Cytokines and endothelial adhesion molecules (sVCAM-1) in preeclampsia

Aleksandr Trunov, Olga Obukhova, Olga Gorbenko, Alya Shvayk, Lilia Trunova

Research Center of Clinical and Experimental Medical, Siberian Branch of the Russian Academy of Medical Sciences, Novosibirsk, Russia
Email: trunov1963@yandex.ru

ABSTRACT

In the purpose of studing the pathogenetic features of the pathological process there were investigated cytokine levels with proinflammatory and anti-inflammatory activity, and also the indicators of endothelial dysfunction in blood serum women with moderate and severe preeclampsia. There were found high concentrations of pro-inflammatory cytokines IL-1β, IL-6 and IL-8, and vascular endothelial adhesion molecule-1 (sVCAM-1), which correlated with the severity of the process. Also a significant decrease in concentrations TGF-β2 and IL-10 in severe preeclampsia was indicated, which may testify the exhaustion of adaptive mechanisms and, apparently, can be an additional diagnostic criterion for the prediction and assessment of the severity of the pathological process.

Keywords: Preeclampsia; Cytokines; Inflammation; Endothelial Dysfunction

1. INTRODUCTION

Preeclampsia, although a close examination of the features of the process remains one of the most sophisticated problems of modern obstetrics and gynecology. It generally determines the structure of maternal and perinatal morbidity and mortality [1,2]. The role of immune mechanisms contributing to the development of a normal pregnancy is widely discussed. Also there was noted their involvement in the pathogenesis of pregnancy complications such as preeclampsia [3]. The analysis of the scientific literature allows us to conclude that many aspects of the pathogenesis of preeclampsia are going into systemic inflammatory response syndrome with the development of a destructive inflammatory process, immune disorders, and the imbalance of cytokine regulation of gestation processes [4-6].

Debated nowadays role of vascular endothelial damage with the development of generalized arteriolar spasm as one of the leading mechanisms in the pathogenesis of preeclampsia is supposed to be significant. These findings have been confirmed by electron microscopy of vascular endothelial of placenta in preeclampsia when there has been a violation of the morphological structure of the endothelium of both arteries and veins. Moreover, these violations were correlated with the severity of preeclampsia [7]. Endotheliosis phenomena lead to systemic disorders in pregnancy, that determines the significance in these processes of the high level expression of intercellular adhesion molecules, indicating the extent of damage of endothelial cells [8,9].

However, the relationship between the development of endothelial dysfunction and violation of cytokine regulation in different clinical forms of preeclampsia also requires further study and is currently represented by single scientific works [10].

The aim of the present work was to study the characteristics of the production of proinflammatory and anti-inflammatory cytokines, vascular endothelial adhesion molecule-1 (sVCAM-1) as markers of endothelial dysfunction and their interactions, with varying degrees of severity of preeclampsia.

2. MATHERIALS AND METHODS

In accordance with the purpose of the study on the basis of Municipal Health Care Institution "Novosibirsk City Perinatal Center" were examined 100 women in III trimester of pregnancy.

All women gave informed consent to participate in the study, which is consistent with the ethical standards developed in accordance with the Declaration of Helsinki. All groups were matched for age and gestational age.

I group—60 patients with moderate preeclampsia (average age 28.2 ± 1.1 years) in the gestation of 26 to 30 weeks;

II group—20 patients with severe preeclampsia (mean age 29.1 ± 2.1 years) in the period from 26 to 31 weeks of pregnancy;

III group (control group)—20 pregnant women with physiological pregnancy (mean age 26.6 ± 1.2 years) in

the period from 26 to 31 weeks of pregnancy.

Inclusion criteria for all groups were the gestation period of 26 to 31 weeks, age 25 to 30 years. For I and II groups, inclusion criterion was the presence of moderate preeclampsia in patients (I group) and the severity of the disease process (II group). For group III criteria for inclusion was physiologically during pregnancy.

Preeclampsia was defined by increased blood pressure (≥ 140 mmHg systolic or ≥ 90 mmHg diastolic on ≥ 2 occasions at least 6 hours apart) that occurred after the 20th week of gestation in a woman with previously normal blood pressure, accompanied by proteinuria (≥ 0.3 g/24h in the absence of urinary tract infection). Preeclampsia was regarded as severe if any of the following criteria was present: blood pressure ≥ 160 mmHg systolic or ≥ 110 mmHg diastolic, or proteinuria ≥ 5.0 g/24h. Pregnant women with eclampsia or HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) were not enrolled in this study.

Exclusion criteria for all surveyed groups considered the presence of endocrine diseases: diabetes mellitus, hyper- or hypothyroidism and other endocrine disorders, requiring hormonal correction. Besides exclusion criterion was also the presence of TORCH-infections markers and other chronic viral or bacterial processes in the acute phase, as well as autoimmune diseases and malignant neoplastic processes.

Determination of the concentrations of interleukins—IL-1β, IL-4, IL-6, IL-8, IL-10 and granulocyte growth factor (G-GSF) in blood serum was performed by using commercial kits for ELISA produced by "Cytokine" company (Russia), following to the manufacturer's instructions.

Determination of the concentration of transforming growth factor-$\beta 2$ (TGF-$\beta 2$) in blood serum performed by enzyme immunoassay using commercial test kits manufacturing DRG International (USA) following the instructions of the manufacturer.

Determination of the concentrations of soluble vascular endothelial adhesion molecule 1 (sVCAM-1) in blood serum performed by enzyme immunoassay using commercial test kits manufacturing Bender Medsystems (Austrian), following the manufacturer's instructions.

The resulting data were statistically analyzed and presented in tabular form. Data analysis was performed using the software package Statgrafics. To overcome the problem of multiple comparisons analysis of variance Kruskal-Wallis test was using. The significance of differences in the variation series of unrelated samples was evaluated using the Mann-Whitney test, the related pairs samples-using Wilcoxon test. Data in tables are presented as M ± m, where M-mean, m-error of the mean. The difference between treatment series was supposed to be significant with a confidence level of 95% ($p < 0.05$).

Correlation analysis was carried out using Spearman rank correlation.

3. RESULTS AND DISCUSSION

The results of the analysis of cytokines content in the blood serum of patients is presented in **Table 1**.

Concentration of the main pro-inflammatory cytokine IL-1β in the blood serum of patients of group I was significantly 2.5 times greater than those figures determined in the control group ($p < 0.05$). There was a significant increase of concentration of IL-1β—3.3 times in the group II of pregnant patients, on the contrary to the values of the patients in group II ($p < 0.05$) and 8.2 times more, compared with the control group ($p < 0.05$).

Concentration of IL-8, which is an active participant in the local inflammatory response in the blood serum of patients of group I was significantly in 4.9 times greater than the value indicator in the control group ($p < 0.05$). There was indicated a double increase in the concentration of IL-8 of pregnant patients of group II as compared with values of the patients in group I ($p < 0.05$) and a ten-fold excess of the values of the control group ($p < 0.05$).

Table 1. The content of IL-1β IL-4, IL-6, IL-8, IL-10, G-GSF, TGF-$\beta 2$ and sVCAM-1 in the blood serum of pregnant surveyed groups (M ± m).

Figures groups	Control group (physiological pregnancy)	Group I (preeclampsia moderate)	Group II (severe preeclampsia)
IL-1β, pg/ml	35.9 ± 5.1	88.7 ± 12.1[*]	296.1 ± 37.8[*,**]
IL-4, pg/ml	130.1 ± 13.4	51.1 ± 6.1[*]	67.5 ± 14.4[*]
IL-6, pg/ml	26.5 ± 1.64	91.7 ± 11.8[*]	85.3 ± 9.7[*]
IL-8, pg/ml	37.8 ± 7.8	187.2 ± 19.6[*]	391.2 ± 41.2[*,**]
IL-10, pg/ml	39.8 ± 6.8	35.3 ± 7.6	14.9 ± 2.3[*,**]
G-GSF, pg/ml	67.9 ± 7.9	102.1 ± 14.7[*]	116.5 ± 17.1[*]
TGF-$\beta 2$, pg/ml	1393.2 ± 35.1	1452.1 ± 28.1	859.1 ± 64.3[*,**]
sVCAM-1, ng/ml	837.5 ± 41.9	1333.2 ± 35.9[*]	1963.1 ± 109.1[*,**]

Note: [*]Significantly different from women with physiological pregnancy ($p < 0.05$); [**]Significantly different from the data of the group of women with preeclampsia moderate ($p < 0.05$).

The concentration of IL-6 in the blood serum of the pregnant patients of group I was significantly, in 3.5 times greater than the value in the control group ($p < 0.05$). Comparative analysis between groups I and II of pregnant women with preeclampsia on the content of IL-6 has not identified the significant differences.

There was also measured the concentration of IL-4-a cytokine that has anti-inflammatory properties and which is involved in regulating the synthesis of blocking antibodies, which are necessary for physiological pregnancy. The content of IL-4 in the blood serum of patients of group I was 2.5 times lower than its concentration in pregnant patients of control group ($p < 0.05$). Patients of group II, with severe preeclampsia, average IL-4 value was also significantly lower than in the control group ($p < 0.05$). In the comparative analysis between groups I and II in pregnant women with preeclampsia on the content of IL-4 significant differences were not detected.

Given the significant role of phagocytes in the development of inflammation, in patients of all groups defined by the maintenance of granulocyte growth factor (G-GSF), which is a marker of phagocytic activity of reactions. The concentration of G-GSF in blood serum of patients in group I was 1.5 times higher than its concentration in the control group ($p < 0.05$). Comparative analysis of significant differences between groups I and II in pregnant women with preeclampsia in content G-GSF was not identified.

Significant reduction of IL-10, which has immuno-suppressive properties, was found only in group II of pregnant—by 2.7 times relative to its concentration in the control group ($p < 0.05$) and 2.6 times relative to content in one group I of the examined patients ($p < 0.05$).

A significant decrease of TGF-β2, cytokine, which is highly proliferative and possess anti-inflammatory properties, was found only in the group II of pregnant. Its concentration in the blood serum was 1.6 times lower than in the control group and 1.7 times lower relative to its concentration in group I surveyed pregnant ($p < 0.05$). This fact probably indicates the exhaustion of adaptive mechanisms intended for the development and maintenance of pregnancy, with severe preeclampsia and may be an additional marker for the differential diagnosis of the severity of the process.

To evaluate the degree of endothelial dysfunction was determined the expression of soluble intercellular adhesion molecule 1 (sVCAM-1). When testing of sVCAM-1 concentration in the blood serum of patients of group I was found a significant increase in their content in the 1.6 times compared to the data obtained in the control group ($p < 0.05$). While pregnant of group II recorded a further significant increase in the concentration levels of sVCAM-1, notably 1.5 times relatively patients of group I ($p < 0.05$) and 2.3 times higher than in pregnant control group ($p < 0. 05$). The above, fact evidence of the growth of endothelial dysfunction and worsening over the study of the pathological process.

In order to identify the relationship between the processes occurring in preeclampsia was carried out a correlation analysis of values of the study parameters.

The most significant in both forms of preeclampsia found a direct significant correlative relationship between the concentrations of IL-8 and sVCAM-1: $r = 0.43$ ($p < 0.05$), with an average severity of the process and $r = 0.63$ ($p < 0.05$) in severe preeclampsia. The presence of such a correlation seems to indicate the relationship between the development of the local immune inflammation and endothelial dysfunction in preeclampsia. It should be noted that this relationship is strengthening with a worsening of the current process.

In addition, the average severity of preeclampsia revealed significant direct correlation between the concentrations of IL-1β and IL-4: $r = 0.53$ ($p < 0.01$), as well as IL-1β and IL-6: $r = 0.51$ ($p < 0.01$). It is noteworthy that in severe preeclampsia, these relationships disappear.

Based on the fact that IL-4 exhibits anti-inflammatory properties, and IL-6 has the ability at certain stages of inflammation inhibit production of IL-1β, and in consideration of their role in the activation of the synthesis of blocking antibodies during pregnancy, it can be assumed that the identified patterns of correlation relationships testify depletion of the woman adaptive reserves, aimed at prolonging pregnancy during the course of worsening preeclampsia.

Summarizing the data, we can draw the following conclusion. In the pathogenesis of preeclampsia significant role plays development of the process of immune inflammation in the complex with endothelial dysfunction. These processes are shown enhanced expression of intercellular adhesion molecule and activation of the immune response in their relationship, growing to a severe disease process. Established correlative relationships between the indicators, reflecting activity of studies processes, confirms this conclusion.

Extremely important, in our view, is a significant decrease in concentration of TGF-β2 and IL-10 in blood serum in severe preeclampsia compared with those indicators in moderate process, and also in physiological pregnancy. This fact can be considered as a major pathogenetic difference of severe preeclampsia not only from the norm, but also from the average degree of severity of pathology. Such a decrease indicates a depletion of adaptive mechanisms, aimed at relief of excessive activity of the inflammatory process and the development of physiological immunosuppression during pregnancy and, apparently, can be an additional diagnostic criterion for the prediction and assessment of the severity of the pathological process.

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