Journal of Advanced Zoology

ISSN: 0253-7214
*Volume **44** Issue **S-7 Year 2023** Page **01:06***

Prognostic Biomarkers of Pre-Eclampsia in Pregnant Women

Musakhodjaeva Diloram A., Eshimbetova Gulsara Z., Sadikova Khanifa Z., Juraeva Dildora M., Azizova Zukhra Sh., Rustamova Nazokat B., Masaidova Iroda B

Institute of Immunology and Human Genomics of the Academy of Sciences of the Republic of Uzbekistan, Center for Development of Professional Qualification of Medical Workers of the Ministry of Health of the Republic of Uzbekistan, Tashkent, Uzbekistan

**Corresponding author's: Musakhodjaeva Diloram A*

Article History

Received: 06 June 2023
Revised: 05 Sept 2023
Accepted: 12 Dec 2023

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Abstract

Summary. *According to various authors, the incidence of preeclampsia (PE) in pregnant women in our country ranges from 8 to 20% and does not tend to decrease. It has been shown that the pathogenesis of preeclampsia fits into the systemic inflammatory response syndrome with disturbances in the immune system and an imbalance in cytokine regulation.* ***The aim*** *of the study was to study pro-inflammatory (IL-1β, IL-6, IL-8) and anti-inflammatory (IL-4, IL-10) cytokines in women with preeclampsia of varying severity.* ***Material and methods****. We examined 76 pregnant women with PE aged from 24 to 36 years at a gestation period of 28–38 weeks. Of these, 42 developed PE (main group), and 34 women had no symptoms of PE (comparison group). 28 women with a physiological pregnancy formed the control group. The levels of pro- and anti-inflammatory cytokines (IL-1β, IL-6, IL-8, IL-4 and IL-10) in blood serum were studied by ELISA.* ***Results.*** *In pregnant women with PE upon admission to the hospital, the blood serum levels of IL-1β increased by 1.3 times ($p < 0.05$), IL-8 by 7 times ($p < 0.001$) and a decrease in the level of IL-4 by 2 times ($p < 0.001$). And in pregnant women without signs of PE, these changes in cytokine synthesis were less pronounced ($p < 0.01$).* ***Conclusion.*** *Maintaining a dynamic balance in the cytokine system plays an important role for the normal development of pregnancy. Th2-type cytokines promote normal trophoblast differentiation and its full invasion and, therefore, mediate the prolongation of physiological pregnancy. Switching to the synthesis of Th1-type cytokines and their enhanced production leads to disruption of trophoblast differentiation and invasion with the development of pregnancy pathology.*

Keywords: *Pregnancy, Preeclampsia, Cytokines*

1. Introduction

Preeclampsia (PE) is a complication of pregnancy that occurs in the second half of pregnancy and is characterized by the appearance of arterial hypertension and proteinuria. To date, the cause of PE has not been fully disclosed, it is generally accepted that this disease is multifactorial. It is a pregnancy-specific complication and its development is associated with the peculiarities of the gestational process itself [1,3,7].

PE is characterized by a triad of typical clinical symptoms: arterial hypertension, proteinuria, not rarely edema. This pathology of pregnancy undoubtedly reveals profound disorders of hemostasis, immunity, vascular function, hemodynamics and microcirculation of the kidneys, liver, lungs, as well as endothelial and fetoplacental system. In general, severe forms of preeclampsia develop severe multi-organ failure and endogenous intoxication [8].

According to statistical studies, the frequency of PE has not practically decreased over the last twenty years and according to different authors it is 7-20% [2,4,5].

According to the literature, the risk factors for the development of this pregnancy pathology include: PE in previous pregnancy, age, first pregnancy, multiple pregnancies, genetic factors, social aspects, occupational hazards, bad habits, unsatisfactory environmental conditions, insufficient and unbalanced nutrition, complicated obstetric and gynecological history, inflammatory diseases of the genitalia, which are usually combined with urinary tract involvement, pathology of the vascular system [6,8].

From the immunologic position, PE is considered as a result of maternal immune system maladaptation and, as a consequence, disruption of placentation processes, which is the first step in the development of the disease. Cytokines, which are signaling proteins that control biological processes throughout pregnancy, from implantation to delivery, are candidates for the role of mediators causing immunological dysfunction [7,9,13,9].

Although implantation and further development of the placenta are pro-inflammatory processes, the maternal immune response controls inflammation through regulatory and anti-inflammatory mediators [5,8]. Several authors have shown that an imbalance between maternal pro-inflammatory cytokines and immune regulatory factors (Tregs and IL-10) is a key contributor to pre-eclampsia [8]. An in-depth understanding of changes in maternal cytokine profile could distinguish successful pregnancy from its complications and help to better understand the immune response during pregnancy.

The aim of the study was to investigate pro-inflammatory (IL-1β, IL-6, IL-8) and anti-inflammatory (IL-4, IL-10) cytokines in women with preeclampsia of varying severity.

2. Materials And Methods

We examined 115 pregnant women in the third trimester of gestation, who made up 3 groups: 28 women with physiologic pregnancy made up the control group; 39 pregnant women with placental insufficiency and risk of PE development made up the 1st group and 48 women whose pregnancy was complicated by the development of preeclampsia made up the 2nd group. The mean age of the studied patients was 27.2 ± 3.8 years, while in the group of pregnant women with physiologic pregnancy the mean age was 26.8 ± 4.2 years.

The levels of pro- (IL-1β, IL-6, IL-8) and anti-inflammatory cytokines (IL-4, IL-10) were studied in serum by ELISA method using test systems of Vector-Best JSC (Novosibirsk, Russia) according to the manufacturer's recommendations.

Statistical processing of research results was carried out by methods of variation statistics. The results are presented as sample mean (M) and standard error (m). Reliability of differences between the mean values (P) of the compared indicators was assessed by Student's criterion (t).

3. Results and Discussion

The analysis of anamnestic data showed that among the examined patients 34 (39.1%) were first-time mothers and 53 (60.1%) were repeat mothers. It should be noted that women with preeclampsia had 5-6 times more frequent inflammatory diseases of the genital organs in the anamnesis than women in the control group, which was statistically significant (56%, 52%, 58% vs. 20%, respectively) ($P<0.01$).

Benign cervical neoplasms were more frequent in the history of women complicated with PE (56.0%), and in women at risk for developing PE, this indicator was -52%. Ectopic pregnancy occurred in 2 (4%) women of the control group and in 5 (10%) pregnant women of group 2. Women with a history of infertility were 3(6%) in the control group and 16% each in group 1 and 2 pregnant women ($P<0.05$).

Somatic diseases are a serious risk factor and unfavorable background for the development of various complications of pregnancy, including PE. Thus, in the group of pregnant women complicated by PE, anemia was observed in 49 (98%) women, while in pregnant women at risk of developing PE in the first and second trimesters, anemia was detected in 90% and 88%, respectively, compared with the control group (78%). Thyroid diseases were also found in 78% of women whose pregnancy was complicated by PE, 92% of pregnant women at risk of PE and 58% of pregnant women in the control group.

Urinary system diseases were registered in the history of almost every second pregnant woman with PE and were more frequent than in the control group, 46% and 6%, respectively.

Studies of the general blood analysis showed a statistically significant decrease in hemoglobin and erythrocyte counts in pregnant women at risk of PE and a trend toward a decrease in the color index in women with PE. Such changes indicate further development of iron deficiency anemia (IDA) in this contingent of patients. Especially pronounced changes were observed in pregnant women of group 2.

Proinflammatory cytokines have a pronounced damaging effect on vascular endothelial cells, leading to the formation of endothelial dysfunction, chronic inflammation. Angiogenesis is an important characteristic of the inflammatory process in placental dysfunction, which can lead to preeclampsia.

The manifestation of signs of inflammation is due to the action of proinflammatory cytokines: IL-1β, IL-6, IL-8 and anti-inflammatory cytokines: IL-4, IL-10, the balance between which is tightly regulated [9,7,10]. Although cytokines act predominantly locally and transiently, the redundancy of the cytokine system is manifested by the fact that each cell type is capable of producing multiple cytokines and each

cytokine can be secreted by different cells, and the measurement of cytokine content in serum represents one of the potential opportunities to assess the inflammatory response [12].

In view of the above, we conducted a study to investigate the level of pro-inflammatory (IL-1β, IL-6, IL-8) and anti-inflammatory (IL-4, IL-10) cytokines in the examined pregnant women and the results are presented in Table 1.

It is known that IL-1β is a cytokine, which is a mediator of inflammation and immunity, is synthesized by many cells of the body, primarily activated macrophages, keratinocytes, stimulated B-cells and fibroblasts [5,22]. It is a central mediator of local and systemic inflammatory reactions. IL-1β binding to receptors in the maternal body is a prerequisite for the process of chorion implantation on the uterine wall. Stimulating the proliferation of endometrial cells forming the placental barrier, it selectively activates the processes of synthesis and secretion of steroid hormones, the level of which affects the course of pregnancy. On the other hand, IL-1β is characterized by the ability to stimulate the production of prostaglandins, thereby triggering the mechanisms of premature fetal rejection [8,14,18].

The results of our study showed that IL-1β level was significantly lower in healthy pregnancy compared to the data of the patients (Table 1).

Table 1. Levels of pro- and anti-inflammatory cytokines in the examined women, (M±m)

Cytokines, pg/mL	Contr. group, n=28	1-st group, n=39	2-nd group, n=48
IL-1β	163,53 ± 8,13	189,46 ± 9,67*	257,42 ± 13,58*^
IL-6	53,35 ± 2,03	68,97 ±3,13*	79,63 ± 3,72*
IL-8	21,92 ± 1,24	27,16 ± 1,24*	32,47 ± 1,58*
IL-4	148,45 ± 10,13	128, 39 ± 9,12*	109,17 ± 8,93*
IL-10	39,8 ± 1,95	28,76 ± 1,64*	22,18 ± 1,21*

Note: *Values are reliable in relation to the control group

^The values are reliable in relation to the 1st group (P<0,05 - 0,001)

Thus, in women at risk of developing PE (group 1), IL-1β level was significantly lower than in women in the control group (P<0.05), and in women who had already developed PE (group 2), there was a more pronounced increase in IL-1β level, (P<0.001), (Fig.1). It should be noted that IL-1β levels in women with PE were significantly higher than in pregnant women at risk of developing PE, (P<0.05). Therefore, IL-1β may be an early predictor of preeclampsia development.

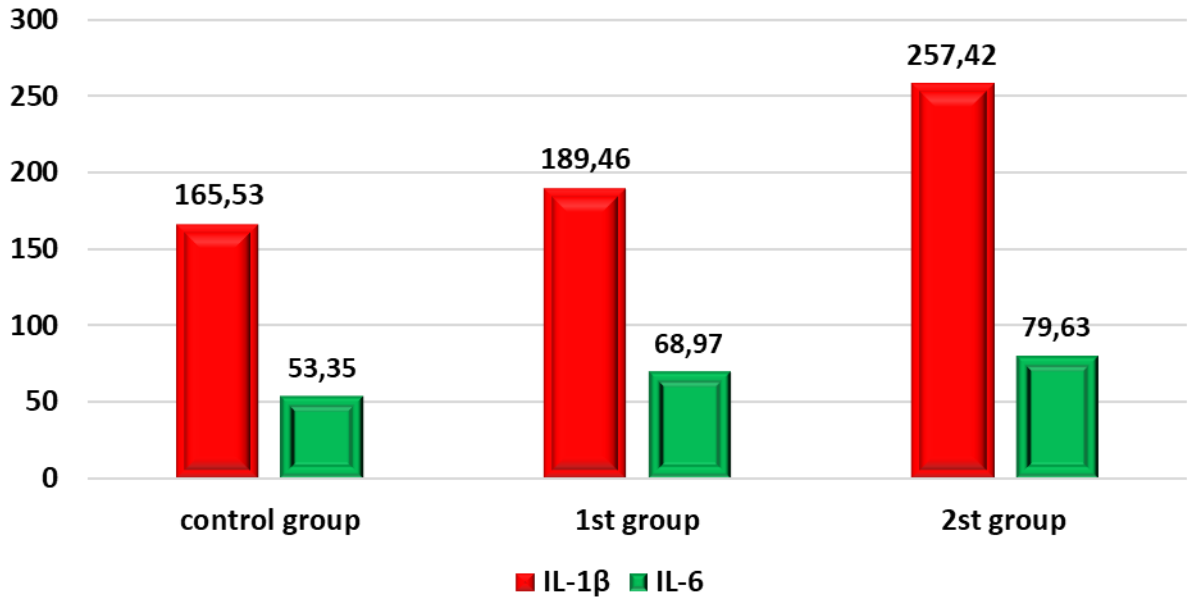

Figure 1. Level of proinflammatory cytokines in the examined pregnant women, (pg/mL).

IL-6 synthesis in women with physiologically normal pregnancy averaged 53.35 ± 2.03 pg/mL, (Fig.1). IL-6 level was significantly increased at risk of PE development, (P<0.05). The maximum value of IL-6 was observed in pregnant women with PE, which averaged 79.63 ± 3.7 pg/mL, (P<0.01). Comparative analysis showed that when PE developed, IL-6 level was significantly higher than in pregnant women at risk of developing PE (P<0.05). Interleukin 6 (IL-6) is known to be an inflammatory cytokine.

Sources of IL-6 production are macrophages, activated T-lymphocytes, B-lymphocytes, as well as cells not directly related to the immune system (fibroblasts, keratinocytes, chondrocytes, endometrial stromal cells, pituitary follicular star cells, smooth muscle cells of blood vessels, endothelial and synovial cells, etc.). IL-6 secretion increases under the action of IL-1 [6].

The level of IL-8 in the risk group, but without the development of PE, was significantly higher than the values of women with physiologic pregnancy ($P<0.05$). And when pre-eclampsia developed, significantly increased IL-8 value was observed ($P<0.001$), reflecting a strong inflammatory environment (Fig.2).

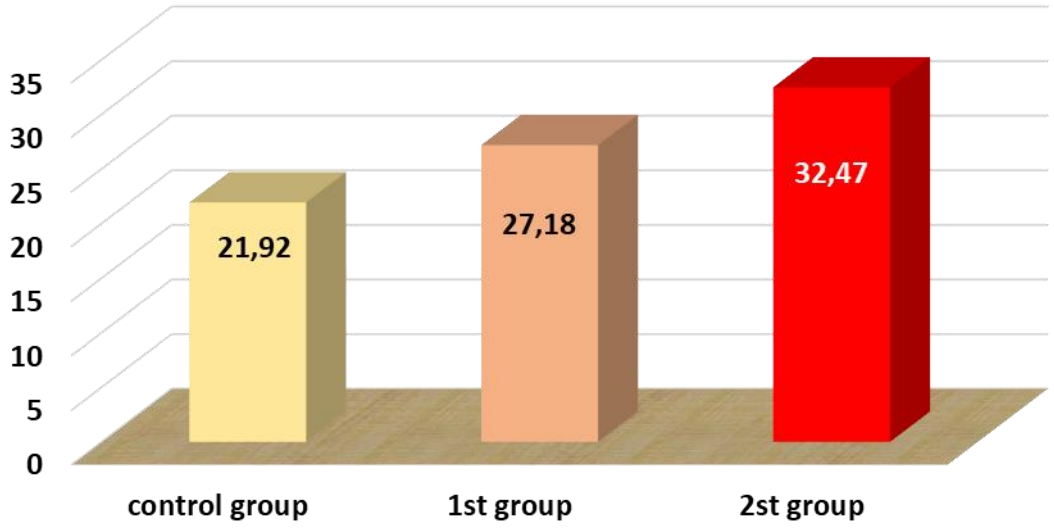

Figure 2. IL-8 level in the examined pregnant women, (pg/mL.)

Neutrophils are known to play a regulatory role in normal placental development and fetal tolerance [8,12,21]. IL-8, which is a chemokine produced by neutrophils and other immune, epithelial and endothelial cells, promotes placental growth and development because it is involved in the regulation of angiogenesis, endothelial activation and cell migration or invasion [22]. However, elevated IL-8 levels also promote neutrophil transmigration and is associated with endothelial dysfunction of both uterine spiral arteries and placental vessels. Consequently, high levels of IL-8, may contribute to the development of PE by promoting neutrophil extravasation into the tissue vascular wall and the release of oxidative stress molecules [18].

Thus, disruption of chorion implantation and subsequently of the placenta, triggers a cascade of events that activate the release of circulating factors such as pro-inflammatory cytokines (IL-1β, IL-6, IL-8) that promote inflammation, create angiogenic imbalance and cause oxidative stress in the mother-placenta-fetus system, which leads to endothelial dysfunction of placental vessels and further development of placental insufficiency, which, in turn, is a predictor of pre-eclampsia [13,15,19].

In addition to proinflammatory cytokines, we studied the indices of anti-inflammatory cytokines, which also play an important role in the development of the inflammatory process. The analysis of the results of the studies showed that the levels of anti-inflammatory cytokines, IL-4 and IL-10 significantly decreased during the development of PE (Fig.3).

As can be seen from the data presented in Fig.3, there was a tendency to decrease the level of IL-4 in women at risk of developing preeclampsia, and in pregnant women with the development of preeclampsia the level of IL-4 was 1.44 times lower than the values of the control group ($P<0.05$).

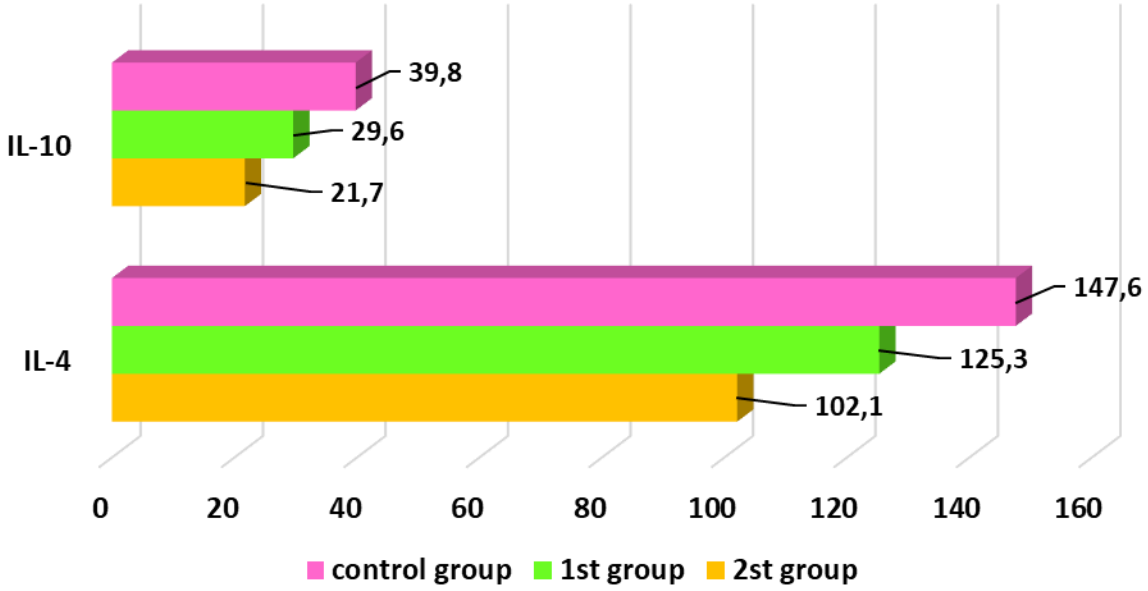

Figure 3. Level of anti-inflammatory cytokines in the examined pregnant women, pg/mL

Analysis of the data on IL-10 showed that its level in women with PE was significantly reduced, with the minimum value observed in pregnant women of group 1 (21.7 ± 1.2 pg/mL vs. 39.8 ± 2.0 pg/mL in the control group, P<0.01). The value of IL-10 in pregnant women with asymptomatic course of PE averaged 29.6 ± 1.7 pg/mL (P<0.05). IL-10 deficiency may amplify into an increased inflammatory response induced by TNF-α and IFN-γ against trophoblast and placental cells. Decreased IL-10 expression by the villous trophoblast is associated with a possible enhanced maternal immune response to fetal antigens and inadequate placental development in PE. IL-10 produced mainly by macrophages and dendritic cells may be a dominant factor in the genesis of PE development, participating in placental vascular damage [16].

The hypothesis that, in normal pregnancy, the maternal immune system tends to move away from a potentially damaging type 1 (Th1 - inflammatory) to type 2 (Th2 - suppressor) response has been repeatedly confirmed. In case of predominance of Th1-type cytokines, miscarriage or insufficient trophoblast insertion into maternal vessels, associated with pre-eclampsia and fetal intrauterine developmental delay, may occur. If low amounts of Th1-type cytokines are produced, excessive trophoblast invasion may occur, associated with bubblegum, placenta accreta, and chorioncarcinoma. Disorders of trophoblast invasion processes in early gestation lead to the realization of late pregnancy complications. Synthesis of proinflammatory cytokines leads to the development of inflammatory reaction and starts systemic dysfunction of maternal vascular endothelium, which can lead to such clinical manifestations of PE as hypertension, proteinuria, edema.

Consequently, the study of cytokine levels in pregnancy can serve as a prognostic criterion for the development of pregnancy pathology, in particular, the development of pre-eclampsia.

4. Conclusion

In pregnant women with preeclampsia, a significant increase in the level of proinflammatory cytokines (IL-1β, IL-6, IL-8) and a significant decrease in anti-inflammatory cytokines (IL-4, IL-10) were detected in plasma, indicating a shift in cytokine regulation in preeclampsia towards the Th1-mechanism. When type 1 T-helper cells are activated, there is an increased production of proinflammatory cytokines, which leads to a disruption of endocrine-immune interactions in the mother-fetus system and cytopathogenic effects on trophoblast and placental cells, which is clinically manifested not only by fetal rejection, but also by the development of PE in late gestation.

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