CONNECTION BETWEEN CYTOKINES AND COMPLICATIONS DERIVED FROM PREECLAMPSIA PREGNANCIES

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ABSTRACT Preeclampsia is a condition of multiorgan involvement that can cause severe complications for the mother's health and endanger the intrauterine development of the fetus. **Purpose:** The purpose is to detect the risk of developing preeclampsia in the second trimester, by examining cytokines and closely monitoring the pregnancy for complications from preeclampsia, whether they are affected by the same cytokines. **Material and methods** A total of 100 patients were monitored in the second trimester between 14 and 20 weeks of gestation. Values of immune biomarkers of their serum were analyzed after obtaining anamnestic data and performing ultrasound examination. With the help of the ELISA methodology, cytokines were verified: TNF-α, IL-1α, IL-2, and IL-6 versus IL-4 and IL-10. **Results** Of the 100 patients examined, 21 patients developed clinical symptoms and were diagnosed with preeclampsia in the third trimester. The interaction of proinflammatory interleukins is in favour of a mutual increase, and a decrease in the values of antiinflammatory interleukins is a significant predictive parameter in the second trimester for the development of preeclampsia. The increase in IL-6 is the largest statistically significant variable in the prediction of preeclampsia. The correlation between IL-6 and pregnancy complications is also with statistical significance (p=0.012). **Conclusion** The impaired immune response can result in consequences such as multiple organic disorders that occur in the clinical preeclampsia syndrome and problems with fetal development. The benefit of analyzing cytokines is highly significant for early diagnose and prevention of further complications.

KEYWORDS preeclampsia, cytokines, prediction, complications

Introduction

Preeclampsia is a complex syndrome with multifactorial etiopathogenesis. It is the leading cause of peripartum morbidity and mortality in the world. It is manifested with hypertension and proteinuria after 20 weeks of gestation, and numerous clinical manifestations have been described.

Demonstrating this condition divided into stages, it has been studied that the placenta plays a primary role in the pathogenesis of preeclampsia.[1] In the first stage, infarct zones and reduced endovascular invasion of the cytotrophoblast and irregular trophoblastic remodelling of the spiral arteries has been detected.[2] Angiogenesis becomes inadequate due to the inability of the cytotrophoblast to change into an endothelial phenotype from an epithelial one, and due to its inability to reduce the expression of epithelial molecules and to accept the adhesional appearance of the endothelium (which is, in fact, pseudovasculogenesis).[3] This leads to placental ischemia. [4-5] The second stage is accompanied by abundant clinical symptomatology and the manifestation of preeclampsia. It is a consequence of affected

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target organs, caused by hypoperfusion and hypoxia, and by a different systemic inflammatory response. It is manifested with increased vascular permeability and glomerular endotheliosis. Severe preeclampsia is also associated with fetal consequences that range from small for gestational age to intrauterine growth restriction, premature birth, and fetal death. [6]

From an immunological point of view, the uterus in pregnancy is mainly colonized by cells of the humoral immune system that include most macrophages and natural killer uNK cells. About 50 to 70 % of the decidual endometrium is covered by uNK [7], 20 to 30% by macrophages, and 10 to 15% by T cells from CD45 + immune cells [8], only about 2% by dendritic cells and T regulatory cells.

The importance of uNK cells is in establishing control of uterine neoangiogenesis, control of remodelling of the spiral arteries, and giving an immune response to the fetal antigen when they are properly activated. Its inadequate activation promotes cytotoxicity, which in turn leads to chronic inflammation characterized by oxidative stress, proinflammatory cytokines, and autoantibodies. [9] The condition worsens as the pregnancy progresses, i.e., due to the excessive placental stimulation, the immune response exacerbates. Placental ischemia in preeclampsia occurs as a result of shallow trophoblastic invasion where proinflammatory CD 4 + T cells are elevated, and T regulatory cells are reduced.

TNF-α (tumor necrosis factor-alpha) usually is produced by placental trophoblast cells and fetoplacental macrophages. It participates in the regulation of endothelial expression of platelet growth factor, endothelin 1 and plasminogen activator inhibitor, inducing structural and functional alteration of endothelial cells. It reduces nitrite oxide synthesis by mRNA, which reduces acetylcholine-induced vasodilation and destabilization of eNOS mRNA, and increases the production of the potent vasoconstrictor endothelin 1 by increasing the expression of preproendothelin 1 mRNA. [10] With that, it contributes to the molecules to adhere between each other, which then attract leukocytes to attach on the vascular tissue and thus contribute to oedema and hypertension.

Elevated TNF and IL-6 values stimulate the production of ROS, endothelin 1, and AT1-AA, contributing to an increase in arterial tension as a cascade. Chatterjee et al. [11] confirmed that reduced values and their absence, specifically IL-10, exacerbated hypertension and endothelial dysfunction. [12] Genetically, in the study of Arngrimsson et al., the gene locus for preeclampsia has been determined to be on the 2p13 chromosome. [13]

Haram K. et al. research a population where it is found that a peak at the 12q chromosome level is associated with HELLP (hemolysis, abnormal values of hepatic enzymes, and thrombocytopenia) syndrome. [14]

In a chronic hypertensive variation, vascular remodelling occurs. Deficiency in the remodelling of the cerebral vascular flow results in the transfer of pressure to the cerebral microcirculatory vessels and causes vasogenic cerebral oedema. When microcirculation or cerebral haemorrhage occurs, circulating factors in the blood are in direct contact with neurons, specifically TNF-α, which causes neural hyper excitability and increased microglial activation.

Present circulating factors elevated in preeclampsia, with increased cerebral perfusion pressure due to impaired myogenic activity, result in cerebrovascular dysfunction, cerebral oedema, and excitation of neurons, resulting in neurological symptomatology of preeclampsia. [15]

The risk of complications from preeclampsia is the development of severe hypertension (10-15%), eclampsia (0.2-0.5%), HELLP (1-2%), placental abruption (0.5-2%), fetal growth restriction (10-12%) and fetal death (0.2-0.5%).[16]

Purpose

The aim is to detect the risk of developing preeclampsia in the second trimester, with the help of immunological biomarkers and to monitor the development of complications and their dependence on the values obtained.

Material and Methods

A total of 100 pregnant patients were followed between 14th and 20th weeks of gestation in an outpatient setting at the University Clinic for Gynecology and Obstetrics in Skopje, following a previously signed informed consent to participate in the study. It is a prospective cohort study. After taking anamnestic data and performing an ultrasound examination of fetal growth and evaluation of the uterine artery flow using the Doppler method, the patients were referred to the Institute of Immunobiology and Human Genetics at the Medical Faculty in Skopje. With the help of ELISA (enzyme-linked immunosorbent assay), the serum methodology of patients was verified with cytokines (TNF-α, IL-1α, IL-2 and IL-6 as proinflammatory versus IL-4 and IL-10 as anti-inflammatory).

Inclusion criteria were single-pregnancies in the second trimester from 14 to 20 gestational weeks, where routine ultrasound examination of the fetus was performed using the Mindray DC-7 and Voluson 8. The Doppler evaluation of the uterine artery was performed for the presence or absence of a notch in the uterine artery and/or a higher value of the pulsatility index. Patients without hypertension were examined.

Exclusion criteria include multiple gestations, proven chromosomopathies, and higher gestational age (over 20 gestational weeks).

Used program for processing the results was STATISTICA 12 and SPSS 21.0 for Windows. All diagnostic criteria for preeclampsia, such as blood pressure, laboratory parameters, and urine with the degree of proteinuria, ultrasound, were also examined. These parameters are needed to monitor the condition of the mother and fetus during pregnancy, including on time for the delivery, for proper management, and to monitor the development of complications from the disease.

Results

Out of 100 patients, anamnestic data were analyzed for age, parity, body mass index, positive family history, and preeclampsia in previous pregnancies. The result is statistically significant only concerning the anamnestic data for previous preeclampsia in pregnancy ($p < 0.05$).

Of all the patients tested, 21 patients developed clinical symptoms and were diagnosed with preeclampsia in the third trimester. They are classified according to the standard diagnostic criteria for preeclampsia, where the elevated blood pressure values, laboratory parameters in addition to hepatic and renal failure. The presence of proteinuria was statistically significant ($p<0.05$) compared with other patients who did not develop preeclampsia syndrome. With the ELISA methodology, the values of TNF-α, IL-1α, IL-2 and IL-6 are analyzed as proinflammatory versus IL-4 and IL-10 as anti-inflammatory cytokines.

The chart below (chart 1) shows the common variation of the mean values of all examined interleukins in preeclamptic and the other, healthy patients.

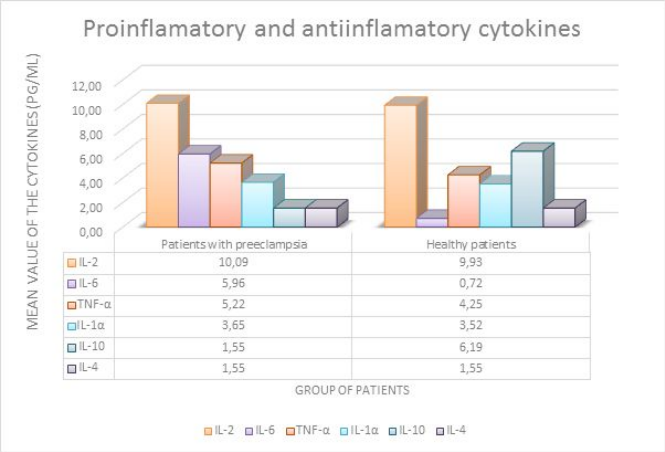


Chart 1: Correlation of proinflammatory versus antiinflammatory interleukins.

Mutual interaction of proinflammatory interleukins is in favour of a mutual increase, and at the same time, a decrease in the values of antiinflammatory interleukins is a significant predictive parameter in the second trimester for the development of preeclampsia. With the help of the ANOVA test and the multivariate logistic regression analysis, it can be seen that the increase in IL-6 is the largest statistically significant variable in the prediction of preeclampsia. The same is expressed in the following three statistical calculation procedures (table 1).

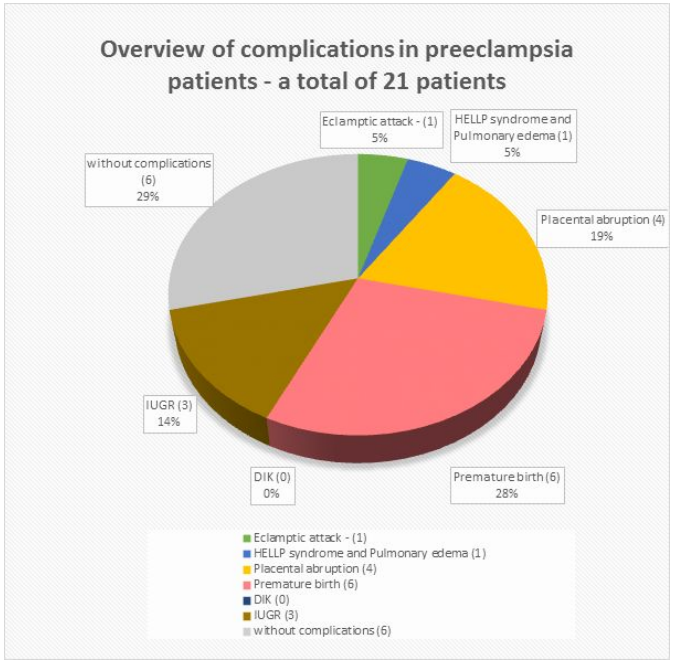
According to the three statistical procedures, the interrelationship of the dependent variable (patients who develop preeclampsia has been assessed as changing concerning the independent variable). It was concluded that of all interleukins with the highest statistical significance, IL-6 had a $p < 0.01$.

The pregnancy was followed until the delivery of the patients, where 15 of the patients with preeclampsia developed complications. They analyzed interleukins, depending on their severe clinical picture and the consequences of the primary preeclampsia syndrome, followed by a graphical representation of the following graph (graph 1).

The graph shows the percentage obtained of: preterm delivery with 28%, placental abruption both before and in term births with 19%. HELLP syndrome and pulmonary oedema develop in one patient or 5%. One patient developed an eclamptic attack. Regarding the condition of the fetus, intrauterine fetal growth restriction is 14%, and 29% of patients are without complications.

The following table (table 2) shows the gestational week of delivery, which means that premature births are more frequent in the interest of the mother's health and then the fetus, with a statistically significant result with $p = 0.0103$. In terms of body weight of the newborn, although fetuses were represented in ultrasound-verified growth restriction, results show the absence of statistical significance regarding the weight of the baby after the mothers' delivery.

The following table (table 3) shows that statistically significant is the interleukin IL-6 with $p = 0.012$. This is of great clinical significance, in terms of the values of previously verified cytokines, to see if their value is related to the severity of the developed complications. This is shown in table number 2.



Graph 1: Graphic presentation of complications in patients with preeclampsia.

It has been shown that there is a link between the changed values of proinflammatory and antiinflammatory proliferation as an impaired immune response reflects the consequences and multiple organ disorders that occur in the clinical syndrome of preeclampsia.

Discussion

Patients with preeclampsia may recover postpartum. However, in some patient, the consequences on the cardiovascular system have been established for up to five years postpartum.

Devika Tayal, [17] as well as our study appropriately confirm the increased values of proinflammatory cytokines in preeclampsia patients compared with a group of normotensive patients. Results are reported also by Founds et al. [18] According to him, TNF-α is actually the most potent for this disease, modifying growth, differentiation, lipid metabolism, coagulation, insulin resistance, and inflammation. The result is structural and functional disorders of the endothelium, reduced acetylcholine induced vasodilation and destabilizes eNOS mRNA. [18]

Similar to our research results, Page et al. [19] and Tosun et al. [20] verified high values of IL-6 and TNF in patients with preeclampsia.

Siddiqui et al. discovered the presence of agonist autoantibodies to the angiotensin type 1 receptor (AT1-AA). [21] Their amount correlated with the severity of the clinical condition in the mother.

Jensen et al. reveal that CD19 (+) CD5 (+) of the B lymphocyte population is a potential source of AT1-AA and is significantly higher in preeclampsia in advanced pregnancy. [22]

Krasnyi AM et al. in 2018 made a detailed and complex analysis of the ratio of total and fetal DNA to cytokines, which resulted in a significant increase in IL-6 value in preeclampsia and the same values insignificant correlation with fetal extracellular DNA in mothers with preeclampsia. [23]

The sensitivity and specificity of Doppler as a predictive method for risk of preeclampsia in the low-risk population

Table 1 Statistical procedures analyzing cytokines. Statistical procedure 1. Summary value.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	0.452[a]	0.204	0.160	0.45976
a) Predictors: (Constants), IL-2, IL-4, IL-6, IL-10, TNF-α				

Statistical procedure 2. ANOVA for analyzing the variations of interleukins at preeclampsia

Model	Sum of squares	Df	Mean square	F	P significance
Regression	4.882	5	0.976	4.619	0.001*
Residual	19.024	90	0.211		
Total	23.906	95			
*The result is significant at p<0.05 Dependent variable: patients with preeclampsia. Predictors (Constant): IL-2, IL-4, IL-6, IL-10, TNF- α					

Statistical precedure 3. Coefficients of multivariate logistic regression analysis of interleukins in preeclampsia.

Model		Unstandardized Coefficients		Standardized Coefficients	T	P
		B	Std. Error	Beta		
1	(Constant)	1.817	0.360		5.044	0.000
	TNF-α	0.008	0.041	0.020	0.197	0.844
	IL-6	-0.072	0.016	-0.432	-4.351	0.000*
	IL-10	0.002	0.002	0.122	1.279	0.204
	IL-4	-0.010	0.008	-0.124	-1.313	0.192
	IL-2	-0.017	0.033	-0.050	-0.519	0.605
*The result is significant at p<0.05 Dependent variable: patients with preeclampsia						

Table 2 Demonstration of patients - delivery characteristics.

Characteristics	Preeclampsia	Mean	Healthy patients	Mean	SD	T	P
Gestational week of delivery	32-38.6	34.4	34.2-40.3	38.3	3.5	4.56	0.0103*
Weight of the newborn	1150-4200	2802	2110-3880	3006	220.5	1.1333	0.2705

*The result is significant at p<0.05.

Table 3 Dependence of interleukins on pregnancy complications with preeclampsia

T-Test Calculator for 2 Independent Means						
Group of patients	21-patients with preeclampsia		15-preeclamptic patients with complications			
	Mean	SD	Mean	SD	T	P
IL-6	2.55	130.84	5.38	299.91	2.35	0.012*
TNF-α	4.79	54.13	5.02	27.13	0.44	0.332
IL-10	0.97	78	1.75	141.95	0.9	0.186
IL-1α	3.76	18.4	3.71	3.55	0.2	0.421
IL-4	4.37	3349.9	1.55	0	0.84	0.203
IL-2	10.14	42.71	10.23	11.8	0.22	0.413

*The result is significant at $p<0.05$.

reaches from 34% to 76%, but with the implementation of biological immune markers of the mother serum, sensitivity and specificity reaches up to 83% to 93%. It has a significant predictive value and is more valuable in detecting early preeclampsia and intrauterine growth restriction than in late preeclampsia. This selects patients who are candidates to start anti-aggregation therapy to prevent preterm preeclampsia. The idea is to ensure proper circulation to avoid possible vascular complications as a result of preeclamptic pregnancies.

Limitations of the study

In terms of immunotherapy, the experimental evaluation of a strategy to increase Treg cells in the human reproductive system must have a very cautious approach and be based on creative principles for clinical trials. Possible side effects that may need to be considered from artificially enhanced maternal Treg cells include reduced pathogenic defence or even reduced immune defence against malignancy. Regardless of the essential work that needs to be done to assess alternative approaches and identify responsible patient groups, there is an imperative to invest in developing immunotherapy options in order to reduce morbidity and mortality associated with preeclampsia if there is sufficient financial support.

Conclusion

Based on the obtained results, it is proposed to continue the research earlier in the pregnancy of asymptomatic patients. Maternal risk factors should be considered, followed by uterine artery Doppler ultrasound in all pregnant patients between 14th and 20th weeks of gestation. It is necessary to determine the values of the significant interleukins in order to predict, diagnose, treat, and manage preeclampsia. The aim of our study was to detect the risk of preeclampsia syndrome earlier to prevent developing complications. In the future, the application of interleukins will arouse interest in their implementation in therapeutic protocols. Exogenous IL-10 treatment has appropriate beneficial effects on endothelial function and is considered to be a possible therapeutic modality appropriate for preeclampsia.

Conflict of interest

The authors declare no conflict of interest.

Patient informed consent

Details relating to individual participants included in the manuscript have received all participants' consent.

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Ethics committee approval

I hereby declare that this article has full compliance with consent committee. The approval was taken before submission of the manuscript.

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