r = 0.5; p <0.0001), as well as the level of CRP (r = 0.3; p < 0.05) and the DAS28 index (r = 0.4; p < 0.001) in patients with RA. In patients with RA, an increase in the concentration of IL-17 was associated with an increase in the level of leptin (r = 0.3; p < 0.05).

Table 4. *Correlations between adipocytokine levels in RA patients and clinical manifestations of the disease, laboratory parameters, disease activity, US-signs of joint inflammation and X-ray stage*

Indicators	Adiponectin (ng/ml)	Leptin (ng/ml)
	r	r
Age (years)	0,2	0,1
BMI (kg/m²)	-0,1	0,6****
Duration of disease (years)	-0,1	-0,1
Morning stiffness (min)	-0,1	0,3*
ЧБС/ТJC	0,1	0,5****
SJC	-0,2	0,1
DAS28-CRP	-0,2	0,38**
CRP (mg/l)	-0,2	0,32*
RF (U/mL)	0,2	-0,1
A-CCP (U/mL)	0,1	-0,2
TNF-α (pkg/ml)	-0,1	0,1
IL-17 (pkg/ml)	-0,2	0,3*
X-Ray stage	0,4***	-0,2
US-signs: Synovitis	-0,2	0,1
Tenosynovitis	-0,2	0,1
Hypervascularization of synovia	-0,2	0,4****
Intake duration of MTX (years)	0,4***	0,1
Intake duration of GCS (months)	0,4**	0,2
Intaking NSAIDs	0,1	0,1

Note: * — p ≤0.05; ** — p <0.01; *** — p <0.001; **** — p <0.0001

However, we did not find any significant correlations between the concentrations of serum adipokines and the levels of RF and anti-CCP (Table 4).

As shown in Table 4, along with BMI, serum leptin showed high correlations with TJC, levels of CRP, as well as the DAS28, indicating disease activity. At the same time, adiponectin had significant correlation with radiological changes in the joints and drugs used (MTX and GCS).

Also, a positive correlation was observed between the concentration of leptin and the presence of the Doppler signal (r = 0.4; p < 0.001) (Table 4).

Discussion

Most of the previous studies have shown that the concentrations of serum leptin [2, 6, 7] and adiponectin [2, 7] are higher in patients with RA than in healthy individuals. At the same time, low levels of these adipokines in RA were also recorded [2, 6]. Our results show a significant increase in adiponectin level in patients with RA (p < 0.0001) compared to the control group. However, there was no tendency to leptin concentration increase in women with RA.

A number of authors note the anti-inflammatory role of adiponectin in the human body. However, in RA, the level of this adipokine does not directly correlate with disease activity [7, 8], as confirmed by our study.

We noted a significant correlation between the level of adiponectin and the radiological stage of RA (p < 0.001), which is consistent with a number of studies that note the relationship between high levels of adiponectin and radiological markers of joint destruction (erosion and narrowing of the joint space) [9, 10].

In the present study, the level of adiponectin in serum significantly correlated with the duration of MTX and GCS treatment, which is consistent with other authors [3, 7]. A number of researchers reported that TNF-α reciprocally inhibits adiponectin production in adipose tissue. Since MTX and GCS inhibit the production of proinflammatory cytokines, including TNF-α, they can cause an increase in serum adiponectin in patients with RA [3].

Most previous studies established a positive correlation between the level of leptin and BMI [2], which is also observed in our study. It has been suggested that leptin may play the role of the pro-inflammatory cytokine in RA [2, 7]. We identified significant positive relationships between the level of leptin and disease activity (TJC, CRP level, DAS28-CRP) and morning stiffness of joints, which is consistent with the results of several authors [2, 6, 7]. Also, in our study, the level of serum leptin positively correlated with the concentration of proinflammatory cytokine (IL-17). Deng J. et al. suggested that

leptin stimulates the differentiation of CD4+ T-lymphocytes along the Th-17 pathway [11], which explains the obtained results.

Such concepts as "subclinical synovitis" and "subclinical tenosynovitis" emerged with the development of imaging techniques [12, 13]. A number of researchers noted the discrepancy between the results of clinical and ultrasound assessments of inflammatory changes in the joints [14, 15], which was confirmed in our study: US showed more joints with signs of inflammation than physical examination in patients with RA (p < 0.0001). At the same time, the proportion of false-negative results was insignificant and amounted to 3.5% in RA.

Ceponis A. et al. note the correlation between the ultrasound signs of synovitis and tenosynovitis in the study in B-mode and PD and SJC [16] in patients with RA, which is consistent with the results of our study.

When determining the relationship between ultrasound and laboratory findings, we found a close correlation between increased blood flow and levels of CRP and anti-CCP (p < 0.0001; p < 0.05) and higher DAS28-CRP (p < 0.0001). Our results were confirmed by studies Xu H. et al. [14], showing that PD reflects the activity of the disease better than the B-mode. Also, a positive correlation was observed between the concentration of leptin and the presence of the Doppler signal (p < 0.001), which is consistent with the results of a study by Sherin H.N. et al. [17], who observed a moderate increase in the average serum leptin level in patients with increased blood flow according to Doppler US.

The obtained data suggest a pathogenetic relationship between adipose tissue hormones and inflammatory and destructive processes in the joints.

Conclusion

There is a significant increase in the level of adiponectin in patients with RA compared with the healthy group, which is associated with severe destructive changes in the joints and the duration of MTX and GCS treatment. The concentration of leptin in patients with RA and healthy individuals is approximately the same. However, there is a positive relationship between the level of leptin and disease activity (TJC, level of CRP and IL-17, DAS28-CRP) and increased blood flow, which suggests its role as a pro-inflammatory cytokine.

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Список литературы/ References:

1. Насонов Е.Л., редактор. Ревматология: клинические рекомендации. Москва, ГЭОТАР-Медиа. 2017; 464 с.
   Nasonov E.L., redaktor. Revmatologiya: klinicheskie rekomendatsii [Rheumatology. Clinical guidelines. Moscow, GEOTAR-Media. 2017; 464 p. [in Russian].

2. Fatel E.C. S., Rosa F.T., Simao A.N. C., et al. Adipokines in rheumatoid arthritis. Advances in Rheumatology. 2018; 58(1): 25. doi.org/10.1186/s42358-018-0026-8.

3. Neumann E., Frommer K.W., Vasile M., et al. Adipocytokines as driving forces in rheumatoid arthritis and related inflammatory diseases? Arthritis & Rheumatology. 2011; 63(5): 1159-1169. doi: 10.1002/art.30291.

4. Дедов И.И., Мельниченко Г.А., Шестакова М.В. и др. Национальные клинические рекомендации по лечению морбидного ожирения у взрослых. Пересмотр 3-й (Лечение морбидного ожирения у взрослых). Ожирение и метаболизм. 2018; 15(1): 53-70. doi: 10.14341/OMET2018153-70
   Dedov I.I., Mel'nichenko G.A., Shestakova M.V. et al. Russian national clinical recommendations for morbid obesity treatment in adults. 3rd revision (Morbid obesity treatment in adults). Obesity and Metabolism. 2018; 15(1): 53-70. doi: 10.14341/OMET2018153-70 [in Russian].

5. Viera A.J., Garrett J.M. Understanding interobserver agreement: the kappa statistic. Family Medicine. 2005; 37(5): 360-363.

6. Tian G., Liang J-N., Wang Z-Y., et al. Emerging role of leptin in rheumatoid arthritis. Clinical & Experimental Immunology. 2014; 177(3): 557-570. doi: 10.1111/cei.12372.

7. Yoshino T., Kusunoki N., Tanaka N., et al. Elevated serum levels of resistin, leptin, and adiponectin are associated with C-reactive protein and also other clinical conditions in rheumatoid arthritis. Internal Medicine. 2011;50(4):269-75. doi: 10.2169/internalmedicine.50.4306.

8. Dessein P.H., Norton G.R., Badenhorst M., et al. Rheumatoid arthritis impacts on the independent relationships between circulating adiponectin concentrations and cardiovascular metabolic risk. Mediators of Inflammation. 2013; 2013: 461849. doi: 10.1155/2013/461849.

9. Giles J.T., van der Heijde D.M., Bathon J.M. Association of circulating adiponectin levels with progression of radiographic joint destruction in rheumatoid arthritis. Annals of the Rheumatic Diseases. 2011; 70(9): 1562-1568. doi: 10.1136/ard.2011.150813.

10. Meyer M., Sellam J., Fellahi S., et al. Serum level of adiponectin is a surrogate independent biomarker of radiographic disease progression in early rheumatoid arthritis: results from the ESPOIR cohort. Arthritis Research & Therapy. 2013; 15(6): R210. doi: 10.1186/ar4404.

11. Deng J., Liu Y., Yang M., et al. Leptin exacerbates collagen-induced arthritis via enhancement of Th17 cell response. Arthritis & Rheumatology. 2012; 64: 3564-3573. doi: 10.1002/art.34637.

12. Sudol-Szopinska I., Jans L., Teh J. Rheumatoid arthritis: what do MRI and ultrasound show. Journal of Ultrasonography. 2017; 17(68): 5-16. doi: 10.15557/JoU.2017.0001.

13. Hernandez Diaz C., Robles-San Roman M., Vargas A., et al. SAT0180 Subclinical inflammation in rheumatoid arthritis (RA) in clinical remission, lack of association between cytokines level and ultrasound-defined synovitis. Annals of the Rheumatic Diseases. 2014; 73(2): 655. doi: 10.1136/annrheumdis-2014-eular.4855.

14. Xu H., ZhangY., Zhang H., et al. Comparison of the clinical effectiveness of US grading scoring system vs MRI in the diagnosis of early rheumatoid arthritis (RA). Journal of Orthopaedic Surgery and Research. 2017; 12(1): 152. doi 10.1186/s13018-017-0653-5.

15. Sun X., Deng X., Xie W., et al. The agreement between ultrasounddetermined joint inflammation and clinical signs in patients with rheumatoid arthritis. Arthritis Research & Therapy. 2019; 21(1): 100. doi.org/10.1186/s13075-019-1892-0.

16. Ceponis A., Onishi M., Bluestein H.G., et.al. Utility of the ultrasound examination of the hand and wrist joints in the management of established rheumatoid arthritis. Arthritis Care & Research. 2014; 66(2): 236-244. doi: 10.1002/acr.22119.

17. Hamza S.H., Younes T.B. Value of Serum Leptin in patients with Rheumatoid arthritis; Its Correlation With disease activity and Musculoskeletal Ultrasound findings. Journal of American Science. 2017; 13(12): 128-137. doi:10.7537/marsjas131217.15.