R e s e a r c h   A r t i c l e

THE CORRELATION OF MATERNAL INTERLEUKIN 4 AND INDICATORS OF MODERATE AND SEVERE PREECLAMPSIA

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ABSTRACT

Aim

The purpose of this research is to study the formation of anti-inflammatory IL-4 cytokine and some indicators of moderate and severe preeclampsia, and normotensive pregnancies in the third trimester of pregnancy

Material and methods

This is a single center study of 100 women with third trimester pregnancies. All patients gave their informed consent prior to inclusion in the study.Patients were divided into two groups: 50 women with pregnancies complicated by varying degrees of preeclampsia and control group of 50 normotensive pregnancies.

Cytokine levels in the serum were measured by the "sandwich" method of solid-phase enzyme immunoassay using double antibody.

Statistical data processing was done using the SPSS13.0 software for Windows. Logistic regression analysis (Binary Logistic Regression) was used to determine the predictive role of the parameters analyzed in the occurrence of severe preeclampsia.

Results

The obtained values of Pearson- coefficients' linear correlation (r) indicate that IL-4 negatively correlates with LDH, creatinine, uric acid and proteinuria, and positively correlates with number of blood platelets. IL-4 in severe preeclampsia has a downward trend, between 28-40 weeks of gestation it may be considered a prognostic marker for the development of severe preeclampsia.

KEYWORDS:Preeclampsia,cytokines,proteinuria,indicators.

INTRODUCTION

Hypertensive disorders in pregnancy occur primarily in humans, and are estimated to cause 10%–15% of maternal deaths [1]. Preeclampsia, defined by hypertension and proteinuria, is a well-described disorder ([2]). It is an important cause of maternal perinatal morbidity and mortality worldwide [3].

Scientific studies suggest that the pathogenesis of preeclampsia is the systemic inflammatory response syndrome with the development of destructive inflammatory process, immune disorders, and the imbalance of cytokine regulation in gestation processes [4].

However, the relationship between the development of endothelial dysfunction and violation of cytokine regulation in different clinical forms of preeclampsia needs to be studied.

Proteinuria has been proposed to be both an indicator of severity as well as a predictor of outcome in preeclampsia [5]. Many clinicians still make major management decisions based on the degree of proteinuria in such patients.

In 1843, John Lever of Guy's Hospital in London discovered the presence of albumin by boiling the urine from pregnant women with puerperal convulsions [6,7]. Preeclampsia is differentiated from gestational hypertension by the presence of proteinuria and is the most common cause of nephrotic syndrome in pregnancy. The quantity of protein that is excreted in the urine varies widely. Significant protein excretion is defined as ≥300 mg in a 24-h urine collection or 1+ or greater on urine dipstick testing of two random urine samples that are collected at least 4 h apart [8].

The serum uric acid level used to be an indicator of preeclampsia, but has been found to lack sensitivity and specificity as a diagnostic tool. However, an elevated serum uric acid level may be of some use in identifying pregnant women with chronic hypertension who have an increased likelihood of having superimposed preeclampsia[9].)

A lactate dehydrogenase (LDH) test is a non-specific test that may be used in the evaluation of a number of diseases and conditions. Thus, the blood level of LDH is a general indicator of tissue and cellular damage and is used as indicator for preeclampsia [10].Several studies have confirmed the accentuation of platelet activation in preeclampsia, which remains an important obstetric complication affecting ~2 to 4% of pregnancies. Detection of aberrations of platelet function and activation appear to have predictive value for preeclampsia diagnosis(11).

The purpose of this research is to study the formation of anti-inflammatory cytokineIL-4 and some indicators in the third trimester of pregnancy in moderate and severe preeclampsia and normotensive pregnancies

MATERIAL AND METHODS

This is a single center study of 100 women with third trimester pregnancies. All patients gave their informed consent prior to inclusion in the study.Patients were divided into two groups: 50 women with pregnancies complicated by varying degrees of preeclampsia and control group of 50 normotensive pregnancies.

Preeclampsia was defined by increased blood pressure (≥ 140 mmHg systolic or ≥ 90 mmHg diastolic on more than 2 occasions at least 6 hours apart)after the 20th week of gestation in a woman with previously normal blood pressure, accompanied by proteinuria (≥ 0.3 g/24h in the absence of urinary tract infection). Preeclampsia was graded as severe if any of the following criteria were present: blood pressure ≥ 160 mmHg systolic or ≥ 110 mmHg diastolic, or proteinuria ≥ 5.0 g/24h.

Exclusion criteria for all surveyed groups were the presence of eclampsia, endocrine diseases, diabetes mellitus, hyper- or hypothyroidism and other endocrine disorders requiring hormonal correction; the presence of TORCH-infection markers and other chronic viral or bacterial processes in the acute phase; as well as autoimmune diseases and malignant neoplastic processes.

The level of maternal IL-4 and other indicators of preeclampsia (proteinuria, LDH, platelets count, creatinin and acidum uricum) were examined.

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, which are part of the test - whale. 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 SPSS13.0 software for Windows. Logistic regression analysis (Binary Logistic Regression) was used to determine the predictive role of the parameters analyzed in the occurrence of severe preeclampsia.

RESULTS

Results of this study showed that the value of anti-inflammatory IL - 4 in serum is a significant factor associated with the occurrence of severe preeclampsia (p═0.003).Increased serum concentrations of IL- 4 reduce the likelihood of developing severe preeclampsia 94.6 % (95.0 % CI 0.008 – 0.367).

Sensitivity of this parameter to predict pregnant women with symptoms of severe preeclampsia was 96 %, specificity was 84 % (Table 1).

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Table 1:.*Univariate logistic regression analysis - IL- 4 in the prediction of severe eclampsia*

Variable	B	S.E.	Wald	Sig.	Exp(B)	95% CI for Exp (B)	
IL – 4	-2.992	0.980	8.895	0.003**	0.054	0.008	0.367
Constant	1.934	0.667	8.398	0.004	6.919		

Dependent variable: IL – 4 severe preeclampsia/moderate preeclampsia, *p<0.01

Sensitivity = 96%

Specificity = 84%Table 2 shows the predictive impact of uric acid and proteinuria in relation to the value of IL - 4 in pregnant women with preeclampsia. Only 19.6% of the change of IL-4 can be explained with the change of uric acid. From both parameters, only proteinuria is confirmed as a significant prediction factor that affects the value of IL-4; with each increase of 1g/L in proteinuria, serum concentrations of IL-4weres reduced by an average of 0.255 (95% CI for B 0.476 to 0.034).

Table 2:*Multiple linear regression models*

	AdjustedRSquare = 0.196R=0.442			F=5.73 df=2 p=0.006		95% CI for B	
	Unstandardized Coefficients		Standardized Coefficients	T	Sig.		
	B	Std.Err.	Beta			Lower Bound	Upper Bound
Constant	3.121	0.789		3.958	0.000	1.535	4.708
Acidum uricum	-0.005	0.003	-0.254	-1.891	0.065	-0.10	0.000
proteinuria	-0.255	0.11	-0.311	-2.317	0.025	-0.476	-0.034

Dependent variable: IL – 4

Table 3:*Multivariate logistic regression analysis for prediction of severe eclampsia*

Variable	B	S.E.	Wald	Sig.	Exp(B)	95,0% CI for Exp (B)	
age	0.2	0.086	5.350	0.021	1.221	1.031	1.446
nuliparity	1.816	1.114	2.657	0.103	6.145	0.692	54.534
Systolic p => 160	3.711	1.053	12.412	0.000	40.900	5.189	322.371
Diastolic p => 100	2.414	0.843	8.192	0.004	11.176	2.140	58.360
proteinuria	3.081	1.307	5.56	0.018	21.785	1.682	282.123
LDH => 450	2.066	0.915	5.102	0.024	7.896	1.314	47.433
albumin	-0.239	0.125	3.66	0.056	0.787	0.616	1.006
creatinine	-0.067	0.035	3.696	0.055	0.935	0.873	1.001
Platelets =< 150	-0.006	0.013	0.236	0.627	0.994	0.97	1.019
IL – 4	-2.992	0.980	8.895	0.003**	0.054	0.008	0.367

Dependent variable/ severe preeclampsia /moderate preeclampsia

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Table 3 presents the results of multivariate logistic regression analysis for determining the independent associations between the analyzed risk factors and severe preeclampsia. In the regression analysis variables or risk factors that were proved as significant in univariate analysiswere included.
Multiple analysis confirmed the systolic blood pressure of 160 mmHg or higher, diastolic blood pressure of 100 mmHg and higher, persistent proteinuria in pregnancy, the values of serum LDH 450 and higher, and reduced serum concentrations of IL- 4as independent significant factors for severe preeclampsia after adjusting age of pregnant women.

Figures 1, 2, 3, 4 and 5 show the results of the examined linear correlation between IL- 4 as the dependent variable,

and the following parameters as independent variables serum levels of LDH, creatinine and uric acid, platelet count and proteinuria.

The resulting values of Pearson's coefficient linear correlation (r) show that IL-4 negatively correlated with LDH, creatinine, uric acid and proteinuria, and positively correlated with the platelet count. Only the correlation of IL-4 and uric acid and proteinuria was statistically significant. These two correlations are negative, or indirect, indicating that by increased serum uric acid concentration and increased amount of proteins in urine, the value of IL-4 in the serum significantly ($p = 0.023$), and highly significantly ($p = 0.009$) decreases
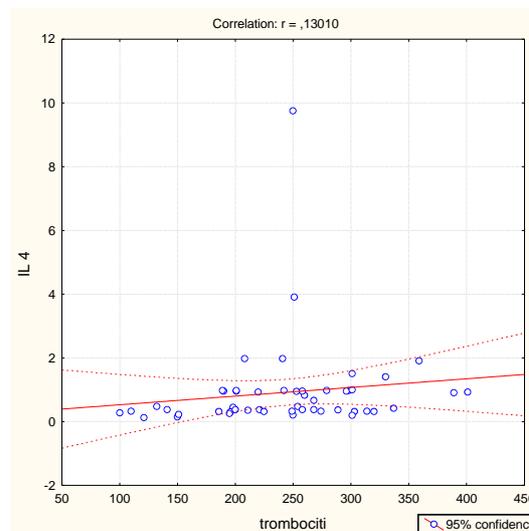
Figure 1:*Correlation of IL-4 / LDH*
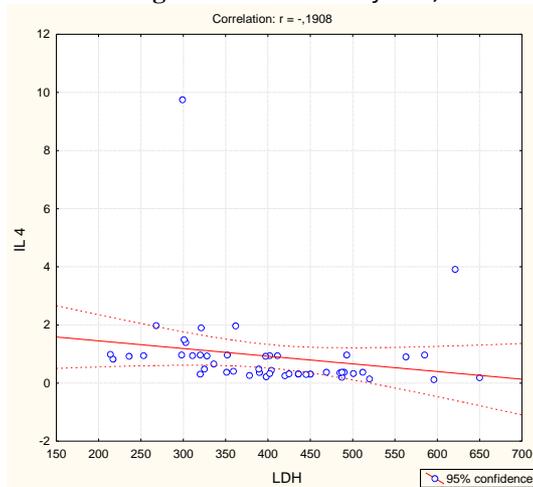
r$=$ - 0.191 p$=$0.184
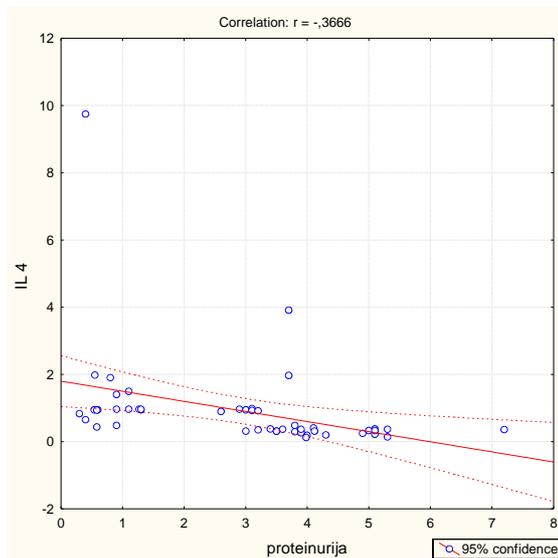
Figure 2:*Correlation of IL-4 / creatinine*
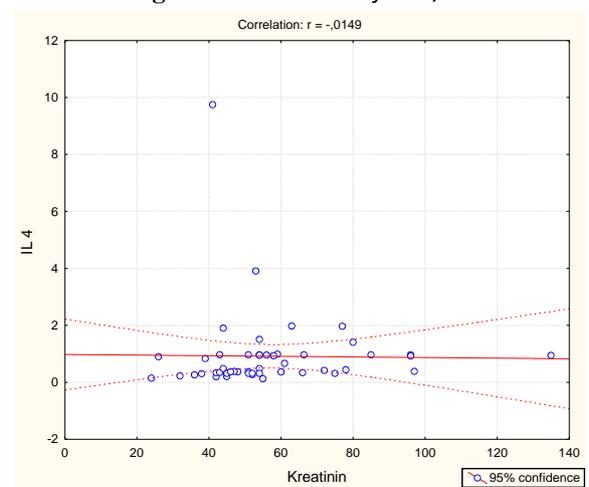
r$=$ - 0.149 p$=$0.918

Figure 3:*Correlation IL-4 / platelets*
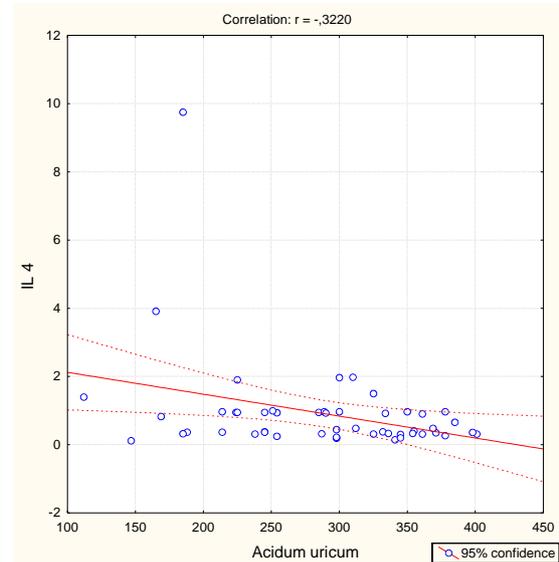

r $=$- 0.130 p$=$ 0.368

Figure 4:*Correlation IL-4 / proteinuria*

r = - 0.367 p=0.009**p<0.01

Figure 5: *Correlation IL-4 / acidum uricum*

r= - 0.322 p=0.023*p<0.05

DISCUSSION

Recent studies showed that the altered immune system could lead to preeclampsia and several epidemiological studies and animal models support this idea. Immune maladaptation has been reported in PE too [12]. There are multiple factors which develop PE and play key roles in its pathophysiology. Several studies demonstrated that Th1/Th2 balance has changed in patients affected with PE [13] and plasma levels of proinflammatory cytokines in PE pregnant women are higher in comparison with normal pregnant women.Furthermore, some evidences indicated that serum levels of interlukine-4 in PE patients have decreased. Interleukin-4 belongs to cytokines released by Th2 and acts as an anti-inflammatory cytokine [14].

This study demonstrates changes in IL-4 in women with preeclampsia compared with those with a normal pregnancy outcome. IL-4 has been identified as an important cytokine in pregnancy.

Some studies suggest a proportional link between the level of proteinuria and adverse clinical outcome. Page et al., in a prospective study of almost 13,000 pregnant women found that significant proteinuria (defined as 2+ or more on dipstick analysis) was associated with an increase in stillbirth rates, fetal growth restriction and neonatal morbidity, when associated with hypertension[15].Other studies suggest that it is the presence of proteinuria rather than the severity, which is associated with poorer outcomes. There is evidence that even the finding of trace proteinuria in pregnant women with hypertension is associated with an increase in adverse outcome [16].

This finding may have contributed to the variation in the diagnostic performance among the studies [17] significant proteinuria of 150mg-180mg/24hrs urine has been established in the preeclamptic group of this study which is similar to finding of Nisell et al. [18], Lindheir et al.[19] and

Davison [20], they reported 300mg/24hrs of protein in urine of preeclamptic women, while Waugh et al.[21] between 150mg/24hrs-200mg/24hrs and Higby et al.[22] who recorded between 120mg/24hrs-200mg/24hrs. The mean platelets count is significantly low when compared to pregnant non hypertensive groups. This study observed decrease of platelets counts among the preeclamptic group and is consistent with findings in other study, they all found that platelet count were significantly low in severe preeclampsia groups. This result proposes a possible relationship between the platelet count and the severity of preeclampsia.In our study the correlation with the platelets count is positive.

Considering the changes of the anti-inflammatory cytokine concentrations in severe preeclampsia caused in the opposite direction, moderate phase can be considered a critical stage in the complicated pregnancy.

Such a decrease indicates a depletion of adative mechanisms, aimed at relief of excessive activity of the inflammatory process and the development of physilogical immunosuppression during pregnancy and, apparently, can be an additional diagnostic criterion for the prediction and assessment of the severity of the pathological process.

With increasing severity of the pathological process, the impact of regulatory factors that limit the systemic effect is reduced.

CONCLUSION

There is a significant decrease in concentration of IL-4 in blood serum in women with severe preeclampsia, compared with other indicators in moderate preeclampsia that are increased. This is a major pathogenetic difference of severe preeclampsia and normal pregnancy.

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