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ACTIVITY OF PROOXIDANT AND ANTIOXIDANT SYSTEMS ENZYMES IN PLASMA OF RHEUMATOID ARTHRITIS PATIENTS

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The **aim** of our study was to characterize the activity profile of prooxidant and antioxidant systems key enzymes in plasma of rheumatoid arthritis (RA) patients.

Methods. 71 RA patients (46 women and 25 men) were enrolled in the study. The diagnosis was verified with ACR/EULAR criteria (2010). All patients were treated in Municipal Clinical Hospital № 25. The control group consisted of 30 healthy individuals. Disease activity was calculated using DAS 28. 24 (33.8%) patients had low disease activity, moderate and high disease activities were determined in 41 (57.7%) and 6 (8.5%) cases, respectively. Extra-articular manifestations were revealed in 30 (42,2%) patients. Plasma xanthine oxidase (XO), xanthine dehydrogenase (XDH) and superoxide dismutase (SOD) activities were measured using previonsly descriped spectrophotometric techniques. XO and XDG activities were expressed as nmol/ml□min, SOD activity – as units of activity.

Results. Reference intervals (M±2σ) of enzymatic activities were 2.28-5.12 nmol/ml□min (for XO), 3.96-7.24 nmol/ml□min (for XDH), and 3.13-6.58 units (for SOD). We heve revealed substantial changes in all the plasma enzymatic activities of RA patients. These changes wore independed with the disease activity autoimmune inflammation and presence of extra-articular manifestations. XO and SOD activities were increased in all RA patients. XO activity have been reached its highest values at maximum disease activity with extra-articular involvemehts, SOD activity – in moderate and high disease activities as well as in patients with articular form of RA. XDG activity was increased at low disease activity and articular form of RA, while moderate, high disease activities and extra-articular manifestations were characterized by decreased activity of this enzyme.

Conclusion. Plasma enzymatic profile in RA patients is characterized by an increase of XO and SOD activities, which indicates the intensification of prooxidant and antioxidant mechanisms. It can be assumed that the excessive generation of reactive oxygen species can stimulate the formation of neutrophil extracellular traps by neutrophil granulocytes as a result of the activation of free radical reactions.

Keywords: rheumatoid arthritis, plasma, enzymatic profile.

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Introduction. Rheumatoid arthritis (RA) is an autoimmune rheumatic disease of unknown etiology which is characterized by chronic erosive arthritis and systemic organ involvement resulting in early disability and shorter life expectancy [6]. Despite significant advances in the diagnostics and treatment of RA over the past 50 years, there still remain unsolved problems. This is due to the fact that RA continues to be one of the most prevalent rheumatic diseases which has significant socioeconomic costs [10]. Moreover, the use of genetically-engineered biological agents frequently leads to RA progression. Therefore, in recent years there has been a growing interest in the molecular and cellular mechanisms of immune-mediated inflammatory pathogenesis of RA. New insights into the mechanisms underpinning RA pathogenesis will lead to improved diagnostics of RA at an early preclinical stage, better assessment of disease activity, prognosis and treatment outcomes, and the development of novel more specific treatment modalities.

At present there is no credible or convincing evidence linking RA with one etiological factor. A combination of genetic and environmental factors has been suggested to cause a cascade of abnormal cellular and humoral immune responses [12]. These responses cause not only the synovial inflammation leading to the destruction of joints but also internal organ involvement. However, the mechanism underpinning the transition of autoimmune response to joint inflammation still remains uncertain. At the same time, numerous studies have shown that anti-citrullinated protein antibodies appear many years before the clinical onset of joint inflammation [11]. The production of anti-citrullinated protein antibodies is considered to play a key role in the pathogenesis of RA.

Neutrophil granulocytes also play a specific role in the induction and promotion of autoimmune inflammation in RA. It is accounted for by the ability of neutrophils to release neutrophil extracellular traps (NETs) through cell death called NETosis [2, 8, 9, 12, 15].

Anti-citrullinated protein antibodies and inflammatory cytokines (IL-17A, TNF-α and IL-8) can stimulate NETosis, whereas NETs externalize citrullinated autoantigens which are potential anti-citrullinated protein antibody targets, thus perpetuating a cycle of citrullinated autoantigen generation and induction of autoimmune responses in RA [12, 13].

NETs are networks of condensed

chromatin (histones and DNA), components of neutrophil granules (neutrophil elastase, myeloperoxidase, cathepsin G, leucocyte proteinase 3, lactoferrin, gelatinase, lysozyme C, calprotectin, defensins and cathelicidins) and cytoskeletal proteins (actin, tubulin) [1]. NET production is commonly accompanied by neutrophil cell death. According to current data, NETs not only induce RA but also initiate autoaggressive response which underlies chronic autoimmune inflammation in RA [12, 15].

One of the factors triggering the production of NETs is overexpression of reactive oxygen species (ROS) [14]. NADPH-oxidase is considered the main source of ROS. The role of NADPH-oxidase in the initiation of NET production is clearly understood. However, ROS can be formed by other reactions. The enzyme xanthine oxidase/xanthine dehydrogenase complex is the most important source of ROS. These two forms of xanthine oxidoreductase (XOR) may be interconverted. NADPH-oxidase plays a leading role in xanthine dehydrogenase (XDH) and xanthine oxidase (XO) regulation. ROS generated by NADPH may promote the conversion of XOR to oxidase [5]. XO is known to act as a prooxidant. XO activation is accompanied by increased formation of superoxide radicals via oxidation of hypoxanthine and xanthine.

Protection from overproduction of ROS in the body is provided by a complex antioxidant system, with superoxide dismutase being the most important. Healthy individuals tend to have prooxidant-antioxidant balance. A shift in the balance between oxidants and antioxidants is accompanied by increased formation of ROS which can result in oxidative stress involved in a number of pathological conditions.

As ROS promotes NET generation, the study of oxidant and antioxidant enzyme activity in patients with RA is of great interest.

The **aim** of our study was to characterize the activity profile of prooxidant and antioxidant systems key enzymes in plasma of rheumatoid arthritis (RA) patients.

Matherials and methods. 71 RA patients (46 women and 25 men) were enrolled in the study. Mean age of patients was 43.2±3.6 years, mean RA duration was 11.9±2.6 years. The diagnosis was verified with ACR/EULAR criteria (2010) [7]. All patients were treated in Municipal Clinical Hospital № 25. The control group consisted of 30 healthy individuals. RA patients and healthy individuals were comparable in gender and age. Disease activity was calculated using DAS 28. 24 (33.8%) patients had low disease activity, moderate and high disease activities were determined in 41 (57.7%) and 6 (8.5%) cases, respectively. Extra-articular manifestations were revealed in 30 (42.2%) patients. Mostly, involvement in the pathological process of the heart (30%), lungs (23.3%) and kidneys (23.3%) was diagnosed. 41 patients (57.8%) had an articular form of the disease. The radiological stage of joint involvement was evaluated using Steinbroker criteria. Most RA patients had II and III stages of joint involvement: 36 (50.7%) and 27 (38.0%), respectively. I stage was determined in 5 (7.0%) cases, IV stage – in 3 cases. Joint functional limitations had 68 (95.8%) patients. 28 (39.4%) of them were related to the first functional class, 35 (49.3%) – to the second functional class, 5 (7.1%) – to the third functional class. Rheumatoid factor antibodies were revealed in 67.6% cases.

Plasma xanthine oxidase (XO; E.C. 1.17.3.2), xanthine dehydrogenase (XDH; E.C. 1.17.1.4) and superoxide dismutase (SOD; E.C. 1.15.1.1) activities were measured using previously described spectrophotometric techniques. XO and XDG activities were expressed as nmol/ml□min, SOD activity – as units of activity [3, 4].

Statistical analysis was performed using Statistica 6.0 software package. Results were presented as (Me, Q25, Q75). The Mann-Whitney U test was used for statistical analysis. Differences were considered significant when $p<0.05$.

Results and discussion. All the plasma enzymatic activities of healthy individuals were not dependent on gender and age. These factors were not considered in the enzymatic activities analysis of RA patients. Reference intervals (M±2σ) of enzymatic activities were 2.28-5.12 nmol/ml□min (for XO), 3.96-7.24 nmol/ml□min (for XDH), and 3.13-6.58 units (for SOD).

We conducted research of the plasma enzymatic activities in RA patients with different clinical manifestations. The dependence of XO, XDG, SOD activities on RA activity and presence of extra-articular manifestations was analyzed. The results are presented in the Table 1.

We have revealed substantial changes in all the plasma enzymatic activities of RA patients. These changes were multidirectional. XO and SOD activities were increased in all RA patients. XO activity have been reached its highest values at maximum disease activity and presence extra-articular involvements, SOD activity – in moderate and high

Activity of enzymes in plasma of rheumatoid arthritis patients (Me (Q25; Q75))

Group	Enzymes			Significance of differences with healthy individuals	Significance of differences between patient's group
	XO	XDG	SOD		
Healthy individuals, n=30	$3.7^{1.2.3.4.6.8}$ (3.2; 4.1)	$5.6^{2.5.7.9}$ (4.9; 6.1)	$4.9^{1.2.3.4.6.8}$ (4.3; 5.1)		
RA patients, n=71	6.3^{1} (4.8; 9.6)	5.5 (4.8; 6.7)	9.2^{1} (7.3; 11.6)	$^{1}p<0.001$	
RA activity (degree)					
1, n=24	$5.3^{2.10.11}$ (4.1; 6.1)	$6.9^{2.13.14}$ (6.3; 7.6)	$6.7^{2.15.16}$ (5.3; 7.4)	$^{2}p<0.001$	$^{10}p<0.001$ $^{11}p<0.001$ $^{12}p=0.015$ $^{13}p<0.001$ $^{14}p<0.001$ $^{15}p<0.001$ $^{16}p<0.001$
2, n=41	$7.2^{3.10.12}$ (5.0; 10.0)	5.2^{13} (4.8; 5.8)	$9.9^{3.15}$ (9.0; 13.2)	$^{3}p<0.001$	
3, n=6	$10.6^{4.11.12}$ (8.3; 11.7)	$4.6^{5.14}$ (3.9; 5.2)	$11.5^{4.16}$ (9.7; 12.9)	$^{4}p<0.001$ $^{5}p=0.008$	
Форма заболевания					
Articular form, n=41	$5.0^{6.18}$ (3.8; 6.2)	$6.5^{7.19}$ (5.2; 6.9)	$10.5^{6.20}$ (6.7; 13.2)	$^{6}p<0.001$ $^{7}p=0.011$	$^{18}p<0.001$ $^{19}p<0.001$ $^{20}p=0.038$
Presence of Extra-articular manifestations, n=30	$9.7^{8.18}$ (7.5; 10.8)	$5.1^{9.19}$ (4.6; 5.5)	$8.4^{8.20}$ (7.5; 9.3)	$^{8}p<0.001$ $^{9}p=0.025$	

disease activities as well as in patients with articular form of RA. XDG activity was increased at low disease activity and articular form of RA, while moderate, high disease activities and extra-articular involvements were characterized by decreased activity of this enzyme.

Plasma enzymatic profile of RA patients is characterized by an increase of XO and SOD activities, which indicates the intensification of prooxidant and antioxidant mechanisms. SOD activity was substantialy lower in RA patients with extra-articular manifestations, that confirms the debilitation of the antioxidant protection with disease progression and visceral organs involvement in the autoimmune rheumatoid process. The progressive increase of XO activity against the background of the decreased XDG activity indicates the intensification of free radical oxidation in more severe forms of RA. It can be assumed that the excessive generation of ROS can stimulate the formation of neutrophil extracellular traps by neutrophil granulocytes as a result of the activation of free radical reactions.

Conclusion.

1. RA is characterized by the activation of the prooxidant and antioxidant systems enzymes.
2. The severe forms of RA accompanied by the intensification of free radical oxidation.
3. The visceral organs involvements are accompanied by the debilitation of the antioxidant protection.

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