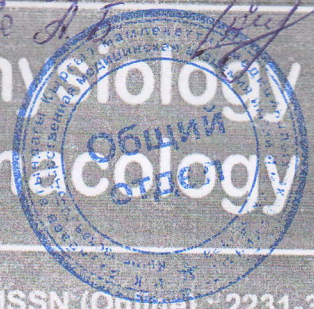


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RESEARCH ARTICLE

Condition of inflammation mediators and antioxidant protection system for endometriosis genitalis in women of reproductive age

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ABSTRACT

**Background:** The paper presents the data on a condition and a role of inflammation mediators and the antioxidant protection system in blood plasma for endometriosis genitalis (EG) in women of reproductive age. The material for the research included results from the survey of 104 women of reproductive age, divided into two groups (control group and clinical group). It is shown in the paper that EG occurs with the development of oxidative stress (OS) caused by activation of lipid peroxidation and the cytokine system with a simultaneous reduction in the antioxidant defense system. **Aims and Objective:** To define the condition and the role of inflammation mediators of lipid and cytokine natures and the antioxidant protection system in the development of EG in women of reproductive age. **Materials and Methods:** The research was carried out among 104 women of reproductive age, divided into control and clinical groups. The clinical groups included women with adenomyosis and EG externa. EG was verified with clinical findings, ultrasound examinations of pelvic organs, hysteroscopies, and diagnostic laparoscopies. Statistik, version 6.0, was used for statistical analysis. Diagnostic techniques included spectrophotometric, biochemical, and enzyme immunodetection methods. **Results:** Imbalance between radical processes and antioxidant protection systems causes the development of OS for EG; imbalance between pro-inflammatory and anti-inflammatory cytokines leads to inflammation activation and increased activities of triggers that develop endometrioid focuses. **Conclusion:** Due to unclear mechanisms of its development, EG is problematic in its respond to treatment while the OS contribution into the pathophysiological basis is highly reasonable. Components of menstrual blood are triggers for OS. With failed mechanisms in the cleansing system, the macrophage reaction gets stronger followed by a release of cytokines.


**KEY WORDS:** Endometriosis Genitalis; Oxidative Stress; Lipid Peroxidation; Antioxidant Protection System; Cytokines; Interleukins

INTRODUCTION

Endometriosis is one of the most pressing challenges in today's gynecology. Its morbidity in women of reproductive

age widely varies and according to different authors, it makes up 7-50% with a steady increase in this pathology in recent years.<sup>[1-3]</sup> Despite a large number of papers on endometriosis diagnostics and treatment, many pathogenic aspects of these problems have remained open, while in almost 50% patients after different treatments, there is a relapse and a further progression of the disease.<sup>[4,5]</sup>

There are several basic theories of endometriosis, including the basic theories of the epithelial metaplasia development influenced by hormonal disorders, inflammation, trauma, and other mechanical effects with a growth of the

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endometriosis-like tissue, and the violation that occurs with abnormal embryogenesis remains in the Mullerian duct.<sup>[6,7]</sup>

In endometriosis diagnostics, it is traditional to apply ultrasonography, morphologic, and laparoscopic methods.<sup>[8-10]</sup> In spite of an informative value of these diagnostic methods, they have not been already sufficient to evaluate etiopathogenetic mechanisms of endometriosis. In recent years, experts have been focused on the role of oxidative stress (OS) in the pathogenesis of endometriosis, which is a consequence of an imbalance between the production of inflammatory mediators and the system to neutralize them. This OS provides the conditions for the pelvic peritoneal inflammation, which occurs in the case of endometriosis.<sup>[11-14]</sup>

The increased growth in heterotopic endometrium may be due to increased levels of lipid peroxidation products in the peritoneal fluid and cytokine derivatives. At the same time, in mesothelium, there are conditions, under which the self-cleaning system fails to dispose of "the menstrual material" that causes failed antioxidant defense mechanisms and the development of the OS.<sup>[15-17]</sup> Thus, in a variety of ways, the OS may break the cell biology and contribute significantly to the histogenesis of endometriosis genitalis (EG), but many mechanisms of this effect have remain unknown.

## MATERIALS AND METHODS

A total of 104 women of reproductive age were an object of research, divided into the control and clinical groups. The control group consisted of 20 healthy women. The clinical groups consisted of 84 women, of these, there were 38 women with adenomyosis (1<sup>st</sup> clinical group), and 46 women had EG externa (2<sup>nd</sup> clinical group). The age of women surveyed in the groups had no significant differences and was as follows: In the control group,  $28.2 \pm 3.4$  years; in the 1<sup>st</sup> clinical group,  $31.4 \pm 4.1$  years; in the 2<sup>nd</sup> clinical group,  $33.2 \pm 4.5$  years. All examined women lived in the Kyrgyz Republic (Bishkek, the Chuy Province). In the research time, women gave their prior consent for diagnostic measures. EG diagnosis was verified with clinical findings, ultrasound examinations of pelvic organs, hysteroscopies, and diagnostic laparoscopies.

There were the following selection criteria for the observed women to include them in the control group or exclude from it: No inflammatory diseases in female reproductive organs at the time of examination; regular menstrual cycle; no clinically significant signs of extragenital diseases. The selection criteria for the clinical groups were as follows: Previously established the diagnosis of EG; EG diagnosed by laparoscopy, ultrasound, and hysteroscopy methods; not more than one suffered surgery on the pelvic and abdominal cavity organs. There were the following criteria to exclude women from clinical groups: Obvious hormone imbalance; adhesions in the pelvis in combination with hormone imbalance.

The diagnostic techniques were as follows:

- Detection of lipid peroxidation products in blood plasma with the spectrophotometric method<sup>[18]</sup>
- Detection of total antioxidant status in blood plasma with the biochemical method<sup>[19]</sup>
- Detection of catalase activity in blood plasma with the spectrophotometric method<sup>[20]</sup>
- Detection of medium molecular peptide (MMP) level in blood plasma with the spectrophotometric method<sup>[21]</sup>
- Erythrocyte membranes (shadows) were obtained with the biochemical method<sup>[22]</sup>
- Detection of cytokine level in blood plasma-interleukin 1 beta (IL-1 $\beta$ ), 4, 6 with the enzyme immunodetection method using sets produced by VECTOR-BIO (Russia).

The findings were processed with the method of variance statistics using Microsoft Excel, MS Windows 6, Statistika 6.0 software spreadsheets. The accuracy coefficient  $P = 95\%$  or  $P < 0.05$ , if  $P = 99\%$  or  $P < 0.01$ , and if  $P = 99.9\%$  or  $P < 0.001$ .

The research was made in accordance with the Declaration of Helsinki.

## RESULTS

As the research shows Table 1, women from the 1<sup>st</sup> clinical group compared to the data from the control group had essential intensification in lipid peroxidation, manifested in an increase in the level of neutral lipids ( $P < 0.01$ ), lipid hydroperoxides ( $P < 0.001$ ), dienketons ( $P < 0.001$ ), respectively, a value of the oxidation index in blood plasma ( $P < 0.01$ ), which is a value saying of a ratio between lipid hydroperoxides and neutral lipids. In the 2<sup>nd</sup> clinical group, in exacerbation time there was a tendency toward further intensification in lipid peroxidation products' processes compared to control values. Thus, with a significant increase in the neutral lipids level ( $P < 0.01$ ), a concentration of lipid hydroperoxides increase more than 6 times ( $P < 0.001$ ), whereas dienketons increase 10 times ( $P < 0.001$ ), a value of oxidation index increase 4 times ( $P < 0.001$ ) and is preferably associated with larger concentration of lipid hydroperoxides compared to neutral lipids. Compared with the data of women from the 1<sup>st</sup> clinical group, there is a verifiable increase in levels of lipid hydroperoxides, dienketons, oxidation index ( $P < 0.05$ ) and a decrease in neutral lipids ( $P < 0.05$ ).

In a parallel way to change to lipid peroxidation products intensity, there is a change to the activity of the antioxidant protection system (Table 2). Thus, in the 1<sup>st</sup> clinical group in exacerbation time compared to the data from the control group, there is a significant reduction in total AS ( $P < 0.01$ ) and catalase activity ( $P < 0.05$ ), whereas the MMP concentration slightly but verifiably increases ( $P < 0.05$ ). In the 2<sup>nd</sup> clinical

**Table 1: Values of lipid peroxidation products in blood plasma in women with EG**

Examined groups of women	Statistical values	Test parameters			
		Neutral lipids, ODU/mL	Lipid hydroperoxides, ODU/mL	Dienketons, ODU/mL	Oxidation index
Control (n=20)	M±m	1.374±0.143	5.537±0.083	0.066±0.011	0.369±0.069
1 <sup>st</sup> clinical (n=38)	M±m	2.93±0.21	2.713±0.371	0.421±0.089	0.906±0.105
	P <sub>2-1</sub>	<0.01	<0.001	<0.001	<0.01
2 <sup>nd</sup> clinical (n=46)	M±m	2.123±0.272	3.41±0.291	0.723±0.145	1.565±0.221
	P <sub>3-1</sub>	<0.01	<0.001	<0.001	<0.001
	P <sub>3-2</sub>	<0.05	<0.05	<0.05	<0.05

**Table 2: Values of antioxidant protection system in blood plasma in women with EG**

Examined groups of women	Statistical values	Test parameters		
		TAS, %	Catalase, mkat/l	MMP, U
Control (n=20)	M±m	25.1±0.97	22.35±1.03	0.221±0.028
1 <sup>st</sup> clinical (n=38)	M±m	16.6±0.84	15.9±0.93	0.286±0.024
	P <sub>2-1</sub>	<0.01	<0.05	<0.05
2 <sup>nd</sup> clinical (n=46)	M±m	14.5±0.67	14.2±0.87	0.294±0.031
	P <sub>3-1</sub>	<0.01	<0.01	<0.05
	P <sub>3-2</sub>	>0.05	>0.05	>0.05

TAS: Total antioxidant status; MMP: Medium molecular peptide

group of women, at this time, there is almost the same tendency toward a change to the antioxidant protection system's function, like in the 1<sup>st</sup> clinical group. Herewith, values between clinical groups do not face significant changes ( $P > 0.05$ ).

The defined cytokine IL-1 $\beta$ , IL-4, and IL-6 values in blood plasma in women with EG revealed (Table 3) that in the 1<sup>st</sup> clinical group, in IL values, there had been a significant increase of IL-1 $\beta$  ( $P < 0.01$ ) and IL-6 ( $P < 0.001$ ) in blood, as well as a slight but verifiable decrease in IL-4 ( $P < 0.05$ ). In the 2<sup>nd</sup> clinical group, we have observed the same picture of dynamics for changes to IL concentration like the 1<sup>st</sup> clinical group, with respect to control values ( $P < 0.001$ - $P < 0.05$ ). In comparison with the data from the 1<sup>st</sup> clinical group, we observe the higher concentration of IL-6 ( $P < 0.01$ ), whereas values of IL-1 $\beta$  and IL-4 do not demonstrate significant changes ( $P > 0.05$ ). As the presented data show, the greatest changes to cytokine values are in the level of IL-6, which for adenomyosis exceeds the control value 8 times and for EG extra 13 times.

## DISCUSSION

The research has shown that because of changes to cells and tissues for EG, there is activation in lipid peroxidation processes with changed functioning of the antioxidant protection system. As one of the reasons, there is inflammation

in endometrioid focuses and adjacent tissues, which with a dissemination of endometrioid focuses with the peritoneum involved in the inflammation process in pelvic organs causes a drop in oxygen tension and progressive tissue hypoxia. For EG, a continuously progressive retrograde menstruation (endometrial cells, menstrual debris, erythrocytes, etc.) leads to activation in inflammation mediators of the lipid nature, while the endometrial tissue starts its active metabolizing that causes more intensive free-radical oxidation processes.

Along with processes of an increase in lipid peroxidation and as a result of interaction with the antioxidant system, there is a reduction in the total antioxidant status. At the same time, the decrease in the total antioxidant status is less than lipid peroxidation products' severity, suggesting possibly long-term mobilization of compensatory capacities in the female body with EG. The reduced catalase activity says of a decreasing respond that prevents accumulation of hydrogen peroxide derived from dismutation of superoxide anion and aerobic oxidation of rehabilitated flavoproteins. MMP accumulation assumes that the lipid peroxidation process assumes the ascorbate-dependent way.

Changes to the cytokine system activity in the EG progress evidence that along with a dysregulation in proliferative processes and differentiation of the immunocompetent cells, the inflammation activation takes place, in particular, a development of an acute inflammation phase. An imbalance between pro-inflammatory and anti-inflammatory cytokines seemingly lead to an increase in triggers for a development of endometrioid focuses. A disparity between inflammation remission and capabilities of the phagocytic system, as well as monocytes' endotoxin tolerance, are mechanisms for its development.

Together with hormones and neurotransmitters, cytokines make a basis for the language of chemical signaling, which in a multicellular organism controls morphogenesis and tissue regeneration. Activation of pro-inflammatory cytokines in the case of endometriosis promote phagocytes, causes their migration into the endometrium and a release of inflammation mediators that are derivatives of lipids, prostaglandin E<sub>2</sub>, and thromboxanes. At the same time, it should be taken into account that endometriosis development is not only possible

**Table 3: Cytokines values in blood plasma in women with EG**

Examined groups of women	Statistical values	Test parameters		
		IL-1 $\beta$ , pg/mL	IL-4, pg/mL	IL-6, pg/mL
Control (n=20)	M $\pm$ m	1.71 $\pm$ 0.11	1.56 $\pm$ 0.147	2.454 $\pm$ 0.201
1 <sup>st</sup> clinical (n=38)	M $\pm$ m	5.64 $\pm$ 0.336	0.946 $\pm$ 0.091	19.66 $\pm$ 2.7
	P <sub>2-1</sub>	<0.01	<0.05	<0.001
2 <sup>nd</sup> clinical (n=46)	M $\pm$ m	6.43 $\pm$ 0.378	0.789 $\pm$ 0.082	32.9 $\pm$ 3.46
	P <sub>3-1</sub>	<0.01	<0.05	<0.001
	P <sub>3-2</sub>	>0.05	>0.05	<0.01

because of proliferation in endometrial tissue cells but also due to a disorder in mechanisms of their programmed death, apoptosis, where the cytokine system plays an important role.

## CONCLUSION

Due to unclear mechanisms in its development, EG is problematic in its respond to treatment, while a contribution of OS into the pathophysiological basis of the disease is highly reasonable. Components of menstrual blood are triggers for OS. With failed mechanisms in the cleansing system, the macrophage reaction gets stronger followed by a release of cytokines.

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