

Correlation between IL-6 (Interleukin 6) and Laboratory Parameters (CRP, Total Proteins and LDH) for Verification of the Inflammatory Origin in Preeclampsia

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Abstract

In normal pregnancy there is a transfer to the Th2 anti-inflammatory type of immunity, directed toward the fetus and the placenta, whereas in pregnancy complications, such as preeclampsia due to absent transfer, Th1 or proinflammatory immune system is dominated. This article analyzes what are the most important circulating biomarkers created by syncytiotrophoblast that together combined and promote oxidative stress. An increase in proinflammatory and decline in the antiinflammatory cytokine is an imbalance that explains the theory that due to impairment of the immunological response, consequences on the mother's circulation are developed and are reflected in the manifestation of pregnancy complications such as preeclampsia and intrauterine growth restriction.

The purpose of the study is to emphasize the importance of the results of the correlation between interleukin 6, measured in the serum of patients from 14 to 20 gestational weeks, with laboratory parameters that are pathognomonic for preeclampsia (LDH, total protein and CRP). This explains the immunological and inflammatory response in preeclampsia serving as a predictor of the condition.

For that purpose, the materials and methods that were used were 100 patients examined at the University Clinic of Obstetrics and Gynecology, divided in two groups. The first or examined group consists of 50 patients with notch of the uterine artery present in the second trimester from 14 to 20 gestational weeks. The second, or control group consists of 50 patients with absent notch of the uterine artery. These patients are then referred to the Institute of Immunobiology and Human Genetics at the Faculty of Medicine in Skopje to examine the levels of cytokines, in particular the relationship between the proinflammatory with antiinflammatory cytokines (TNF- α , IL-6 vs. IL-10) using ELISA (enzyme-linked immunosorbent assay) methodology, with Magnetic Luminex Assay multiplex kit.

The results between the examined and the controlled group verified an increase in proinflammatory (TNF- α and IL-6) and a decrease in the antiinflammatory cytokine (IL-10), from which the variance of the level IL-6 showed important statistical significance $p < 0.01$. Positive correlation was of great statistical significance between levels of cytokine IL-6 and increased levels of LDH and CRP, and with decreased levels of the total proteins of the preeclamptic patients. (LDH $p < 0.00001$, CRP $p < 0.025$ and total proteins $p < 0.0012$).

The role of the inflammatory response is obtained by the correlation of the interleukins in the second trimester and laboratory examinations in the third trimester as a sufficient predictive method in detection in early stages the preeclampsia syndrome.

Keywords: Preeclampsia; prediction; interleukins, predictive metod.

1. Introduction

Preeclampsia is a hypertensive disorder in pregnancy, followed by proteinuria, after the 20th gestational week with multifactorial etiology. The etiopathogenesis includes genetic, immunological, hematological, angiogenic theory, induced oxidative stress, maternal endothelial dysfunction and inappropriate remodelling of the spiral arteries. These contributes to the development of ischemia and hypoxia which subsequently induce promotion of bioactive substances and immunological biomarkers.

There is a complex relationship persisting between immunology, inflammation and preeclampsia. That connection is presented in pregnancy through adaptation of the immunological response needed for the mother to tolerate the different immunological system of the fetus throughout the entire pregnancy. Laura A. Mageea et al. (2014) stated that preeclampsia can be result of an inflammatory cause [1].

In the process of trophoblast invasion, the decidua, which forms the mother's side of the placenta, contains a large number of immune cells necessary for proper migration of trophoblast cells. Macrophages, NK (natural killer) cells, DC (dendritic cells), T regulatory cells are present in the decidua and are required for normal invasion of the trophoblast cell for placentation [2, 3].

These cells jointly infiltrate the decidua and together with the trophoblast cells make a conglomerate allowing it to reach the endometrium through controlled removal of the spiral artery's native cells. Uterine NK cells, T regulatory cells and regulatory cytokines provide adequate control and function of proinflammatory cells in their activity to ensure proper invasion. Namely, DC cells from the decidua through their function promote Th2 cells to make the mother immunotolerant to the fetus. In summary, immune cells in pregnancy produced by the decidua have the role of providing proper implantation and promoting trophoblast invasion, which is neither superficial nor overly invasive. This is achieved by the production of cytokines and angiogenic factors, which are necessary for normal pregnancy [4].

Damage to this process, especially in the myometrial part of the blood vessels, is predisposed to complications such as preeclampsia, intrauterine growth restriction, preterm birth or fetal death [5]. It is manifested with increased resistance in the blood flow and appearance of notch of the uterine artery.

In order to properly invade the walls of the myometrium, the distal column cytotrophoblast expresses a number of extravilial trophoblast markers such as HLA-G, T cell factor 4 (TCF-4), integrin $\alpha 5$ (ITG $\alpha 5$) and $\beta 1$, Notch2, proteoglycan 2 and ErbB2 [6].

Taking a genetic overview, TNFSF13B is a member of tumours necrosis factor ligands. It is located on chromosomal region 13q32-q34 and is responsible for regulating the immunological response to infections, autoimmune diseases and inflammation. It is assumed that it modulates the mother's immunological tolerance to the fetus.

TLRs (toll-like receptors) are involved in host defence against external microbes. They are central components of the humoral immune system. The allelic variants of the humoral immune system TLR-4 (toll-like receptor-4) and the NOD2 gene (apoptosis regulator) are correlated with high IL-6 values in patients with early onset preeclampsia. They also have immunosuppression of the T regulatory cells [7].

TNF- α (tumour necrosis factor α) stimulates the production of angiotensin II in the female reproductive system, while in combination with IL-6, it participates in increased production or expression of angiotensin II type 1 receptors in the smooth muscle of blood vessels [8].

IL-6 (interleukin 6) is an equally important proinflammatory cytokine, produced by mononuclear phagocytes, endothelial cells, fibroblasts, and T cells and is involved in immunological activation, i.e., the function of the vascularisation in the modulation of the immunological response. The imbalance caused by the increase in proinflammatory and decline in the antiinflammatory cytokine explains the theory that due to impairment of the immunological response develops overall consequences on the mother's circulation which are reflected in the manifestation of pregnancy complications such as preeclampsia and intrauterine growth restriction.

In preeclampsia, due to placental ischemia and consequently present endothelial dysfunction, the balance of their proper synthesis and function is disturbed. Increased levels of the proinflammatory cytokines correlate with endothelial cell permeability, manifested by increased blood pressure and decreased renal function (pathologically renal tissue is glomerulosclerotic, glomeruli show hardened outlook with major constriction or occlusion). The capillary endothelium is edematous with thickening of the capillary basal membrane and enlargement of interstitial spaces with intense edema as proven by Sien Yee Lau et al. [9].

2. Purpose

There is a correlation between the increased value of interleukin 6, proinflammatory cytokine, measured in the serum of patients from 14 to 20 gestational weeks, and laboratory parameters that are divergent and are pathognomonic for preeclampsia (LDH, total protein and CRP). The purpose of this study is how reflects the immunological and inflammatory response in preeclampsia serving as a predictor and signal for her preventive treatment.

3. Materials and Methods

The study is a prospective cohort, comprising of 100 patients examined at the University Clinic of Obstetrics and Gynecology, who have previously written informed consent to participate in the study. They are divided into two groups:

The first or examined group consists of 50 patients with notch of the uterine artery present in the second trimester from 14 to 20 gestational weeks as the main inclusion criterion. The second, or control group consists of 50 patients with absent notch of the uterine artery. The inclusion criteria are the presence of a notch of the uterine artery, and the exclusion criteria are twin pregnancies, chromosomal abnormalities, and dead fetuses.

These patients are then referred to the Institute of Immunobiology and Human Genetics at the Faculty of Medicine in Skopje, for analysis of circulating immune biomarkers in the patient's serum, i.e. to examine the levels of cytokines, in particular the relationship between the proinflammatory with antiinflammatory cytokines (TNF- α , IL-6 vs. IL-10). They are developed by ELISA (enzyme-linked immunosorbent assay) methodology, using the Magnetic Luminex Assay multiplex kit (figure 1).

The results were subjected to statistical analysis and processing with computer programs: STATISTICA 12 and SPSS 21.0 for Windows. On this basis, tests for significance of differences between the comparison groups of all the parameters analyzed were performed.

Patients are monitored further in the third trimester if they will develop clinical symptomatology for preeclampsia verified by basic diagnostic criteria, and laboratory deviations are compared and set in correlation with the results of the cytokines obtained.

With the correlation, the idea is to see how deviations in the second trimester in the ratio of the interleukin values can be a predictive parameter for the development of preeclampsia.



Figure 1: Kit used for development of interleukins

4. Results

The results of the two investigated groups, examined (48) and control (48) patients, and were processed. Four patients (2 from the examined group and 2 from the control group) left the study for medical malcondition and pregnancy loss.

Anamnestic data such as age, positive family history, parity, BMI, pre-comorbidities, preeclampsia in previous pregnancy were analyzed and a statistical significance was found only in relation to the anamnestic data of previous pregnancy with preeclampsia ($p < 0.05$). Comparison of the other parameters showed no statistically significant result.

Using the ELISA methodology, values of cytokines (TNF- α IL-6 versus IL-10) were obtained from both the control and the examined group to obtain appropriate conclusions.

Table 1: Analysis of interleukins between the examined and control groups (standard deviation and standard mean error)

| Group of patients | Number | Mean Value | Std. Deviation | Std. Error Mean |
|---------------------------|--------|------------|----------------|-----------------|
| Examined (TNF- α) | 48 | 4.4965 | 1.42655 | 0.21266 |
| Control (TNF- α) | 48 | 4.2547 | 0.98405 | 0.13779 |
| Examined (IL-6) | 48 | 3.2177 | 4.00221 | 0.59661 |
| Control (IL-6) | 48 | 0.7166 | .53494 | 0.07491 |
| Examined (IL-10) | 48 | 1.3943 | 3.49554 | 0.52108 |
| Control (IL-10) | 48 | 6.1853 | 40.28594 | 5.64116 |

TNF- α in patients in the examined group, i.e. those with verified notch on the uterine artery, ranged from the lowest 1.59 to the highest 8.29 pg/ml with a mean of 4.49 and SD of 1.42 which is 5% higher value than the control group.

Then, IL-6 in the patients of the examined group variates in span from 0.64 at the lowest to 15.25 pg/ml, as the highest with a mean value of 3.2 pg/ml and SD of 4.0 which is three times higher value than the control group.

The IL-10 in the examined group is presented with a mean value of 1.39 pg/ml with SD 3.49, ranging from 0.54 to 20.70 pg/ml which in comparison with the control group has a tendency of a sharp decrease with five times lower value.

Using the T-test for significance of the differences between the two comparison groups (with and without notch), the following table shows that the only statistically significant result is in the value of IL-6, ($t = 4.422$, $p < 0.01$), where it is evident that patients who have notch from the examined group have higher IL-6 values than patients from the control group.

No statistically significant differences were observed among the other parameters ($p > 0.05$).

Table 2: P-test for significance and T-test for significance of differences between test and control group

| | T-test for significance of differences | | | | | | |
|---------------|--|--------|---------------------------------------|--------------------|--------------------------|--|---------|
| | t | Df | P-test (Significa t -(2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | Lower | Upper |
| TNF- α | 0.976 | 94 | 0.332(NS) | 0.24182 | 0.24777 | -0.25014 | 0.73379 |
| | 0.954 | 76.792 | 0.343(NS) | 0.24182 | 0.25340 | -0.26278 | 0.74643 |
| IL-6 | 4.422 | 94 | 0.000* | 20.50116 | 0.56568 | 1.37799 | 3.62433 |
| | 4.160 | 45.388 | 0.000* | 2.50116 | 0.60130 | 1.29037 | 3.71195 |
| IL-10 | -0.795 | 94 | 0.429(NS) | -4.79099 | 6.02910 | -16.76192 | 7.17994 |
| | -0.846 | 50.853 | 0.402(NS) | -4.79099 | 5.66518 | -16.16510 | 6.58312 |

Statistical analysis using multivariate regression analysis of the three obtained statistical procedures revealed that, of all the interleukins that differ, IL-6 had the highest statistical significance at $p < 0.01$.

Table 3: Statistical analysis, coefficients of multivariate logistic regression analysis of interleukins in preeclampsia

| Model | | Unstandardized Coefficients | | Standardized Coefficients | T | P significance |
|-------|---------------|-----------------------------|------------|---------------------------|--------|-------------------|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | 1.817 | 0.360 | | 5.044 | 0.000 |
| | TNF- α | 0.008 | 0.041 | 0.020 | 0.197 | 0.8449(NS) |
| | IL-6 | -0.072 | 0.016 | -0.432 | -4.351 | 0.000* |
| | IL-10 | 0.002 | 0.002 | 0.122 | 1.279 | 0.204(NS) |

*The result is significant at $p < 0.05$

(NS) The result is not significant at $p > 0.05$

Dependent variable: patients with preeclampsia

Based on the above, the patients were examined in the third trimester, of which preeclampsia was developed after 31 gestational weeks. Of the 48 patients, 32 had abnormalities and interleukin imbalances, and 21

developed clinical preeclampsia syndrome. The other 11 patients had other comorbidities and a false positive result.

Regarding systolic, diastolic blood pressure, lactate dehydrogenase, C-reactive protein, total proteins, proteinuria and 24-hour proteinuria, there was a statistically significant result in the $p < 0.05$ groups studied.

Correlation between IL-6 as the most significant predictive parameter of the statistical multivariate regression analysis and some of the deviations in laboratory parameters in addition to preeclampsia were made that resulted in statistical significance.

The aim is to establish the fact that previously raised values of proinflammatory interleukins in the serum in the second trimester correspond to the values of laboratory parameters that are stigmatized for advanced preeclampsia in the third trimester.

Table 4: Correlation between IL-6 with LDH, CRP, and Total Proteins

| Correlation | | LDH | CRP | Total proteins |
|-------------|------------------------------------|-----------|-----------|----------------|
| IL-6 | Pirsons coefficient of correlation | 0.9108 | 0.486 | -0.6563 |
| | P – significance | <0.00001* | 0.025501* | 0.001242* |
| | Number of patients with PE | 21 | 21 | 21 |

*The result is significant at $p < 0.05$

From the results shown in Table 4. where the Pearson coefficient of correlation between the variables is used, it can be concluded that in preeclampsia patients with increasing IL-6 value and growth of the LDH value (0.91) show a statistically significant result with $p < 0.001$, i.e. there is a positive correlation between them.

The same is obtained by the correlation between the increase in IL-6 values and the increase in CRP values, which is an explanation that the etiology of preeclampsia is dependent on the patient's immune response (0.48 i.e. $p < 0.025$).

Conversely, showing a correlation between increased IL-6 and decreased total protein results, which is in favour of hypoproteinemia in preeclampsia, which is also a statistically significant result ($p < 0.0012$).

The course of pregnancies and birth has been followed, i.e. how many patients have developed maternal and fetal complications according to the urgency and measures taken for a better perinatal outcome. That is a subject of other study.

5. Discussion

Immunological modulators represent a direct complement for early detection of preeclampsia. Its symptoms of hypertension, proteinuria, deviations in laboratory parameters in addition to an increase in degradation products,

and a decrease in protein derivatives in the blood and the presence of proteinuria have been diagnosed. Subsequently rich symptomatology from sight disorder, edema to fulminant endangerment of the mother's condition or endangerment of the fetus have been diagnosed. 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 immunological system and the presence of immune factors Redman analyzes in detail [10].

According to him, the inflammatory response is induced by placental particles, ranging from large deposited multinuclear fragments to subcellular fragments distributed along the surface of the human placenta. Changes in the number and magnitude of syncytiotrophoblastic exosomes and blood vessel damage with microdimensions are very important in maternal preeclampsia syndrome. Yanfang Guo et al. through numerous studies elaborate the immunological base as a trigger factor in the maternal systemic circulation [11]. Walker JJ writes on the same topic, stating that it is a failure or deficiency in the normal defence mechanism to the fetus. Interleukins such as IL-6, IL-8, and TNF- α grow at the same time with lipid peroxidase, proving their monocyte origin [11]. 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. Genetic modification and change in differentiation in the production of TNF- α and nitric oxide, also modifies the development of the disease [12].

In our study, proinflammatory interleukin TNF- α is increased, as well as nitric oxide and by Pearson correlation between the variables it is verified that not only does it grow, but it grows at the same time with IL-6, $p = 0.017$ which is a statistically significant result.

The results of this study were compared with those of Teran et al., [13] and Hentschke et al., [14] who also analyzed the increase in plasma cytokine levels and verified an increase in IL-6 in preeclampsia. Afshar et al, [15] found an increase in IL-6 but not a common increase in TNF- α while Olusi et al. [16] found at the same time a symmetrical increase in both IL-6 and TNF- α and in some of the normal pregnancies. The comparison in these investigations is significant to be performed at the same time. It is of very importance the impact of gestational age, the multiparity, the amount of blood derivatives sample taken, because of the differences in the dilutions and the kits with which the interleukins themselves are pronounced and by which methodologies and whether patients are undergoing some therapy.

In the study by Ifeoma Udenze [17], he reported a statistically significant result in the levels of proinflammatory cytokines, IL-6 and TNF- α and in CRP levels in women with severe preeclampsia compared with women with normotensive pregnancy. The high levels of IL-6 and TNF- α found in association with CRP values in severe preeclampsia have a significant clinical contribution to the theory that preeclampsia is indeed due to exacerbation of the inflammatory response. His analyzes are consistent with those of Cui Xie et al., in order to confirm the above theory [18]. The results of this study correspond to the results of Udenze because a correlation was made and a statistically significant correlation was found between IL-6 as most significant with CRP with $p < 0.05$ $p = 0.025$ which is in favour of impaired immune response. A correlation of IL-6 with LDH and total protein was also performed, which also resulted in a statistically significant result corresponding to $p < 0.01$ and is a risk signal for the development of organic affection and the development of hypoproteinemia

with proteinuria in addition to the development of preeclamptic symptoms. The present imbalance in the immune response, i.e., higher values of proinflammatory parameters are reflected as follows. High levels of IL-6 and TNF- α systemically induce the inflammatory response through the hepatal cells to stimulate the synthesis of CRP, or C reactive protein, which is a sensitive marker for tissue damage and inflammation of tissues, which is an indication of the rate of inflammation, oxidative stress and their correlation with preeclampsia.

6. Conclusion

From the results obtained and the discussions, it can be seen that with correlation of the interleukins in the second trimester and laboratory examinations in the third trimester, insight into patients at increased risk for development of clinical preeclampsia syndrome can be provided. It was confirmed and in our investigation. This enables careful follow-up of these patients for the purpose for prevention, prediction, diagnosis and appropriate early treatment of preeclampsia.

The whole point is through investigation of the correlation between the interleukins and laboratory parameters for verification of the inflammatory origin in preeclampsia to have a better perinatal outcome and well-being for both the mother and the fetus.

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