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PATHOGENETIC SUBSTANTIATION OF MEDICAL IMMUNE SYSTEM CORRECTION IN PAROUS WOMEN WITH TUMOR-LIKE FORMATIONS

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ABSTRACT

Endometrioid ovarian cysts in women of childbearing age take a leading position in the structure of gynecological diseases, which is 30 - 40% of cases dramatically reduces the chances of getting pregnant.

The methods of treatment applied (surgical, hormonal, anti-inflammatory and others) do not always give the desired effect, so the tactics of treatment for these patients remains controversial.

The imbalance between cytopoiesis in the body and timely elimination of altered cells from the body underlies the pathogenesis of genesis of any cysts. Therefore, immune disorders are the cause of formation and functioning of any cysts. On the basis of this, we conducted a study with the objective to identify effective treatment in parous women with ovarian tumor-like formations with consideration for the characteristics of their immune status. Eighty women were surveyed: 50 healthy non-pregnant women, 15 women with a confirmed diagnosis of endometrioid ovarian cyst receiving conventional treatment, 15 women with the same diagnosis who received comprehensive treatment, including the use of immunomodulators and adaptogens Galavit and Indogreen (indole-3-carbinol).

Research results showed that in parous women with ovarian tumor-like formations on pretreatment stage irregularities were detected in the immune system, which were manifested by incomplete phagocytosis, decreased neutrophil microbicidal potential, increase in the number of B-lymphocytes and immunoglobulins of basic groups, indicating an impairment of the immune mechanisms of protection of the woman's body and confirming their involvement in the pathogenesis of the disease.

At the end of the traditional course of treatment microbicidal capacity of neutrophils has been still reduced, and there was observed activation of humoral immune system indicating the need for well-grounded immune system correction targeting key pathophysiological processes. Following a comprehensive treatment including the use of immunomodulators and adaptogens Galavit and Indogreen, normalization of the body immune homeostasis took place, which indicates an increase in the effectiveness of the treatment and the possibility for recommending this regimen for future use.

KEYWORDS: *tumor-like formations of ovaries, pregnancy, immune system correction, immunodiagnostics.*

INTRODUCTION

The presence of tumor-like formations of ovaries is one of the leading problems of modern gynecology and occurs in 20% of women of reproductive age. It was found that in 30-40% of cases in this pathology infertility is observed, which is not absolute, but dramatically reduces the chances of getting pregnant [*Narzullaeva E, 2003; Popov N et al., 2005; Burke T et al., 1997; Abdurahmanova N, Khushvakhtova E, 2011*].

For the majority of women, diagnosed with endometrial ovarian cysts, who are determined to bear a second child, the most pressing issue is the possibility of the next pregnancy.

Despite the negative prognosis, endometriosis does not always cause problems with conception. A certain percentage of women suffering from this disease can become pregnant without treatment (depending on the stage of the disease) and bear a child.

For many years, pregnancy was considered and is considered a natural method of treatment of this disease. However, a complete cure in pregnancy and lactation does not occur, only remission and suppression of lesions without reverse development of endometriotic cysts is possible, i.e. after birth these women need to be treated.

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Presently, the proposed methods of treatment of ovarian cysts are not effective enough, so the tactics for these patients, according to some researchers, remains controversial.

Immunological disorders play one of the main and perhaps the leading part in the pathogenesis of endometriosis [*Kammer-Bartosinska A et al., 2003; Song M et al., 2003; Dmowski W, Braun D, 2004; Linde V et al., 2008*], which affects the process of ovulation. Identification of these disorders is important, especially for therapeutic (surgical and nonsurgical) point of view, since in previously parous women endometriotic lesions are often not local, their dimensions are not always sufficient for surgical manipulation (which in turn may lead to a decrease in ovarian reserve and necessitate further inefficient auxiliary reproductive technologies), and the results of clinical studies indicate that the use of only the drugs that suppress estrogen synthesis gives a high rate of relapse [*Nikolaev I et al., 2005; Budinetz T, Sanfilippo J, 2010*].

In this regard, improvement of treatment results of women with previously fulfilled reproductive function currently remains urgent, taking into account the state of the immune system, which was the goal of our work: to identify the effectiveness of different treatments of ovarian tumor-like formations in previously parous women with the consideration for the features of the immunological status.

Material and Methods

We examined 80 women aged 17-40 years who gave written informed consent to participate in the study. The study was approved by the Bioethics Committee of the SI "Zaporozhye Medical Academy of Postgraduate Education of Ministry of Health of Ukraine" and conforms to the ethical, moral and legal requirements according to the Order of the Ministry of Health of Ukraine No. 281 as of 01.11.2000.

The first group (control) consisted of 50 healthy non-pregnant women of reproductive age, with no signs of gynecological and chronic somatic pathology at the time of the examination, who went to the medical facility for options of contraception. The 2nd group women with a confirmed diagnosis of endometrioid ovarian cyst who were not treated after pregnancy that ended in a live birth were divided into 2 subgroups (2a, 2b) depending on the treatment. Group 2a consisted of 15 women who have previously given birth with a confirmed diagnosis of endometrioid ovarian cyst treated with conventional treatment. Group 2b consisted of 15 women who have previously given birth to a confirmed diagnosis of endometrioid ovarian cyst treated with comprehensive treatment. Comprehensive treatment consisted of supplemental drugs with the immunomodulatory and adaptogenic action: Galavit and Indogreen.

Treatment efficiency was assessed after 3 months of the treatment. In all patients the indicators of nonspecific and specific parts of the immune system were studied.

To investigate the non-specific immune responsiveness the following was determined: phagocytic activity of blood neutrophils based on the method of determining their absorptive and digestive ability in relation to a microbial culture test after a joint preincubation [*Frimel N, 1984*]; oxygen-dependent metabolism of neutrophils (nitroblue tetrazolium test or NBT-test) and functional reserve of cells (stimulated NBT-test) [*Viksman M, Mayanski A, 1979*]; myeloperoxidase activity of neutrophils (MPO) [*Narcissov R, 1964*]; the content of cationic proteins (CP) in neutrophils [*Shubich M, 1974*].

Subpopulations of lymphocytes were determined by using monoclonal antibodies against antigens CD_3^+ (total number of T-lymphocytes), CD_4^+ (T-helper), CD_8^+ (T-suppressors), CD_{16}^+ (NK cells), CD_{19}^+ (B cells) produced by Granum R&D Company (Kharkiv, Ukraine).

Humoral immunity of IgA, IgM, IgG was determined by using monospecific sera against said immunoglobulins [*Mancini G et al., 1965*].

Statistical data was processed with STATISTICA computer software package (StatSoftStatistica v.6.0). Assessment of the nature of the distribution of the analyzed indicators by fitting criterion of Kolmogorov-Smirnov determined that most of them are not subject to the normal law. Statistical significance of compared parameters was determined by using a criterion of the Wald-Wolfowitz series at a significance level of $p<0.05$. The analyzed data are presented as the median (Me) and quartile range (RQ), which represents the difference between the values of the 75th and 25th percentiles (RQ = 75% UQ – 25% LQ), where UQ is the upper quartile and LQ is the lower quartile.

Results and Discussion

Earlier in the study of the immune status of women with ovarian tumor-like formations, we identified changes that were characterized by incomplete phagocytosis of neutrophils in the presence of intact functional-metabolic reserve, reduced microbicidal capacity, intact number of T-lymphocytes and their subpopulations, a slight increase in the number of B-lymphocytes, and hyperglobulinaemia.

Based on these results, a comparative analysis of the immune status of women treated with traditional and comprehensive treatment, which was mainly immunomodulatory therapy.

In assessing the indicators characterizing the functional and metabolic status of neutrophils, statistically insignificant, but clinically significant changes in the absorptive and digestive ability, as well as the functional activity of neutrophils with respect to both the control group and the comparison group (from 2% to 5%) were found in 2a group. Functional-metabolic reserve, characterized by an indicator of spontaneous and stimulated NBT test increased by 8% and 7% compared to the control group and matched the comparison group parameters (Table 1).

The indicators of microbicidal system – cationic protein and myeloperoxidase (CP, MPO) were reduced by 9% and 8% relative to the control group and increased by 5% and 5%, respectively, relative to the comparison group.

In the women of 2a group treated with conventional techniques the level of indicators of T-cell and NK-system cells – CD_3^+, CD_4^+, CD_8^+, CD_{16}^+ values significantly matched those of the control and the comparison groups. All the changes were not statistically, but clinically significant (Table 2).

When evaluating the performance of humoral immunity, the level of CD_{19}^+ increased relative to the control group by 10 % and nearly matched the comparison group values. The concentration of Ig A, M, G showed 11%, 54%, 48% increase relative to the control group and 17%, 11% and 3% decrease relative to the comparison group, which is

Table 1.

State of functional-metabolic status of neutrophils in parous women with endometriosis, depending on the treatment received, Me (75%Q–25%Q=RQ)

Indicators	Groups			
	1 (n=50)	2 (n=30)	2a (n=15)	2b (n=15)
NPI on 30th *min*, %	67.5 (74.2–49.1=25.1)	64.1* (81.7–42.2=39.5)	65.7 (83.4–41.3=42.1)	69.4*.** (86.7–51.0=35.7)
NPN on 30th *min, c.u.*	3.1 (6.5–1.3=5.2)	3.3 (5.0–1.3=3.7)	3.2 (5.7–1.5=4.2)	3.1 (6.2–1.7=4.5)
NPI on 120th *min*, %	58.4 (68.3–39.8=28.5)	57.4 (60.1–30.8=29.3)	57.4 (62.3–36.7=25.6)	58.2 (67.6–37.2=30.4)
NPN on 120th *min, c.u.*	5.7 (6.1–4.0=2.1)	5.3 (7.1–3.4=3.7)	5.5 (7.4–3.9=3.5)	5.8 (6.7–4.2=2.5)
NBTsp, c.u.	1.2 (1.3–1.0=0.3)	1.5* (2.5–0.7=1.8)	1.3 (2.0–0.6=1.4)	1.2 (1.4–0.9=0.5)
NBTst, *c.u.*	1.3 (2.2–0.8=1.4)	1.4 (2.3–0.6=1.7)	1.4 (2.1–0.9=1.2)	1.3 (2.0–0.7=1.3)
Cationic proteins, *c.u.*	2.2 (3.0–1.4=1.6)	1.9 (2.7–0.4=2.3)	2.0 (2.9–0.8=2.1)	2.1 (2.9–1.1=1.8)
Myeloperoxidase, *c.u.*	2.3 (3.4–0.7=2.7)	2.0 (2.9–0.6=2.3)	2.1 (3.1–0.9=2.2)	2.2 (3.0–0.7=2.3)

Notes: ** – statistically significant differences ($p<0.05$) relative to the control group,*
*** – Statistically significant differences ($p<0.05$) relative to Group 2,*
NPI – neutrophils phagocytic index, NPN - neutrophils phagocytic number.

Table 2.

State of cell immunity in parous women with endometriosis depending on the treatment received, Me (75%Q–25%Q=RQ)

Indicators, %	Groups			
	1 (n=50)	2 (n=30)	2a (n=15)	2b (n=15)
CD_3^+	63.7 (67.1–59.8=7.3)	62.9 (67.7–56.2=11.5)	63.9 (68.2–57.7=10.5)	63.9 (67.9–58.5=9.4)
CD_4^+	38.7 (44.2–31.8=12.4)	38.2 (43.4–30.3=13.1)	39.5** (46.3–31.7=14.6)	39.7* (45.1–32.6=12.5)
CD_8^+	25.6 (39.0–21.3=17.7)	23.8* (33.1–20.4=12.7)	24.7 (37.2–20.6=16.6)	25.0** (38.4–21.7=16.7)
CD_{16}^+	16.5 (24.6–11.9=12.7)	16.0 (22.5–12.3=10.2)	16.5 (24.9–11.3=13.6)	16.9 (25.1–12.6=12.5)

***Notes**: * - statistically significant differences (p<0.05) relative to the control group,*
*** - Statistically significant differences (p<0.05) relative to Group 2.*

not statistically, but clinically significant in the subsequent case (Table 3).

Thus, the presence of immune disorders after conventional therapy (which is fraught with the development of secondary bacterial infections) and activation of humoral immune system prove the need for use of immunomodulators, which is pathogenically well-grounded.

Based on these results it seemed necessary to correct the traditional treatment by prescribing immunomodulatory drugs.

The drug of choice for this group of patients was Galavit (aminodihydrophthalasindione sodium), which mainly targets phagocytosis and antibody response (especially the synthesis of Ig G). It was prescribed at 0.1 *g* twice daily for a 20-day course. Comprehensive treatment was complemented by prescription of a minor immunomodulatory agent Indogreen (indole-3-carbinol) at 300 *mg* daily during meals for a 3-month course.

In assessing the indicators characterizing the functional and metabolic status of neutrophils in group 2b, the indicators characterizing the absorptive and digestive ability were not very different from the values of the control group; they increased by 8% and 2% versus the comparison group, which in the latter case is not statistically, but clinically significant. The indicators of functional activity of neutrophils on the 30th minute and 120th minute correspond to the index of the control group; on the 30th minute they decreased by 6% and on the 120th minute they increased by 9% versus the com-

Table 3.

State of humoral immune system in parous women with endometriosis, depending on the applied treatment, Me (75%Q–25% Q=RQ)

Indicators	Groups			
	1 (n=50)	2 (n=30)	2a (n=15)	2b (n=15)
CD_{19}^+, %	17.2 (23.1–15.6=7.5)	19.4* (25.3–16.1=9.2)	19.0 (24.7–15.7=9.0)	18.2** (23.9–14.8=9.1)
Ig A, *g/l*	1.8 (2.3–0.9=1.4)	2.4 (2.7–1.8=0.9)	2.0 (2.5–1.6=0.9)	2.0 (2.6–1.4=1.2)
Ig M, *g/l*	1.1 (2.5–0.4=2.1)	1.9 (3.1–0.7=2.4)	1.7* (2.9–0.6=2.3)	1.4** (2.6–0.5=2.1)
Ig G, *g/l*	10.2 (13.1–6.7=6.4)	11.3 (13.4–7.8=5.6)	11.0 (12.8–7.3=5.5)	11.0 (13.0–7.1=5.9)

***Notes**: * – statistically significant differences (p<0.05) relative to the control group,*
*** – statistically significant differences (p<0.05) relative to Group 2.*

parison group parameters. The indicators of functional metabolic reserve (NBTsp and NBTst) corresponded to those of the control group and decreased relative to the comparison group by 20% and 7%, respectively.

The indicators of microbicidal system of cationic proteins and myeloperoxidase activity decreased by 5% and 4%, respectively, versus the control group and increased by 10% and 10%, respectively, versus the comparison group (Table 1).

The women of group 2b who underwent the comprehensive treatment the indicators of cellular immunity CD_3^+, CD_4^+, CD_8^+ - corresponded the values of the control and comparison groups. The level of CD_{16}^+ almost matched the values of the control group, exceeding the value of the comparison group by 6%, respectively (Table 2).

The indicators describing the humoral immunity status – CD_{19}^+ – exceeded the value of the control group by 6% and decreased by 6% versus the comparison group. Concentration of Ig A, M, G showed 11%, 27% and 7% increase relative to the control group and 17%, 26%, 43% decrease relative to the comparison group (Table 3).

The therapy with the drugs Galavit and Indogreen that we proposed demonstrated recovery of microbicidal potential of the phagocytic system as well as normalization of humoral immunity.

Thus, the effect of the proposed method of treatment was achieved by prescribing immunomodulators and adaptogens, which led to the restoration of physiological values of the immune status parameters.

CONCLUSION

In parous women with ovarian tumor-like formations in pretreatment stage, irregularities were detected in the immune system, manifested by incomplete phagocytosis, decreased neutrophil microbicidal potential, increase in the number of B-lymphocytes and immunoglobulins of basic groups, indicating an impairment of the mechanisms of immune protection of women and confirming their involvement in the pathogenesis of the disease.

At the end of the conventional treatment course the microbicidal capacity of neutrophils still remained reduced. Also, the treatment allowed us to observe activation of humoral immune system, indicating the need for well-grounded immune system correction designed to target key pathophysiological processes. Following the comprehensive treatment *including prescribed immunomodulators and adaptogens Galavit and Indogreen, normalization of the body immune* homeostasis was observed, which indicates an increase in the effectiveness of the treatment and the possibility for recommendations of this regimen for future use.

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