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THE ACTIVITY OF CYTOTOXIC LYMPHOCYTES IN PATIENTS WITH PREECLAMPSIA

With preeclampsia, the average levels of biochemical parameters in mild and severe form did not differ significantly from those in the group with uncomplicated preeclampsia of pregnancy. There is a tendency to a decrease in total protein and an increase in bilirubin, creatinine, urea, and blood transferases as it becomes heavier. In the hemostatic system, there was also a tendency toward a decrease in fibrinogen and platelet count as gestosis worsened. The average hemostasis in the groups with pre-eclampsia did not differ from the group with uncomplicated pregnancy. However, thrombocytopenia was observed in 28% and 32% of cases with mild and severe forms of preeclampsia. Moreover, a pro-nounced decrease in platelet count (almost 10 times) was recorded in 5.4% of patients with severe pre-eclampsia. In patients with preeclampsia, the production of intracellular perforin in the regulatory cells
- CD4+Perf+ lymphocytes was significantly reduced and increased - in natural killers CD16+Perf+ and CD56+Perf+.

Key words: preeclampsia, cytotoxic lymphocytes, perforin.

Introduction

Preeclampsia is a leading obstetric pathology that determines the level of reproductive loss and perinatal complications [1]. The frequency of preeclampsia in the general population of pregnant women is 5-10% and is one of the main causes of maternal mortality - 12-30% [2]. The frequency of preeclampsia in the Republic of Kazakhstan ranges from 14.5-35%.

According to modern concepts, pre-eclampsia is considered as a “disease of adaptation” to pregnancy with all stages inherent in the general adaptation syndrome. Indicators of developing dysfunction in the body of a pregnant woman are changes in the immune, neurohumoral and other systems responsible for the regulation of vascular tone and the state of microcirculation [3]. The pathogenesis of preeclampsia is based on imperfect adaptation, in which insufficient release of Th1 cytokines, proteolytic enzymes, and free radicals causes a small invasion of the spiral arteries by cytotrophoblast and systemic dysfunction of endothelial cells [4]. Hypertension developing in the III trimester is a compensatory response with adequate placental perfusion for fetal growth [5].

Physiological pregnancy is characterized by the activation of an inflammatory cellular response, which was demonstrated by the analysis of inflammatory markers (CD11b, CD64, CD62L, HLA-DR and intracellular types of reactive oxygen). The leukocytes of healthy pregnant women showed significantly higher levels of CD11b +, CD64 + cells and oxygen radicals compared with samples from non-pregnant. At the same time, women with preeclampsia showed lower expression of CD62L and significantly higher levels of production of reactive oxygen species compared to healthy pregnant women, indicating generalized changes in circulating leukocytes, however, there were differences in many respects between pregnant women with preeclampsia and gestosis smaller than those between the control groups of pregnant and non-pregnant women [6].

The most obvious changes in gestosis were noted in the level of lymphocytes with a phenotype characteristic of cells with cytotoxic activity [3]. It was shown that in the peripheral blood of women with this pathology, the contents of CD16 +, CD8 + HLA-DR + and CD8 + CD16 + lymphocytes were higher than during normal pregnancy [7]. Analysis of cellular immunity indices showed an increase in natural killers of CD16 + CD56 + in the peripheral blood during preclampsia [8]. It is known that, upon activation of natural killers, perforin production increases [9]. Therefore, the level of intracellular

production of perforin in effector lymphocytes can be regarded as a marker indicating the cytotoxic potential of killer cells, and as a possible predictor of the development of preeclampsia.

In this regard, we conducted a study evaluating the cytotoxic potential of peripheral blood lymphocytes in patients with preeclampsia.

Material and Methods

We examined 25 patients with mild preeclampsia, 37 patients with severe preeclampsia and 15 patients with uncomplicated preeclampsia during pregnancy with a gestational age of 34-38 weeks (III trimester). All patients underwent a comprehensive examination, including determination of hemoglobin level, biochemical tests (ALT, AST, bilirubin, total protein), platelet count, hemostasiogram indicators, protein level in urine analysis. A subpopulation analysis of peripheral blood lymphocytes was performed by flow cytometry on a FacsCalibur instrument (Becton Dickenson / USA) in the CellQuest program using monoclonal antibodies to surface lymphocyte antigens (NPO Sorbent / Moscow, Russian Federation): mature T-lymphocytes – CD3 +, T helper cells – CD4 +, cytotoxic lymphocytes CD8 +, natural killers CD16 +, CD56 + stained with phycoerythrin. The level of intracellular perforin in the cytotoxic cells was evaluated, and the expression of Perf + stained with FITC was evaluated.

Results and Discussion

It was found that the average levels of biochemical parameters in mild and severe form did not differ significantly from those in the group with uncomplicated pre-eclampsia of pregnancy (table 1).

Table 1 – Blood biochemical parameters in patients with preeclampsia (PE)

Indicators	Control, n = 15	Light PE group, n = 25	Heavy E group, n = 37
Total protein, g / l	66,5±2,9	67,2±5,4	63,5±5,1
Bilirubin, mmol /l	6,2±0,7	8,7±3,8	9,0±2,6
Creatinine, mmol /l	57,3±5,0	70,3±13,7	65,2±13,3
Urea, mmol /l	3,1±0,5	4,1±1,2	7,2±4,8
Glucose, mmol /l	6,3±2,4	4,5±0,4	4,8±0,6
ALT, Units /l	16,4±6,4	11,5±4,5	14,6±7,4
AST, Units /l	15,4±7,6	16,9±7,1	18,9±7,8

Despite the tendency for a decrease in total protein and an increase in bilirubin, creatinine, urea with preeclampsia, there was a pronounced scatter of indicators (there were both reduced and increased indicators). The level of blood transferases also showed a tendency to increase them. In patients with uncomplicated pre-eclampsia pregnancy, a 2-fold increase in ALT was recorded in 10% of cases, while in patients with a mild form of preeclampsia, an increase in ALT and AST by 2 norms was detected in 8% and 12% of cases, respectively, and in patients with a severe form of preeclampsia in 21% and 19% of cases, respectively.

In the hemostasis system (table 2), a tendency toward a decrease in fibrinogen and platelet count as gestosis worsened was also recorded.

Table 2 – Hemostasis in patients with preeclampsia (PE)

Indicators	Control, n = 15	Light PE group, n = 25	Heavy PE group, n = 37
Fibrinogen, g / l	4,3±0,1	4,0±0,1	3,4±0,5
Prothrombin time, sec	16,2±2,4	17,4±1,1	16,7±0,8
Prothrombin index	90±4,8	90,6±5,8	94,1±3,7
Platelets, x 10_9 g/l	235,2±55,0	221,2±56,9	224,5±73,8

However, the average hemostasis in the groups with preeclampsia did not differ from the group with uncomplicated pregnancy. However, thrombocytopenia was observed in 28% and 32% of cases with mild and severe forms of preeclampsia. Moreover, a pronounced decrease in platelet count (almost 10 times) was recorded in 5.4% of patients with severe preeclampsia.

Data characterizing the characteristics of the population composition of peripheral blood lymphocytes with a mild form of preeclampsia are presented in table 3.

Table 3 – Relative content of lymphocyte's subpopulations in patients with light PE

Indicators, %	Control, n = 10	PE, n=10	P < 0,05
CD3+Perf-	64,7±2,7	67,3±2,8	
CD4+Perf-	37,8±1,6	34,3±8,3	
CD8+ Perf-	31,4±1,0	24,9±6,3	
CD16+Perf-	0,61±0,3	4,07±3,4	
CD56+Perf-	0,7±0,1	3,2±1,4	

It should be noted that according to the average values of the lymphocyte subpopulations, there were no significant differences in comparison with the control group. Although there was a downward trend in CD4 + lymphocytes and an increase in CD16 + and CD56 + lymphocytes.

We also studied the production of perforin by cytotoxic cells of peripheral blood (table 4).

Table 4 – The relative content of perforin-positive lymphocytes in patients with PE

Indicators, %	Control, n = 10	PE, n=10	
CD3+Perf+	9,1±0,9	10,5±1,3	
CD4+Perf+	9,6±0,5	4,9±1,2	P < 0,05
CD8+Perf+	6,8±0,4	13,3±7,1	
CD16+Perf+	1,0±0,02	9,1±0,7	P < 0,05
CD56+Perf+	0,6±0,03	9,8±2,4	P < 0,05

Conclusion

In patients with preeclampsia, the production of intracellular perforin in the regulatory cells - CD4+Perf+ lymphocytes was significantly reduced and increased - in natural killers CD16+Perf+ and CD56+Perf+. The content of CD3+Perf+ and CD8+Perf+ in patients with preeclampsia did not differ from those in the group with uncomplicated preeclampsia pregnancy. From the second half of physiological pregnancy, a second wave of trophoblast invasion into the muscle segment of the spiral arteries of the uterus is observed, and activation of regulatory T-helpers is necessary [10]. It would expect an increase in the level of cytotoxic lymphocytes - CD4+, CD8+ lymphocytes and natural killers that limit trophoblast invasion. However, in the pathological course of pregnancy, in our study, significant differences were found only in the level of perforin-positive natural killers. Apparently, during preeclampsia, the functional properties of cytotoxic cells are redistributed, therefore, their study must be carried out with the study of the intracellular cytokine content.

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