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The Role of Lipid Peroxidation and Activity of The Blood Antioxidant System in The Development of Postpartum Endometritis and Their Prognostic Significance

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Abstract: Postpartum endometritis is one of the most common infectious complications in the postpartum period. According to various authors, the incidence of endometritis after natural childbirth is 1-3%, while after cesarean section, the rate is 27% [3,5,12,13,18]. The frequency of cesarean sections in Uzbekistan has been steadily increasing from 9.58% in 1999 to 16.10% in 2019. In perinatal centers of Uzbekistan in 2017-2020. Thus, the determining of predictor factors which play huge role in the development of postpartum endometritis is relevant. In this article are given the results of scientific work which was done with 89 women who were after delivery in order to predict postpartum endometritis.

Keywords: Endometritis, postpartum period, lipid peroxidation, antioxidant system, systemic inflammation, postpartum sepsis, malon dialdehyde.

Introduction: Postpartum endometritis is one of the most common infectious complications in the postpartum period. According to various authors, the incidence of endometritis after natural childbirth is 1-3%, while after cesarean section, the rate is 27% [3,5,12,13,18]. At the same time, postpartum endometritis is the main cause of death associated with purulent-septic complications occurring on the 3-7th day after childbirth. [15]. Cesarean section is associated with a 5- to 10-fold increase in the incidence of postpartum infections and endometritis compared with vaginal delivery [15]. Moreover, the incidence of postpartum endometritis is approximately ten times higher in cesarean sections performed after the onset of labor than in elective ones [16]. To reduce obstetric complications, the frequency of caesarean sections recommended by WHO should not exceed 15–30% of the total number of births.

The frequency of cesarean sections in Uzbekistan has been steadily increasing from 9.58% in 1999 to 16.10% in 2019. In perinatal centers of Uzbekistan in 2017-2020. Moreover, the frequency of indications for CS also increased and was noted: the frequency of CS operations in perinatal centers in 2017 was 29.5%, in 2018 - 33.2%, in 2019 - 34.5% and in 2020 - 34.3%.

Although there are many factors that increase the risk of developing intrauterine infection in the postpartum period, the most common of them are: young maternal age; different somatic diseases in the anamnesis: immunosuppressive disorders, obesity, diabetes, taking corticosteroid supplements; complications during antenatal period or delivery: decreased frequency of antenatal care, chorioamnionitis, history of repeat cesarean section, emergency cesarean section, stapled wound closure, and excessive blood loss [1].

Inflammation is the basis of most diseases associated with postpartum infectious pathology. The inflammatory process is initiated by microbial, viral or fungal infections and / or systemic pathology of various

genesis, leading to tissue damage or autoimmune reactions. Currently, in obstetrics and gynecology, the prognostic potential of hematological markers of inflammation and processes associated with inflammation is widely used, studies are carried out on the role of the systemic inflammatory state of a pregnant woman and a woman in labor in the prenatal and postpartum periods in the development of PE. The severity of the inflammatory state is assessed using immunological, biochemical and physiological changes, markers of which are proinflammatory chemical mediators of inflammation, such as chemokines, cytokines, vasoactive amines, eicosanoids and proteolytic cascade products, the levels of which are important prognostic markers of PE development.

Systemic inflammation is associated with changes in the number and function of blood cell components. Hematological markers are considered to be a reflection of the systemic inflammatory environment. The neutrophil-to-lymphocyte ratio is widely used to assess the severity of inflammation in postpartum infectious pathology. The role of neutrophils and lymphocytes in maintaining or spreading inflammatory cascades in the most common pregnancy complications and the effectiveness of assessing the neutrophil-to-lymphocyte ratio for diagnosing and predicting pregnancy-related complications as potential predictors of acute and chronic inflammatory gynecological and reproductive disorders have been proven [1,2,4]. Factors causing an inflammatory response are expressed by activation of circulating leukocytes, increased production of neutrophil activators and complement activity. In assessing the risk of developing infection after CS, a high prognostic potential was demonstrated by a complete blood count and different full blood count variables. They include hemoglobin level, red blood cell volume, red blood cell distribution width, hematocrit, blood cells with their morphology, and systemic markers of inflammation based on the ratios of various forms of leukocytes [6,9].

However, the information content of clinical blood analysis is low and changes characteristic of the inflammatory process are detected in no more than 60% of women in labor with endometritis [8], while the content of leukocytes and neutrophils, as predictors of postpartum endometritis in women in labor, there is not any test to exclude purulent-inflammatory diseases in women in labor, which will be able to estimate clinical condition and give prognostic value as a screening [6]

Aim of the work

Thus, the determining of predictor factors which play huge role in the development of postpartum endometritis is relevant and the aim of our work was to study the prognostic significance of lipid peroxidation and the activity of the antioxidant system of the blood in the early postpartum period in the development of postpartum endometritis.

METHODS

During the study were collected 89 women who were after delivery. Main group consist of 66 women after labor with postpartum endometritis and they were divided in to three subgroups according to the severity of endometritis: 23 with mild; 22 with moderate and 21 with severe type. The control (comparison) group consisted of 23 women after labor with a physiological course of the postpartum period.

All parturient women were assessed for lipid peroxidation levels and AOS enzyme activity on day 3 postpartum. Using standard aseptic precautions, 5 ml of venous blood from the antecubital vein was collected into a polyethylene Stoppard tube containing 60 µl of the anticoagulant k3 EDTA (tripotassium ethylenediaminetetraacetic acid). After centrifugation at 3000 rpm for 10 min, plasma was separated and stored at -40°C until analysis in the research laboratory. Data were collected using a pre-designed, validated data collection sheet. The concentration of lipid peroxidation products was estimated by the level of malonic aldehyde (MDA) in a reaction with thiobarbituric acid [8]; the total antioxidant activity of blood plasma was estimated by the oxidation reaction with paraphenylenediamine [7].

Statistical analysis was performed using SPSS. The results are presented as the mean (M), dispersion (õ) and standard deviation (± m); median (25% lower quartile – 75% upper quartile). The assumption of normal distribution was tested by the Shapiro- Wilk test (α=0.05). Comparison of intergroup differences for independent samples was performed using Tukey's pairwise comparison HSD / Tukey Kramer ANOVA program, for comparing quantitative data having a distribution different from normal, the Kruskal method was used Wallis , Spearman's method was used to identify correlations . Results were considered statistically significant at an error level of $p < 0.05$. ROC analysis was used to calculate the threshold level of values, concentrations of LPO products and activity of AOS enzymes, specificity and sensitivity of the methods.

RESULTS

During our scientifis work the criteria for diagnosing postpartum endometritis in women who gave the delivery were the results of clinical and laboratory examination of women in labor (table 1).

TABLE I.

CLINICAL CHARACTERISTICS OF POSTPARTUM ENDOMETRITIS OF VARYING SEVERITY IN COMPARISON GROUP (M,M)

Indicator	Severity of postpartum endometritis			Control group m = 23
	Light m = 23	Moderate m = 22	Heavy m = 21	
T ° body °C	37.6±1.3 5^{1}	38.4±1.62 ^{12}	38.8±1.13^{1} ^{23}	36.5±1.2 3
Pain on palpation	0.77±0.0 3^{1}	1.25±0.04 ^{12}	2.45±0.12^{1} ^{23}	0.3±0.01
Lochia	0.69±0.0 2^{1}	1.37±0.06 ^{12}	2.65±0.12^{1} ^{23}	0.20±0.0 1
Symptoms of intoxication	0.34±0.0 1^{1}	1.15±0.05 ^{12}	2.33±0.10^{1} ^{23}	0.35±0.0 2
Involution of the uterus	0.47±0.0 2^{1}	0.85±0.03 ^{12}	1.77±0.08^{1} ^{23}	0.25±0.0 1
Hemostasisogram	0.55±0.0 2^{1}	1.08±0.04 ^{12}	2.41±0.4^{123}	0.25±0.0 1
Blood biochemistry	0.40±0.0 1^{1}	1.25±0.06 ^{12}	1.92±0.09^{1} ^{23}	0.21±0.0 1
Blood formula	0.65±0.0 3^{1}	1.90±0.08 ^{12}	2.35±0.10^{1} ^{23}	0.37±0.0 2

Note: [1]- p<0.05 in relation to the control group; [2]- p<0.05 in relation to the mild course group; [3]- p<0.05 in relation to the moderate and severe course group.

Despite the large number of primary etiological factors, the immediate cause is microbial invasion followed by inflammatory damage to the endometrium [2], an indispensable attribute of inflammation is oxidative stress [13], which determines the importance of studying free radical oxidation processes. The content of the final product of lipid peroxidation of cell membranes is malonic dialdehyde (MDA) in the blood plasma of women in labor with endometritis statistically significantly exceeded the corresponding control values and progressively increased in women in labor with a more severe course (table 2).

TABLE 2.

LEVELS OF THE POL-AOS SYSTEM INDICATORS IN WOMEN IN LABOR WITH DIFFERENT SEVERITY OF ENDOMETRITIS

Indicator	Stat parameters	Severity of postpartum endometritis			Control group m=23
		Light m = 23	Moderate m = 22	Heavy m = 21	
MDA mmol /l	Msr	4.24	5.38	8.95	3.71
	S	0.50	0.56	0.76	0.36
	± m	0.10	0.12	0.17	0.08
95% DI	Min	3.9	5.2	8.7	3.5
	Max	4.5	5.7	9.5	40
Tukey-Kramer : x1-x2, x1-x3, x1-x4, x2-x3, x2-x4, x3-x4					
AOA %	Msr	57.8	48.0	34.5	60.6
	S	3.30	4.56	1.91	1.50
	± m	0.73	0.97	0.41	0.32
95% DI	Min	55	45	33	57
	Max	61	51	35	62
Tukey-Kramer : x1-x2, x1-x3, x1-x4, x2-x3, x2-x4, x3-x4					

Post hoc comparisons of mean group MDA levels using Tukey's method showed that statistically significant differences in the analysis of variance were associated with differences in mean MDA values in all three groups (table 3).

TABLE 3.

EVALUATION OF THE RELIABILITY OF INTERGROUP DIFFERENCES IN PAIRWISE COMPARISON OF MEAN GROUP VALUES OF MDA USING THE TUKEY-KRAMER CRITERIA IN ANOVA ANALYSIS

PAIR	DIFFERENCE	SE	Q	Lower CI	Upper CI	Critical mean	p-value
x1-x2	11,02	1,17	9,52	6,84	15,56	4,3	≤0,001
x1-x3	46,95	1,19	39,43	42,54	51,37	4,4	≤0,001
x1-x4	5,47	1,16	4,70	1,16	9,79	4,3	≤0,001
x2-x3	35,75	1,203	29,7	31,28	40,2	4,4	≤0,001
x2-x4	16,68	1,17	14,1	12,3	21,04	4,3	≤0,001
x3-x4	52,43	1,19	44,03	40,02	56,85	4,4	≤0,001

The difference in the results of the study of MDA concentrations in the comparison groups is clearly seen in Fig. 1. Moreover, with an increase in the severity of postpartum endometritis, the concentration of MDA in the blood increased reliably. As can be seen from Fig. 1, the concentration of MDA in moderate PE exceeded the level of mild PE by 1.12 µmol / l ($p \leq 0.001$); in severe PE, this difference was 4.70 µmol / l ($p \leq 0.001$); and the difference between mild PE and the control was 0.55 µmol / l ($p \leq 0.001$); between moderate PE and the control 1.67 µmol / l ($p \leq 0.001$); and between severe PE and the control 5.24 µmol / l ($p \leq 0.001$).

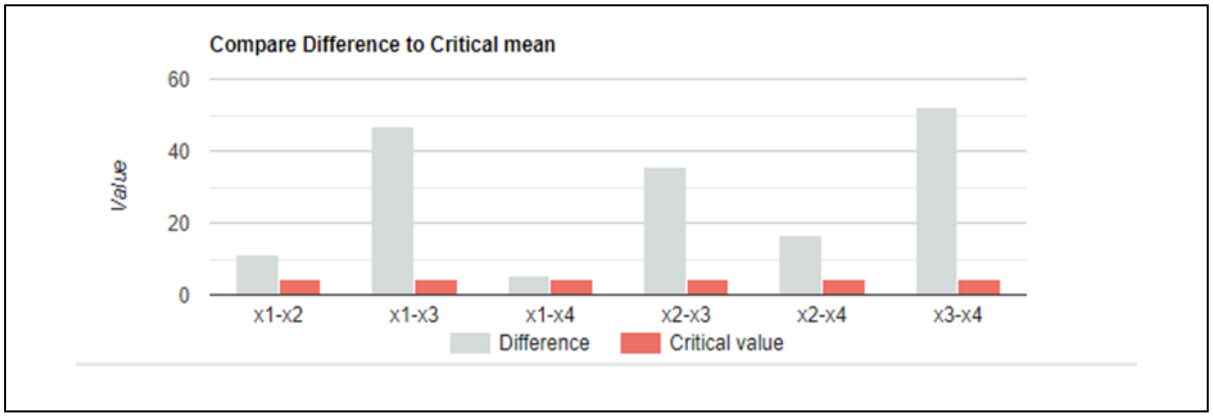

Fig. 1. Difference between the critical mean and mean group values of MDA in comparison groups

Oxidative stress is a condition in which the antioxidant system fails to cope with the overproduction of reactive oxygen species, leading to oxidation of key cellular macromolecules and proteins of cell membranes and molecular dysfunction. MDA is the end product of peroxidation of polyunsaturated fatty acids of cell membranes and is recognized as a marker of oxidative stress and antioxidant status [10,14]. Thus, the progression of PE contributes to an increase in the production of reactive oxygen species (ROS) and an increase in the severity of endometritis itself.

The assessment of antioxidant activity (AOA) demonstrated the opposite direction: with increasing severity of PE, the total AOA activity significantly decreased (Table 2).

Post-hoc comparisons of mean group indicators of AOA activity using the Tukey - Kramer method in ANOVA analysis showed that statistically significant differences in the analysis of variance are associated with differences in the mean values of AOA in the comparison groups (table 4).

TABLE 4.

EVALUATION OF THE REABILITY OF INTERGROUP DIFFERENCES IN PAIRWISE COMPRISON AVERAGE GROUP VALUES OF AOA (IN %) ACCORDING TO THE TURKEY-KRAMER CRITERION IN THE ANOVA ANALYSIS

PAIR	DIFFERENCE	SE	Q	Lower CI	Upper CI	Critical mean	p-value
x1-x2	3,39	0,6	5,2	1	5,7	2,3	≤0,001
x1-x3	12,6	0,64	19,5	10,2	15	2,4	≤0,001
x1-x4	26,1	0,65	39,8	23,6	28,5	2,4	≤0,001
x2-x3	9,2	0,64	14,2	6,8	11,6	2,4	≤0,001
x2-x4	22,7	0,65	34,6	20,3	25,1	2,4	≤0,001
x3-x4	13,4	0,66	20,3	11	19,9	2,4	≤0,001

The difference between the critical average and average group AOA activity (in%) in the comparison groups demonstrates a decrease in AOA with increasing severity of PE (fig. 2).

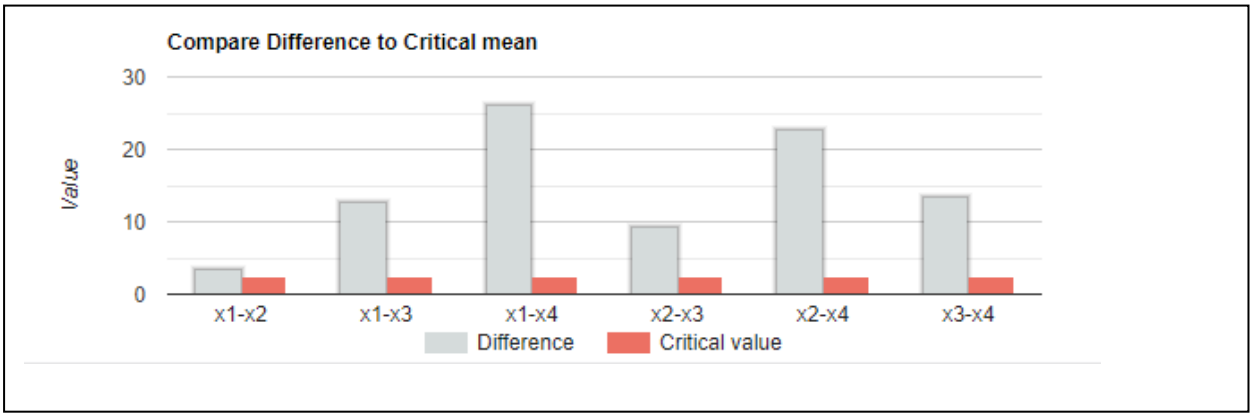

Fig. 1. Difference between the critical mean and mean group values of AOA in comparison groups

In fig. 2, the critical average value of AOA in % in group 4 – severe course of PE – is taken as the critical average, while the difference between the critical average and average group activity of AOA in women in labor without PE and with severe course (X1 – X4) is -26.13 AOA units; with moderate and severe course (X2 – X4) – 22.74 AOA units; the corresponding difference between moderate and severe course is 13.46 AOA units, etc. (fig. 2).

The priority of studying oxidative stress in PE is determined by the need to understand the pathophysiological mechanisms underlying conditions associated with the development of endometrial inflammation in the postpartum period. The imbalance between reactive oxygen species (ROS) and antioxidants played as a marker role int the oxidative stress, as with the help of them can be predected the pathophysiology of endometritis and the general inflammatory response of the body.

Total antioxidant capacity (activity) (AOA) is a biological parameter representing the sum of antioxidant effects of enzymatic antioxidants and molecules with antioxidant properties in a living organism, reflecting the ability to negate the negative impact of free radicals at the cellular level [16,17]. Determination of total antioxidant activity as the integration activity of several antioxidants in plasma is important in the analysis of biological systems. A decrease in AOA with increasing severity of PE reflects the role of pathogenetic mechanisms of oxidative stress in the severity of PE.

Prediction of the risk of developing postpartum endometritis based on MDA and AOA levels was performed using ROC analysis curves. The ratio of the number of correctly classified positive examples (true

positive) and the number of incorrectly classified negative examples (false negative) was estimated, as a result of which diagnostically significant levels of MDA and AOA in blood plasma were established.

ROC curves represent graphical ranges of possible cut-off points with sensitivity versus 1-specificity (i.e., false positive rate). This illustrates the strengths of a particular predictor/prognostic model, allowing different cut-off points to be defined for specific applications - depending on the "cost" of misclassification. Area under the curve (AUC) estimates provide insight into the usefulness of a predictor and are a means of comparing (testing) two or more predictive methods.

The diagnostic performance of a test is the accuracy of the test in distinguishing disease cases from normal controls. ROC curves can also be used to compare the diagnostic performance of two or more laboratory tests.

TABLE 5.

ESTABLISHING THE THRESHOLD CONCENTRATION OF MDA IN BLOOD PLASMA TO FORM A PROGNOSIS FOR THE DEVELOPMENT OF POSTPARTUM ENDOMETRITIS

Diagnostic level of MDA µmol/l	Detected frequencies in %		Diagnostic accuracy	
	Control	Women in labor with PE	Specificity	Sensitivity
8.00-8.40	4.35	40.91	4.35	39.71
8.39-7.78	4.35	27,27	8.70	66.18
7.77-7.18°	4.35	13.64	13.04	79.41
7.17-6.56	4.35	7.58	17.39	89.71
6.55-596	4.35	3.03	21.74	92.65
5.95-5.91	4.35	1.51	26.05	94.12
5.91-5.30	4.35	1.51	30.34	95.59
5.29-4.71	13.04	1.51	43,43	97.06
4.70-4.11	21.74	1.51	65.22	98.53
4.10-3.50	35.78	1.51	100,0	100,0

If the MDA concentration in the blood is more than 7.18 µmol/l, a conclusion can be made about a high risk of developing postpartum endometritis with a diagnostic sensitivity of 79.41% and a diagnostic specificity of 13.04%

TABLE 6.

ESTABLISHING THE THRESHOLD CONCENTRATION OF AOA IN BLOOD PLASMA TO FORM A PROGNOSIS FOR THE DEVELOPMENT OF POSTPARTUM ENDOMETRITIS

Diagnostic level of MDA µmol/l	Detected frequencies in %		Diagnostic accuracy	
	Control	Women in labor with PE	Specificity	Sensitivity
30-32	1	17	4.35	25.76
33-35	1	15	8.70	48,48
36-38	1	13	13.04	60.18
40-42°	1	8	17.39	80.30
43-45	1	4	21.74	86.36
47-49	2	3	30.43	90,91
52-54	2	2	39.13	93.41
55-58	2	2	47.83	96,97
59-60	3	1	60.87	98.48

>60	9	1	100,0	100,0

If the AOA concentration is less than 40%, a conclusion can be made about a high risk of developing postpartum endometritis with a diagnostic sensitivity of 80.30% and a diagnostic specificity of 17.39%.

To compare the effectiveness ROC (Receiver Operator Characteristic) analysis was performed on the MDA level and AOA concentration, which is an analysis of characteristic curves with the calculation of the area under the ROC curves AUC (Area Under Curve). Clinical informativeness of the MDA level and AOA concentration using ROC analysis: the AUC (area under the curve) value for MDA was 88.27%, R^2 = 85.67%; for AOA, the AUC value was 82.40%, R^2 = 93.52%.

The AUC value of 88.27% for MDA corresponds to a good model; the predictive value of AOA with an AUC of 82.40% is lower than that of MDA.

The area of the AUC curve in the range of 0.9-1.0 is considered the highest information content of the diagnostic method, in the range of 0.8-0.9 - good information content, in the range of 0.7-0.8 - satisfactory, in the range of 0.6-0.7 - mediocre information content, and below - useless classification.

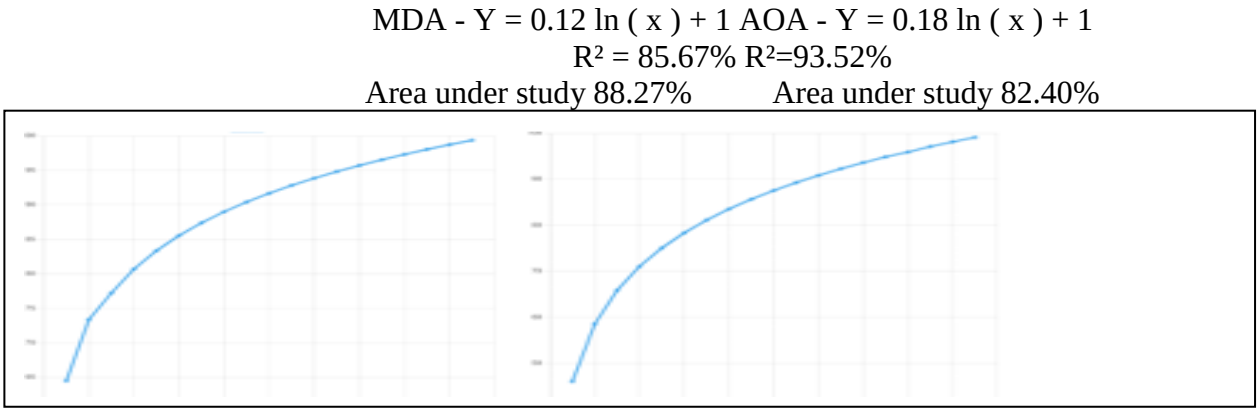

Fig. 3. ROC curve of the ratio of sensitivity and specificity of the prediction of the occurrence of postpartum endometritis based on the level of MDA and AOA activity (in%) in blood plasma.

Linear regression models for the risk of developing PE at different concentrations of MDA have the form: Y = 0.12ln(x) + 1; and for known values of AOA activity - Y = 0.18ln(x) + 1.

The quality of the predictability of the models was assessed using the Nagelkerke determination coefficient R^2, which is the main indicator of the quality of the regression model describing the relationships between the dependent and independent variables. The Nagelkerke determination coefficient R^2 reflects the specific weight of the contribution of changes in the studied inflammatory factors to the prognosis of the development of postpartum endometritis.

The R^2 value should be in the range from zero to one: $0 \le R2 \le 1$. The model is considered to be of higher quality if the value of the determination coefficient is close to 1. The statistical indicator shows what part of the variability of the observed variable can be explained by the constructed model, i.e. the value of the determination coefficient determines the share (in percent) of changes due to the influence of factor characteristics in the total variability of the resultant characteristic. R^2 for an increase in MDA concentration is 85.67%; and for a decrease in AOA activity - 93.52%, which proves the higher predictive significance of the obtained regression models.

DISCUSSION

The presence of increased concentrations of free radicals and decreased antioxidant potential leads to oxidative stress. The development of oxidative stress may be one of the links in the chain of events leading to endometritis. Levels of oxidation -reduction potential can modulate the severity and dynamics of endometritis, and disease progression, biomarkers are associated with the level of oxidative stress with the severity of endometritis.

In uncomplicated pregnancy, there is a balance between the antioxidant system and reactive intermediates, but this balance can be disrupted by pregnancy complications or delivery with an unfavorable outcome [19]. The imbalance between prooxidant and antioxidant factors leads to oxidative stress, which contributes to the development of many diseases. This oxidative aggression can be a precursor to pathologies in the pregnant woman [5].

Analysis of the concentration of the end product of lipid peroxidation of cell membranes - MDA in the blood plasma of women in labor with endometritis of varying

severity showed a statistically significant excess of control levels and a progressive increase in women in labor with a more severe course: the concentration of MDA in moderate PE exceeded the level of mild course by 1.12 µmol / l ($p \leq 0.001$); in severe course this difference was 4.70 µmol / l ($p \leq 0.001$); and the difference between mild course and control - 0.55 µmol / l ($p \leq 0.001$); between moderate course and control 1.67 µmol / l ($p \leq 0.001$); and between severe course and control - 5.24 µmol / l ($p \leq 0.001$).

It was found that against the background of increasing MDA concentration, blood AOA progressively decreased. Thus, if the AOA value in % in group 4 (severe PE) is taken as the critical average, then the difference between the critical average and average group AOA activity in women in labor without PE and with a severe course is -26.13 AOA units ($p \leq 0.001$); with moderate and severe course 22.74 AOA units ($p \leq 0.001$); the corresponding difference between moderate and severe course is 13.46 ($p \leq 0.001$) AOA units, etc. AOA activity in women in labor in all groups was lower than in the control group ($p \leq 0.001$). The obtained results are consistent with the literature data. It is recognized that oxidative stress plays a central role in the pathophysiology of many disorders of pregnancy and the postpartum period, including pregnancy complications such as placental pathology, preeclampsia (PE), intrauterine growth restriction (IUGR), gestational diabetes and miscarriage [1,2] in the pathophysiology of oxidative stress in obstetric complications, the role of harmful habits, including alcohol abuse, is high [1,2,11, 19].

At the end of the research our results demonstrated that compared to the control group there was a significant increase in lipid peroxidation and a significant decrease in antioxidant status in parturient women with postpartum endometritis. This disrupted balance leads to a remarkble increase in the oxidative stress index in parturient women with postpartum endometritis.

Oxidant-antioxidant system disturbances during pregnancy in adverse pregnancy outcomes has been proven. Oxidative stress can lead to numerous pathological conditions during female reproductive processes, contributing to the development of endometriosis, polycystic ovary syndrome, and various forms of infertility. Excessive ROS production can lead to fetal developmental disorders and increases the risk of miscarriage, intrauterine growth retardation, preeclampsia, premature birth, and gestational diabetes [5,7].

Thus, oxidative stress during pregnancy and in the postpartum period can be a consequence of primary etiologic factors that aggravate the development of pathological processes and lead to inflammation of the endometrium against the background of microbial invasion. From a clinical point of view, it is important to evaluate the threshold levels (cutoff points) of diagnostically significant indicators in the prognosis of pathology development. To assess the clinical informativeness of MDA and AOA in blood plasma for postpartum endometritis, their diagnostically significant levels were established using ROC analysis curves. At a concentration of MDA in the blood of more than 7.18 ng / ml, a conclusion can be made about a high risk of postpartum endometritis with a diagnostic sensitivity of 79.41% and a diagnostic specificity of 13.04%. At the AOA concentration less than 40%, a conclusion can be made about a high risk of developing postpartum endometritis with a diagnostic sensitivity of 80.30% and a diagnostic specificity of 7.39%. The clinical information content of the MDA level by AUC was 88.27% and the AOA concentration - 82.40%. The AUC value of 88.27% for MDA corresponds to a good model; the prognostic significance of AOA with an AUC of 82.40% is lower than the MDA value. The linear regression models of the risk of developing PE at different MDA concentrations are as follows: $Y = 0.12\ln(x) + 1$; and with known values of AOA activity - $Y = 0.18\ln(x) + 1$.

The quality of the predictive ability of the models was assessed using the Nagelkerke determination coefficient R^2, R^2 for an increase in the concentration of MDA is equal to = 85.67%; and for a decrease in the activity of AOA - 93.52%, which proves the higher predictive significance of the obtained regression models

CONCLUSION

The development of postpartum endometritis is characterized by a complicated obstetric-gynecological, somatic, social history and postpartum history with a high level of proinflammatory markers.

The developed method for predicting the development of postpartum endometritis allows identifying women in labor with a high risk of endometritis in an obstetric hospital at the preclinical stage of the disease, preventing the occurrence of severe purulent-septic complications after childbirth and is one of the measures to reduce maternal mortality.

Biochemical studies with determination of MDA and AOA in blood serum are useful as diagnostic methods for determining the risk of postpartum endometritis, significantly narrowing the range of studies and allowing to assess the risk of endometritis with high diagnostic accuracy using a limited range of studies

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