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EVALUATION OF CYTOKINE PRODUCTION IN PATIENTS WITH INFERTILITY AT THE STAGE OF PREPARING FOR EXTRACORPORAL FERTILIZATION

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Being one of the relevant areas of modern biology and medicine, the study of the immune system in the implementation of the human reproductive function is important and there is no complete picture reflecting changes in the concentrations of the most known cytokines in blood serum. The study has involved 40 women with infertility and 20 of them with a physiological menstrual cycle examination. The concentration of cytokines in the blood serum of the women has been determined by the immunoenzyme method using commercial BioSOURCE kits. Analysis of the dynamics of changes in IFNγ has revealed that its average level in the group of patients with infertility has been 7.07±1.01 pg/ml, and significantly exceeded the values in the control group by 1.8 times. High concentrations of IFNγ are determined in many complications of the physiological course of pregnancy. It also acts as a mediator of pathological immunoinflammatory processes in various human diseases. It has been found that in women with infertility, the TNF-α level has been 32.82±9.14 pg/ml, which has significantly exceeded the values in the control group by 6.7 times. The significant predominance of proinflammatory cytokines in pregnants' serum is an unfavorable prognostic sign. And further studies of cytokine production open up prospects for substantiating and increasing the effectiveness of assisted reproductive technologies.

Key words: reproductive function, pregnancy, interleukin-2, tumour necrosis factor-α, interferon-γ

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ОЦІНКА ПРОДУКЦІЇ ЦИТОКІНІВ У ПАЦІЄНТОК З БЕЗПЛІДДЯМ НА ЕТАПІ ПІДГОТОВКИ ДО ЕКСТРАКОРПОРАЛЬНОГО ЗАПЛІДНЕННЯ

Вивчення ролі імунної системи у здійсненні репродуктивної функції людини є одним із актуальних напрямків сучасної біології та медицини. Мета роботи: оцінити продукцію цитокінів у пацієнток із безпліддям на етапі підготовки до екстракорпорального запліднення. У дослідженні взяли участь 40 жінок з безпліддям (основна група), які звернулися до клініки для проведення процедури ЕКЗ та 20 практично здорових жінок з фізіологічним менструальним циклом (контрольна група). Концентрацію цитокінів (IL-2, TNFα та IFNγ) у сироватках крові жінок досліджуваних груп визначали імуноферментним методом за допомогою комерційних наборів BioSOURCE (Бельгія) відповідно до протоколів, що додаються. Аналіз динаміки зміни IFNγ виявив, що його середній рівень в основній групі становив 7,07±1,01 пг/що у 1,8 рази вище, ніж у контролі (p=0,01). Встановлено, що у жінок з безпліддям рівень TNF-α склав 32,82±9,14 пг/мл, що у 6,7 разу вище, ніж у контрольній групі (p=0,003). Було проведено оцінку продукції цитокінів у пацієнток із безпліддям на етапі підготовки до ЕКЗ. У пацієнток основної групи виявлено достовірне підвищення концентрації всіх вивчених цитокінів периферичної крові. Виявлені зміни цитокінового профілю у пацієнток із безпліддям можуть бути причиною невдач при проведенні ЕКЗ. Значна перевага у сироватці крові вагітних прозапальних цитокінів є несприятливою прогностичною ознакою. Подальші дослідження продукції цитокінів відкривають перспективи для обґрунтування нових підходів до прогнозу та підвищення ефективності допоміжних репродуктивних технологій.

Ключові слова: репродуктивна функція, інтерлейкін-2, фактор некрозу пухлин-α, інтерферон-γ

A review of the role of the immune system in the execution of the human reproductive function is one of the current trends in modern biology and medicine. Moreover, the immune mechanisms affecting different stages of this process are not well understood. Understanding the interaction of the immune and reproductive systems is necessary to uncover the causes of impaired fertility and develop treatments for male and female infertility [1, 2].

Immunological factors in the structure of female infertility occupy about 3 % [8]. Immunological factors considered to lead to violations of the reproductive process at its various stages: folliculogenesis, ovulation, and implantation. Immune disorders may be involved in the etiology and pathogenesis of various forms of infertility [3, 8].

According to modern concepts, the stages of egg maturation, implantation and development of the embryo are cytokine-dependent processes and are controlled by the immune system. Currently, the cells of the immune system and the cytokines produced by them are given special attention, since the normal course of the gestational period largely depends on the effectiveness of the immune mechanisms in maintaining tolerance to fetal alloantigens [4, 12].

Interest in the issues of cytokine regulation at the initial stages of the reproductive process is not purely theoretical and is largely associated with clinical requests. The effectiveness of in vitro fertilization (IVF) currently does not exceed 35 %, and about a third of all pregnancies are terminated in the early stages.

Therefore, the study of the level of cytokines is of undoubted interest in terms of substantiating new approaches to the prognosis of implantation and the successful course of pregnancy during the IVF procedure [3, 7].

In spite of complete and intensive research in the field of reproduction immunology, information about the importance of various cytokines in the reproductive function of women is ambiguous to date [6, 11]. At present, there is no complete picture reflecting changes in the concentrations of the most known cytokines (such as interferon gamma – IFNγ, interleukin-2 – IL-2, and tumor necrosis factor α (TNFα)) in blood serum in case of infertility and their possible role in the outcome of IVF programs [12].

The purpose of the study was to access the content of cytokines IL-2, TNFα, and IFNγ in the blood serum of women with infertility in the program of in vitro fertilization.

Material and methods. 40 women with infertility (the main group) who applied to the Baku Central Clinical Hospital (the Republic of Azerbaijan) for an IVF procedure have taken part in the research. According to inclusion criteria, the formation of a group of examined women has been carried out. The main inclusion criterion was considered a verified diagnosis of infertility in the observed patients.20 practically healthy women with a physiological menstrual cycle have been examined, their serum has been used as a control group. All of them have been married and had a history of labour ending in the birth of a healthy child. Clinically examined patients have included acquisition of medical history, initial examination, and special gynecological examination. Immunological examination of patients has included determination of the level of cytokines in blood serum. Concentration of cytokines (IL-2, TNFα, and IFNγ) in the blood serum of women of the main and control groups has been determined by immunoenzyme method using commercial BioSOURCE kits (Belgium) according to the protocols attached to them. Serum IL-2 level has been studied in 34 patients, TNFα, and IFNγ in 36 patients with infertility. In the control group, the level of all serum cytokines has been determined in 20 women.

Taken blood sampling for laboratory testing has been performed due to a standard technique: peripheral blood obtained by puncture of ulnar vein has been incubated for 30-40 minutes at room temperature after which the blood serum has been separated. The allotted serum samples have been frozen and stored in Eppendorf-type plastic tubes until testing at a temperature of -20°C before analysis, for no more than 6 months.

Sensitivity of determination during measurement has been estimated at the minimum reliably determined concentration calculated on the basis of the arithmetic mean of ten measurements of the optical density of the calibration sample from the test system. Cytokine values in both standard calibration dilutions and in test samples have been well reproducible.

Mathematical processing of the results has been carried out by methods of variation statistics in the statistical analysis system STATISTICA 10 (STATISTICA USA software package, Version 10 for Windows 8). In all samples, the nature of distribution to normality has been determined by the criteria of Kolmogorov-Smirnov, Shapiro-Wilkie, and Leven.The Mann-Whitney method has been used in distribution of indicators other than normal. Their arithmetic mean values (M) and their standard errors (m) have been determinedto characterize the group of homogeneous units. In order to compare the data between the groups obtained during the study, methods have been used to assess the differences between two independent samples - the Student's t-criterion and Fisher's exact method. As a result differences have been considered significant at $p<0.05$.

Results of the study and their discussion. The study permitted to assess concentration of these cytokines in the blood serum of patients with infertility. Table 1 shows the results of determining the content of proinflammatory cytokines IL-2, TNFα, and IFNγ in peripheral blood in the selected clinical groups. The activity of these cytokines in blood serum has been significantly different from the corresponding indicators of the control group. The following patterns have been revealed as a result of the research.

Interleukin-2 (IL-2) induces T cell immunity, enhances secretion of IFNγ by T-lymphocytes. Definition of IL-2 is the best indicator of T cells activation. Analysis of IL-2 concentration in blood serum has showed that initially its average content in women with infertility has been 56.34±7.85 pg/m1 (fluctuation limit of 16.8-200.0 pg/m1), which has significantly exceeded the value in the control group 1.6 times (36.1±5.89 pg/m1, p=0.04) (Table 1).

Table 1

Serum cytokines in infertility patients

Observation groups	Indices	Cytokine concentration, pg/ml		
		IL-2	TNFα	IFNγ
Patients with infertility	M±m	56.34±7.85	32.82±9.14	7.07±1.01
	min – max	16.8–200.0	9.6–282.0	3.0–21.4
p		p=0.04	p=0.003	p=0.01
Control	M±m	36.1±5.89	4.91±0.66	3.86±0.73
	min – max	16.1–93.6	1.03–9.7	1.3–13.1

Tumor necrotizing factor alpha (TNF-α) is one of the main proinflammatory cytokines. On the one hand, it plays an important role in regulation of normal differentiation, growth and metabolism of different cells, and on the other hand, it acts as a mediator of pathological immunoinflammatory processes in various human diseases. It has been found that in women with infertility, the TNF-α level has been 32.82±9.14 pg/ml (fluctuation range of 9.6–282.0 pg/ml), which has significantly exceeded the values in the control group by 6.7 times (4.91±0.66 pg/ml, p=0.003).

In the patients of the main group, a significant increase in concentration of interferon-gamma (IFNγ), the most important endogenous immunostimulant necessary for development of a specific immune response, has been detected, the increase in the synthesis of which is associated with activation of Th1 pathway of the immune response and development of inflammation. Analysis of the dynamics of changes in IFNγ has revealed that its average level in the group of patients with infertility has been 7.07±1.01 pg/ml (fluctuation range of 3.0-21.4 pg/ml), which has significantly exceeded the values in the control group by 1.8 times (3.86±0.73 pg/ml, p=0.01). High concentrations of IFNγ are determined in many complications of the physiological course of pregnancy, which is also considered as an unfavourable factor for the processes of conception and gestation.

In the patients with infertility can be the cause of failures during IVF, identified changes in the cytokine profile. In general, it should be stated that a group of women with infertility included in the IVF program has been characterized by a wide variability of individual serum cytokine values.

Immunocompetent cells and the regulatory molecules they produce play an important role in the functioning of various organs and tissues, including the reproductive system. Our results suggest that infertility is initially characterized by increased immunoreactivity. However, the type of immune response may be different. Similar studies to determine the factors affecting the effectiveness of ART procedures were carried out by Motovilova N.O. et al., who studied the effect of certain cytokines on the effectiveness of infertility treatment using in vitro fertilization [3]. The authors found a correlation between the efficiency of IVF and the content of cytokine IL1b-13, however, unlike our study, the determination of cytokines was carried out not in the blood serum, but in the follicular fluid, which, in our opinion, somewhat complicates the diagnostic procedure in comparison with a more accessible blood test.

Closer to our results were the data of Osakue N et.al., who, when studying the content of cytokines in the blood of women with infertility who applied to clinics for the conduct of the IVF procedure, found a significant increase in the level of TNF-α and IFN-γ in the second trimester of pregnancy compared to the first trimester of pregnancy. However, this did not take into account the level of IL-2, as in our study, which is certainly important for predicting inflammatory processes, especially in the pelvic organs, which may affect the course of pregnancy and IVF results [10].

The results of the study indicate that women with infertility have hyperproduction of pro-inflammatory cytokines in the blood serum. Thus, according to the results of our studies, we recorded the level of all studied cytokines in the blood serum (in the absence of null results) in the group of women with infertility, which significantly differed from healthy individuals.

According to the results of the analyzes, a sharp increase in the levels of pro-inflammatory cytokines IL-2, TNF-α and IFNγ in the blood serum of patients with infertility in relation to the control group of healthy women may indicate the activation of the macrophage link of the immune system, since the release of immunoregulatory factors characterizes the functional state of mononuclear phagocytes. Studies of pro-inflammatory cytokines prior to our study were generally fragmented; did not include a cumulative assessment of outcomes. Thus, Alijotas-Reig J et al. paid attention to TNF- and its content in the blood of women, suggesting that a high level of this cytokine in combination with IVF failure may

be a predictor of an autoimmune process that is hidden. The authors even noted the possible efficacy of TNF-blockers as targeted therapy in the treatment of women with obstetric complications associated with TNF-α hyperproduction, such as miscarriage, early and severe preeclampsia, and recurrent failed implantation syndrome, all "idiopathic" cases [5]. However, other cytokines were not evaluated in this study, and the potential risk of embryo-fetal toxicity of these drugs during pregnancy should not be forgotten.

Against the background of resistance to programs of assisted reproductive technologies, violations of the cytokine profile of blood serum in women with infertility indicate the participation of the immune link in the formation of reproductive dysfunction. This confirms the data of other studies, and indicates the need to determine the cytokine profile at the stages preceding implantation in order to timely detect immuno-inflammatory disorders that may affect the outcome of IVF [9, 11, 12]. Because a considerable predominance of proinflammatory cytokines in the blood serum of pregnants is an unfavourable prognostic sign, the study of the immunopathogenetic mechanisms of infertility open up prospects for optimizing approaches to the management of patients.

Conclusion

1. The average level of IFNγ in the group of patients with infertility was 7.07±1.01 pg/ml, and significantly exceeded the values in the control group by 1.8 times.

2. The TNF-α level in women with infertility was 32.82±9.14 pg/ml, which has significantly exceeded the values in the control group by 6.7 times. Thus, further studies of cytokine production open up prospects for substantiating new approaches to forecasting and increasing the effectiveness of assisted reproductive techniques.

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