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IMMUNOGENETIC ASPECTS OF THE ORIGIN OF PREECLAMPSIA

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ABSTRACT

The combinations of genes of the fetal HLA-C and the maternal KIR affects the pregnancy outcome.

Objective: to examine patients in second trimester of pregnancy by predicting that a change in the immune response may be demonstrated before the development of preeclampsia symptomatology.

Material and Methods: For the purpose of this study, 100 patients were examined in the second trimester, at the University Clinic of Gynecology and Obstetrics and at the Institute of Immunobiology and Human Genetics in Skopje, which analyzed serum levels of cytokines, proinflammatory compared to antiinflamatory antibodies.

Results: The results were obtained using ELISA methodology. Of the above 100 patients, 21% developed clinical preeclampsia syndrome. In them, there was a change in values in addition to an increase in TNF - α, IL-6, IL-2, IL-1α at the same time a tendency for a decrease in IL-10 was observed. IL-4 showed no variation in its value.

Conclusion: In our research paper, proinflammatory interleukin TNF-α increases, and by means of Pearson's correlation between variables it is verified that not only does it grow, but it grows concurrently with IL-6, which is a statistically significant result, or $p < 0.05$. IL-1α increases concurrently with TNF-α, which corresponds to the following studies in support of our study.

Key words: immunogenic aspects, prediction of preeclampsia, interleukins

INTRODUCTION

In healthy pregnancies, reduced Th1/Th2 ratio in favor of higher Th2 cell counts in the peripheral circulation of the mother maintains an immune tolerance to the fetus (1).

Immune balance is the most important and from its maintenance derive excreted circulatory cytokines, both proinflammatory and antiinflammatory (2).

The major histocompatibity complex MHC I class over expressed by extravillous trophoblast, interacts directly with numerous receptors for natural killer cells (uNK) and thereby transmit either inhibitory or activating signal to their cytotoxicity and production of cytokines (3).

The MHC I class express a unique combination of the classical HLA-C (human leukocyte antigen) and the nonclassical HLA-E, HLA-F and HLA-G class I ligands each with their respective roles in the immune acceptance of the fetus (4). HLA-C promotes cell degranulation and secretion of granulocyte macrophage colon-stimulating factor (GM-CSF) and tumor necrosis factor (TNF). HLA-E has a role in early implantation from the 5th to 7th weeks of gestation. HLA-G has a controlling or that is a restrictive role on the cytotoxic effect of uNK cells, i.e. it promotes immunotolerance by inhibiting the role of proinflammatory cytokines including IFN- γ, TNF, IL-1α, IL-6 (5).

uNK cells are responsible for producing cytokines and several growth factors, but are also responsible for

producing multiple factors whose receptors are located precisely on the primary extravillous trophoblast. For example, uNK cells produce high levels of IL-8, INF-, TNF, TGFB1, CXCL10 as well as angiogenic factors such as VEGF-A, VEGF-C, and PGF (6). Maternal-fetal interphase produces receptors for these ligands by the extravillous trophoblast itself. The IL-8 receptor is CXCR1, the CXCR3 is CXCL10 receptor (7), while TNFR1 as well as VEGFR-1 and VEGFR-3 later bind to VEGF-A and VEGF-C respectively (8). TNF and IFN- have the potential to inhibit trophoblast migration and invasion by inducing an increase in PAI expression and promoting MMP-induced proteolysis (9).

Macrophages, on the other hand, are divided into two groups: M1, representing the proinflammatory group, more specifically secreting IL-6, TNF and IL-8, as well as the M2, i.e. the anti-inflammatory phenotype with its typical M2 markers as CD209 and CD206 that directly secrete IL-10 and TGF- (10). Decidual macrophages have the ability to suppress T cell activity and induce T regulatory cells. The association of HLA-G homodimers with macrophage-associated leukocyte immunoglobulin like receptor B1 (LILRB1) has been shown to increase secretion of IL-6, IL-8, and TNF.

There is some evidence to suggest that in cases of preeclampsia, polarization to the M1 type of macrophages is likely to exacerbate proinflammatory cytokine production (11).

In contrast, M2, i.e., anti-inflammatory cytokines such as IL-10 and TGF-, restrict extravillous trophoblast to its migratory potential.

Disruption of the KIR (killer-cell immunoglobulin-like receptor) interaction with uNK cells with HLA-C on the interstitial trophoblast gives an abnormal decidual immune response resulting in inappropriate remodeling of the spiral arteries (12). It is presented on Figure 1. Vinketova et al. (2016). Human Decidual Stromal Cells as a Component of the Implantation Niche and a Modulator of Maternal Immunity (25)

In preeclampsia, there is a limited invasion of the spiral arteries only to the superficial layers of the decidua.

Failure of trophoblast invasion results in decreased uterine perfusion pressure and consequently placental ischemia.

In that function, blood samples as well as placenta samples at genetic level are analyzed in details.

Numerous homologous KIR genes are mapped to the 19q chromosome and the two basic genetic clusters are classified as haplotypes A and B. The first type encodes KIR, thus inhibiting natural killer cells, while the second type stimulates it.

Preeclampsia is more common in homozygous and inhibitory A haplotypes (AA) than in homozygous B (BB) (13).

The HLA-G gene polymorphism is a gene located at 6p21.3 and belongs to the HLA1 class and has an immunosuppressive effect. It is an important feature implicated in modulating the mother's immune system in terms of inhibiting pregnancy when the mother makes contact with the fetus. It is associated with recurrent miscarriage and preeclampsia (14).

Trophoblast expression of CD200 and CD200R promotes the production of inflammatory cytokines in the preeclamptic placenta (15).

In preeclampsia, trophoblast produces significantly more TNF-, sTNFR-1, IL-6, and IL-8, as well as significantly less IL-10, compared to trophoblast in normal placenta. That is, reduced regulation of CD200 expression results in an imbalance of elevated Th1 cytokines and decreased Th2 cytokines in the production of placental trophoblast in preeclampsia.

The risk of complications from preeclampsia during conservative (expectative) treatment is development of severe hypertension (10-15%), eclampsia (0.2-0.5%), HELLP (hemolysis, elevated liver enzymes, thrombocytopenia 1-2%), abruption of the placenta (0.5-2%), fetal growth restriction (10-12%), and fetal death (0.2-0.5%) (16).

In contrast, emergency delivery is associated with preterm neonates and thus the need for stay and treatment at the intensive care unit, neonatal respiratory complications and an increase in neonatal mortality.

With the hypothesis that a change in the immune response may be demonstrated before the development of preeclampsia symptomatology, patients in the second trimester were examined.

MATERIAL AND METHODS

For the purpose of this study, 100 patients were examined in the second trimester, at the University Clinic of Gynecology and Obstetrics and at the Institute of Immunobiology and Human Genetics in Skopje, which analyzed serum levels of cytokines, proinflammatory

compared to antiinflamatory antibodies. Patients signed informed consent to participate in the study. Important anamnestic data were obtained and an ultrasound examination was performed.

The results were obtained using ELISA methodology. Of the above 100 patients, 21% developed clinical preeclampsia syndrome. In them, there was a change in values in addition to an increase in TNF - , IL-6, IL2, IL-1 at the same time a tendency for a decrease in IL-10 was observed. IL-4 showed no variation in its value. A statistically significant result was obtained, with $p < 0.05$ in relation to the values obtained from patients who were affected, compared to those who did not develop clinical signs of preeclampsia. It is presented in Table 1: Results from the examined interleukins, with correlation of proinflamatory and antiinflamatory and in the Table 2: Pearson correlation coefficient in women with developed clinical preeclampsia syndrome..

The sensitivity and specificity of the interleukins were calculated individually, sensitivity of TNF- -91% and specificity of 41%, IL-6 85% with 40% respectively, IL-4 46/49%, IL-10 95/25 %, IL-1 83/29%, IL-2 77/35%.

Among them, i.e., the mutual increase of proinflammatory cytokines is 78-91.2% sensitivity for a predictive parameter.

DISCUSSION

Considering feto-maternal microchimerism as clearly recognized in normal pregnancy, fetal cells are those that continuously induce maternal immune activation verified by detecting anti-fetal HLA antibodies in the mother's serum during pregnancy. The fetal cells themselves are clearly separated from the mother's immune system and are contacted by the fetal extravillous trophoblast which in turn has a low antigenic effect due to poor expression by classical MHC class I (except HLA-C) and MHC class II.

Fetal antigens are presented through the maternal antigen presenting cells (APCs) of the fetal maternal interphase, that is, the decidua. In fact, up to 50% of the decidual cells make up the maternal immune cells. Therefore, the decidua is an important site of events where the maternal immune system encounters fetal antigens and creates a mechanism of tolerance. It is therefore not surprising that recurrent miscarriages as well as preeclampsia occur due to impaired immune tolerance.

The most commonly studied variant of preeclampsia is the -308G> A transition region of the promoter region, which is associated with increased production of TNF- and increased risk of preeclampsia, but also diabetes mellitus type II, coronary artery disease and dyslipidemia.

On the other hand, variation in IL-10 values in the pathogenesis of preeclampsia have been investigated, providing an appropriate inflammatory response to trophoblast cells resulting in appropriate invasion and remodeling of the spiral arterioles.

Studies have suggested that preeclampsia may be a consequence of the development of cardiovascular disease, renal disease, several years after the end of pregnancy (17).

Increased values of microalbuminuria up to 5 years after pregnancy have been demonstrated in women with preeclampsia. This finding is compatible with the presence of underlying unrecognized renal disease or the damaging effect of preeclampsia on the kidney (18).

It has been diagnosed so far when symptoms of hypertension, proteinuria, deviations in laboratory parameters in addition to an increase in degradative products, and a decrease in protein derivates in the blood and the presence of proteinuria, subsequently rich symptomatology of sight disorder, have been developed. Edema, develops or worsening of the mother's condition or endangerment of the fetus.

Many authors and colleagues appreciate Professor Redman as one of the founders of the understanding of the etiology, pathology, diagnosis and management of preeclampsia. The importance of the immune system and the presence of immune factors Redman analyzes in detail (19). The inflammatory response according to him, is induced by placental particles, ranging from large deposited multinuclear fragments to subcellular fragments distributed along the placenta surface. Changes in the number and magnitude of syncytrophobic exosomes and microscopic dimensional damages of the blood vessel are very important in maternal preeclampsia syndrome. Yanfang Guo is al. through numerous studies elaborates the immunological base as a trigger in the maternal systemic circulation (20). Walker JJ elaborates on the same topic, arguing that it is a failure or deficiency in the normal defense mechanism to the fetus. Interleukins such as IL-6, IL-8, and TNF- co-grow with lipid peroxidase, proving their monocyte origin (20). Stimulated monocytes produce free radicals that cause oxidative damage. Maternal cells are protected from plasma and intracellular oxidants.

The very imbalance between oxidants and antioxidants and subsequent change in membrane oxidation leads to instability of membrane permeability which is the basis of clinical manifestations of preeclampsia. To modify both genetic modification and differentiation in the production of TNF-α and nitric oxide enables modification in the development of the disease (21).

In our study, proinflammatory interleukin TNF-α increases, and by means of Pearson's correlation between variables it is verified that not only does it grow, but it grows concurrently with IL-6, which is a statistically significant result ($p < 0.05$). IL-1α increases concurrently with TNF-α, which corresponds to the following studies in support of our study.

Siddiqui et al. have found the presence of agonist autoantibodies to the angiotensin type 1 receptor (AT1-AA). (22) Their amount correlates with the severity of the mother's clinical condition. Jensen et al. find that CD19 (+) CD5 (+) from the B lymphocyte population are a potential source of AT1-AA and are significantly higher in preeclampsia and in advanced pregnancy (23).

Krasnyi AM et al. in 2018 performed a detailed and complex analysis of the ratio of total and fetal DNA to cytokines, which resulted in a significant increase in IL-6 in preeclampsia and the same values are in significant correlation with fetal extracellular DNA in mothers with preeclampsia (24).

CONCLUSION

In preeclampsia, an increased amount of circulating proinflammatory cytokines activate the endometrium, provoking exacerbation of the systemic immune response.

The immunogenetic aspects of the origin of preeclampsia are very important and they need to be emphasized and to be in the final results of the investigation of this condition. In view of the above, there is a need for additional methods in the early diagnosis of pregnant women with preeclampsia before it is even manifested.

Predictive importance is perceived in order to prevent the pathological condition by appropriately prescribing therapy, counseling, a hygienic diet and careful monitoring of the health of both the mother and the fetus.

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Table and Legends

Table 1: Results from the examined interleukins, with

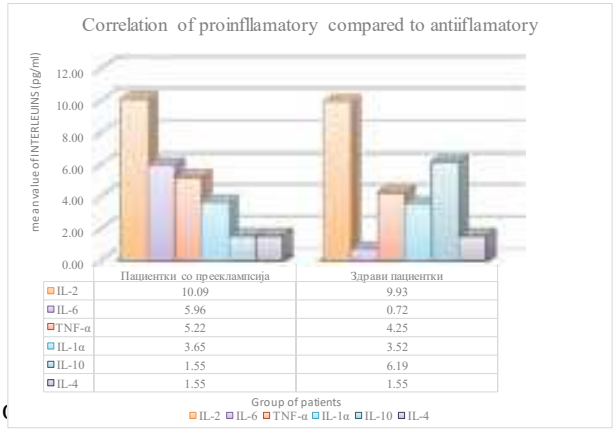

Figure 1. Vinketova et al. (2016). Human Decidual Stromal Cells as a Component of the Implantation Niche and a Modulator of Maternal Immunity (25)

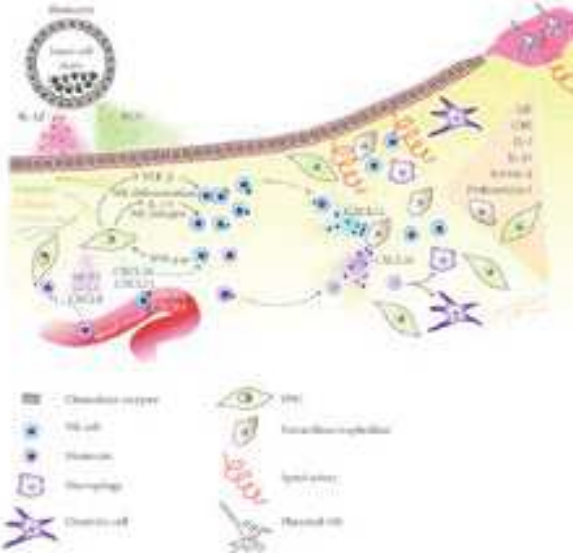

Table 2: Pearson correlation coefficient in women with developed clinical preeclampsia syndrome.

		TNF-α	IL-6	IL-10	IL-1α	IL-4	IL-2
TNF-α	Pearson correlation coefficient	1	0.256	-0.053	0.505	.b	-0.138
	P significance		0.305	0.833	0.032	.	0.586
	Number	21	21	21	21	21	21
IL-6	Pearson correlation coefficient	0.256	1	-0.356	-0.193	.b	0.273
	P significance	0.305		0.146	0.442	.	0.274
	Number	21	21	21	21	21	21
IL-10	Pearson correlation coefficient	-0.053	-0.356	1	0.004	.b	0.016
	P significance	0.833	0.146		0.987	.	0.950
	Number	21	21	21	21	21	21
IL-1α	Pearson correlation coefficient	0.505	-0.193	0.004	1	.b	0.392
	P significance	0.032*	0.442	0.987		.	0.107
	Number	21	21	21	21	21	21
IL-4	Pearson correlation coefficient	.b	.b	.b	.b	.b	.b
	P significance	.	.	.	.		.
	Number	21	21	21	21	21	21
IL-2	Pearson correlation coefficient	-0.138	0.273	0.016	0.392	.b	1
	P significance	0.586	0.274	0.950	0.107	.	
	Number	21	21	21	21	21	21