ИММУНОЛОГИЧЕСКИЕ ФАКТОРЫ РАЗВИТИЯ НАРУЖНОГО ГЕНИТАЛЬНОГО ЭНДОМЕТРИОЗА

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Резюме. Наружный генитальный эндометриоз (НГЭ) – одно из распространенных гинекологических заболеваний женщин репродуктивного возраста с рецидивирующим, прогрессирующим течением, ухудшающим качество жизни пациенток из-за болевого синдрома, эмоциональной неуравновешенности, страха рецидива и возможного оперативного вмешательства. В настоящее время эндометриоз признан одним из наиболее распространенных заболеваний, связанных с бесплодием. Так, среди фертильных женщин с сохраненной детородной функцией заболевание в целом диагностируется примерно у 6-7%, тогда как среди пациенток, страдающих бесплодием, его частота может достигать 20-48%.

Однако причины, определяющие репродуктивную дисфункцию у больных с НГЭ, изучены недостаточно. Большое внимание в настоящее время уделяется роли иммунитета в формировании эндометриоза. У больных с НГЭ отмечаются изменения как факторов местного иммунитета, так и иммунологических компонентов циркулирующей крови.

Цель исследования – изучение факторов врожденного и адаптивного иммунитета у пациенток репродуктивного возраста с наружным генитальным эндометриозом (НГЭ).

В исследование была включена 71 пациентка с различными стадиями наружного генитального эндометриоза, в контрольную группу вошли 24 пациентки, без эндометриоза. Определение популяционного состава лимфоцитов периферической крови, уровня моноцитов, экспрессирующих TLR, активационных маркеров, проводили методом лазерной проточной цитометрии – Immunotex (Франция), Caltag (США), FITC (изотиоцинат флуоресцеина) – меченые CD3, CD4, CD8, CD16, CD19, HLA-DR, CD282, CD284 и PE(фикоэритрин) – меченые CD25, CD69, CD95, CD107a, CD14.

Наружный генитальный эндометриоз характеризуется: при I-II стадии заболевания – нарушением ранних этапов врожденного иммунного ответа (повышение количества моноцитов, экспрессирующих TLR-4, нарушением процессов активации и дифференцировки иммунокомпетентных клеток, что отражается в снижении экспрессии CD16, CD8, $CD16^+HLA\text{-}DR^+$, $CD16^+CD107a^+$, $CD8^+CD107a^+$,

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а при III-IV стадии заболевания отмечается снижении уровня CD16 и маркеров активации CD69, HLA-DR, CD107a на их поверхности, что сочетается со снижением экспрессии CD8, CD16, HLADR и CD107a на их поверхности. Повышение $CD16^+CD95^+$ и $CD8^+CD95^+$ выявлено при различных стадиях НГЭ.

Полученные результаты позволяют понять особенности функционирования врожденного и адаптивного иммунитета на различных стадиях наружного генитального эндометриоза, а изученные иммунологические показатели могут быть использованы в качестве диагностических критериев формирования различных стадий НГЭ. Эти данные могут служить теоретической основой для дальнейшей идентификации маркеров прогрессирования НГЭ, а также механизмов, лежащих в основе иммунного воспаления.

Ключевые слова: врожденный иммунитет, адаптивный иммунитет, TLR-рецепторы, цитотоксичность, маркеры активации, наружный генитальный эндометриоз

IMMUNOLOGICAL FACTOR DEVELOPMENT OF EXTERNAL GENITAL ENDOMETRIOSIS

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Abstract. External genital endometriosis (EGE) is one of the common gynecological diseases of women of reproductive age with a relapsing, progressive course that worsens the quality of life of patients due to pain, emotional imbalance, fear of relapse and possible surgical intervention. Currently, endometriosis is recognized as one of the most common diseases associated with infertility. Thus, among fertile women with preserved childbearing function, the disease is generally diagnosed in approximately 6-7%, while among patients suffering from infertility, its frequency can reach 20-48%.

However, the causes that determine reproductive dysfunction in patients with EGE are not well understood. Much attention is currently paid to the role of immunity in the formation of endometriosis. Patients with EGE show changes in both local immunity factors and immunological components of circulating blood.

Purpose of the study: the study of factors of innate and adaptive immunity in patients of reproductive age with external genital endometriosis (EGE).

The study included 71 patients with various stages of external genital endometriosis, the control group included 24 patients without endometriosis. Determination of the population composition of peripheral blood lymphocytes, the level of monocytes expressing TLR, activation markers, was carried out by laser flow cytometry – Immunotex (France), Caltag (USA), FITC (fluorescein isothiocynate) – labeled CD3, CD4, CD8, CD16, CD19, HLA -DR, CD282, CD284 and PE (phycoerythrin) – labeled with CD25, CD69, CD95, CD107a, CD14.

External genital endometriosis is characterized by: at stages I-II of the disease – a violation of the early stages of the innate immune response (an increase in the number of monocytes expressing TLR-4, a violation of the activation and differentiation processes of immunocompetent cells, which is reflected in a decrease in the expression of CD16, CD8, $CD16^+HLA\text{-}DR^+$, $CD16^+CD107a^+$, $CD8^+CD107a^+$, at III-IV stages of the disease, there is a decrease in the level of CD16 and activation markers CD69, HLA-DR, CD107a on their surface, which is combined with a decrease in the expression of CD8, CD16, HLADR and CD107a on their surface. $CD95^+$ and $CD8^+CD95^+$ were found at various stages of EGE.

The results obtained allow us to understand the features of the functioning of innate and adaptive immunity at various stages of external genital endometriosis, and the studied immunological parameters can be used as diagnostic criteria for the formation of various stages of EGE. These data can serve as a theoretical basis for further identification of markers of EGE progression, as well as the mechanisms underlying immune inflammation.

Keywords: innate immunity, adaptive immunity, TLR receptors, cytotoxicity, activation markers, external genital endometriosis

Introduction

External genital endometriosis (EGE) is one of the common gynecological diseases of women of reproductive age with a relapsing, progressive course that worsens the patient's quality of life due to pain, emotional imbalance, fear of relapse and possible surgical intervention. Currently, endometriosis is recognized as one of the most common diseases associated with infertility. Thus, among fertile women with preserved childbearing function, the disease is generally diagnosed in approximately 6-7%, while among patients suffering from infertility, its frequency can reach 20-48% [1].

However, the causes that determine reproductive dysfunction in patients with EGE are not well understood. Much attention is currently paid to the role of immunity in the formation of endometriosis [3, 4]. Patients with EGE show changes in both local immunity factors and immunological components in the circulating blood [2, 5].

To date, it is obvious that immune system disorders play an important role in the development of EGE, however, despite research in this area, there is practically no information on markers of positive and negative activation, cytotoxicity of T lymphocytes, NK cells. Insufficiently studied are the mechanisms of innate immunity, in particular, the level of monocytes expressing TLR-2, TLR-4.

In this regard, **the purpose of our study** was to study the factors of innate and adaptive immunity in patients of reproductive age with external genital endometriosis.

Materials and methods

Under observation there were 71 patients with EGE, who were divided into two groups: group 1 – women with I-II stages of EGE (n = 31); Group 2 – patients with III-IV stages of EGE (n = 40). The control group included 24 patients who were examined in the Department of Operative Gynecology, who underwent therapeutic and diagnostic laparoscopy. In this group of patients, neither EGE, nor acute or chronic inflammatory diseases of the pelvic organs, nor benign neoplasms were detected intraoperatively. Criteria for inclusion of patients in the study: the reproductive age of patients 18-49 years, the presence of complaints of infertility and/or pain syndrome, body mass index 18.5-25 kg/m^2, normal body temperature, laparo- and hysteroscopy. with morphological verification of the diagnosis. The exclusion criteria from the study were: pubertal and perimenopausal age of patients, malignant neoplasms, severe extragenital pathology at the stage of decompensation, acute infectious diseases or exacerbation of their chronic forms. Determination of the population composition of peripheral blood lymphocytes, the level of monocytes expressing Toll receptors (TLR), activation markers was carried out by laser flow cytometry – Immunotex (France), Caltag (USA), FITC (fluoresceinisothiocyanate) – labeled CD3, CD4, CD8, CD16, CD19, HLA-DR, CD282, CD284 and PE (phycoerythrin) labeled with CD25, CD69, CD95, CD107a, CD14. The results were taken into account on a BECKMAN COULTER EPICS XL-II laser flow cytometer (USA). To form a database and conduct a statistical study, the capabilities of the Excel 2003 spreadsheet processor and application packages ("Megastat" and Statistica 6.0) were used. Descriptive statistics was carried out with the determination of the values of the median and interquantile range: Me ($Q_{0.25}$-$Q_{0.75}$). A p value < 0.05 was considered statistically significant.

Results and discussion

Analysis of clinical data in observed women with EGE showed a high percentage of primary infertility – 69%. In patients with stages I-II of the disease – in 67.7% of cases, in patients with stages III-IV – in 70%. Secondary infertility occurs in 31% of patients with EGE, while with minimal forms of the disease – in 32.3%, and with severe forms of endometriosis – in 30%. In women with EGE, chronic inflammatory processes were detected in the same percentage of cases (67.7%), and sexually transmitted infections in patients with stage I-II EGE were detected in 32.2% of women, while in patients with EGE III-IV stages are 2 times less.

Our work showed that the formation of external genital endometriosis was accompanied by significant changes in immunity parameters. When studying the parameters of the immune status in the 1st and 2nd groups, compared with the control group, a statistically significant decrease in CD8 was revealed, the most pronounced deviations were noted in the 2nd group, the indicators were 1.7 times lower than in the control group ($p < 0.05$).

According to current data, NK cells are divided into two types: typical CD56bright CD16-NK cells producing cytokines, and CD56dimCD16$^+$ NK cells characterized by high cytotoxicity. Analyzing the indicators of the content of natural killers (CD16$^+$) in the 1st and 2nd groups, a statistically significant decrease in their relative number was noted compared to the control group (by 2.9 and 1.3 times, ($p < 0.05$); indicators in the 2nd group were lower than in the

1st ($p < 0.05$). The role of B lymphocytes in the development of endometriosis and their relationship with clinical symptoms and disease severity are not well understood. In the 2nd group, the CD19 values were higher than in the control group ($p < 0.05$), which indicates the activation of the B cell link of immunity.

When analyzing the functional state of immunocompetent cells, it was found that the level of $CD16^+CD69^+$ in group 2 was lower than the values in the control group and in group 1 by 2.0 times ($p < 0.05$). The content of $CD8^+CD25^+$ in the 2nd group was statistically significantly lower than in the control group ($p < 0.05$). When studying the expression of markers of late activation, a decrease in $CD16^+HLADR^+$ was revealed at various stages of EGE, the most pronounced deviations were noted in the 2nd group, the indicators were 2 times lower control ($p < 0.05$). The study of the content of $CD8^+$, expressing HLA-DR, showed their decrease in the 2nd group compared with the control group and the 1st group (1.7 and 1.89 times ($p < 0.05$).

$CD107a^+$ is a marker for degranulation of $CD8^+$ and $CD16^+$ lymphocytes.

The study of the number of CD16 lymphocytes expressing the CD107a molecule involved in immune inflammation through the mechanism of cellular cytotoxicity revealed that in the 2nd group the content of $CD16^+CD107a^+$ was 2.6 times lower than in the control group ($p < 0.05$), and 1 – 1.8 times ($p < 0.05$). Similar data were found for the content of $CD8^+CD107a^+$, the indicators in the 2nd group were lower than the control level and group 1 by 1.7 and 1.1 times, respectively, ($p < 0.05$), which indicates a decrease in functional activity natural killer cells and cytotoxic T lymphocytes. The study of the content of cytotoxic lymphocytes and NK cells expressing the Fas receptor $CD95^+$ revealed an increase in $CD16^+CD95^+$ at various stages of ege. Similar data were found for $CD8^+CD95^+$, their level exceeded the control values in the 1st group by 1.2 times, in the 2nd – by 1.46 times ($p < 0.05$).

It is known that the proliferation of endometrioid lesions is regulated by the innate immune system. Important components of the innate immune system are Toll-like receptors (TLRs), which are type 1 transmembrane proteins with an N-terminal extracellular domain. TLRs can be activated by endogenous ligands, including lipopolysaccharide (LPS), heat shock protein, S100, fibronectin, fatty acids, neutrophil elastase, etc. In our study, groups 1 and 2 showed a statistically significant increase in the number of peripheral blood monocytes expressing TLR-4 ($CD14^+CD284^+$), compared with the control group. The most significant deviations were noted in the 2nd group.

Thus, external genital endometriosis is characterized by: at stages I-II of the disease, a violation of the early stages of the innate immune response (an increase in the number of monocytes expressing TLR-4, a violation of the activation processes of immunocompetent cells, which is reflected in a decrease in the expression of CD16, CD8, $CD16^+HLA\text{-}DR^+$, $CD16^+CD107a^+$, $CD8^+CD107a^+$, at III-IV stages of the disease, there is a decrease in the level of CD16 and activation markers CD69, HLA-DR, CD107a on their surface, which is combined with a decrease in the expression of CD8 and CD16 lymphocytes, $HLADR^+$, CD107a on their surface. An increase in $CD16^+CD95^+$ and $CD8^+CD95^+$ was found at various stages of EGE.

Aberrant immune responses of cytotoxic T lymphocytes and NK cells are trigger factors for the development of endometriosis. Violation of the work of innate immunity mediated by natural killers leads to disruption of the functioning of adaptive immunity, the development and progression of endometriosis and infertility. Immune dysfunction in endometriosis is probably formed both due to a decrease in the number of cytotoxic T lymphocytes and NK cells, and due to a functional defect [7]. The decrease in cytotoxicity mediated by T lymphocytes and NK cells in women with EGE may also be associated with the induction of apoptosis along the Fas-FasL pathway and contributes to the formation of endometrial heterotopias.

The revealed increase in the content of $CD14^+CD284^+$ in women with EGE suggests that TLR-4-dependent signaling can lead to an increase in the synthesis of pro-inflammatory cytokines and chemokines, stimulates the proliferation of endometrial cells, activates macrophages, DC, natural killer cells, forming an inflammatory microenvironment, contributing to the progression EGE.

Conclusion

The results obtained allow us to understand the features of the functioning of innate and adaptive immunity at various stages of external genital endometriosis, and the studied immunological parameters can be used as diagnostic criteria for the formation of various stages of EGE. These data may provide a theoretical basis for further identification of markers of EGE progression as well as underlying immune inflammation. Further research is needed to explore the role of cytotoxic T lymphocytes, NK cells, and TLR signaling pathways in the pathogenesis and molecular mechanisms of EGE.

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