



Original Article

Relationship of Immune Indicators and Structural and Functional Properties of Erythrocytes in Chronic Endometritis

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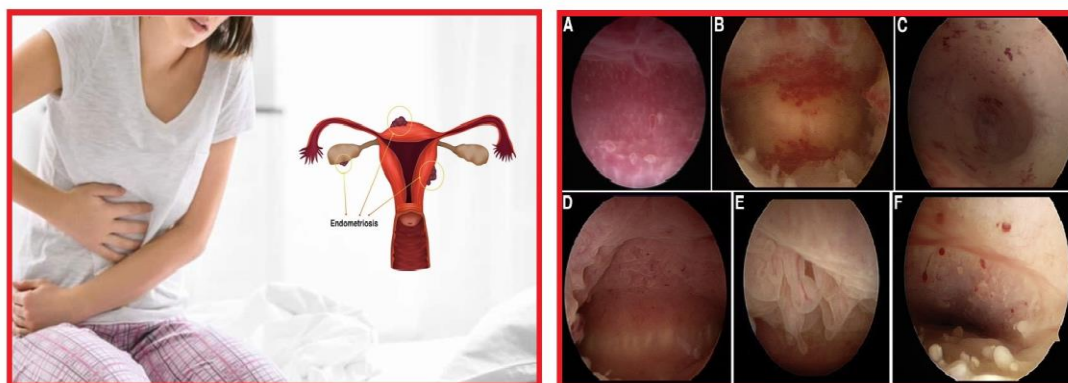
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ABSTRACT

This study dealt with women of childbearing age with infertility against the background of the chronic inflammatory process in the stage of incomplete clinical remission, pronounced changes from pro, anti-inflammatory, regulatory cytokines, components and inhibitors of the complement system. Changes in the architectonics of proteins lead to serious functional disorders properties of peripheral blood erythrocytes in chronic kidney disease even in remission. The composition of blood plasma changes significantly during pathology, which in turn has a significant effect on cell morphology, lipid-protein interactions and the latter-related activity of its enzyme systems. There is a "tension" of the immune and metabolic statuses, which can serve to assess the severity of the disease, its prognosis, treatment effectiveness and preventive measures. "Women with infertility in chronic endometritis and infertility in kidney disease with infertility" was published by the European Society for Human Reproduction and Developmental Disorder (ESDD) in May 2013. The study found that despite the absence of clinical manifestations of inflammation, there was a destructive immune-inflammatory process that requires mandatory correction.

GRAPHICAL ABSTRACT

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Introduction

In connection with the demographic situation in our country, special attention is being paid to the problem of reproductive health. According to the WHO, about 15% of married couples are infertile, and the effectiveness of their assisted reproductive technology (ART) methods does not exceed 25% [1–7]. The most common causes of female infertility and unsuccessful attempts at in vitro fertilization are endometrial pathology, the morpho functional state of which is one of the key factors determining the success of implantation and the full development of the embryo in ART cycles [8,9]. Among the pathological conditions of the endometrium, a significant place is occupied by chronic endometritis (CE), the frequency of detection of which in patients with infertility, according to different authors, ranges from 12 to 68%, reaching its greatest importance in women with tubal-peritoneal form of infertility [10–15].

Despite the enormous amount of knowledge currently accumulated on the development of pathological processes in the endometrium, there is still no single concept of the pathogenesis of CE, not only explaining the phased formation of this pathology, but also revealing the pathogenetic basis of reproductive dysfunction on its background. It is well known that chronic inflammation in the endometrium at the initial stages of its development is supported by a constant persistence of the microbial agent, which in itself is caused by pathogenic characteristics of the microorganism, on the one hand, and various defects in the microorganism's immune defence system, on the other [16,17].

A reflection of the latter is the development of a local immunodeficiency state with the deposition of pathogenic immune complexes on the walls of the glands and blood vessels of the endometrium, which, in turn, due to the activation of the compliment system and other inflammatory mediators, contributes to the maintenance of the inflammatory process in the endometrium. In the future, with a prolonged course of CE, the immunological changes occurring in the

endometrium (pathological lymphohistioplasmocytic infiltration of the stroma, changes in the phenotypic composition of cells and the ratio of Th1- and Th2-type cytokines) themselves begin to act as an etiological factor in the violation of implantation processes and the development of chorion, with subsequent formation of women infertility autoimmune genesis [18,19].

In this regard, in-depth data in the study of immune changes will make it possible to develop new methods for the diagnosis and treatment of this pathology, and reduce the level of chronicity of the process, and the economic costs in the treatment of this pathology [20–22].

Material and methods

Under constant supervision in the gynaecological department of the Kursk City Maternity Hospital there were 30 patients of reproductive age (18-35 years old). Patients were included in the study on the basis of informed consent. All patients underwent a comprehensive clinical and instrumental examination according to generally accepted standards, and in all cases, there was a verification of the diagnosis of chronic endometritis in the stage of incomplete remission. Women were divided into two equal groups, randomized by age, minimal concomitant diseases in remission, predicted disease severity, those who all underwent endoscopic surgery for infertility. Patients of the first group received traditional treatment, antibacterial, taking into account the sensitivity of the pathogen, antimycotic, antiviral, non-steroidal anti-inflammatory drugs, probiotics, funds aimed at restoring the vaginal microflora. The second part of the patients received an additional course of intravenous laser blood irradiation (VLOK) using the Mulat laser therapy apparatus (Russia) with a wavelength of 0.63 microns and an output power of 2 mW at the end of the OS-2 (KIVL-01) single-use optical fibre. Laser irradiation was carried out for 25 minutes in a continuous mode of radiation; the course of treatment was 7 daily procedures.

Laboratory examination was carried out immediately upon admission to the hospital and upon discharge on the 10th day. Levels of TNF, IL-1 β , IL-8, IL-18, IL-4, IL-10, IF γ , G-CSF, complement components (C₃, C₄, C₅, C_{5a}), C₁ inhibitor, immunoglobulins of classes M, G, A (IgM, G, A), sIgA were determined in blood plasma and vaginal cervical flush using reagent kits of Vector-Best CJSC, NPO Tsitokin, Protein Contour LLC, St. Petersburg by enzyme-linked immunosorbent assay analysis. Before and after complex treatment, red blood cells were obtained from 10 ml of heparinized blood, for which it was defatted twice in 10 mm Na-phosphate buffer (pH = 7.4), containing 0.9% sodium chloride and 3% T-500 dextran, within 30 minutes at a temperature of 37°C. After this, the blood was centrifuged, after separation of the supernatant, the erythrocyte mass was subjected to additional purification on a chromatographic column through HBS cellulose, after which the sorption capacity of erythrocytes (SCE) and the sorption capacity of their glycocalyx (SCG) were determined [6]. Erythrocyte membranes were isolated by G.T. Dodge membrane lipids were determined by thin layer chromatography. Protein electrophoresis was performed in the presence of sodium dodecyl sulphate in vertical plates of a polyacrylamide gel according to the method of U.K. Laemmli, proteins stained Kumashi with blue R-250.

The intensity of lipid peroxidation (LPO) was evaluated by the content of acyl hydroperoxides (AHP) and malondialdehyde (MDA) in erythrocytes formed with a coloured complex of thiobarbituric acid [23,24]. The determination of MDA and AHP was carried out using the TBK-Agat kit (Agat-Med Russia) using the Apel-330 spectrometer (Japan) at a wavelength of 535 nm and 570 nm. To assess the state of the antioxidant system, we focused on direct / competitive enzyme-linked immunosorbent assay (ELISA) with the detection of reaction products in the wavelength range 405-630 using ready-made commercial kits: Bender Med systems superoxide dismutase (SOD) activity (Austria) and Cayman

Chemical catalase "(USA). Total antioxidant activity (OAA) was determined by a method based on the degree of inhibition of ascorbate and ferro induced oxidation of Tween-80 to MDA. The concentration of stable nitric oxide metabolites (CM_{ON}) was detected in the Griss reaction at a wavelength of 540 nm using an ELISA kit from R&D (England). All ELISA results were recorded using a Sunrise microplate photometer, Tecan (Austria).

Result and Dissection

Statistical processing of the research results was carried out according to generally accepted criteria of variational-statistical analysis with calculation of average values (M) and arithmetic mean errors (m) using the Microsoft Excel 2010 software package. The significance of differences was evaluated by the U-criterion, and the relationships were established based on the coefficient Spearman's rank correlation. Differences with p <0.05 were considered statistically significant.

Upon admission to the clinic, patients showed an increase in plasma levels of pro-inflammatory cytokines (TNF by 29.4 times, IL-1 β by 4.3 times, IL-8 by 8.6 times, IL-18 by 1.7 times), compensatory anti-inflammatory (IL-4 by 8.8 times, IL-10 by 6.2 times), IF γ by 2.2 times and G-CSF by 1.5 times). Before treatment, in this category of patients there was an increase in the concentration of components (C₃, C₄, C₅, C_{5a}) and the C₁ inhibitor of the complement system. In addition, an increase in the concentration of IgM with a decrease in immunoglobulins of classes G and A was detected.

At the local level, an increase in the level of TNF, IL-8, C₃ and C₄ was also established in the vaginal-cervical flush, but, unlike the system level, a decrease in IL-4, IL-10 and C₁-inhibitor of the complement system was observed. In addition, a decrease in the concentration of secretory immunoglobulin sIgA was detected. Standard plasma treatment was partially normalized, but not to the level of healthy donors, the content of TNF, IL-1 β , IL-8, IF γ , C₃, C₄, C_{5a}

components of complement, IgM and IgA, even more increased the level of anti-inflammatory cytokines and C₁ inhibitor of the complement system. The concentration of IL-18, G-CSF, C₅ remained unchanged, and the IgG content was higher than that of donors.

At the local level, standard treatment corrected the content of all investigated cytokines and the C₃ component of complement, significantly increased the concentration of sIgA, but did not affect the level of C₄ and C₁ inhibitor of the complement system.

The results obtained showed that the standard pharmacotherapy in this category of patients did not have an adequate corrective effect on the impaired immune status parameters, indicating the need for additional means and methods of immunorehabilitation in the postoperative period.

As for the changes occurring in red blood cells, when admitted to patients with CE, a decrease in the level of α - and β -spectrin, ankyrin, anion transport protein (ATB), pallidine, dematin, glyceraldehyde-3-phosphate dehydrogenase (G-3PD) was found in the erythrocyte membrane - FD) and glutathione-S-transferase (G-S-T), an increase in the protein content of band 4.1, actin and tropomyosin with a normal level of protein of band 4.5. The performed operative and TT normalized the representativeness of α - and β -spectrin and tropomyosin in the erythrocyte membrane was corrected, but not to the normal parameters, the content of ankyrin, pallidine, dematin and actin, did not affect the level of ATB, protein of the band 4.1, G-3-PD and G-S-T. The use of VLOK additionally, in comparison with pharmacological therapy, normalizes the representativeness of ATB, actin, G-3-PD, G-S-T and, to an even greater extent, corrected the level of ankyrin, band 4.1 protein, pallidine and dematin.

In patients with CE, upon admission to the clinic, a decrease in the content of phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), glycerophospholipids (GPL - the sum of LPC, PC, PE, PS and PI),

sphingomyelin (SM) was revealed in the erythrocyte membrane phospholipids (PL - the sum of GPL and SM), an increase in the level of lysophosphatidylcholine (LPC), cholesterol (C), cholesterol esters (EC) and triacylglycerols (TAG), with a normal content of phosphatidylethanolamine (PE), the sum of mono- and diacylglycerol,) and unesterified fatty acids (NEFA). The performed TT was closer to the parameters of healthy donors LPC, PS, PI, GPL, SM and FL, but did not affect the representativeness of PC, C, EC and TAG. The addition of pharmacological therapy of the postoperative period of VLOK additionally normalized the content of LPC, PI, SM, EC, TAG and, to an even greater extent, correcting the level of PC, FS, GPL and FL.

When studying the indicators of red blood cell metabolism before treatment, the activation of LPO processes (increase in the concentration of MDA and AHP), a decrease in antioxidant defence factors (OAA, SOD and catalase activity) were established. In addition, an increase in the level of CMON and a decrease in the sorption parameters of the erythrocyte membrane (SCG and SCE) were revealed. The pharmacotherapy did not affect the changed indicators of the antioxidant protection of erythrocytes (SOD and OAA) and corrected the remaining parameters of the metabolic activity of red blood cells in the direction of indicators of healthy donors. Supplementation of VLOK treatment normalized the antioxidant defence parameters and sorption indices and, to an even greater extent, correcting the LPO parameters and CM_{ON} level.

Thus, it can be stated that in patients with CE before surgery and TT, 87.9% of the indicators of the structural and functional properties of red blood cells were found to be altered from the values of healthy donors, respectively. Conducted comprehensive treatment normalized 10.3% of the parameters changed before treatment, but not the norm, 55.2% and left unchanged 34.5% of the indicators. The use of VLOK was significantly more effective, since it normalized 58.6%,

corrected 37.9% and left unchanged only 3.5% of the parameters.

A definite proof of the integrative processes between the terms of the laboratory status is the presence of reliable correlation between them. To do this, we analysed the matrix of multiple

correlation between the immune parameters and the structural and functional properties of red blood cells in chronic endometritis at the systemic and local levels before treatment, the results of which are given below (Table 1).

Table 1: The relationship between immune parameters and structural and functional properties of red blood cells in chronic endometritis at the local level

Indicators	TNF	IL-8	IL-4	IL-10	C ₃	C ₄	C ₁ -ing.	sIgA
α-spectrin	0,87	0,33	0,46	0,74	0,44	0,37	0,58	0,52
β-spectrin	0,41	0,25	0,53	0,53	0,51	0,36	0,38	0,4
Ankirin	0,63	0,39	0,61	0,4	0,53	0,3	0,77	0,46
ATB	0,43	0,44	0,3	0,29	0,28	0,38	0,49	0,41
4.1	0,42	0,29	0,4	0,52	0,29	0,33	0,43	0,54
Pallidine	0,53	0,42	0,33	0,36	0,77	0,54	0,62	0,36
4.5	0,52	0,36	0,54	0,41	0,3	0,52	0,5	0,28
Damatin	0,54	0,4	0,77	0,63	0,77	0,63	0,53	0,59
Actin	0,69	0,28	0,77	0,49	0,68	0,77	0,36	0,7
G-3-PD	0,41	0,59	0,29	0,3	0,77	0,63	0,41	0,57
Tropomyosin	0,33	0,7	0,5	0,68	0,42	0,33	0,59	0,3
G-S-T	0,4	0,57	0,51	0,54	0,41	0,41	0,42	0,59
PC	0,61	0,3	0,5	0,68	0,59	0,59	0,41	0,28
LPC	0,4	0,3	0,67	0,3	0,59	0,43	0,59	0,36
PE	0,6	0,43	0,63	0,7	0,33	0,87	0,68	0,41
PS	0,28	0,28	0,29	0,43	0,59	0,59	0,62	0,59
PI	0,45	0,41	0,48	0,69	0,41	0,7	0,42	0,5
GPL	0,43	0,5	0,62	0,86	0,59	0,57	0,68	0,57
SM	0,52	0,5	0,6	0,39	0,36	0,3	0,63	0,3
PL	0,39	0,36	0,49	0,36	0,59	0,6	0,68	0,33
C	0,42	0,82	0,69	0,53	0,42	0,28	0,62	0,41
EC	0,75	0,51	0,33	0,48	0,36	0,36	0,62	0,59
TAG	0,38	0,28	0,53	0,41	0,77	0,49	0,3	0,43
DAG + MAG	0,41	0,28	0,58	0,38	0,29	0,43	0,49	0,36
NEFA	0,48	0,54	0,42	0,28	0,53	0,3	0,54	0,3
MDA	0,41	0,63	0,63	0,62	0,52	0,52	0,62	0,52
AHP	0,52	0,65	0,47	0,54	0,33	0,53	0,64	0,33
OAA	0,77	0,82	0,3	0,36	0,43	0,39	0,59	0,48
SOD	0,47	0,57	0,52	0,36	0,51	0,36	0,32	0,53
Catalase	0,63	0,28	0,54	0,77	0,54	0,3	0,56	0,67
CM _{NO}	0,45	0,63	0,77	0,64	0,33	0,26	0,8	0,37
SCG	0,33	0,48	0,53	0,72	0,42	0,55	0,47	0,36
SCE	0,36	0,47	0,54	0,47	0,33	0,52	0,5	0,42

When analysing the correlation relationships between the immune parameters and the structural and functional properties of red blood cells in chronic endometritis [25] at the systemic level, 20 strong reliable correlation relationships

were identified before treatment. Most of the connections were found between the indicators of the immune status of C₁-ing, TNF, IL-8, IL-1β, and the following indicators of the structural and functional properties of red blood cells - Actin,

Tropomyosin, LPC, C, EC, TAG. As for the oxidative status of red blood cells, 10 reliable connections were established with AHP, MDA, and CM_{NO} indicators, with 4 links for the level of AHP (C1-ing, IF γ , IL-8, IL-10), 3 connections with MDA (IF γ , IL-18, C1-ing), CM_{NO} (TNF, IL-1 β , IL-10).

Estimating the matrix of Spearman's multiple correlations between the immune parameters and the structural and functional properties of red blood cells in chronic endometritis at the local level, 41 reliable relationships were established. At the same time, 6 relationships were identified for TNF (α -spectrin, ankyrin, actin, PC, PE, EC,); IL-8 has 2 relationships (Tropomyosin, C); IL-4 (ankirin, dematin, actin, LPC, PE, GPL, SM, C,); IL-10 (α -spectrin, dematin, tropomyosin, PC, PE, PI, GPL,); C₃ (pallidine, G-3-PD, actin, dematin, TAG,); C₄ (G-3-PD, actin, dematin, PE, PL); C₁-ing (ankyrin, Pallidin, PE, GPL, SM, PL, C, ES, PL,); sIgA (Actin).

When studying the correlation between oxidative indices and structural and functional properties of red blood cells, 16 reliable strong relationships were determined. The TNF indicator had 2 relationships (OAA, catalase), IL-8 had 4 relationships (CM_{NO} , SOD, OAA, MDA), IL-4 two relationships (CM_{NO} , MDA), IL-10 corrected with two indicators (CM_{NO} , MDA, catalase), indicators C₃, C₄ and sIgA had no correlation, C₁-ing. was corrected with two indicators (MDA, AHP).

The study, as well as the results of modern literature showed that, despite the absence of pronounced clinical manifestations of inflammation and the normative values of laboratory tests, in women of childbearing age with infertility against the background of the chronic inflammatory process of the reproductive sphere in the stage of incomplete clinical remission, pronounced changes from pro, anti-inflammatory, regulatory cytokines, components and inhibitors of the complement system, immunoglobulins on both systemic and at the local level, indicate the presence of a destructive immune-inflammatory process that requires mandatory correction.

On the basis of the current inquiry, red blood cells indicate significant changes in the proteins responsible for the structure formation and stabilization of the erythrocyte membrane (α - and β -spectrin, dematin - the main proteins of the cytoskeleton, ankyrin, band protein 4.1, pallidine), the formation and flexibility of the membrane (actin, tropomyosin), intracellular metabolism (G-3-PD, G-S-T).

The results revealed changes in the content and ratio of the lipid composition of the membrane, primarily, a decrease in the content of GFL and SM membranes, which form the basis of the double lipid skeleton of the cell membrane and play a major role in the ordering of protein macromolecules and the normal metabolism of red blood cells, along with a change in the architectonics of proteins, leading to serious functional disorders properties of peripheral blood erythrocytes in chronic kidney disease even in remission, as evidenced by an increase in lipid peroxidation processes and the content of CM_{NO} , which are an indirect indicator of the level of NO.

In addition, a significant decrease in the activity of key antioxidant enzymes (SOD and catalase) in red blood cells indicates the development of oxidative stress. Mature red blood cells are not able to synthesize proteins and lipids; the maintenance and changes in their content and ratio is due to the microenvironment of red blood cells, namely the composition of blood plasma, which changes significantly during pathology, which in turn has a significant effect on cell morphology, lipid-protein interactions in the erythrocyte membrane and the latter-related activity of its enzyme systems.

Conclusion

In sum, there are pronounced immune and oxidative disturbances in CE both at the systemic and local levels, which significantly changes the qualitative and quantitative composition of blood plasma.

The revealed close correlation between the studied immune indices and the structural and

functional properties of red blood cells in chronic endometritis allowed us to conclude that there is a "tension" of the immune and metabolic statuses. It is proved that a clear correlation is traced between the indicators. These correlations between indicators of the immune status and structural and functional properties of erythrocytes indicate their close relationship and interdependence in the development of the inflammatory process in endometritis and can serve to assess the severity of the disease, its prognosis, treatment effectiveness and preventive measures.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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