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Evaluation of Leukocytes and Neutrophils, Markers of Inflammatory Syndrome in Preeclampsia

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Abstract

Introduction: In pregnancies with preeclampsia, there is a generalized inflammatory response, which is much more intense than in normal pregnancy. *Aim:* To evaluate leukocytes and neutrophils in the serum of pregnant women with preeclampsia and to compare these values to normal pregnancy. To investigate a possible relation between the detected values and the severity of preeclamptic syndrome. *Material and method:* A transversal study was performed in three groups of patients: group 1 (preeclampsia), group 2 (normal pregnancy), group 3 (control). The samples were processed using a multichannel automated hematology analyzer – ACCOS 319. The results obtained were processed by descriptive and comparative statistical methods. *Results:* A significant increase in leukocyte and neutrophil values was found in preeclampsia compared to normal pregnancy. Also, for group 2 (PE), there was no correlation between the number of leukocytes, neutrophils, respectively, and SBP and uric acid values, but a significant correlation was found between the studied inflammatory markers and DBP values. *Conclusions:* Leukocyte and neutrophil values can be considered markers of the inflammatory syndrome present in preeclampsia. These parameters were positively correlated with DBP values.

Keywords: Preeclampsia; Pregnancy; Leukocytes; Neutrophils.

Introduction

Preeclampsia remains "a disease of theories", as many uncertainties persist regarding its etiopathogenic and pathophysiological mechanisms, aspects that are reflected by the difficulty of some screening tests and effective prophylactic treatment [1].

Preeclampsia is the consequence of deficient trophoblast invasion at the level of spiral arteries. The resulting uteroplacental ischemia is responsible for the appearance of chronic hypoxia and generalized endothelial dysfunction, which cause arterial hypertension and maternal organic impairment.

Normal pregnancy is characterized by a controlled innocuous inflammatory state, located in the implantation area during early stages and generalized in the last trimester of pregnancy.

Although the majority of the authors support the presence of a generalized maternal inflammatory response in pregnancies with preeclampsia, which is much more intense compared to normal pregnancies, there are major differences between the results of studies evaluating various components of the inflammatory reaction [2, 3].

The main objective of our study was the evaluation of leukocytes and neutrophils as components of the inflammatory response in the serum of pregnant women with preeclampsia during the last trimester of pregnancy. Another objective of the study was the evaluation of a possible correlation between the intensity of the inflammatory syndrome and the severity of preeclampsia, assessed based on blood pressure and uric acid values.

Material and Method

In order to achieve the proposed objectives, we performed a transversal study in three groups of patients. The study was performed between 1st of November 2009 and 1st of July 2010 in "Dominic Stanca" Clinic of Obstetrics and Gynecology Cluj-Napoca.

In order to interpret the changes in the values of leukocytes and neutrophils in preeclampsia, we measured their levels in pregnant women with a physiological evolution of pregnancy during the third trimester, as well as in non-pregnant patients.

Group 1 (preeclampsia – PE) included 30 pregnant women selected according to the following criteria:

- Inclusion criteria:
  - Pregnant women with PE (moderate or severe form)
  - Third trimester of pregnancy (28 – 41 week of amenoreea- WA)
  - Single pregnancy
- Exclusion criteria:
  - Pregnant women in labor or with ruptured chorioamniotic membranes
  - Pregnant women with clinically manifest infections
  - Pregnant women with diseases associated with a chronic inflammatory response (autoimmune and chronic inflammatory diseases) prior to pregnancy
  - Recent treatment with nonsteroidal anti-inflammatory drugs or corticosteroids (≤ 14 days).

Group 2 (normal pregnancy – NP) – included 30 pregnant women selected according to the following criteria:

- Inclusion criteria:
  - Pregnant women with normal blood pressure values throughout the pregnancy
  - Third trimester of pregnancy (28 – 41 WA)
  - Single pregnancy
  - Evolution of pregnancy and birth within physiological limits.
- Exclusion criteria:
  - Pregnant women in labor or with ruptured chorioamniotic membranes
  - Pregnant women with clinically manifest infections
  - Pregnant women with diseases associated with a chronic inflammatory response (autoimmune and chronic inflammatory diseases) prior to pregnancy
  - Recent treatment with nonsteroidal anti-inflammatory drugs or corticosteroids (≤ 14 days).

Group 3 (control group - C) – included 30 patients selected according to the following criteria:

- Inclusion criteria
  - Non-pregnant women
  - Age 20 – 40 years
- Exclusion criteria
  - Primary or secondary arterial hypertension
  - Renal diseases
  - Chronic inflammatory and autoimmune diseases

- Clinically manifest infections
- Recent treatment with nonsteroidal anti-inflammatory drugs or corticosteroids (≤ 14 days)
- Oral estrogen treatment

From each subject included in the study, 2 ml blood were taken by venous puncture under fasting conditions in EDTA test tubes for a complete hemoleukogram, which was performed with a multichannel automated hematology analyzer that sorts the cells depending on size and electrical impedance - the ACCOs 319 Analyzer. The values considered to be normal were: for leukocytes 4000 - 10000 / mm^3, and for neutrophils 2000 - 7000 / mm^3.

Uric acid was determined using the Dunalyser blood analyzer. The values considered to be normal were 2.5 – 7 mg / dl.

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

Descriptive statistics. For numeric 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 analysis.

Comparative statistics. In order to compare the quantitative variables and the differences between their means, the Student t and ANOVA tests were used. For the evaluation of the relation between two quantitative variables, regression and correlation calculations were used. The correlation coefficient (Pearson index, r) for numeric data can take values between -1 and 1. For the illustration of the relation between two or more evaluated variables, scatterplots were used, the presence of a linear correlation of the point cloud being enhanced by the illustration of a regression line. For statistical significance, the threshold value $p < 0.05$ was considered.

Results

The descriptive analysis of leukocytes and neutrophils

The mean values in the three groups were:

- Group 1 (PE) – the mean leukocyte count was 12985.5 / mm^3, with range values between 9500 – 18200 (95% CI: 12468 - 13798); SD ± 2128.9.
- Group 2 (NP) the mean leukocyte count was 10908.5 /mm^3, with range values between 6100 - 15900 (95% CI: 10189.98 - 11586.38); SD ± 2259.25.
- Group 3 (C) the mean leukocyte count was 6728 / mm^3, with range values between 4400 - 8600 (95% CI: 6448 - 7018.68); SD ± 738.02 (Figure 1).

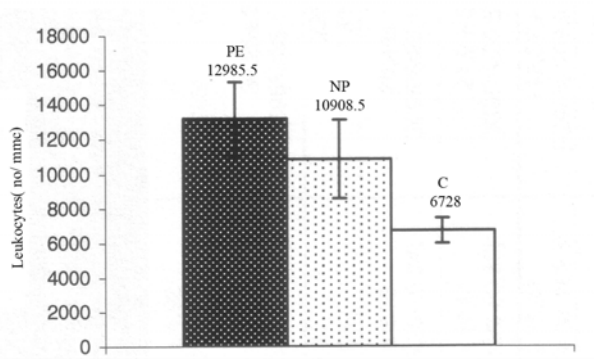

Figure 1. Mean leukocyte count for the study groups; $p < 0.001$

Statistical calculations: Student t and ANOVA tests.

- Group NP vs group C: difference - 4180.5, $p < 0.001$.

- Group PE vs group NP: difference - 2077.0, $p < 0.001$. ANOVA test: highly statistical 79.12, $p < 0.001$.
- Group 1 (PE) the mean neutrophil count was 10098.5 / mm^3 (95% CI: 9490.84 - 10768.25); SD ± 1924.54.
- Group 2 (NP) the mean neutrophil count was 7898 / mm^3 (95% CI: 7228.68 - 8598.68); SD ± 2179.89.
- Group 3 (C) the mean neutrophil count was 4448.96 mm^3 (95% CI: 4189.86 - 4710.34); SD ± 684.62 (Figure 2).

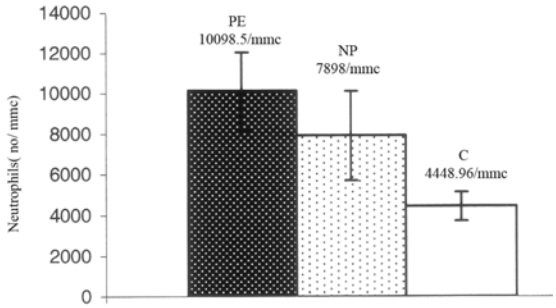

Figure 2. Mean neutrophil count for the study groups; $p < 0.00$

Statistical calculations: Student t and ANOVA tests.

- Group NP vs group C: difference - 3449.04, $p < 0.001$.
- Group PE vs group NP: difference - 2200.5, $p < 0.001$.

ANOVA test: highly statistical 53.89, $p < 0.001$.

The comparative analysis of the studied parameters

Relation between the leukocyte count and the severity of the preeclamptic syndrome.

In preeclampsia, there was no significant correlation between the leukocyte count and systolic blood pressure (SBP), while a highly significant correlation was found between the leukocyte count and diastolic blood pressure (DBP) (Figure 3).

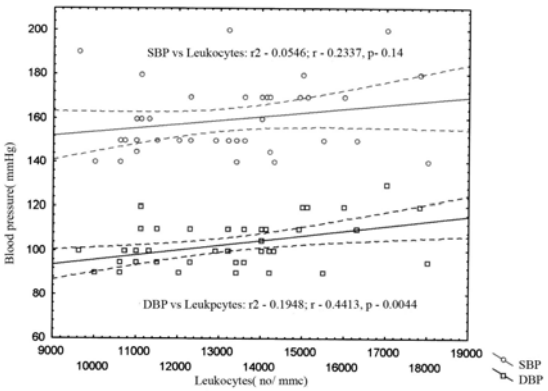

Figure 3. Relation between the leukocyte count and blood pressure values in preeclampsia

No significant correlation was found between the leukocyte count and serum uric acid concentrations in the group with preeclampsia (Figure 4).

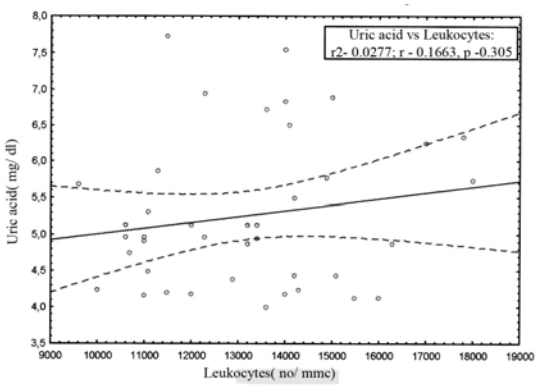

Figure 4. Relation between the leukocyte count and serum uric acid concentration in preeclampsia

Relation between the neutrophil count and the severity of preeclamptic syndrome.

For group 1 (PE), no correlation was found between the neutrophil count and SBP, but there was a highly significant positive correlation between the neutrophil count and DBP (Figure 5).

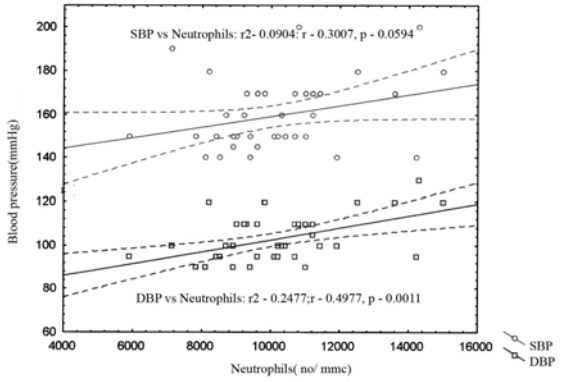

Figure 5. Relation between the neutrophil count and blood pressure values in preeclampsia

For group 1 (PE), no significant correlation was found between the neutrophil count and serum uric acid concentrations (Figure 6).

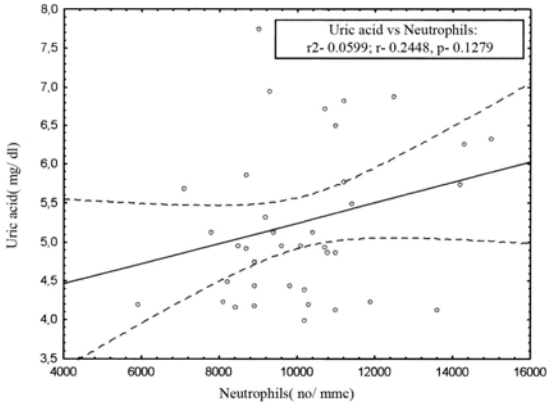

Figure 6. Relation between the neutrophil count and serum uric acid concentration in preeclampsia

Discussion

Following the researches performed, we detected a significant increase in the leukocyte and neutrophil count in the third trimester of normal pregnancy compared to the non-pregnant status. These observations confirm the results of other literature studies (Table 1).

Table 1. Results of studies evaluating leukocyte and neutrophil values in normal pregnancy

Study		Non-pregnant women	Pregnant women 1st trimester	Pregnant women 2nd trimester	Pregnant women 3rd trimester
Rebelo 1995	Leukocytes	5.6 ± 0.2	10.2 ± 0.2	9.7 ± 0.4	9.8 ± 0.3
	Neutrophils	4.3 ± 0.5	4.4 ± 0.2	6.8 ± 0.2	7.3 ± 0.3
Belo 2005	Leukocytes	6.38 ± 1.41	9.04 ± 2.18	10.29 ± 2.26	10.04 ± 2.1
	Neutrophils	3.70 ± 1.43	6.46 ± 1.64	7.70 ± 1.67	7.37 ± 1.76
Teran 2001	Leukocytes	6.6 ± 1.2			8.5 ± 2.8
	Neutrophils	4.2 ± 0.9			6.1 ± 2.6

Leukocytosis is obvious starting with the first trimester of pregnancy and increases with the evolution of pregnancy. The increase in the number of circulating leukocytes is most probably due to the high cortisol levels in pregnancy, which stimulate their mobilization at the level of marginal pools [4-6].

A number of studies support leukocyte activation during normal pregnancy compared to the non-pregnant status. Sacks et al. demonstrated for the first time in 1998, using whole blood flow cytometry, a significantly increased expression of CD 11 b and CD 64 at the surface of granulocytes and monocytes, and an increased production of reactive oxygen species in granulocytes, monocytes and lymphocytes compared to the non-pregnant status. Phagocytosis and the chemiluminescent responses of neutrophils after stimulation with serum opsonized zymosan are also increased during the course of normal pregnancy [6, 7].

In our study, we detected in pregnant women with preeclampsia a significantly increased leukocyte and neutrophil count compared to normal pregnancy ($p < 0.001$). However, the literature data are controversial, reporting an increased, unchanged or even decreased leukocyte / neutrophil count in preeclampsia (Table 2).

Table 2. Results of studies evaluating leukocyte and neutrophil values in preeclampsia compared to normal pregnancy

Study		Normal pregnancy	Preeclampsia	p
Barden, 2001	Leukocytes ($\cdot 10^9$/l)	9.67 ± 0.42	12.24 ± 0.94	<0.05
	Neutrophils ($\cdot 10^9$/l)	7.13 ± 0.38	9.57 ± 0.87	<0.05
Bowen, 2001	Leukocytes ($\cdot 10^9$/l)	13.76 ± 0.89	13.76 ± 0.89	<0.01
	Neutrophils ($\cdot 10^9$/l)	12.52 ± 0.80	9.12 ± 0.95	<0.001
Aly, 2004	Leukocytes ($\cdot 10^9$/l)	12.7 ± 0.8	12.7 ± 1.6 moderate PE 13.3 ± 0.3 severe PE	>0.05
Kumru, 2006	Leukocytes ($\cdot 10^9$/l)	9.65 ± 3.29	11.84 ± 4.73	>0.05

There is evidence in the literature supporting a higher activation of neutrophils in preeclampsia compared to normal pregnancy both in the placental bed and in maternal circulation [8-11].

Some authors have reported an increased superoxide production in neutrophils. Recent studies have demonstrated that in preeclampsia there is a modulation of neutrophil function towards a higher production of superoxide compared to NO, which results in endothelial damage and dysfunction [12, 13].

An increased infiltration of neutrophils has been demonstrated in the vascular system of pregnant women with preeclampsia. The flattened neutrophils adhere to the endothelium and infiltrate the intima. Neutrophil infiltration is associated with inflammation markers at endothelial level: activation of nuclear factor – KB; increased expression of ICAM-1, COX-2, and IL-8.

Although circulating factors might initially activate the endothelium, the adhesion of neutrophils to the endothelium exacerbates this situation by promoting endothelial lipid peroxidation and the release of TNF-α and myeloperoxidase [14].

The role of hypoxia in the activation of neutrophils is supported by in vitro studies which have demonstrated that neutrophils modulate the expression of cell adhesion molecules when exposed to low oxygen concentrations. Hypoxia can activate leukocytes directly in the intervillous spaces or can stimulate the production of lipoperoxides and proinflammatory cytokines by the placenta, which can activate the leukocytes during their passage through placental circulation [15].

By evaluating the relation between the number of leukocytes, neutrophils, respectively, and the severity of preeclamptic syndrome, we detected a significant positive correlation between the leukocyte and neutrophil count and DBP values ($p < 0.01$, $p < 0.005$, respectively).

The number of leukocytes and neutrophils did not significantly correlate with SBP values or with serum uric acid concentrations.

Ozkaya et al. found in 2006 that DBP levels > 120 mmHg and a leukocyte count >16.000/microL are independent predictors of the eclamptic crisis [16].

Terrone et al. evaluated leukocytosis in pregnant women with various severity degrees of the HELLP syndrome established based on the number of thrombocytes, compared to pregnant women with severe preeclampsia, without thrombocytopenia. The authors reported a significant increase in the leukocyte count in the HELLP syndrome ($12.5 \pm 0.442 \cdot 10^9$ / l) versus severe preeclampsia ($10.3 \pm 0.288 \cdot 10^9$ / l).

The increase in the number of leukocytes was proportional to the severity of the HELLP syndrome, with a significant negative correlation between the leukocyte count and the thrombocyte count ($r = -0.22$; $p < 0.05$) [17].

Inflammatory injury mediated by neutrophils can occur by several mechanisms:

1. The integrin dependent activation of the hyperadhesion of neutrophils promotes homotypic aggregation that results in blood vessel obstruction and distal ischemia.
2. The increased concentrations of inflammatory stimuli determine the degranulation of neutrophils with the release of a protease variety that causes the destruction of the underlying extracellular matrix.
3. The generation of oxidative metabolites at the level of neutrophils can result in direct endothelial damage or can stimulate the destructive effects of the proteases released from granules either by the activation of latent matrix metalloproteinases or by the activation of oxidant sensitive antiproteases [18].

Conclusions

1. The leukocyte and neutrophil count in peripheral blood is significantly increased in the last trimester of pregnancy in preeclampsia compared to normal pregnancy.
2. Compared to the non-pregnant status, normal pregnancy is characterized by a physiological increase in the leukocyte count, mainly by an increase in the number of neutrophils.
3. The number of leukocytes and neutrophils was not significantly correlated with SBP and uric acid values, but a highly significant correlation was found between these inflammatory syndrome markers and DBP.
4. In preeclampsia, there is an exacerbation of a generalized inflammatory response, physiologically present during the last trimester of gestation.

Ethical Issues

The study was accomplished based on a grant, which grant had the approval of the Etics Committee of "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca. The patients have signed the informed consent regarding the inclusion in the study.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgements

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