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Article

The Role Intercellular Cytokines in Prediction of Preeclampsia

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Abstract

Background: Preeclampsia (PE) is a multisystem obstetric complication, accompanied by changes in the immunological status. Immune cells such as regulatory T cells, macrophages, natural killer cells and neutrophils play an important role in the pathogenesis of preeclampsia, but the contribution of other immune cells is now also recognized. Therefore, studying the production of cytokines by different cell types will allow the identification of potential biomarkers for the prediction of preeclampsia. **Objectives:** to investigate the levels of intracellular cytokine production by lymphocytes of peripheral blood in preeclampsia. **Methods**: 35 pregnant women admitted to labor with physiological pregnancy (28) and with PE (7). The multicolor immunophenotyping with intracellular cytokine production of TNF, GM-CSF, VEGFR-2, and IGF by different immunocompetent cell types were evaluated on a BD FACS CALIBUR flow cytometer. **Results:** Women with preeclampsia had a significant increase in the level of CD8+GM-CSF+ cells (28.5 ± 3.7 vs. 17.3 ± 1.5 in the control group, p<0.001), and CD56+VEGF2+ (14.2±3.47 vs 0.62±0.55, p<0.001), and CD14+IL-10+ cells (55.7 ± 15.7 vs. 0.89 ± 0.61 in the control group, p<0.001), and level of CD19+IGF+ (12.0 ± 3.99 vs 1.10±0.46 in the control group, p<0.001). In contrast, the content of CD56+TNF+ cells was significantly reduced in the PE group compared to the control group (2.26 ± 1.15 vs. 15.7 ± 1.74, p<0.001). **Conclusions:** These data will not only expand existing knowledge about the role of intracellular cytokines in the pathogenesis of preeclampsia, but will also help to obtain new markers for predicting preeclampsia.

Keywords: preeclampsia; CD-phenotyping; cytokines; IL-10+; TNF+; GM-CSF+; IGF

1. Introduction

Preeclampsia (PE) is a multisystem obstetric complication that contributes significantly to maternal and neonatal mortality. In Kazakhstan, PE ranks second in the structure of maternal mortality, accounting for 11% of all obstetric causes. According to the International Society for the Study of Hypertension in Pregnancy (ISSHP), preeclampsia (de novo) is a hypertensive condition (systolic BP ≥ 140 mmHg, diastolic BP ≥ 90 mmHg), accompanied by one or more new complications after 20 weeks of pregnancy: proteinuria, neurological complications, renal and hepatic damage, hematological complications, pulmonary edema, uteroplacental dysfunction [1]. There is also evidence that women who have had preeclampsia have an increased risk of developing cardiovascular complications throughout their lives [2].

According to the literature, preeclampsia is divided into early and late PE, but these terms are not yet officially used in clinical protocols. Early PE occurs before 34 weeks of pregnancy and

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accounts for approximately 5-20% of all cases of PE worldwide, and is also characterized by greater danger to both the mother and the fetus. Late PE occurs after 34 weeks of pregnancy and accounts for 80-95% of cases worldwide [3,4]. The mechanisms of early and late development of PE may not be entirely the same. It is known that the pathogenesis of PE is associated with reduced blood supply to the placenta, which in turn leads to fetal growth disorders and increases the risk of stillbirth. It is believed that preeclampsia develops in two phases: 1) abnormal placentation in the first trimester, followed by 2) "maternal syndrome" in the late second and third trimesters, which is characterized by an excess of angiogenic factors [5].

Despite intensive research, the pathogenesis of PE remains poorly understood, and universal biomarkers for early prediction and diagnosis have not yet been approved in international guidelines. In recent years, particular attention has been paid to immunological mechanisms, in particular, immune disorders are considered a key link: there is a shift towards Th1/Th17 (pro-inflammatory cytokines TNF-α, IL-17) and a decrease in the Th2/Treg response (IL-10, IL-4), creating an unproductive, persistent inflammatory background [6]. Studies show that TNF-α is a key pro-inflammatory cytokine that plays a central role in the pathogenesis of preeclampsia. A review by LaMarca et al. emphasizes that preeclampsia activates innate and adaptive immune cells and increases TNF-α production, leading to endothelial dysfunction, vasoconstriction, and hypertension [7].

Unlike TNF-α, IL-10 is a key anti-inflammatory cytokine that maintains immune tolerance during pregnancy. The study of Salvany-Celades et al. demonstrated the existence of several functional subtypes of regulatory T cells (Treg) at the maternal-fetal interface, which suppress effector T cell responses through IL-10 production, ensuring immune balance and preventing excessive inflammation [8].

The role of granulocyte-macrophage colony-stimulating factor (GM-CSF) in PE remains a subject of debate. A number of studies have shown increased levels of GM-CSF in both peripheral blood and placental tissue in women with PE, indicating its possible involvement in immune dysregulation and the formation of an inflammatory response [9,10]. However, our recent study showed a decrease in GM-CSF expression in the placenta in PE [11], which is consistent with the data of Tian et al., where it was shown that a decrease in PD-L1 in trophoblasts is associated with the suppression of GM-CSF through the activation of the JAK2/STAT5 pathway; an increase in PD-L1 in a PE-like model in rats partially alleviated hypertension and proteinuria [12].

Insulin-like growth factor 1 (IGF-1) is associated with cell growth, metabolism, angiogenesis, and differentiation [13]. One study showed that IGF-1 may inhibit the development of preeclampsia by decreasing miR-183 expression by increasing ZEB1 expression. Other molecular mechanisms may also contribute to improved prediction of preeclampsia and suggests new promising therapeutic targets for the treatment of PE [14]. The impact of the Covid pandemic on changes in immunoreactivity, including during pregnancy, cannot be ruled out. Thus, in pregnant women with SARS-CoV-2 infection, the risk of developing preeclampsia was significantly higher than in women without infection [6].

Thus, TNF-α, IL-10, GM-CSF, IGF and are key intracellular mediators involved in the pathogenesis of PE. Studying them at the level of individual cell subpopulations using intracellular cytokine staining will allow for a deeper understanding of the immunological mechanisms of preeclampsia in the post-COVID period and identify possible diagnostic markers.

2. Materials and Methods

2.1. Test Subjects

Peripheral blood analysis was performed in 35 women (7 from the preeclampsia group and 28 from the control group) admitted for labor to the Scientific Center of Obstetrics, Gynecology and Perinatology, Almaty, Kazakhstan in November-December 2024.

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The criteria for inclusion in the PE group were as follows: pregnant women aged 18 years and older, gestational hypertension, blood pressure >140/90 mm Hg after 20 weeks, proteinuria >0.3 g/day, as well as the presence of anamnestic, laboratory, and instrumental signs and clinical manifestations of organ damage against the background of hypertension.

Inclusion criteria for the control group: pregnant women over 18 years of age, pregnancy not complicated by hypertension.

Exclusion criteria for both groups: acute and chronic inflammatory diseases, severe extragenital pathology, previous organ transplantation, previous cancer, diabetes mellitus, blood transfusion, systemic autoimmune diseases, tuberculosis, HIV, chronic arterial hypertension. All patients signed an informed consent form to participate in the study.

2.2. Ethical Approval

The work was carried out in accordance with the principles of voluntariness and confidentiality based on the Code of the Republic of Kazakhstan "On Public Health and the Health Care System" (dated July 7, 2020, No. 360-VI SAM) and the Helsinki Declaration. The study was approved by the Local Ethics Committee of the Scientific Center for Obstetrics, Gynecology, and Perinatology (No. 2 dated November 9, 2022). All participants gave written informed consent for the use of biomaterials in this study. The material was anonymized.

2.3. Sample Processing

The peripheral blood in a volume of 5 ml in a special lilac test tube with EDTA was collected and sent to the immunology laboratory of the Scientific Center for Obstetrics, Gynecology, and Perinatology (Almaty, Kazakhstan).

2.4. Immunophenotyping

The blood was conducted according to the manufacturer's protocol (www.bdbiosciences.com).

The samples were stained with monoclonal antibodies (mAb) using Becton Dickinson (BD) reagents against CD4, CD8, CD56, CD14, and CD19 for staining and binding surface receptors (Table 1).

Table 1. Characteristics of mAb used for staining surface receptors.

Cellular markers	Fluorochrome	Clone
CD4	FITC	RPA-T4
CD8	FITC	RPA-T8
CD14	FITC	M5E2
CD56	FITC	B159
CD19	FITC	HIB19

5 µl of mAb was added to a tube containing 50 µl of peripheral blood sample, mixed on a Vortex, and incubated for 15 minutes at room temperature in the dark. After incubation, erythrocytes were lysed with Lysing solution, mixed on a Vortex and incubated for 10 minutes at room temperature in the dark. The mixture was centrifuged for 5 minutes at 300 g/min, after which the supernatant was removed. Permeabilization of membranes with BD Cytofix/Cytoperm™ Plus Fixation/Permeabilization Kit (with BD GolgiStop™ protein transport inhibitor contains monensin) (Cat. No. 554715) was also carried out, followed by the introduction of mAb for staining and binding of intracellular receptors against TNF, GM-CSF, VEGFR-2, and IGF (Table 2).

Table 2. Characteristics of mAb used for staining surface intracellular receptors.

Cellular markers	Fluorochrome	Clone
TNF	PerCP-Cy5.5	Mab11

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IL-10	PE	JES3-19F1
GM-CSF	PE	BVD2-21C11
VEGFR-2 (CD309)	PE	89106
IGF (CD221)	PE	1H7

2.5. Flow Cytometric Analysis

The total population of leukocyte cells was isolated using the CD45+ marker, then lymphocytes and monocytes were isolated from this fraction. Concentration-matched isotype controls were used to set the gates and single-fluorochrome stained controls were used to compensate for spectral overlap. The immunophenotyping with cytokine production of cells were evaluated on a BD FACS CALIBUR flow cytometer (USA) and the data was analyzed using the CELL Quest program.

2.6. Statistical Analysis

Statistical calculations and analysis were performed using the Jamovi program (Version 2.3, available online: https://www.jamovi.org) and R Core Team. R: A language and environment for statistical computing (Version 4.1, available online: https://cran.r-project.org). To compare the characteristics of the study groups, given the small sample size, Fisher's criterion (for nominal data) was used. The statistical significance of differences in quantitative data between groups was calculated using the Mann-Whitney U test. To assess the accuracy of the estimates, confidence intervals (CI) were calculated for clinical variables. Values of $p < 0.05$ were considered statistically significant. To assess the degree of differences between groups, in addition to the standard test of statistical significance (p-values), effect size indicators were used in the study.

3. Results

3.1. Clinical Data

The study included 7 women with preeclampsia and 32 women from a control group matched for age and anthropometric parameters (Table 3).

Table 3. Clinical data and pregnancy outcomes of the PE and control groups.

Indicators	PE (n=7)	95% CI	Control (n=28)	95% CI	P value
Age	32.7±3.95	(26;37)	32.5±6.48	(21;45)	0.923
Weight	82.1±9.37	(66;92)	80.9±15.3	(61;132)	0.843
Height	166±1.5	(163;167)	164±5.65	(153;175)	0.332
Blood pressure					
systolic	139±11	(130;160)	102±9.57	(80;120)	<0.001
diastolic	84.3±7.87	(70; 90)	67.5±12.4	(60;120)	0.002
Gestational age at diagnosis	36.2±2.14	(34.1±40.1)	-	-	
Gestation period	38±1.48	(36.6;41.0)	39.4±1.26	(37.4;41.3)	0.018
Child's weight in g	3041±643	(2170;3900)	3618±401	(2680;4270)	0.006

The mean age of patients with PE was 32.7 ± 3.95 years, which was not statistically different from the control group (32.5 ± 6.48 years; p=0.923). Body weight (92 ± 19.37 kg vs. 90.1 ± 25.61 kg; p=0.843) and height (166 ± 1.5 cm vs. 164 ± 5.65 cm; p=0.332) were also comparable between the groups. In pregnant women with PE hemodynamic parameters had significant difference: systolic blood pressure in women with PE was significantly higher (139 ± 11 mmHg) compared to the control group (102.9 ± 9.57 mmHg; p<0.001). Similarly, diastolic pressure was significantly higher (84.3 ± 7.87 mmHg) compared to the control group (67.5 ± 12.4 mmHg; p=0.002). The mean gestational age at the time of diagnosis of preeclampsia was 36.2 ± 2.14 weeks, which was lower than in the control group

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(39.4 ± 1.26 weeks; p=0.018). These differences are also reflected in perinatal outcomes: the weight of newborns in the PE group was significantly lower (3041 ± 643 g) compared to the control group (3618 ± 400 g; p=0.006).

The clinical profile of women with preeclampsia was characterized by significantly higher blood pressure values, shorter gestation duration at delivery, and lower birth weight of newborns, confirming the negative impact of PE on the course of pregnancy and outcomes for the child.

3.2. Immunological Parameters in Peripheral Blood

The study analyzed the intracellular cytokine profiles of the main immune cell subpopulations isolated from the peripheral blood of women with preeclampsia and a control group with physiological pregnancies. Double phenotypes were used for evaluation: CD8+GM-CSF+, CD56+TNF+, and CD14+IL-10+. Immunological parameters in peripheral blood are shown in Table 4.

Table 4. Results of statistical analysis using the nonparametric Mann-Whitney test.

Double phenotyping markers	PE (n=7)	Control (n=28)	p-value
CD8+GM-CSF+	28.5±3.71	7.4±1.59	<0.001
CD56+TNF+	2.3±1.15	15.7±1.83	<0.001
CD56+VEGF2+	14.2±3.47	0.62±0.55	<0.001
CD14+IL-10+	55.7±15.7	0.89±0.61	<0.001
CD19+IGF+	12.0±3.99	1.10±0.46	<0.001

The data obtained demonstrated marked differences between the groups. Women with preeclampsia had a significant increase in the level of CD8+GM-CSF+ cells (28.5 ± 3.71 vs. 7.4 ± 1.59 in comparison with the control group, p<0.001) and CD56+VEGF2+ (14.2±3.47 vs 0.62±0.55). A significant increase in the level of CD14+IL-10+ cells was also found in women with PE (55.7 ± 15.7 vs 0.89 ± 0.61 in the control group, p<0.001) and level of CD19+IGF+ (12.0 ± 3.99 vs 1.10±0.46 in the control group, p<0.001). In contrast, the content of CD56+TNF+ cells was significantly reduced in the PE group compared to the control group (2.3 ± 1.15 vs. 15.7 ± 1.83, p<0.001).

The results obtained confirm the involvement of immune cell imbalance and intracellular cytokines in the pathogenesis of preeclampsia.

4. Discussion

In our study, women with PE showed an increase in the level of CD8+GM-CSF+ cells and CD56+VEGF2+ cells, a significant increase the level of CD19+IGF+ and CD14+IL-10+ cells compared to the control group, a decrease in the level of CD56+TNF+ expression. These data are consistent with the hypothesis of impaired regulation of innate and adaptive immunity at the maternal-fetal interface.

The identified changes in the intracellular cytokine profile confirm the key role of the imbalance between pro-inflammatory and anti-inflammatory mediators in the pathogenesis of preeclampsia and can be considered as potential immunological markers of the disease.

Increased expression of GM-CSF+ in $CD8^+$ cells may reflect activation of the cytotoxic immune system and an enhanced inflammatory response. A significant increase of CD14+IL-10+ cells level indicates compensatory activation of anti-inflammatory mechanisms; however, judging by the severity of clinical manifestations, this regulation is insufficient to prevent the development of PE. At the same time, a decrease in TNF production in CD56+ cells (natural killer cells) may indicate a disruption of their effector function, which can affect placentation processes and control of local inflammation.

TNF-α. TNF-α is known to be a key pro-inflammatory cytokine, the level of which increases in complicated pregnancies, including PE. A number of studies have shown that excessive production

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of TNF-α in the placenta and peripheral blood is associated with endothelial dysfunction and increased vascular tone [7]. Recent data on the genetic mechanisms regulating TNF-α production are of particular interest. According to a meta-analysis by Hossen et al. (2024), which included 32 publications, TNF-α rs1800629 (G/A) polymorphism is significantly associated with an increased risk of developing preeclampsia. Moreover, this association is particularly pronounced in Asian populations (OR up to 2.3 in the dominant model), which emphasizes the ethnic specificity and genetic predisposition to TNF-α hyperproduction in pregnant women [15]. These data suggest that the differences we identified in intracellular TNF-α expression in women from the Almaty cohort may be partly due to genetic factors, including this polymorphism. However, a large cohort study by Adomi et al. (2024), which included more than 4.3 million pregnancies, showed that the use of TNF-α inhibitors in the first and second trimesters does not reduce the risk of early or late preeclampsia (RR 1.25 [0.93–1.67] and RR 0.99 [0.81–1.22], respectively) [16]. This indicates that, despite the pathogenetic significance of TNF-α, systemic blockade of this cytokine does not prevent the development of the disease. Consequently, the identified imbalance should be considered a biomarker of a pathological phenotype rather than a direct therapeutic target.

IL-10. The immunoregulatory cytokine IL-10 plays a central role in maintaining tolerance during pregnancy. Experimental studies have shown that the addition of IL-10 increases the number of Tregs and reduces blood pressure in the RUPP (Reduced Uterine Perfusion Pressure) model in rats [17]. A review by Cubro et al. (2018) highlights its role as a potential therapeutic factor: IL-10 regulates vascular response and enhances cellular interactions at the maternal-fetal interface [18]. According to a meta-analysis by Nath et al. (2020), circulating IL-10 levels do not differ before the onset of PE (SMD ≈ –0.01, P=0.76), but decrease significantly at the time of disease onset (SMD ≈ –0.79, P=0.0004), confirming its role as a potential biomarker of PE activity [19]. Interestingly, our cohort showed an increase in CD14/IL-10 cells, which can be interpreted as an attempt at a compensatory anti-inflammatory response.

GM-CSF. GM-CSF plays a key role in regulating the immune response during pregnancy: it stimulates the differentiation of macrophages and dendritic cells and influences the balance of proinflammatory and regulatory T cells in the uteroplacental interface. However, this cytokine has been less studied in the context of PE. In the works of Jancsura et al. (2023) and Jancsura et al. (2025) showed that GM-CSF levels in overweight women who subsequently developed preeclampsia were significantly elevated as early as the first trimester compared to women without PE. This indicates that GM-CSF may be an early marker of inflammation activation. At the same time, the dynamics differed from the control group: while in healthy women the concentration of inflammatory markers (including GM-CSF) increased as pregnancy progressed, in women with PE there was no such "physiological rise". This may reflect early depletion or disruption of immune regulation, which creates the conditions for placental dysfunction [20,21].

Our data also revealed an increase in CD8+GM-CSF+ cells in women with PE. This indicates that T lymphocytes contribute to the hyperinflammatory background of the disease by enhancing GM-CSF production. This finding supports the hypothesis that GM-CSF is not just a systemic serum marker, but also the result of cellular restructuring of the immune response.

Our study showed increased IGF-1 production by B cells in preeclampsia. However, other studies have shown low IGF-1 expression in women with preeclampsia compared to women in normal pregnancy [14]. In patients with preeclampsia, the placental IGF-1 (insulin-like growth factor 1) expression was significantly reduced compared with healthy pregnancy, associated with a downregulation of ZEB1 [22].

5. Conclusions

Preeclampsia remains one of the leading causes of maternal and perinatal mortality, and its molecular mechanisms continue to be studied. An analysis of intracellular cytokines showed that women with preeclampsia have increased production of growth factors, particularly GM-CSF, VEGF2+, and IGF+ by different types of cells. This imbalance reflects a shift in the immune response

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toward inflammation and may contribute to the disruption of adaptive mechanisms at the maternal-fetal interface. The results confirm the importance of assessing the cytokine profile as an additional approach to understanding the pathogenesis of preeclampsia.

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Informed Consent Statement: Informed consent was obtained from all subjects who participated in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical issues.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

PE – Preeclampsia
BP – blood pressure
ISSHP – International Society for the Study of Hypertension in Pregnancy
GM-CSF – Granulocyte-Macrophage Colony-Stimulating Factor
TNF-α – Tumor Necrosis Factor alpha
CI - Confidence Intervals
BD – Becton Dickinson
FACS – Fluorescence-Activated Cell Sorting

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