ORIGINAL ARTICLE

Pro-inflammatory Cytokines, Endothelial Dysfunction and Hemocoagulation Disorders in Late-Stage Rheumatoid Arthritis with Antiphospholipid Syndrome

Yuliya A. Sarycheva[1], Tatyana V. Chernysheva[1]

ABSTRACT

The activation of both cellular and humoral links of innate and acquired immunity with an imbalance between pro-inflammatory and anti-inflammatory cytokines can be observed at the various stages of the pathogenesis of rheumatoid arthritis. In some cases, dysregulation of the "cytokine cascade" leads to significant changes in the blood coagulation system, platelet activation, changes in the vascular wall, and the formation of blood clots in vessels of various sizes. The present study observed changes in the levels of interleukin-6, interleukin-18, and tumor necrosis factor -α and their effect on the main parameters of the hemostatic system and endothelial dysfunction in patients with late-stage of rheumatoid arthritis in condition of comorbidity with antiphospholipid syndrome. This cross-sectional study included 80 patients aged 40 to 65 years with a diagnosis of rheumatoid arthritis – 40 people with antiphospholipid syndrome and 40 without it. There was a direct correlation between increased levels of pro-inflammatory cytokines and an increase in platelet count, prothrombin index, fibrinogen, D-dimer, and endothelin-1; and an inverse correlation with values of activated partial thromboplastin time. It was found that an increase in the concentration of interleukin-6, interleukin-18 and tumor necrosis factor-α is associated with increased hypercoagulation, increased markers of endothelial dysfunction and thrombosis, which undoubtedly worsens the course of the underlying disease.

Keywords: Pro-inflammatory cytokines, rheumatoid arthritis, antiphospholipid syndrome, hemocoagulation

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INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease characterized by prolonged destructive joint inflammation, systemic disorders in the functioning of internal organs, comorbid pathology, leading to premature disability.[1,2] In current scientific publications, RA is described as an autoimmune pathology characterized by chronic inflammation, and a key aspect of its pathogenesis is the disruption of the cytokine balance. Cytokines are an important and universal group of factors of the immune system. Their main role is to act as mediators of intercellular interactions and to regulate immune responses and inflammation.[3] At various stages of RA pathogenesis, activation of both cellular and humoral components of innate and acquired immunity can be observed, with an imbalance between pro-inflammatory and anti-inflammatory cytokines.[4,5] The overproduction of the latter leads to significant inflammatory reactions and the production of acute phase proteins, rheumatoid factor, antibodies to cyclic citrullinated peptide, and others. It is believed that pro-inflammatory cytokines play a significant role in the pathogenesis

1. Orenburg State Medical University, Orenburg, Russian Federation.

Correspondence to: Yuliya A. Sarycheva, Orenburg State Medical University City, Orenburg, Russian Federation. Email: yualsarycheva@mail.ru

of RA, determining the development of clinical symptoms and laboratory changes in the early stages of the disease. At the same time, their influence in the advanced and late stages of the disease remains insufficiently studied.[5,6] Evidence showed that the results of cytokine damage in RA can lead to endothelial dysfunction, to a greater extent at the microcirculatory level, and excessive production of interleukin-6 (IL-6) in combination with C-reactive protein (CRP) in the course of the disease for more than 2 years potentiates the development of macrovascular endothelial dysfunction.[6,7] New laboratory biomarkers are currently being actively developed that can help improve the diagnosis of immune-inflammatory rheumatic diseases.[8] One of the options being considered is the measurement of serum interleukin-18 (IL-18) levels. The results obtained in laboratory animal models and clinical studies indicate that IL-18 may play a role in the pathogenesis of various human diseases: inflammatory processes in the intestine, systemic vasculitis, etc.[9-11] Moreover, IL-18 is a promising target for anti-cytokine therapy, especially in individuals with pronounced inflammatory activity, which is caused by the overactivation of the innate immune response.[12] The study of cytokine concentrations in patients with RA complicated by concomitant diseases is of significant scientific and clinical interest, especially in the context of comorbidity. The analysis of the cytokine profile in the presence of concomitant pathology is an important task.[4] In recent years, the cellular immune mechanisms of antiphospholipid syndrome (APS) have been intensively studied. It is believed that dysregulation of the normal cytokine balance with an increase in the production of pro-inflammatory cytokines is an important factor in the immunopathogenesis of APS. The study of the clinical and pathogenetic significance of the latter is limited to isolated reports of increased serum levels of tumor necrosis factor alpha (TNF-alpha) and IL-6 in this condition.[13] The study of the effect of interleukin-1, IL-6, interleukin-8, and TNF-α in women with APS and a history of pregnancy loss showed that they can initiate pathological autoimmune processes under certain circumstances. In these cases, there is a disruption in the regulation of the "cytokine cascade", which leads to serious changes in the coagulation system, platelet activation, vascular wall disorders, and the formation of blood clots.[14-16] Studying the effect of pro-inflammatory cytokines on blood clotting parameters can serve as an important link in the diagnosis, prediction, and monitoring of treatment effectiveness. Understanding the role of specific cytokines contributes to the development of new targeted therapeutic strategies, including for patients who are resistant to standard therapy.

Therefore, the objective of this study was to observe changes in the levels of interleukin-6, interleukin-18, and tumor necrosis factor-α and their effect on the main parameters of the hemostatic system and endothelial dysfunction in patients with late-stage of rheumatoid arthritis in condition of comorbidity with antiphospholipid syndrome.

METHODS

80 patients with late-stage RA aged from 40 to 65 years were examined: 40 people with antiphospholipid syndrome (APS) and 40 people without APS. The diagnosis of APS was verified based on the classification criteria for APS, 2023 ACR/EULAR. The groups were comparable in terms of gender, age, and duration of RA. Hematological parameters were examined using automatic hematological analyzers Medonic and Swelab. Coagulogram was performed using an automatic coagulometer CoaLAB 1000. The levels of IL-6, IL-18, TNF-α, fibrinogen, D-dimer, and endothelin-1 were determined by enzyme-linked immunosorbent assay (Bio-Rad Model 680 Microplate Reader). Statistical data processing was performed using the Statistica 10.0 program (StatSoft Inc., USA). Quantitative variables were represented by the following statistical characteristics: the arithmetic mean (M), the standard deviation from the arithmetic mean (σ), the 25th and 75th percentiles, and the median. Qualitative variables were described by absolute and relative frequencies (percentages). The results were considered statistically significant at $p<0.05$.

RESULTS

The changes in the levels of pro-inflammatory cytokines, such as IL-6, IL-18, and TNF-α, in RA patients with APS and without APS are presented in Table 1. In the context of RA and APS comorbidity, significantly higher levels of pro-inflammatory cytokines were detected, compared to patients suffering from RA only.

Increased concentrations of these inflammatory mediators may indicate a more pronounced activity of immunopathological processes in the combination of these two pathologies. In both groups, there was a direct correlation between the levels of pro-inflammatory cytokines and the erythrocyte sedimentation rate, CRP, and the activity of the inflammatory process (RA and APS: $p<0.01$; RA without APS: $p<0.05$). Additionally, the effect of pro-inflammatory cytokines on the change in the concentration of endothelin-1 (ET-1), a powerful vasoconstrictor and inflammatory mediator with thrombotic activity, was evaluated. Changes in ET-1 levels are presented in Table 2. Patients with APS have severe endothelial dysfunction associated with damage to endothelial cells by antiphospholipid antibodies (aPL) and inflammation, leading to hypercoagulation and thrombosis. There is a "two-hit" model: aPL initially causes endothelial dysfunction, and a secondary environmental factor triggers clinical manifestations. The mechanism of thrombosis in APS involves the recognition of aPL antigen on endothelial cells, followed by signal transduction and changes in cellular activity. This is manifested by the production of adhesion molecules and increased procoagulant activity (TF- expression). It has been established that the cytokines under study can affect the stimulation of ET-1 production, which can lead to the development of endothelial dysfunction in patients with RA, especially in combination with APS. The increased level of ET-1 induced by pro-inflammatory cytokines can have pathological consequences, contributing to the development and progression of cardiovascular diseases. The correlation between changes in the levels of IL-6, IL-18, TNF-α and the main parameters of the hemostatic system and the level of ET-1 is presented in Table 3.

Table 1: Changes in the levels of IL-6, IL-18, TNF-α in RA patients with APS and without APS

Variables	Group A (n=40) RA with APS	Group B (n=40) RA without APS	p-value
IL-6 (Me), pg/mL	3.93 [1.54;13.52]	2.99 [0.84;8.32]	<0.05
IL-6 (min), pg/mL	-	-	-
IL-6 (max), pg/mL	1889.0	41.38	<0.001
IL-6 (increased), n (%)	22 (55%)	11 (27.5%)	<0.01
IL-6 (increased), pg/mL	18.33 [12.41;47.64]	13,42 [10.53;19.44]	<0.05
IL-18 (Me), pg/mL	293.56 [174.27;457.49]	281.27 [149.57;368.22]	<0.05
IL-18 (min), pg/mL	106.05	103.93	-
IL-18 (max), pg/mL	4157.12	1741.62	<0.001
IL-18 (increased, more than 370 pg/mL), n (%)	17 (42.1%)	12 (30%)	<0.05
IL-18 (increased, more than 650 pg/mL), n (%)	12 (30%)	7 (17.5%)	<0.01
IL-18 (increased), pg/mL	495.22 [410.87;843.92]	439.45 [389.56;656.45]	<0.05
TNF-α (Me), pg/mL	2.66 [0.88;11.64]	1.11 [0.67;6.91]	<0.05
TNF-α (min), pg/mL	-	-	-
TNF-α (max), pg/mL	29.57	9.98	<0.01
TNF-α (increased), n (%)	9 (22,5%)	6 (15%)	<0.05
TNF-α (increased), pg/mL	9.54 [7.01;13.78]	7.88 [6.51;10.87]	<0.05

Abbreviations: IL-6- Interleukin-6; IL-18- Interleukin-18; TNF-α-Tumor necrosis factor-α

Table 2: Changes in ET-1 levels in RA patients with APS and without APS

Variables	Group A (n=40) RA and APS	Group B (n=40) RA without APS	p-value
ET- 1 (M±σ), pg/mL	2.25±0.61	1.82±0.59	<0.05
ET -1 (increased), pg/mL	2.67±0.31	2.28±0.37	<0.05
ET -1 (increased), n (%)	24 (60%)	10 (25%)	<0.01
ET-1 (increased above average values), n (%)	40 (100%)	24 (60%)	<0.01

Abbreviations: ET-1- endothelin-1

Table 3: Correlation of IL-6, IL-18, TNF-α with the main parameters of the hemostatic system and ET-1

Variables	Group A (n=40) RA and APS			Group B (n=40) RA without APS		
	IL-6	IL-18	TNF-α	IL-6	IL-18	TNF-α
Platelets count	r=0.176, p<0.05	r=0.122, p<0.05	r=0.127, p<0.05	r=0.131, p<0.05	r=0.119, p<0.05	r=0.016, p<0.05
Activated partial thromboplastin time	r=-0.352, p<0.05	r=-0.349, p<0.05	r=-0.394, p<0.05	r=-0.298, p<0.05	r=-0.197, p<0.05	r=-0.291, p<0.05
Prothrombin index	r=0.557, p<0.01	r=0.667, p<0.001	r=0.576, p<0.01	r=0.467, p<0.01	r=0.332, p<0.05	r=0.430, p<0.01
Fibrinogen	r=0.668, p<0.001	r=0.765, p<0,001	r=0.654, p<0.001	r=0.411, p<0.01	r=0,402, p<0.05	r=0.378, p<0.05
D-dimer	r=0.584, p<0.01	r=0.593, p<0.01	r=0.499, p<0.01	r=0.356, p<0.05	r=0.255, p<0.05	r=0.230, p<0.05
ET-1	r=0.677, p<0.01	r=0.654, p<0.001	r=0.547, p<0.01	r=0.316, p<0.05	r=0.288, p<0.05	r=0.256, p<0.05

Abbreviations: IL-6- Interleukin-6; IL-18- Interleukin-18; TNF-α-Tumor necrosis factor-α; ET-1-endothelin-1

DISCUSSION

According to our data as presented in the tables, both groups show relationships between IL-6, IL-18, TNF-α, and increased levels of platelets, decreased activated partial thromboplastin time, increased prothrombin index, fibrinogen, D-dimer, and ET-1. Moreover, these relationships are more pronounced in the group of RA patients with APS. This difference may indicate a synergistic effect of APS on the pro-inflammatory response and its consequences for the hemostatic system. It is likely that the presence of aPL, in addition to the chronic inflammation characteristic of RA, enhances the activation of the endothelium, platelets, and coagulation factors, resulting in more significant changes in hemostatic parameters in response to increased levels of the cytokines under study. Mechanisms that may potentially participate in the development of complications include: mutual activation of the inflammatory process, which enhances the damage of the endothelial wall, increased platelet activity, reduced anticoagulant mechanisms, secretion of vasoactive substances, vascular spasm, etc. Damaged endothelium loses its ability to effectively regulate blood flow, causes the formation of platelet aggregations and activates coagulation pathways. Clinically, this may manifest itself in an increased frequency of arterial and venous thrombosis.

When studying RA, we often encounter the fact that its course can be complicated by other conditions that affect the overall picture of the disease, including APS, which is insufficiently studied in RA. Pro-inflammatory cytokines play a key role in changing the parameters of hemostasis in patients with late-stage RA. Their influence is enhanced in the presence of APS, which leads to more pronounced changes. The study revealed the specific changes in IL-6, IL-18, and TNF-α in RA, both in combination with APS and without it. The results showed that an increase in the concentration of these mediators is associated with an exacerbation of the hypercoagulable state, an increase in markers of endothelial dysfunction, and thrombus formation. More pronounced relationships between the presented changes were observed in the group of RA patients with APS. Therefore, it is necessary to develop criteria for more thorough monitoring of such patients. A personalized approach based on the assessment of the cytokine profile and blood clotting parameters can help improve the prognosis and reduce the incidence of life-threatening vascular complications. The results of the study open up new prospects for the development of therapeutic strategies aimed at correcting cytokine imbalance. Further research is needed to determine the optimal algorithm for monitoring inflammatory markers in RA patients, including those with comorbidities.

CONCLUSION

Our study revealed a clear correlation between the levels of the most important pro-inflammatory cytokines and hemostatic disorders in patients with advanced RA and APS. These results not only deepen our understanding of these diseases, but also can improve to understand for the specific targets for therapy and developing personalized treatment approaches that can prevent dangerous thrombotic conditions.

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