Interleukin-6 - a Marker of the Inflammatory Syndrome in Preeclampsia

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Abstract

Introduction. *Normal pregnancy is characterized by a controlled inflammatory state, localized at the level of the implant area during early stages, and generalized in the last trimester of pregnancy. Preeclampsia represents an exacerbation of systemic inflammatory response.* ***Aim.*** *Evaluation of IL-6 in the serum of preeclamptic pregnant women in the last trimester of pregnancy compared to normal pregnancy. Investigation of a possible relationship between the intensity of the inflammatory syndrome, the severity of preeclampsia, and fetal status at birth.* ***Material and method.*** *A transversal study was performed in three groups of patients: group 1 (preeclampsia); group 2 (normal pregnancy); group 3 (control). The serum samples for the determination of IL-6 were processed using the HS human IL-6 Elisa kit.* ***Results.*** *A significant positive correlation between serum IL-6 concentration and blood pressure values was found. A significant positive correlation was also obtained in preeclampsia between serum IL-6 concentration and uric acid concentration, as well as proteinuria. A significant negative correlation between serum IL-6 concentration, fetal weight at birth and the Apgar score of the newborn was found.* ***Conclusions.*** *Serum IL-6 concentration represents a marker of the severity of preeclamptic syndrome, being at the same time an accuracy predictor of fetal weight and status at birth.*
Keywords: *preeclampsia, interleukin-6, proinflammatory state, pregnancy*

Introduction

The absence of the rejection of the semi-allogenic fetus by the activated maternal immune system is a real immunological paradox, which is not completely understood.

Preeclampsia and recurrent abortion are considered by the majority of the authors as the result of the alteration of immune physiological processes that maintain pregnancy.

During the course of normal pregnancy, there are two immunologically distinct maternal-fetal interfaces, which are spatially and even temporally separated. The first interface is situated at decidual level and includes the interactions between the immune maternal decidual cell population (NK uterine cells, macrophages, dendritic cells and T lymphocytes) and the invasive extravillous cytotrophoblast. This interface is dominant in early pregnancy and almost completely disappears in the third trimester of pregnancy, with the regression of the invasive trophoblast.

The second interface includes interactions between the circulating maternal immune cells (T lymphocytes, NK cells, monocytes and dendritic cells) and the syncytiotrophoblast that forms the villous surface of the hemochorial human placenta[(1)]. This interface is activated at the onset of utero-placental circulation (6-8 AW) and increases with the growth of the placenta, becoming dominant in the third trimester of pregnancy[(2)].

Normal pregnancy is characterized by a controlled innocuous inflammatory state, localized at the level of the implant area during early stages and generalized in the last trimester of pregnancy[(3)]. Thus, at the level of the first interface, leukocyte infiltration and an increased production of proinflammatory cytokines (IL-12, IL-15, IL-18, IFN-Y) are found, while at systemic level, the following are evidenced in normal pregnancy compared to non-pregnant status: leukocytosis and leukocyte activation, endothelial activation, complement, coagulation and thrombocyte activation, phenomena accompanied by an increased synthesis of proinflammatory cytokines (IL-12, IL-6, IL-18, TNF-α, IFN-Y).

The aim of the study was the evaluation of IL-6 as a component of systemic inflammatory response in the serum of preeclamptic pregnant women in the last trimester of pregnancy compared to normal pregnancy. In order to interpret IL-6 changes in preeclampsia, we initially deter-

mined physiological IL-6 levels in the third trimester of pregnancy compared to non-pregnant status.

Another objective of the study was the investigation of a possible relationship between the intensity of inflammatory syndrome and the severity of preeclampsia, evaluated by arterial blood pressure, proteinuria and uric acid values. We also aimed to evaluate the relationship between serum IL-6 levels, fetal weight and fetal status at birth.

Material and method

In order to attain the proposed objectives, we performed a transversal study in the following three groups:

Group I (preeclampsia - PE) included 30 pregnant women selected based on the following criteria:

- **Inclusion criteria:**
  - ✓ pregnant women with PE (moderate or severe form)
  - ✓ third trimester of pregnancy (28-41 AW)
  - ✓ monofetal pregnancy
- **Exclusion criteria:**
  - ✓ pregnant women in labor or with ruptured chorioamniotic membranes
  - ✓ pregnant women with clinically manifest infections
  - ✓ pregnant women with diseases prior to pregnancy associated with a chronic inflammatory response (autoimmune and chronic inflammatory diseases)
  - ✓ recent treatment with non-steroidal antiinflammatory drugs or corticosteroids (≤14 days).

Group II (normal pregnancy - NP) - included 30 pregnant women selected based on the following criteria:

- **Inclusion criteria:**
  - ✓ normotensive pregnant women throughout the course of pregnancy;
  - ✓ third trimester of pregnancy (28-41 AW);
  - ✓ monofetal pregnancy;
  - ✓ the evolution of pregnancy and birth were within physiological limits.
- **Exclusion criteria:**
  - ✓ pregnant women in labor or with ruptured chorioamniotic membranes;
  - ✓ pregnant women with clinically manifest infections;
  - ✓ pregnant women with diseases prior to pregnancy associated with a chronic inflammatory response (autoimmune and chronic inflammatory diseases);
  - ✓ recent treatment with non-steroidal antiinflammatory drugs or corticosteroids (≤ 14 days).

Group III (control group - C) – included 30 patients selected based on the following criteria:

- **Inclusion criteria:**
  - ✓ non-pregnant women;
  - ✓ age 20-40 years.
- **Exclusion criteria:**
  - ✓ primary or secondary arterial hypertension;
  - ✓ renal diseases;
  - ✓ chronic inflammatory diseases and autoimmune diseases;
  - ✓ clinically manifest infections;
  - ✓ recent antiinflammatory or corticosteroid treatment (≤ 14 days);
  - ✓ oral estrogen treatment.

IL-6 concentrations in the maternal serum were determined according to the following study protocol: 1 ml blood was collected on anticoagulant; the plasma was separated by centrifugation for 15 minutes at 1000 xg. The samples were stored in a freezer at -20^{0}C. When processed, the samples were gradually thawed on ice.

The samples were processed using the HS human IL-6 Elisa kit - the Quantikine HS Human IL-6 Immunoassay.

Statistical analysis. The results obtained were experimented as representative indices and indicators, which were illustrated depending on relevance as figures or diagrams.

- **Descriptive statistics.** For numerical variables, dispersion and centrality indices were calculated: mean, median, standard deviation, standard error. For the illustration of the conclusions of the determinations performed, column diagrams were used, according to the results and the type of the analysis.
- **Comparative statistics.** For the comparison of the quantitative variables and of the differences between their means, the Student t test and the ANOVA test were used. For the evaluation of the relationship between two quantitative variables, regression and correlation calculations were used. The correlation coefficient (Pearson index, r) for numerical data can take values between -1 and 1. In order to illustrate the relationship between two or more evaluated variables, scatter plots were used, the presence of a linear relationship of the point cloud being increased by the illustration of a regression line. For statistical significance, the threshold value $p<0.05$ was considered.

Results

Descriptive analysis of IL-6

The mean serum IL-6 concentrations in the three analyzed groups were :

- Group 1 (PE) = 12.94 pg/ml; range values 0-38.11 (95% CI: 9.95-15.68; SD ± 8.98.
- Group 2 (NP) = 5.22 pg/ml; range values 0-14.83 (95% CI: 3.83-6.2); SD ± 3.2.
- Group 3 (C) = 1.86 pg/ml; range values 0-4.83 (95% CI: 1.24-2.46; SD ± 1.68.

Comparative analysis of the studied parameters

Relationship between serum IL-6 concentration and the severity of the preeclamptic syndrome

In PE, a highly significant positive correlation was found both between serum IL-6 concentration and systolic BP (SBP), and between serum IL-6 concentration and diastolic BP (DBP) .

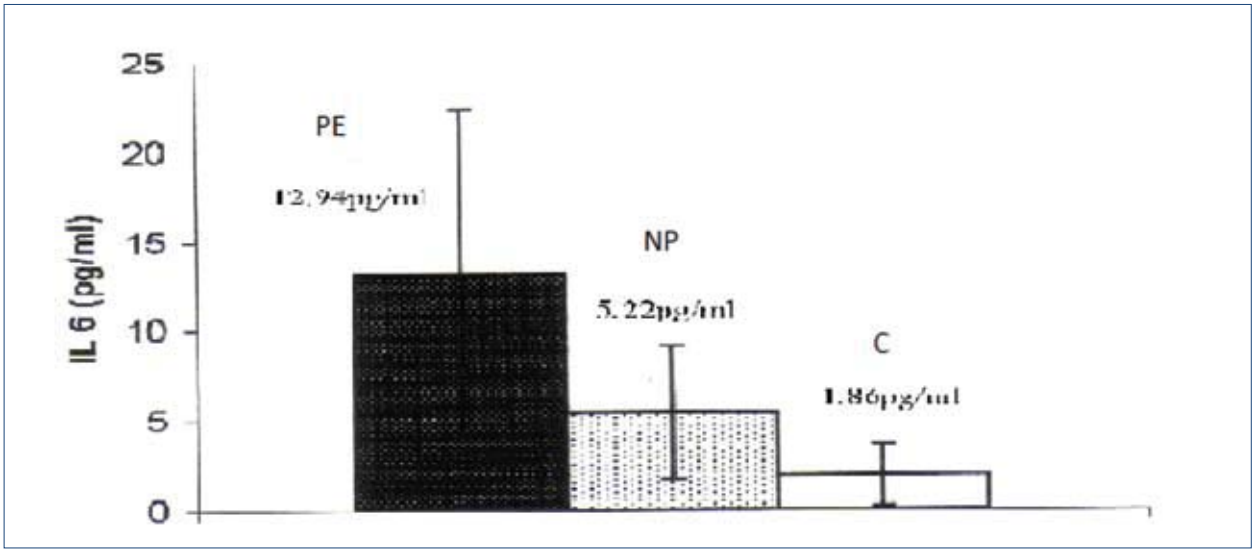
A significant positive correlation was also obtained in preeclampsia between serum IL-6 concentration and uric acid concentration .

For group 1 (PE), a significant positive correlation between serum IL-6 concentration and proteinuria was found.

Relationship between serum IL-6 concentration and some fetal parameters at birth

For group 1 (PE), a significant negative correlation was found between serum IL-6 concentration and fetal weight at birth.

Figure 1. Mean serum IL-6 concentration for the studied groups



For group 1 (PE), a significant negative correlation was found between serum IL-6 concentration and the Apgar score of the newborns at birth .

Discussion

Following the researches performed, we detected in the serum of normal pregnant women significantly increased IL-6 concentrations compared to non-pregnant women ($p<0.001$). Longitudinal studies have demonstrated elevated circulating IL-6 levels as early as the first trimester of pregnancy, which increase with the progress of pregnancy[5,6,7] (Table 1).

According to our results, preeclampsia is characterized by significantly increased IL-6 values compared to normal pregnancy ($p<0.001$).

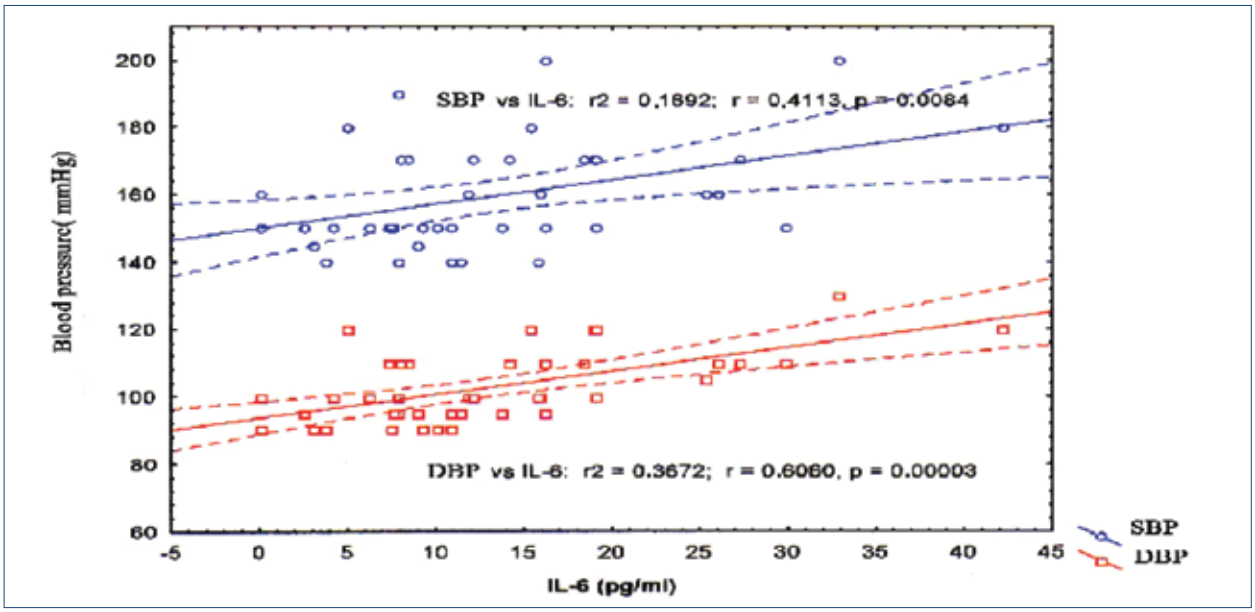
Similar results have been reported by the majority of the literature studies[6,8,9,10] (Table 2).

The tissues responsible for the increased IL-6 production are not completely known to date.

Although the syncytiotrophoblast has the capacity to synthesize IL-6, studies have demonstrated that the placenta is most probably not a source of IL-6 in preeclampsia[11,12,13]. Possible sources of IL-6 in preeclampsia might be the activated leukocytes or maternal endothelial cells after exposure to stimuli such as TNF-α[14].

It has been demonstrated that monocytes from preeclamptic pregnant women synthesize IL-6 spontaneously. IL-6 expression in monocytes in preeclampsia is significantly increased compared to normal pregnancy. Cytokine production varies depending on the cell activation status,

Figure 2. Relationship between serum IL-6 concentration and blood pressure values



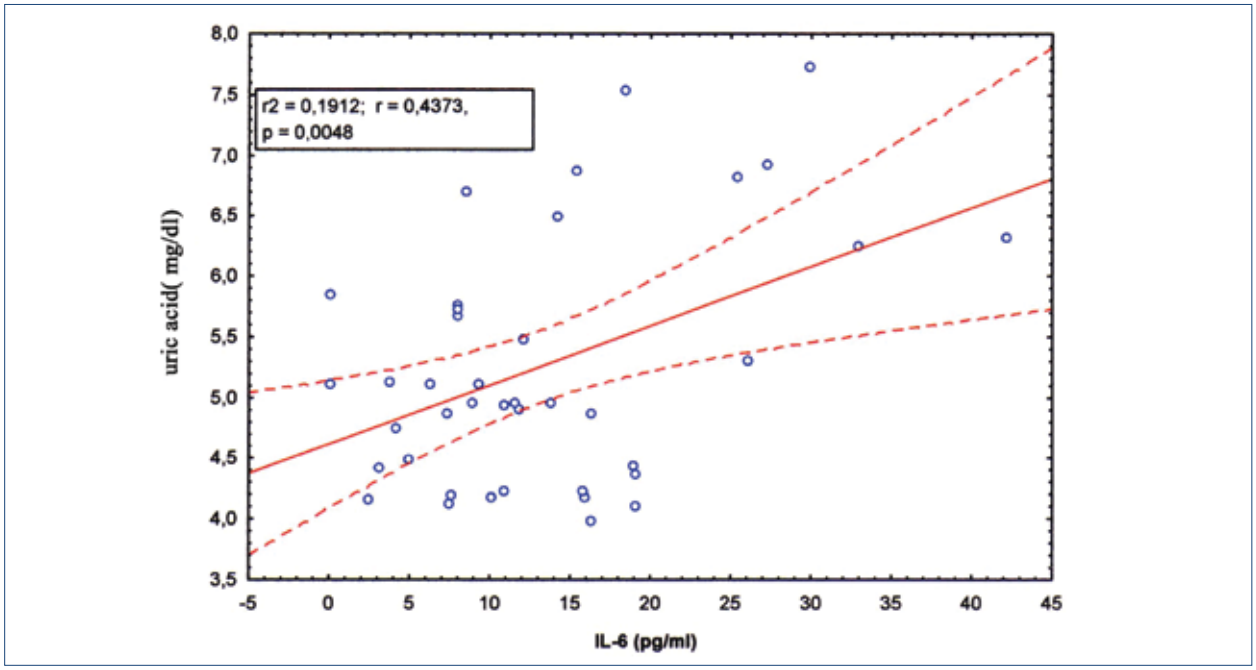


Figure 3. Relationship between serum IL-6 concentration and the serum titer of uric acid

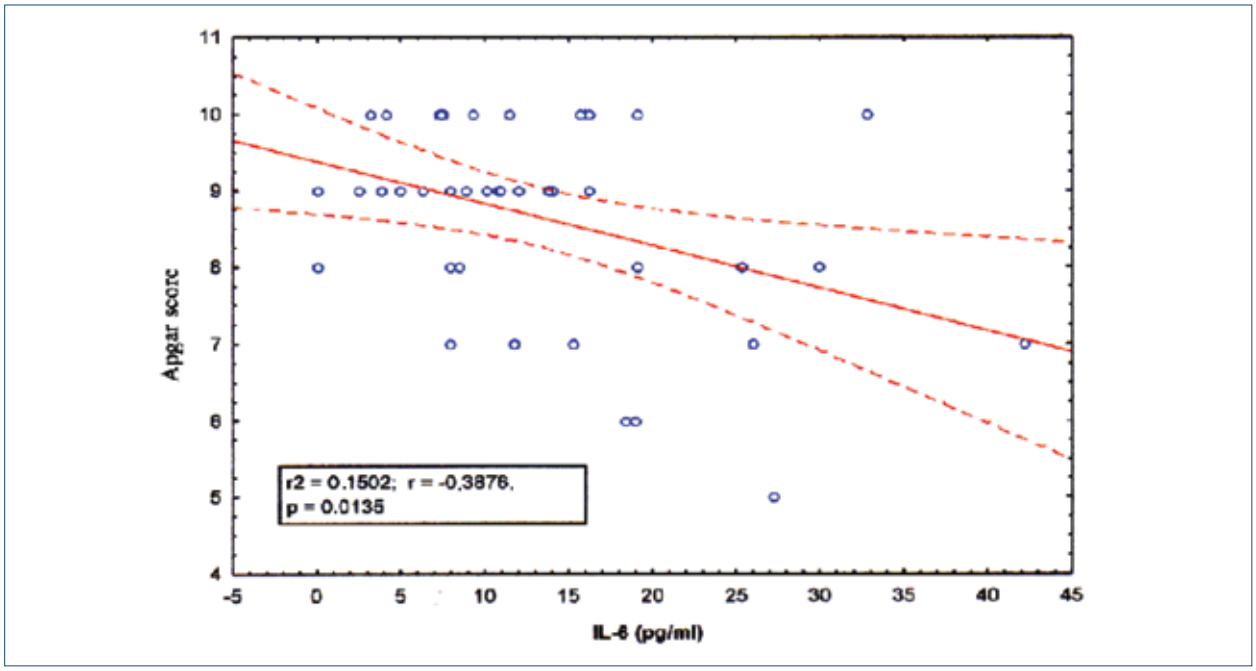


Figure 4. Relationship between serum IL-6 concentration and the Apgar score of the newborns

Table 1 Circulating IL-6 levels in non-pregnant women and during normal pregnancy

Study	Non-pregnant women	Pregnant women 1st trimester	Pregnant women 2nd trimester	Pregnant women 3rd trimester	p
Vassiliadis 1998	2.70 ± 0.30 pg/ml	2.55 ± 0.24 pg/ml	2.91 + 0.38 pg/ml	2.91 ± 0.34 pg/ml	<0.05 (ANOVA)
Teran 2001	1,25 ± 0,13 pg/ml	-	-	5.07 ± 0.423 pg/ml	<0,001 (t)
Coussons - Read 2007	-	1.7 ± 0.9 pg/ml	1.83 ± 0.7 pg/ml	3.03 ± 0.3 pg/ml	<0.05 (ANOVA)

Table 2 Circulating IL-6 levels in preeclampsia, compared to normal pregnancy

Study	Technique	Normal pregnancy	Preeclampsia	p
Greer 1994	ELISA plasma	2.06 pg/ml	2.56 pg/ml	0.01
Teran 2001	ELISA serum	5.07±0.423 pg/ml	12.91±1.29 pg/ml	0.0002
Jansson 2006	multiplex bread array ser	3.3 pg/ml	13 pg/ml	0.002
Afshari 2005	high sensitivity indirect ELISA serum	3.01 ± 2.45 pg/ml	5.8 ± 4.85 pg/ml	0.02

and the activation of circulating maternal monocytes is higher compared to normal pregnancy. In preeclampsia, monocytes can be activated during the uteroplacental passage or in systemic circulation, under the influence of syncytiotrophoblast microparticles[15].

In the performed study, IL-6 concentration was significantly correlated with the severity of preeclampsia, evaluated by arterial blood pressure values, proteinuria and serum uric acid values.

A negative correlation was found between serum IL-6 values, fetal weight at birth and the Apgar score of the newborn. These results have also been reported by other studies[16].

IL-6 is a multifunctional cytokine, which plays an important role in host defense mechanisms, being involved in the recruitment of T lymphocytes at the site of inflammation by increased lymphocyte-endothelial adhesion, and in the stimulation of the hepatic synthesis of acute phase reactions, such as C reactive protein.

It is not known whether IL-6 plays a direct role in the pathogenesis of preeclampsia or is just a marker of endothelial cell activation[17].

It has been recently demonstrated that IL-6 has the capacity to interfere with the mechanisms of contraction and relaxation at the level of systemic vessels in pregnancy, playing a role in the modulation of vascular resistance and implicitly, of arterial blood pressure[18].

Conclusions

Preeclampsia is an exacerbation of a generalized inflammatory response, which is physiologically present in the last trimester of pregnancy.

The evaluation of the inflammatory response in pregnancy has deep implications regarding the prediction, screening and prophylaxis of preeclampsia.

Serum IL-6 concentration is a marker of the severity of the preeclamptic syndrome, being at the same time an accuracy predictor of fetal weight and status at birth. ■

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