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Correlation of the system of cytokines in moderate and severe preeclampsia

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Summary

Objective of the study: To study the production of pro-inflammatory (IL-1β, IL- 2, IL-6, IL-8) and anti-inflammatory (IL-4, IL-10) cytokines in pregnancy complicated by preeclampsia in the third trimester. *Institution:* University Clinic of Gynecology and Obstetrics, Skopje, Republic of Macedonia. *Material and Methods:* Fifty women with pregnancies complicated by preeclampsia in the third trimester and 50 women with physiological pregnancy. Levels of IL-1β, IL- 2, IL-6, IL- 8, IL- 4, and IL- 10 were measured by using a solid -phase enzyme immunoassay. Statistical data processing was done using the application program SPSS for Windows 13, 0. To describe the distribution of analyzed variables, descriptive methods (mean, median, minim and max) were used . *Results:* In pregnancies complicated by preeclampsia, there are increased levels of proinflammatory cytokines and a change in the behaviour of opposing pools. Most pronounced changes in the levels of proinflammatory cytokines were observed in mild preeclampsia. In severe preeclampsia there was reduction of the concentration of anti-inflammatory cytokines IL- 4 and IL-10. *Conclusion:* The use of assessment cytokine profile monitoring of health status of women with preeclampsia is expedient.

Key words: Cytokines; Severe preeclampsia; Moderate preeclampsia; Correlation.

Introduction

One of the major concerns of modern medicine and molecular biology is the examination of the role of cytokines in the pathogenesis of various diseases. In clarifying the nature of immune responses, cytokines have participated in all the immune mechanisms and inflammatory reactions [1]. System disorders of cytokines leads to changes of immune competent cells, changes in immune homeostasis, and generally to disruption of the normal functioning of the immune system [2-4].

Changing spectrum of cytokines in the dynamics of gestational process deserves special attention due to their important function of immunomodulation. The process of change in cytokine levels during pregnancy is an important telltale of body's adaptive reaction in pregnant women. The regulation of the synthesis of cytokines during pregnancy is aimed at restructuring the intracellular interactions links to allow normal functioning of organs and body system of the mother and the genetically different fetus [5, 6]. Especially important is not only the change in the level of certain cytokines, but also the ratio of opposite pools, because it may reflect the activity and severity of the pathological process and the level of adaptation - compensatory reactions [7-12]. Imbalance of cytokines plays an integral role in the development of functional inferiority of immune competent cells and the pathogenic mechanisms of many diseases [13].

The evaluation of cytokine status in various forms of pathological pregnancy bears special interest owing to the changes in serum concentrations of cytokines, which have numerous biological effects, for they may be indicators of immune system diseases. [14-16].

In recent years, the leading role of immunological disorders in the pathogenesis of preeclampsia has often been evaluated. According to the literature, the development of preeclampsia is accompanied by pronounced changes in cytokine profile [17-19]. According to the modern concepts, preeclampsia is regarded as systemic and local level development to inflammatory response conditioned by hyper- activation of phagocytes [20]. Many researchers state that the pregnancy complicated by preeclampsia activates an entire range of pro-inflammatory cytokines, the high concentration of which is an unfavorable factor reflecting the activity and severity of the pathological process [21, 22]. Sequence authors inform that the preeclampsia ratio of pro-inflammatory and anti-inflammatory cytokines in peripheral blood increases substantially [23].

The purpose of this research is to study the formation of pro-inflammatory (IL-1β, IL-2, IL- 6, IL-8) and anti-inflammatory (IL-4, IL-10) cytokines in pregnancy complicated by preeclampsia of different degree in the third trimester.

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Materials and Methods

Examination of the cytokine profile in serum was conducted in 50 women with pregnancies complicated by varying degrees of preeclampsia in the third trimester of gestation hospitalized at the University Clinic of Gynecology and Obstetrics, Skopje, Republic of Macedonia. The severity of preeclampsia was determined according to the definition of the World Health Organization. Control group consisted of 50 women in the third trimester of normal pregnancy. Both groups were comparable for age, number of pregnancies, and births.

The level of IL-1β, IL-2, IL-4, IL-6, IL-8, and IL-10 was determined using a commercial test, using reagents from ELISA research kits. Cytokine levels in the serum were measured by the "sandwich" method of solid -phase enzyme immunoassay using double antibody. As a standard for comparison of each reaction used were recombinant cytokines. The detection was done by "Victor" immunoassay analytics. According to the titration of standard samples calibration graphs were made for each cytokine, as determined by their level in the range of detected concentrations (1-2000 pg/ml). Statistical data processing was done using the SPSS 13.0 software for Windows.

Descriptive methods (mean, median, minim and max) were used to describe the distribution of analyzed variables. Categorical variables were analyzed with chi-square test and Fisher's exact test, whereas quantitative variables were analyzed with Student's t-test for independent samples, Mann-Whitney U test (Z), Analysis of Variance (F), and Kruskal-Wallis ANOVA (H) test. To be considered statistically significant, differences between groups were set at $p < 0.05$, and to be highly significant, value was at $p < 0.01$.

Table 1. — *Comparative values of IL-2, IL-4, and IL-6 in moderate, severe preeclampsia, and control groups.*

Variable	Groups			
	PE N=50	Moderate PE (mPE) N=25	Severe PE (sPE) N=25	Control (CO) N=50
IL-2	41.6±34.9	37.6±27	45.7±41.6	16±8.3
mean±SD,	35.4	31.5	40,5	13,7
median, range	7.4 – 220.6	7.45 – 108.8	8.2 – 220.6	4.3 – 36.9
PE/CO Z=5.6 $p < 0.01$**				
mPE/ sPE / CO/		mPE/CO	sPE/CO	mPE/ sPE
H=31.9 $p < 0.01$**		$p < 0.01$**	$p < 0.01$**	$p = 0.4$
IL-4	0.62±1.02	0.74±1.26	0.51±0.71	0.39±0.12
mean±SD,	0.36	0.37	0.35	0.36
median, range	0.14 – 6.75	0.25 – 6.75	0.14 – 3.91	0.27 – 0.87
PE/CO Z=1.49 $p = 0.14$				
mPE/ sPE / CO/		mPE/CO	sPE/CO	mPE/ sPE
H=10.1 $p = 0.006$**		$p = 0.003$**	$p = 0.6$	$p = 0.01$*
IL-6	16±42.1	18.3±42.1	13.8±42.9	2.8±9.6
mean±SD,	1.38	1.74	1.15	1.02
median, range	0.66 - 185	0.66 - 185	0.73 - 185	0.42 – 68.4
PE/CO Z=2.6 $p = 0.01$*				
mPE/ sPE / CO/		mPE/CO	sPE/CO	mPE/ sPE
H=7.8 $p = 0.02$*		$p < 0.01$**	$p = 0.1$	$p = 0.2$

*$p < 0.05$; **$p < 0.01$

Results

A survey showed that in pregnancy complicated by preeclampsia, the level of all cytokines essentially changes compared with their level in physiological pregnancy. Thus, directional change was identified even in a lighter form of preeclampsia, i.e., elevated levels of pro- and anti-inflammatory cytokines except for IL-10, where in a downward trend in severe preeclampsia is recorded. Table 1 shows the comparative values of serum concentrations of IL-2, IL-4 and IL-6 in the studied groups.

The average concentrations of IL-2 in the group with preeclampsia was 41.6 ± 34.9 pg/ml, 37.6 ± 27 pg/ml in the group with moderate preeclampsia, and 45.7 ± 41.6 pg/ml in the group with severe preeclampsia. Average value of IL-2 was the lowest in the control group, at 16 ± 8.3 pg/ml. Statistical analysis as highly significant ($p < 0.01$) confirmed differences in serum concentrations of IL-2 among pregnant women with preeclampsia and healthy pregnant women. Healthy pregnant women have highly significant lower values of IL-2 compared to pregnant women with preeclampsia, both in regards to pregnant women with symptoms of moderate preeclampsia, and to those with severe symptoms of preeclampsia. Group with moderate preeclampsia showed insignificant ($p = 0.4$) lower values of IL-2 in serum as compared with pregnant women with severe preeclampsia.

The group of patients with preeclampsia, which included those with medium and with severe preeclampsia, had in-

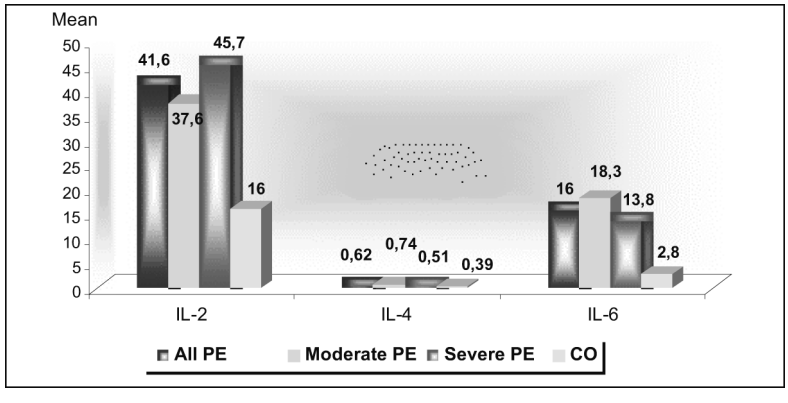

Figure 1. — Mean value of IL-2, IL-4, and IL-6 in moderate, severe preeclampsia, and control groups.

Table 2. — *Comparative values of IL-8, IL-10, and Il-1β in moderate, severe preeclampsia, and control groups.*

Variable	Groups			
	ALL PE (PE) N=50	Moderate PE (mPE) N=25	Severe PE (sPE) N=25	Control (CO) N=50
IL-8	46.4±117.2	57.1±93.6	35.7±138	13.6±33.1
Mean ±SD,	6.31	8.11	5.99	6.03
median, range	2.39 - 697	3.05 - 259	2.39 - 697	1.2 - 237
PE/CO Z=0.4 $p = 0.7$				
mPE/ sPE / CO/ H=3,6 $p = 0,17$				
IL-10	23.2±40.7	45.5±48.4	0.8±0.4	4.2±6.7
Mean ±SD,	1.36	27.28	0.75	1.47
median, range	0.2 - 164	0.56 - 164	0.2 – 2.12	0.44 – 26.28
PE/CO Z=0.6 $p = 0.5$				
mPE/ sPE / CO/ H=37.68 $p < 0.01$**		mPE/CO $p < 0.01$**	sPE/CO $p < 0.01$**	mPE/ sPE $p < 0.01$**
IL-b	6.6±25.7	1.9±4.9	11.3±35.7	1.8±7.4
Mean ±SD,	0.78	0,67	0.95	0.665
median, range	0.42 - 131	0.42 – 24.67	0.49 - 131	0.13 - 53
PE/CO Z=2.3 $p = 0.02$*				
mPE/ sPE / CO/ H=9.8 $p < 0.01$**		mPE/CO $p = 0.5$	sPE/CO/ $p < 0.01$**	mPE/ sPE $p = 0.04$*

*$p < 0.05$; **$p < 0.01$

significant ($p = 0.14$) higher values of IL-4 in serum, compared with the control group. Insignificant was the difference between the group with severe preeclampsia and the control group ($p = 0.6$), while the group with moderate preeclampsia had highly significant values higher than the control group ($p < 0.003$), comparing the group with severe preeclampsia ($p < 0.01$). Average serum concentrations of IL-4 was the lowest in the group of healthy pregnant women - 0.39 ± 0.12 pg/ml, while the group of pregnant women with symptoms of moderate preeclampsia had the highest average values of 0.51 ± 0.71 pg/ml. The group which included all pregnant women with symptoms of preeclampsia had averages of IL-4 from 0.62 ± 1.02 pg/ml.

Pregnant women with preeclampsia had highly significant ($p = 0.01$) higher values of IL-6 in serum compared to normotensive pregnant women, a variation due to the highly significant difference between pregnant women with moderate preeclampsia and healthy pregnant women. The difference between women with severe preeclampsia and healthy pregnant women was insignificant ($p = 0.1$). Both subunits of pregnants, medium, and severe preeclampsia, insignificantly differed in IL-6 values in serum. The average serum concentrations of IL-6 amount was 16 ± 42.1 pg/ml or 18.3 ± 42.1 pg/ml in the group with moderate preeclampsia, and 13.8 ± 42.9 pg/ml in the group with severe preeclampsia. Normotensive pregnant women had the lowest average value of IL-6 in serum from 2.8 ± 9.6 pg/ml. Mean value of IL-2, IL-4 and IL-6 in patients with moderate and severe preeclampsia, and control group are presented in Figure 1.

Pregnant women with preeclampsia and healthy pregnant women had insignificant ($p = 0.7$) different values of IL-8 in serum, and pregnant women with moderate and with severe preeclampsia had insignificant higher values compared to the control group ($p = 0.17$). Average values, median, and lowest and highest values of IL-8, IL-10, and IL-1β in the analyzed groups are presented in Table 2.

Statistical analysis confirmed insignificant differences in the values of IL-10 in serum among pregnant women with moderate preeclampsia and healthy pregnant women ($p = 0.5$), but the statistically highly significant ($p < 0.01$) difference among moderate preeclampsia / severe preeclampsia / control group was due to the lower values of this interleukin in severe preeclampsia group, comparing moderate preeclampsia in relation to the control, and due to the highly significant lower values when comparing control in relation to moderate preeclampsia group. Average concentrations of IL-10 in serum amounted to 23.2 ± 40.7 pg/ml in the group with preeclampsia, that is, 45.5 ± 48.4 pg/ml in the group of moderate preeclampsia, and 0.8 ± 0.4 pg/ml in the group with severe preeclampsia. In normotensive group, the average serum concentration of IL-10 was 4.2 ± 6.7 pg/ml.

Patients with preeclampsia had significantly ($p = 0.02$) higher serum concentrations of IL-b compared to nor-

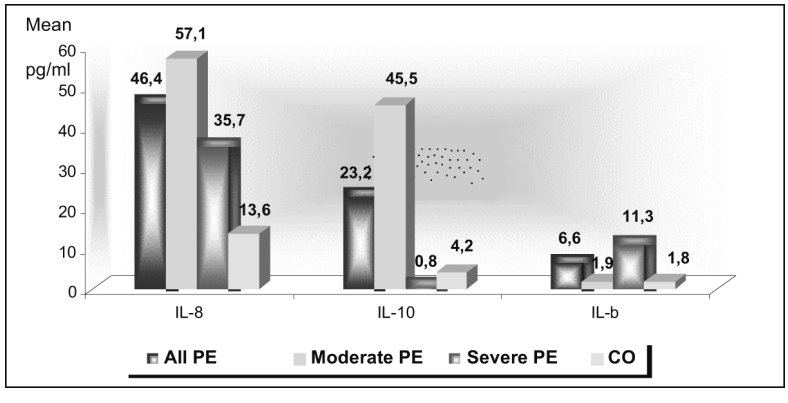

Figure 2. — Mean levels of IL-8, IL-10, and IL-1β in moderate, severe preeclampsia, and control groups.

motensive, due to the highly significant (p <0.01) higher concentrations in the group of severe preeclampsia versus control group, while the difference observed in moderate form of preeclampsia and control group was statistically insignificant (p = 0.5). Statistically significant, whereas p = 0.04, was the difference between the two subgroups of preeclampsia. The lowest average value of IL-1β was 1.8 ± 7.4 pg/ml, as registered in the control group, with similar average values measured in the group with moderate preeclampsia, while the highest average value in the group with severe preeclampsia measured 11.3 ± 35.7 pg/ml. Mean levels of IL-8, IL-10 and IL-1β in patients with moderate and severe preeclampsia and control group are presented in Figure 2.

Discussion

An analysis of the results of the present research, showed that the largest changes in the concentrations of pro-inflammatory cytokines were seen in moderate preeclampsia. In moderate preeclampsia there was increased synthesis of these cytokines, as the level of IL- 10 and IL-8 reaches maximum values. In severe preeclampsia, the level of pro-inflammatory cytokines (IL-4) either remained elevated or did not differ from the values characteristic of physiological pregnancy. Thus, the survey showed that the cytokine profile in pregnancies complicated by preeclampsia did not only increase the levels of pro-inflammatory cytokines, which coincides with the results of other studies [24, 25], but it also amended the ratio of opposite pools. However, changes in the cytokine level depending on the gravity of preeclampsia in the present study did not differ from the dynamics identified in other studies. Thus, many researchers argue that the concentration of IL-1β significantly rises with increasing severity of preeclampsia and reaches maximum values during severe preeclampsia [26]. An analogous situation was the change in the concentration of other pro-inflammatory cytokines (IL-6 and TNF-α), which increased with the worsening of the disease [27]. Based on elevated concentrations of pro-inflammatory cytokine array, researchers come to the conclusion that there are signs of systemic inflammatory response of preeclampsia [26, 28]. Some authors emphasize the increased synthesis of IL-2 in the third trimester of pregnancy complicated by preeclampsia, thereby significantly increasing the proportions of TNF-α / IL-4 and IL-2 / IL- 4 [29]. Based on the data obtained, they are drawing conclusions about the prevalence of Th- 1 immune response in this pathology.

A comparison of changes in cytokine profile with increasing severity of preeclampsia allow us to determine the levels of compensation of this pathological condition which reflect the degree of implementation and functional reserve of various mechanisms to maintain homeostasis. The first phase of the changes seen in mild preeclampsia is manifested by an increase when creating all studied cytokines, except IL-4 and IL-10. During the second phase IL-1β and TNF-α begin to decrease, ceding to further increment in the levels of IL-8 and IL- 6, which suppress the inflammatory reaction occurring as antagonists of IL-1β and TNF-α. In that period, the limiting role of IL-10 weakens and manifests itself by reduced levels compared with values in normal pregnancy, and hence indicates weakening of the compensatory mechanisms. The third phase, which can be called decompensated, is characterized by the absence of significant differences in the levels of IL-1β and IL-6, compared with the same level during normal pregnancy. This occurs in the background of increased concentrations of other pro-inflammatory cytokines and reduced levels of anti-inflammatory cytokines IL-4 and IL-10.

In regards to changes whereby anti-inflammatory cytokine concentrations in severe preeclampsia take place in the opposite direction, moderate phase can be considered a critical stage during complicated pregnancy, which comes as the most functional strain to the homeostatic system. It can be assumed that with the effect of moderately aggressive factors acting as initiators of mediator synthesis for intercellular interaction (with moderate preeclampsia), the development of the immune response is regulated by the interaction of cytokines and their antagonists. Through the increasing severity of the pathological process, there is reduction in the impact of regulatory factors that limit the systemic effect, thus causing enhanced creation of cytokines that are activated immunocytes. At a certain stage of this process, spending of functonal reserves of mononuclear cells occurs, resulting in a state of decompensation characterized by “leukocyte depression” in which the synthesis of immunoregulatory factors significantly reduces.

Conclusion

Sufficient evidence from animal and human studies has now been gathered to reveal the pathogenesis of preeclampsia on the basis of the influence of cytokines both in the placenta and in the periphery. A unifying hypothesis for preeclampsia is that inadequate trophoblast invasion and remodeling of spiral arteries stimulate placental ischemia and hypoxia via intermittent perfusion of the placenta; this results in an increased release of trophoblast microparticles into the maternal circulation followed by increased production of maternal pro-inflammatory cytokines and activation of maternal endothelial cells. This is proposed to eventually lead to “systemic, diffuse endothelial cell dysfunction” - the fundamental pathophysiological feature of this syndrome.

While understanding the etiology and pathophysiology of preeclampsia is certainly of interest from a basic medical science perspective, it also has important implications for the treatment and management of this dangerous complication. There is renewed optimism that basic and clinical

research, which was instrumental in elucidating the pathogenesis of this disease, will lead to the rational design of interventions for the management and treatment of this important and common complication of pregnancy. In this way, the results of the present study show that pregnancy complicated by preeclampsia are taking significant immunological changes. Disorders in the immune system are associated with the severity of a particular complication of gestational process.

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