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STATE OF CELLULAR AND HUMORAL SYSTEMIC IMMUNITY IN WOMEN OF REPRODUCTIVE AGE UNDER THE DEVELOPMENT OF PROLIFERATIVE PROCESSES IN THE ENDOMETRY OF THE UTERUS AND BREAST GLANDS

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The immune system plays an important role in the pathogenesis of endometrial hyperplasia (EH) and benign breast tumors, as in the body of women this system interacts closely with the reproductive system. Due to the fact that the transformation of endometrial cells and mammary glands is controlled by the immune system, it is important to study the redistribution of components of cellular and humoral immune components in women with combined pathology.

The aim of the study was to study the state of cellular and humoral parts of the immune system in women of reproductive age, patients with endometrial hyperplasia and benign breast tumors.

Materials and methods. Studies of the state of the immune cell were performed in peripheral blood to determine the subpopulation composition of blood lymphocytes using monoclonal antibodies to antigens CD3 + (total number of T lymphocytes), CD4 + (T-helpers), CD8 + (T-suppressors), CD16 + (NK cells), CD19 + (B-lymphocytes). Indicators of humoral immunity – immunoglobulins (Ig) of classes A, M and G were determined using monospecific sera against these immunoglobulins.

Results of the research. There was a decrease in the mean values of T-lymphocytes, T-suppressors, T-helpers and B-lymphocytes with a simultaneous increase in NK cells in the peripheral blood in patients with GE and mastopathy compared with the control group. There was a decrease in the immunoregulatory index - the ratio of CD4 + / CD8 +. An increase in the content of Ig G and a decrease in the levels of Ig M and Ig A in the groups of patients with GE and in the combination of GE and mastopathy in comparison with healthy women is shown.

Conclusions. Immunological homeostasis, which is characterized by changes in cellular and humoral immunity at the systemic level, is involved in the violation of reproductive function in women with hormonal imbalance, which leads to the development of GE and mastopathy

Keywords: hyperplasia, endometrium, mastopathy, leukocytes, T-lymphocytes, T-helpers, T-suppressors, B-lymphocytes, NK-cells, immunoglobulins

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1. Introduction

Irregular growth of the endometrium leads to an abnormal ratio of glands and stroma and is a continuum of the spectrum of changes in endometrial tissues [1]. Impaired cell proliferation of endometrial glands leads to endometrial hyperplasia (EH) [2]. The development of these processes is accompanied by varying degrees of histopathological complexity and atypical features in cells and nuclei, which is manifested by the development of different types of GE – atypical or without atypia [3]. Such changes in the body are the result of continuous estrogenic stimulation of endometrial tissue with a relative lack of balancing effects of progesterone. Such hormonal imbalance is observed in several conditions when the cause of excess estrogen is endogenous or exogenous [4].

Pathogenetic changes in a woman's body with EH may directly depend on the state of the body's immune system, as hormonal fluctuations during the menstrual cycle modulate immune function [5]. The immune sys-

tem supports the processes during menstruation, and it can help stimulate the endometrium of the uterus by increasing the number of leukocytes [6]. Immune system dysfunction can lead to a local physiological inflammatory process, resulting in changes in the components of cellular and humoral immunity not only at the local but also at the systemic level [6, 7].

Changes in the immune system may be exacerbated by concomitant proliferative processes in the mammary glands [8]. Since the transformation of endometrial cells and mammary glands is controlled by the immune system, it is important to study the redistribution of components of cellular and humoral immune defense in patients. The relationship between the reproductive and immune systems in women of childbearing age with proliferative processes in the uterine endometrium and mammary glands remains unclear.

The aim of the research. To study the state of cellular and humoral parts of the immune system in

women of reproductive age, patients with endometrial hyperplasia and benign breast tumours.

2. Materials and methods

The study was conducted based on the State Institution “Zaporizhzhya Medical Academy of Postgraduate Education of the Ministry of Health of Ukraine” for the period 2020–2022.

A comprehensive clinical examination of patients with hyperplastic processes in the endometrium and benign tumours in the breast was performed. The study included women of reproductive age from 18 to 47 years (mean age of patients was 28.6 ± 2.3 years). The main group (30 patients – 33.3 % of the total) consisted of patients diagnosed with EH and benign breast disease. The comparison group consisted of patients diagnosed with EH without concomitant breast pathology (30 patients – 33.3% of the total number of women). The control group included 30 healthy women.

In all patients of the study groups, the diagnosis was morphologically verified. The diagnosis of EH was made based on the results of a comprehensive examination, which consisted of clinical, instrumental, hardware and clinical and laboratory studies. All patients with suspected benign breast disease underwent mammography. Women diagnosed with mastopathy were assigned to the main group.

Studies of the cellular and humoral parts of the immune system were performed in peripheral blood. Determination of subpopulation composition of blood lymphocytes was performed using monoclonal antibodies to antigens CD3 + (total number of T-lymphocytes), CD4 + (T-helpers), CD8 + (T-suppressors), CD16 + (NK

cells), CD19 + (B-lymphocytes) produced by NVO “Granum” (Ukraine) by flow cytofluorimetry [9] using reagents to determine clusters of differentiation, as well as by calculating the immunoregulatory index – CD4+/CD8+. Indicators of humoral immunity IgA, IgM, IgG were determined using monospecific sera against these immunoglobulins by the method of G. Manchini [10].

Statistical processing of the obtained results was processed using the program “Statistica for Windows” version 13.0, Stat Soft Inc (USA). The distribution of quantitative indicators in the groups was defined as the arithmetic mean ($M \pm m$) under the condition of normality of the distribution (the normality test was performed using the Shapiro-Wilks test). Statistically significant difference between the indicators in the experimental groups for relative numbers in the normal distribution was determined using the Student's t test (parametric statistics method). Under conditions of uneven distribution of indicators in the experimental groups, the Mann-Whitney U-test (non-parametric statistics method) was used. A statistically significant difference between the comparators was considered at $p < 0.05$.

4. Research results

The results of the studies showed that patients with EH had severe leukocytosis, as the total number of leukocytes in the peripheral blood was 1.7 times higher than the control group ($p < 0.05$) (Table 1). Similar changes were observed in patients with EH and concomitant mastopathy, in whom the level of leukocytes in the peripheral blood was 2.2 times higher than the control index ($p < 0.05$) (Table 1).

Table 1

The number of immunocompetent cells in the peripheral blood of patients with endometrial hyperplasia and mastopathy ($M \pm m$)

Indicator	Comparison group (n=30)	Main group (n=30)	Control group (n=30)
Leukocytes, $10^9 / l$	$8.25 \pm 0.442^*$	$10.83 \pm 0.609^{*, \#}$	5.01 ± 0.421
Eosinophils, %	$1.65 \pm 0.091^*$	$2.1 \pm 0.234^{*, \#}$	1.26 ± 0.124
Stick neutrophils, %	4.85 ± 0.462	5.1 ± 0.504	4.41 ± 0.524
Segmental neutrophils, %	58.4 ± 5.467	$49.5 \pm 4.87^{*, \#}$	63.3 ± 6.04
Monocytes, %	9.4 ± 0.799	9.9 ± 0.767	8.1 ± 0.966
Lymphocytes, %	21.6 ± 1.904	$19.3 \pm 1.041^*$	24.8 ± 1.208

Note: * – statistically significant difference compared to the control group; # - statistically significant difference compared to the comparison group, $p < 0.05$

Since the different forms of leukocytes are in a certain ratio, which is called the leukogram, and the most practical is the analysis of the leukocyte profile of blood to determine granulocytes and agranulocytes in peripheral blood, it is appropriate to analyze changes in different types of leukocytes.

The shift of the leukocyte formula to the left was observed to a greater extent in patients with EH and mastopathy. Thus, the leukocyte formula shows that the level of rod-shaped non-neutrophils did not change in all study groups compared to the control, and the number of mature (segmental) neutrophils decreased only in the main group – 1.3 times compared to the control group ($p < 0.05$) (Table 1).

During the study of agranulocyte blood cells it was found that in the leukograms of patients with EH the level of lymphocytes in the leukogram corresponded to the statistical norm. However, in patients with EH and mastopathy their level was the lowest and was at the level of the lower limit of normal, which was statistically significantly different from the corresponding group of healthy women ($p < 0.05$) (Table 1). Therefore, the increase in white blood cell counts in patients with combined EH pathology and mastopathy was mainly due to increased eosinophils. At the same time, the level of lymphocytes was at the level of the lower limit of the norm, which does not exclude the imbalance between different lymphocyte populations (Table 1).

Since lymphocytes include B-lymphocytes and different subpopulations of T-lymphocytes, it would be appropriate to determine their level. The results of the studies showed that in patients with EH and EH and combined pathology of the mammary glands, the indicators of immunological status differed from those of patients in the control group. The studies showed a decrease in the mean values of T-lymphocytes (CD3 +), T-suppressors (CD8 +), T-helpers (CD4 +) and B-lymphocytes (CD19 +) with a simultaneous increase in

NK cells (CD16 +) in peripheral blood in patients of all study groups in accordance with the control (Fig. 1). It should be noted that the direction of these changes is more pronounced in patients with combined pathology. Thus, in patients with EH and mastopathy, the level of CD3 +, CD4 +, CD8 + and CD19 + decreased 1.6 times, 1.5 times, 1.5 times and 1.8 times compared to controls, respectively ($p < 0.05$) (Fig. 1). At the same time, the value of CD16 + level was 1.5 times higher than the norm ($p < 0.05$) (Fig. 1).

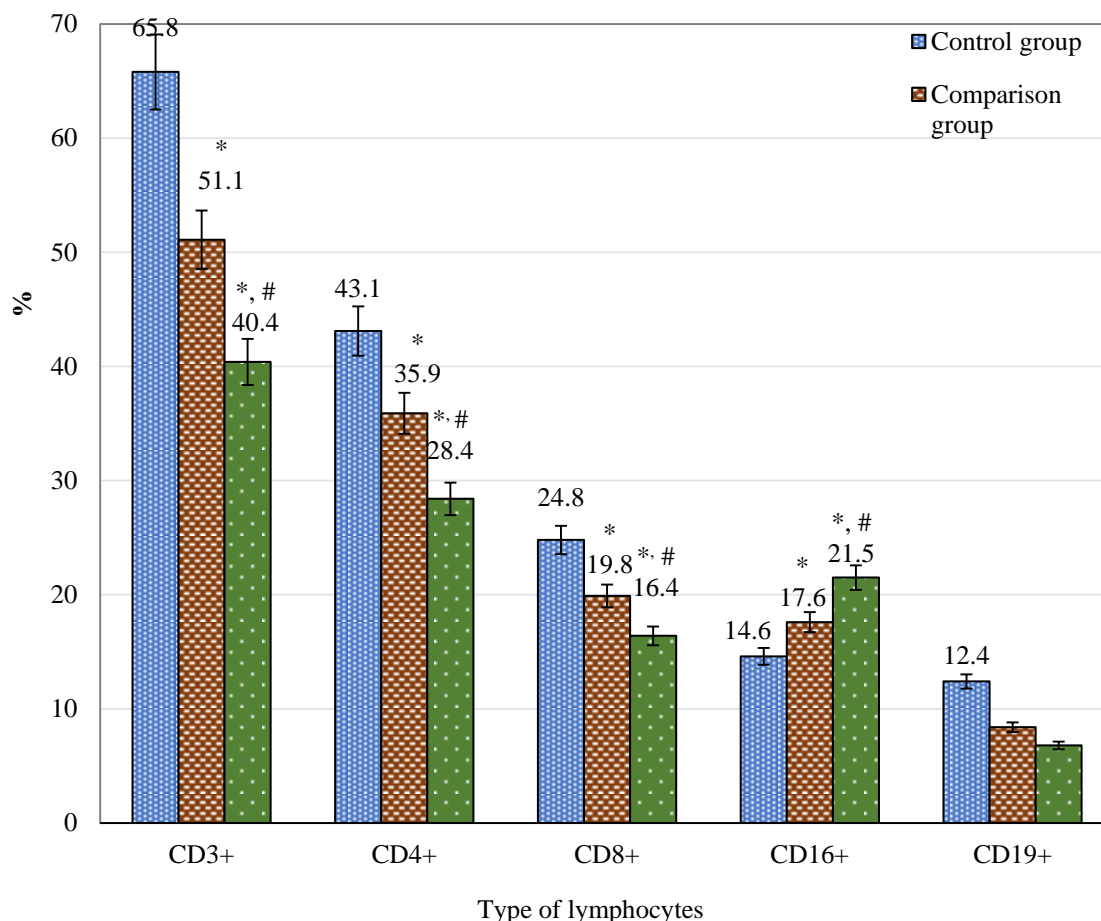


Fig. 1. The content of different populations of lymphocytes in the peripheral blood of women with hyperplastic processes in the endometrium and mastopathy

The results indicate low functional activity of T-helpers, and hence a decrease in the immune response in general, because T-helpers activate both T-killer cells and B-lymphocytes.

It should be noted that in patients with different types of EH statistically significant differences in the mean absolute values of T- and B-lymphocytes (CD3 + and CD19 +), T-suppressors (CD8 +), T-helpers (CD4 +), and NK cells CD16 +) was not detected in peripheral blood. In addition, no differences were found in patients of different age groups within one study group.

For a more reliable analysis of the T-cell component of immunity, it would be appropriate to determine the immunoregulatory index – the ratio of CD4 + / CD8 +.

The results showed that the ratio of CD4 + / CD8 + in EH decreased both in concomitant pathology and without it compared with the control group (Fig. 2),

which indicates an imbalance in the immune system in the development of proliferative processes in the reproductive organs.

In the next stage, we studied circulating antibodies in the blood, which play an important role in the body in preventing the formation of tumours and can be considered as mediators of intersystem interactions. Therefore, in the analysis of systemic immunity, the determination of the level of immunoglobulins in blood plasma is quite appropriate.

Determination of the humoral component of systemic immunity revealed a statistically significant increase in the content of class G immunoglobulins (Ig G) in groups of patients with EH and in the combination of EH and mastopathy compared with healthy women. It was found that in EH the content of Ig G was 1.3 times, and in EH and mastopathy 1.5 times higher than the control index ($p < 0.05$) (Fig. 3).

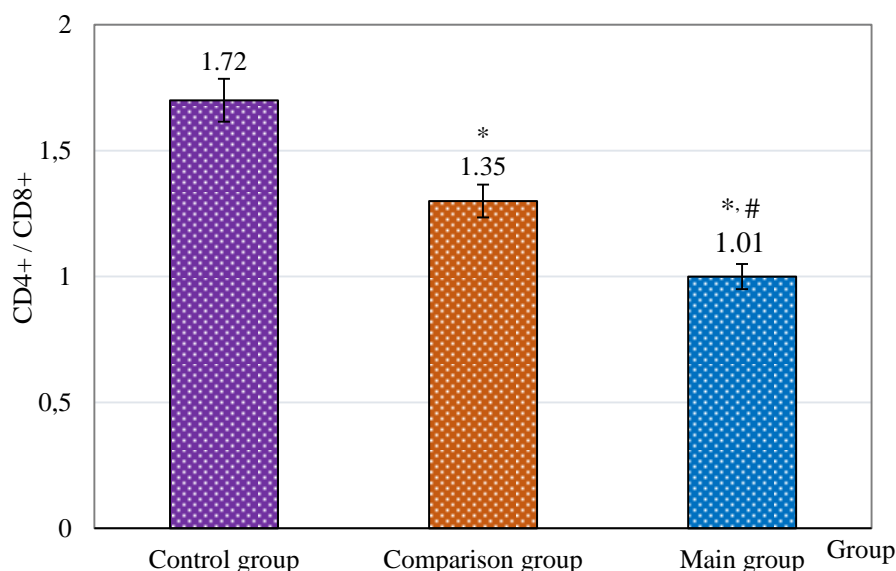


Fig. 2. The ratio of CD4 + / CD8 + in the peripheral blood of patients with endometrial hyperplasia and mastopathy

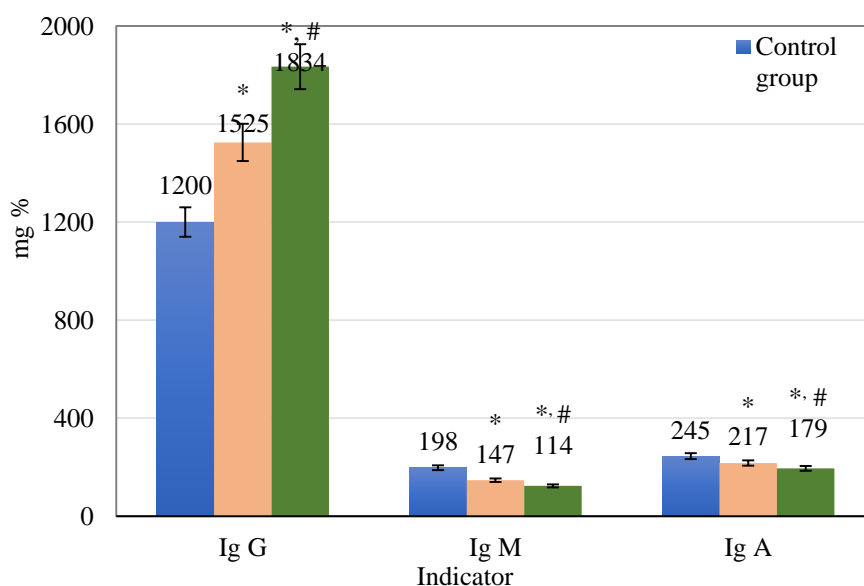


Fig. 3. The content of immunoglobulins in the blood plasma of women with hyperplastic processes in the endometrium and mastopathy

Less pronounced changes were observed in the mean values of IgM and IgA levels between the study groups and the control group (Fig. 3), which apparently indicates a lower role of these markers of immune status in pathogenetic changes in women in the development of proliferative processes in reproductive bodies.

Thus, the study of immune parameters at the systemic level revealed a tendency to reduce the relative content of T lymphocytes, reduce the number of T helpers, T suppressors and B lymphocytes, as well as increase the content of NK cells and class G immunoglobulins in patients with GPE and especially in the presence of benign neoplasms of the mammary glands.

5. Discussion of research results

The development of proliferative processes in the body may be accompanied by immunopathological conditions, which are characterized by a decrease in the

body's overall resistance. In this case, the body becomes sensitive to diffuse hyperplastic processes in the endometrium [11] and sensitive mammary glands [12], resulting in the appearance of focal proliferates in these organs. The state of cellular and humoral immunity is one of the main factors determining the course of EH and benign tumors in the breast.

It was found that the state of T- and B-links of the immune system in women with EH and especially with concomitant mastopathy is characterized by a decrease in the number of T-cells and probably suppression of their functional activity. This increases the level of natural killers. The content of T-effectors (CD8 + cells), which perform the functions of both suppressors and killers, decreases, along with a decrease in the level of T-helpers. These studied cells are able to suppress the activity of cellular and humoral adaptive immunity, while influencing the mechanisms of intersystem interaction [13]. Decrease

in plasma of patients with EH of these cells testifies to inactivation of immunological reactivity of an organism.

The decrease in the ratio of CD4 + to CD8 + cells found by us indicates an imbalance between T-helpers and cytotoxic T-lymphocytes. The decrease in the number of T-helpers will be accompanied by the inefficiency of the cellular and humoral parts of the immune response in EH against the background of hormonal changes in the body. In addition, reducing the number of T-helpers will not promote the activation and maturation of B-lymphocytes, which synthesize antibodies in the body [14].

Decreased immunoregulatory index indicates the second degree of immune disorders under proliferative processes [15] and indicates reciprocal function and recognition within autonomous immunocorrection in the internal reproductive organs of women of reproductive age [16]. These disorders in the body could lead to changes in the endotoxin-antiendotoxin system, as the ratio of different types of leukocytes shows an increase in the leukocyte index of intoxication in women with EH and mastopathy compared to the control group of women. The results show that the decrease in the reactivity of the cellular component of the systemic immunity leads to intoxication of the body, probably by products of impaired metabolism of transformed endometrial cells and mammary glands. The established fact should be considered when choosing the tactics of treatment of women with the studied pathologies.

In general, the results suggest that factors of hormonal regulation of the menstrual cycle and changes in cellular and humoral links of systemic immunity play a significant role in the pathogenesis of EH and benign breast diseases. The phenomenon of metabolic immunosuppression probably occurs due to the adverse effects on lymphocytes of hyperestrogenemia, hyperprolactinemia and hypoprogesteronemia [1, 3]. Decreased stimulating effect on the immune system by thyroid hormones was observed. This makes it possible to re-evaluate the principles of prevention of this disease in women of childbearing age.

Thus, in the violation of reproductive function in women with hormonal imbalance, which leads to the development of EH, involved immunological homeostasis, which is characterized by changes in specific and non-specific immunity at the systemic level. Such changes are exacerbated in women with EH and benign breast disease. Examination of the number of leukocytes and their types, as well as the number of different types of lymphocytes could serve as one of the diagnostic criteria

for early detection of the studied pathologies in women of reproductive age, which is likely to maintain fertility. Given the results obtained, a necessary condition for the implementation of reproductive function is, above all, the correction of the identified changes by optimizing the treatment and prevention complex.

Study limitations. The state of cellular and humoral immune systems in patients with endometrial hyperplasia and benign breast tumors has been studied, but the relationship between these parameters and cytokine levels in patients with various types of endometrial hyperplasia has not been studied.

Prospects for further research. Against the background of changes in cellular and humoral parts of the immune system, the next step will be to study the direction of hormonal changes in different types of endometrial hyperplasia under the conditions of combined pathology of benign breast diseases.

6. Conclusions

1. In women with endometrial hyperplasia and benign neoplasms of the mammary glands, an increase in the total number of leukocytes and eosinophils with a simultaneous decrease in segmental neutrophils and lymphocytes.

2. The revealed imbalance of subpopulation content of T-cells was manifested by a decrease in the average values of T-lymphocytes (CD3 +), T-suppressors (CD8 +), T-helpers (CD4 +) mainly due to a decrease in circulating T-helpers / inducers, which was accompanied by a decrease CD4 + / CD8 + and indicated the formation of secondary immunodeficiency. At the same time, a decrease in B-lymphocytes (CD19 +) with a simultaneous increase in NK cells (CD16 +) in peripheral blood was detected.

3. The study of the humoral part of the immune system revealed an increase in the content of class G immunoglobulins and a slight decrease in the levels of IgM and IgA in the plasma of patients with EH and mastopathy.

Conflict of interests

The author declares there is no conflict of interest.

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