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CHANGES IN CELL-MEDIATED AND HUMORAL IMMUNITY INDICES AND LEVELS OF PRO- AND ANTI-INFLAMMATORY CYTOKINES IN CERVICAL MUCUS IN PREGNANT WOMEN OF HIGH RISK FOR PREECLAMPSIA

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62 pregnant women were examined, of which 30 were healthy pregnant ones (control group) and 32 women at high risk of preeclampsia (main group – MG) at 18–20 + 6 weeks and 28–32 weeks of pregnancy. The content of pro- and anti-inflammatory cytokines (TNFα, INFγ, IL10) in cervical mucus and indices of cellular-humoral immunity in the blood, including the concentration of circulating immune complexes (CIC) in the serum were studied. The analysis of the subpopulation composition of T - cells revealed a decrease in the concentration of T-helpers (CD4+) with a significant increase in the concentration of T-suppressors/killers (CD8+) with a corresponding decrease in the immunoregulatory index. A significant decrease in the number of B cells (CD22+) was registered. An increase in CIC in the main group of subjects was demonstrated, and an increase in CIC levels above 100 IU/ml was closely associated with the development of preeclampsia. IgM in women with preeclampsia was significantly elevated, which may indicate the presence of trophoblastic stimulation of the immune system in these women. IgG levels were significantly reduced. In women with preeclampsia, a significant predominance of pro-inflammatory cytokines has been found in anti-inflammatory cytokine deficiency.

Key words: preeclampsia, cell-mediated and humoral immunity, cytokines.

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ЗМІНИ ПОКАЗНИКІВ КЛІТИННО-ГУМОРАЛЬНОГО ІМУНІТЕТУ ТА РІВЕНЬ ПРО- І ПРОТИЗАПАЛЬНИХ ЦИТОКІНІВ У ЦЕРВІКАЛЬНОМУ СЛИЗУ У ВАГІТНИХ ГРУПИ ВИСОКОГО РИЗИКУ ВИНИКНЕННЯ ПРЕЕКЛАМПСІЇ

Були обстежені 62 вагітні, з яких 30 – здорові вагітні (контрольна група) та 32 жінки групи високого ризику по виникненню прееклампсії (основна група – ОГ) в терміні 18–20^{+6} тижнів та 28–32 тижні вагітності. Вивчали вміст про- та протизапальних цитокінів (TNFα, INFγ, IL10) у цервікальному слизу і показники клітинно – гуморального імунітету в крові, включаючи концентрацію циркулюючих імунних комплексів (ЦІК) в сироватці крові. При аналізі субпопуляційного складу Т – клітин виявлено зниження концентрації Т – хелперів (CD4+) при достовірному підвищенні концентрації Т – супресорів/кілерів (CD8+) із відповідним зменшенням показника імунорегуляторного індексу. Зареєстроване достовірне падіння кількості В-клітин (CD22+). Продемонстровано збільшення ЦІК в основній групі обстежених, причому зростання рівня ЦІК вище 100 од/мл мало тісний зв'язок з розвитком прееклампсії. Показник IgM у жінок з прееклампсією був достовірно підвищений, що може вказувати на наявність трофобластичної стимуляції імунної системи цих жінок. Рівні ж IgG були достовірно знижені. У жінок з прееклампсією знайдено значне домінування прозапальних цитокінів при дефіциті протизапальних цитокінів.

Ключові слова: прееклампсія, клітинно – гуморальний імунітет, цитокіни.

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Preeclampsia is a complex neurohumoral pathophysiological process, which is manifested by various disorders in the central and peripheral nervous, cardiovascular and endocrine systems, as well as metabolic disorders, immune response and the emergence of multiple organ and system failure [2, 8–9].

The immunological theory of the preeclampsia connects the occurrence of this pathology with a violation of implantation of the fertilized egg. During normal pregnancy, trophoblasts interact with the decidual membrane and with uterine natural killer (NK) cells, changing their cytokine profile, regulating the adhesion of molecules and matrix metalloproteinases [1, 3, 13]. Cytokines interact with VEGF-R1 (Flt-1) receptors on the surface of monocytes, enhancing the expression of integrins (CD11a, CD11b, CD18, CD51), mRNA, TNFα, IL1β, MCP1, IL8, MIP1β and stimulate the process of their transendothelial migration (TEM) [5, 10, 12]. The level of monocyte production of proinflammatory cytokines Th1 is reduced in the peripheral blood of pregnant women, which is caused by inhibition of NF-κB and increased production of Th2 cytokines [3, 5, 7].

It is believed that the onset of preeclampsia is a process similar to allograft rejection [4, 6]. The immune response switching hypothesis, which is the leading concept for changing regulatory mechanisms during pregnancy, is explained by the indirect effect of T-helpers type 1 and type 2 (Th_1, Th_2) on immune response [7, 11].

There is currently no single mechanism for explaining the complex pathogenesis of preeclampsia, there is no reliable marker or predictor of this disease. Therefore, there is an urgent need for further study, in particular, of clinical and immunological mechanisms of preeclampsia.

The purpose of the study was to determine the features of cell-mediated and humoral immunity and levels of pro- and anti-inflammatory cytokines in cervical mucus in pregnant women at high risk of preeclampsia.

Materials and methods. 62 pregnant women were examined. The selection of patients for the study was carried out at 18–20^{+6} weeks of pregnancy among women with a history of risk factors for preeclampsia during the second ultrasound screening by detecting a decrease in blood flow in the spiral arteries of the uterus in the placental area by Doppler method. In the future, a second examination of pregnant women at 28–32 weeks of pregnancy was performed in dynamics. The main group (MG) consisted of 32 pregnant women who refused our proposed secondary prevention of preeclampsia; in this group, 14 women (43.8 %) showed preeclampsia. Control group (CG) – 30 healthy pregnant women with no risk factors for preeclampsia, intact blood flow in the spiral arteries of the uterus in the placental area, in which no infection was detected in the cervical canal.

The content of pro- and anti-inflammatory cytokines (TNFα, INFγ, IL10) in cervical mucus and indices of cell-mediated and humoral immunity in the blood serum were determined in the Immunological Department of the Clinical and Diagnostic Laboratory of the ME "M.V. Sklifosovsky Poltava Regional Clinical Hospital".

Cervical mucus was collected with a sterile brush during examination of the cervix in mirrors, dissolved in a buffer solution and stored frozen at -40° C until the time of the study. Cytokine concentrations were determined by enzyme-linked immunosorbent assay using appropriate standard commercial reagent kits from the company "Vector BEST" (Russian Federation) according to the instructions of the manufacturer.

Immunological parameters characterizing the functional state of cellular and humoral parts of immunity and phagocytic cells were also studied. Phenotyping of peripheral blood lymphocytes to determine cellular immunity was performed by indirect immunofluorescence after their specific binding to monoclonal antibodies (MCAB) to surface antigens – clusters of differentiation. We used commercial erythrocyte diagnostic preparations of antibodies of CD3+, CD4+, CD8+, CD16+, CD22+ classes certified in Ukraine by LLC RPL "Granum" (Kharkiv, Ukraine). At the same time, MCABs of the CD3+ class were considered to characterize the total population of T-lymphocytes, CD4+ – the population of T-helpers/inducers, CD8+ – T-suppressors/killers, and CD22+ – B cells. The CD4/CD8 immunoregulatory index was calculated, which was interpreted as the ratio of lymphocytes with helper and suppressor activity. The functional activity of T-lymphocytes was studied using the blast transformation reaction of lymphocytes (BTRL) using PHA as a nonspecific mitogen [5, 7, 13].

The level of the main classes of serum Ig was determined using a test system based on the principle of competitive enzyme-linked immunosorbent assay with immobilized antigens (Ig A, M and G), introducing test samples and conjugates (anti-Ig A, anti-Ig M, anti-Ig G labeled with peroxidase) [5].

The concentration of circulating immune complexes (CICs) in blood serum was studied by immune turbidimetric assay (a kit of CIC-Xema reagents). It was considered that the severity of the immunotoxicity syndrome can be judged by the level of CICs, and the fact that CICs are eliminated relatively quickly due to their phagocytosis by cells of the mononuclear phagocytic system was also taken into account [5].

Statistical processing of the obtained results was performed by the method of variation statistics for unrelated observations using the software package Statistica 6.0. Distribution of almost all variation series was subject to the criteria of normality, so Student's t-test was used to determine the significance of differences that were considered significant at $p<0.05$.

The study was guided by the Rules of Humane Treatment of Patients in accordance with the requirements of the Tokyo Declaration of the World Medical Association, International Recommendations of Declaration of Helsinki on Human Rights, the Council of Europe Convention on Human Rights and Biomedicine, Laws of Ukraine, orders of the Ministry of Health of Ukraine and the requirements of the Code of Ethics of Physicians of Ukraine. All patients agreed in writing to participate in the study, which was approved by the Commission on Ethical Issues and Biomedical Ethics of the Poltava State Medical University.

Results of the study and their discussion. The study of cell-mediated and humoral immunity and levels of pro- and anti-inflammatory cytokines in cervical mucus in high-risk pregnant women with preeclampsia was performed at 18–20^{+6} weeks of pregnancy (with the formation of the main group of

women) and at 28–32 weeks of pregnancy. However, at 18–20^{+6} weeks of pregnancy, the main studied immune parameters did not differ significantly from those in the control group. Therefore, we present the results of the examination at 28-32 weeks of pregnancy, when changes in the indices of cell-mediated and humoral immunity and levels of pro- and anti-inflammatory cytokines in cervical mucus in women MG-I, who refused our proposed treatment, which is intended for secondary prevention of preeclampsia.

Indices of the number of white blood cells in MG-I (in general) and in MG-I (in women with preeclampsia) did not differ from the group of healthy women. The absolute number of lymphocytes in both divisions of MG-I fluctuated slightly around the rate in healthy pregnant women (CG). The absolute number of T-lymphocytes ($CD3^+$) tended to decrease.

When analyzing the subpopulation composition of T-cells, a decrease in the concentration of T-helper cells – CD4+ was revealed, both in MG–I (in general) – 0.50±0.03 per 10^9/L ($p>0.05$) and in MG−I (women with preeclampsia) – 0.40±0.02 per 10^9/L ($p<0.001$) (in the control group – 0.58±0.03 per 10^9/L).

The level of T-suppressors/killers – CD8+ in MG-I gradually increased, reaching the reliability in MG-I (women with preeclampsia) – 0.48±0.05 per 10^9/L, in CG – 0.37±0.02 per 10^9/L ($p<0.05$). Accordingly, the immunoregulatory index (CD4+/CD8+) significantly decreased in women with MG-I to 0.83±0.07 in pregnant women with preeclampsia against 1.56±0.10 in healthy pregnant women ($p<0.001$).

There was a moderate decrease in the absolute amount of CD22+ in women with MG-I in general (0.60±0.02 per 10^9/L; $p<0.05$) and in the presence of preeclampsia (0.57±0.04 per 10^9/L; $p<0.05$) compared with the control one (0.68±0.03 per 10^9/L).

Thus, changes in cellular immunity in women MG-I, who refused our proposed preventive treatment, and in which in 43.8 % of cases there was preeclampsia, manifested by its suppression in the form of:

1. Trends towards a decrease in the absolute number of T-lymphocytes (CD3+) and B cells (CD22+);
2. A decrease in the concentration of T-helpers (CD4 +) with a significant increase in the concentration of T-suppressors/killers (CD8+) with a corresponding decrease in the immunoregulatory index.

The peculiarities of humoral immunity were also studied, namely the content of class A, M and G immunoglobulins in the blood serum in the studied groups. The concentration of IgA, tending to decrease in MG-I (1.89±0.04 g/L vs. 2.08±0.11 g/L in CG; $p>0.1$), did not reach the level of reliability. The IgM index in MG-I (in women with preeclampsia) was significantly increased (1.66±0.03 g/L versus 1.49±0.06 g/L in the control group; $p<0.02$). Increased IgM levels in uninfected women with preeclampsia may indicate the presence of trophoblastic stimulation of the immune system of these women [3, 5, 13].

IgG levels were significantly reduced both in MG-I in general (11.06±0.3 g/L at a rate of 13.97±0.4 g/L; $p<0.001$) and in pregnant women with preeclampsia (9.28±0.4 g/L; $p<0.001$). Our correlation analysis showed a significant relationship between a decrease in the level of B-lymphocytes (CD22+) and a decrease in the concentration of IgG in both MG-I in general ($r=0.47$; $p<0.05$) and in women in this group, in whom preeclampsia occurred ($r=0.58$; $p<0.01$).

Given the fact that in healthy pregnant women the level of IgG in the serum increases, which is associated with increased synthesis of this immunoglobulin to neutralize the trophoblast specific antigen, a significant drop in IgG concentration in women with preeclampsia is probably caused by depletion of the reactivity of the humoral part of the general immunity in this disease [5, 13].

Our study demonstrated an increase in circulating immune complexes in MG-I (95.5±4.6 U/mL at a rate of 84.1±3.5 U/mL; $p<0.05$), especially in pregnant women with preeclampsia (103.7±5.1 U/mL). An increase in CICs levels above 100 U/mL was closely associated with the development of preeclampsia ($r=0.76$, $p<0.05$).

Studying the dynamics of changes in proinflammatory and anti-inflammatory cytokines in physiological pregnancy and pregnancy complicated by preeclampsia (Table 1), it was found that pregnant women at high risk of preeclampsia (MG-I) showed a significant increase in levels of proinflammatory cytokines INFγ and TNFα. Thus, the concentration of INFγ in cervical mucus in MG-I was 13.06±0.70 pg/ml versus 10.42±0.51 pg/mL in CG ($p<0.01$), and the TNFα content in MG-I was 7.05±0.54 pg/mL with a value in CG of 3.40±0.24 pg/ml ($p<0.001$). And in women of MG-I, who developed preeclampsia, the level of pro-inflammatory cytokines significantly increased not only in relation to women in the control group, but also in relation to pregnant women of MG-I in general (INFγ was 15.80±0.85 pg/ml TNFα – 8.79±0.48 pg/ml).

Table 1

The level of pro- and anti-inflammatory cytokines in cervical mucus in pregnant women who refused the proposed treatment (MG-I), at 28–34 weeks of pregnancy

Pro- and anti-inflammatory cytokine levels	Groups of women		
	Control group n=27	Main group-I (in general) n=26	Main group-I (women with preeclampsia) n=14
INFγ, pg/mL	10.42±0.51	13.06±0.70 $P_1<0.01$	15.80±0.85 $P_1<0.001$ $P_2<0.02$
TNFα, pg/mL	3.40±0.24	7.05±0.54 $P_1<0.001$	8.79±0.48 $P_1<0.001$ $P_2<0.05$
IL10, pg/mL	9.56±0.63	6.14±0.19 $P_1<0.001$	4.90±0.25 $P_1P_2<0.001$
TNFα/IL10	0.36±0.02	1.15±0.06 $P_1<0.001$	1.80±0.09 $P_1P_2<0.001$
INFγ/IL10	1.09±0.10	2.13±0.14 $P_1<0.001$	3.22±0.12 $P_1P_2<0.001$

In contrast to the increase in pro-inflammatory cytokine levels in the examined pregnant women, the concentration of anti-inflammatory cytokine IL-10 significantly decreased, reaching 4.90±0.25 pg/mL in women with preeclampsia (against 9.56±0.63 pg/mL in the control group and 6.14±0.19 pg/mL in MG-I in general; $p_1p_2<0.001$). The immunosuppression phenomena demonstrated in our study in pregnant women with preeclampsia probably contribute to the inability of trophoblasts to perform a full-fledged invasion of the spiral arteries of the uterus with the subsequent development of endothelial dysfunction.

According to the opposite changes in the content of pro- and anti-inflammatory cytokines, the TNFα/IL10 ratio progressively increased from 0.36±0.02 in CG to 1.15±0.06 in MG-I in general ($p<0.001$) and up to 1.80±0.09 in pregnant women of MG-I, with preeclampsia ($p_1p_2<0.001$). The ratio of pro-inflammatory and anti-inflammatory cytokines increased 3.2-fold in MG-I in general and 5-fold in MG-I women with preeclampsia.

The INFγ/IL10 ratio also increased significantly from 1.09±0.10 in CG to 2.13±0.14 in MG-I (in general) and 3.22±0.12 in MG-I (women with preeclampsia) ($p_1p_2<0.001$), which indicates a likely activation of cellular aggression in pregnant women with preeclampsia who refused preventive treatment.

That is, our studies of the content of pro- and anti-inflammatory cytokines in cervical mucus have shown that women with preeclampsia are characterized by a significant predominance of pro-inflammatory cytokines.

Preeclampsia is a multisystem disorder based on a cascade of immunopathological events originating from the placenta [2, 8, 10, 13]. There is no single mechanism to explain the complex pathogenesis of preeclampsia. There is currently no reliable marker or predictor of preeclampsia.

During normal pregnancy, trophoblasts interact with the decidual membrane and with unique uterine NK cells, modifying their cytokine spectrum, regulating the adhesion of molecules and matrix metalloproteinases [4–6, 10]. Several proinflammatory (Tumour Necrosis Factor alpha, gamma-interferon) and anti-inflammatory (interleukins 4 and 10) cytokines produced by the maternal-fetal interface affect trophoblast invasion [5]. The inability of trophoblasts to perform a full invasion can be a critical factor for the onset of preeclampsia [4, 8]. Existing hypotheses about the etiology of preeclampsia are focused on maladaptation of immune responses and defective introduction of trophoblasts.

And it is the advantage of immunosuppression or immunological aggression during pregnancy, as well as the depth of these immune imbalances are key in pregnancy and the development of complications, including preeclampsia [5, 8]. Therefore, this question requires, in our opinion, further in-depth research to better understand all the pathological processes that may occur in a pregnant women.

The presented results of the study of cell-mediated and humoral immunity showed that in pregnant women of MG-I, who did not receive our proposed therapeutic and prophylactic complex of drugs (and in which almost half of the cases manifested preeclampsia), deepened immunosuppression inherent in pregnancy in general [1, 3, 5, 7–8]. Of particular importance in the development of preeclampsia is a decrease in the production of anti-inflammatory cytokines with probable suppression of type 2 T-helpers.

We believe that excessive inflammatory response of the maternal organism (in the form of excessive release of pro-inflammatory cytokines in the deficiency of anti-inflammatory cytokines, increased levels of T-suppressors/killers, increased IgM production) [3, 13], which is probably directed

against foreign fetal antigens, leads to a chain of events, including defective invasion of the trophoblast into the spiral arteries of the uterus with the subsequent formation of systemic endothelial dysfunction and the occurrence of preeclampsia [2, 5, 8].

Conclusions

1. In pregnant women of the main group, who did not receive our proposed therapeutic and prophylactic complex of drugs (and in which almost half of the cases showed preeclampsia), the phenomena of immunosuppression inherent in pregnancy in general deepened.

2. An imbalance of the T-cell subpopulation was revealed: a decrease in the concentration of T-helpers (CD4 +) with a significant increase in the concentration of T-suppressors/killers (CD8+) with a corresponding decrease in the immunoregulatory index.

3. A significant drop in IgG concentration in women with preeclampsia (which correlates with a decrease in the number of B cells) is probably caused by depletion of the reactivity of the humoral part of the general immunity in this disease.

4. A significant dominance of pro-inflammatory cytokines was predominant in women with preeclampsia with a deficiency of anti-inflammatory cytokines.

Prospects for further research lie in studying the mechanisms of immunosuppression in pregnant women with preeclampsia by studying changes in nitric oxide metabolism in this group of patients.

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