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RESEARCH ARTICLE

The role of inflammatory markers hs-CRP, sialic acid, and IL-6 in the pathogenesis of preeclampsia and intrauterine growth restriction

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ABSTRACT. ***Objective:*** **The aim of our study was to evaluate serum high-sensitivity C-Reactive Protein (hs-CRP), sialic acid (SA), and interleukin-6 (IL-6) levels in pregnancies complicated with preeclampsia (PE) and intrauterine growth restriction (IUGR) and to compare with healthy pregnancies.** ***Materials and Methods:*** **This study was conducted at a tertiary-level maternity hospital with 80 pregnant women. Fasting blood samples were taken from 44 consecutive women with pregnancies complicated by PE (*n*: 20) and IUGR (*n*: 24), and 36 were from normal pregnancies. Serum hs-CRP, SA, and IL-6 concentrations were measured in all participants.** ***Results:*** **Serum mean hs-CRP, SA, and IL-6 levels were higher in the PE and IUGR group when compared with the control group, but this difference was statistically insignificant (*P*>0.05). No significant correlation was observed between these inflammatory markers (*P*>0.05).** ***Conclusion:*** **The serum levels of hs-CRP, SA, and IL-6 were not elevated in pregnancies complicated with PE and IUGR compared with normal pregnancies. Since pregnancy is already a process with inflammation, fluctuations in some markers related to inflammation may be masked by the gestation itself. A local subclinical inflammation may have a role in the pathogenesis of PE and IUGR rather than systemic inflammation.**

Key words: preeclampsia, fetal growth restriction, inflammation, pregnancy

Pregnancy is a condition in which the natural immune system is activated and this activation may be exaggerated in complicated situations such as preeclampsia (PE) and intrauterine growth restriction (IUGR) [1]. PE usually occurs after 20 weeks of gestation in nulliparous and is characterized by hypertension, concomitant proteinuria, and/or end organ dysfunction. It is one of the most important causes of maternal and fetal morbidity and mortality [2]. IUGR is described as the inability of the fetus to gain appropriate weight according to gestational age and sex, as determined by population standards. The most common cut-off point is under the 10th percentile [3]. IUGR is the second most important cause of perinatal death after premature birth [4]. High risk of perinatal death has led to a very intensive investigation of the pathophysiology of these two clinical entities by researchers. Therefore, understanding the pathogenesis and predicting PE and IUGR are very important for taking the necessary measures timely and for preventing possible complications.

In these pathologies, several studies have shown that a number of released cytokines and inflammatory markers are responsible for endothelial damage, but the results are conflicting and no consensus has yet been reached on which markers may be better predictive of PE and IUGR [5, 6]. When we look at the literature, we have not found any study investigating the relationship with the above pathologies by combining acute phase reactants such as hs-CRP and SA with IL-6, a marker of cellular immune response. Therefore, in this study, with the help of the above-mentioned markers we aimed to investigate the role of exaggerated immunological mechanisms in those two important pregnancy complications.

MATERIALS AND METHODS

This prospective study was conducted in the Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara, Turkey between August 2009 and March 2010. The study group consisted of 80 patients who applied to antenatal and perinatology outpatient clinics and services on these dates. The intuitional review board approved the study and informed consent was obtained from all the patients. All of the information belonging to the patients was taken through a questionnaire form. All pregnant women in the study group were healthy without any previously known disease. Women in active labor and women who have essential hypertension, gestational diabetes, multiple pregnancy, collagen tissue diseases, additional

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pathologies such as renal and autoimmune diseases, fetal malformation, early membrane rupture, clinical sings of chorioamnionitis (maternal fever, vaginal discharge, fetal tachycardia) and women who were treated with magnesium sulfate were excluded from the study. Smokers, corticosteroids/non-steroidal anti-inflammatory, and illegal drug users were also excluded.

20 of 80 patients were PE, while 24 were isolated IUGR patients, and 36 were normal pregnant women without any complicated condition accepted as control group. PE was diagnosed after 20 weeks of gestation, 140 mmHg of systolic blood pressure measured at rest twice every 4 hours and 90 mmHg of the diastolic blood pressure measured after 4 hours interval and detection of proteinuria over 300 mg /dl in 24-hour urine measurements of patients. The IUGR diagnosis was made in serial fetal biometrics, where measurements or birth weight were determined below the 10th percentile. All patients in the IUGR group had a fetus with unexplained IUGR. Patients with features such as congenital structural anomaly, chronic intrauterine infection and chromosomal aberration that may cause IUGR were excluded from the study.

The last menstrual date was used to determine the gestational week of the patients and was confirmed by first trimester USG measurements. Venous blood samples were taken from all patients after one night of fasting at the time of initial diagnosis. Blood samples for IL-6 and SA were placed on jelly-free tube while blood samples for hs-CRP were placed in the biochemical tube, and the sera were stored at -80 ° C until the assay time. Routine hematological and biochemical tests were run and 24-hour urinalysis was performed in all women at the time of the study enrollment. During the collection of 24-hour urine samples after the patients make their first urine out, they began to collect their urine until the next morning including the same-day first morning urination.

Hs-CRP was measured on Cobas Integra 400 Plus using a latex particle-enhanced immunoturbidimetric assay according to the manufacturer's instructions (Roche Diagnostics, Indianapolis, IN). Serum levels of IL-6 were analyzed by the solid phase enzyme-linked chemiluminescence immunometric assay on the IMMULITE 1000 analyzer following the manufacturer's instructions (Siemens Diagnostic Product Corporation, Los Angeles, CA). SA levels were measured by the enzyme-linked immunosorbent assay (ELISA) method using a commercially available diagnostic kit (Cusabio Biotech Co., Ltd., Wuhan, China).

STATISTICAL ANALYSIS

Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 15 was used for statistical analysis. Compliance with the normal distribution of data was analyzed considering the Kolmogorov-Smirnov test. Parametric methods were used to analyze the variables with normal distribution whereas in the analysis of non-normally distributed variables non-parametric methods were used. In order to compare the independent groups, one-way analysis of variance and Kruskal Wallis tests were used. Partial correlation analysis was performed to analyze the correlation between the inflammatory markers measured. Mean ±standard deviation and median (minimum-maximum) for quantitative data were computed. Data were examined in the 95% confidence level, and statistical significance was set at $P<0.05$.

RESULTS

The average maternal age in the control group was calculated as 24.5±5.9 years. The average age was 30 ±5.3 years and 23.5±5.6 years in the PE and IUGR groups, respectively (*P*: 0.001). There were no significant differences between the groups in terms of mean gravidity and parity ($P>0.05$) (*table 1*). Mean BMI was significantly higher in the PE group compared to controls and IUGR group [30.2±5.6 kg/m^2, 29.4±1.8 kg/m^2, and 29.3±2.0 kg/m^2, respectively ($P<0.05$)]. No significant difference was observed between the groups with regard to gestational age at admission (*P*: 0.058). The average birth weight of the PE group was calculated as 2465 ± 792 gr. The birth weight of the IUGR group was calculated as 2550 plusmn;439 ($P>0.05$).

37.5% of the PE group, 40% of the IUGR group, and 38.9% of the control group used prenatal vitamins. There was no difference in the use of vitamins among the groups ($P>0.05$). The mean systolic/diastolic blood pressures of the PE group were 150±11.1/110 ±10 mmHg while it was 90±13/70±7.4 and 118 +10.8/70+10.1 mmHg in the control and IUGR group, respectively ($P<0.001$).

The mean value of hs-CRP, one of the markers of inflammation in the PE group, was 5.1±6.2 mg/L whereas in IUGR group this value was calculated as 4.7±5.1 mg/L. In the control group, mean value was found as 4.6±7.9 mg/L. There was no significant difference between the groups in terms of hs-CRP levels ($P>0.05$). The mean value of IL-6 in the PE group was 3.7±20.1 pg/mL. In the IUGR group this

Table 1
Demographic features of the subjects.

	Preeclampsia (*n*: 20)	**Control (*n*: 36)**	**IUGR (*n*: 24)**	***P*-value**
Age (years)	30±5.3	24.5±5.9	23.5±5.6	0.001
Gravity	2±1.1	2±0.9	1±1.2	0.519
Parity	1±0.8	1+0.8	0±0.8	0.147
BMI (kg/m^2)	30.2±5.6	29.4±1.8	29.3±2.0	0.031
Gestational age (weeks)	36±5.3	36±3.0	37±1.1	0.058

value was calculated as 4.2±16.3 pg/mL, and it was calculated as 2.4±8.9 pg/mL in the control group. There was no significant difference between the groups in terms of IL-6 levels ($P > 0.05$). SA values were also compared between the groups. Its serum levels were measured as 23.1±24.2 mg/dL, 19.5±13.1 mg/dL, and 18.0±68.9 mg/dL in the PE, IUGR, and control group, respectively. No significant difference was detected between the groups in terms of SA levels ($P > 0.05$). When the white blood cell levels were compared between the groups, the mean value in the PE group was calculated as 10300±2861 whereas in the IUGR group it was calculated as 11950 ±4345. In the control group, the mean white blood cell level was 9500±1856. There was no significant difference between the groups in terms of white blood cell levels ($P > 0.05$) (*table 2*). In partial correlation analysis, no significant difference was observed between these inflammatory markers even after adjusting for maternal age ($P > 0.05$).

DISCUSSION

In this study our aim was to strengthen the hypothesis that the natural immune system is activated in pregnancy and that this activation increases even more in complicated cases such as PE and IUGR. Therefore, we investigated changes in three well-known inflammatory markers such as IL-6, hs-CRP and SA in PE, IUGR and healthy normal pregnancy groups. In our study we have seen that none of the aforementioned markers remarkably increased in the sera of the PE, IUGR, and control pregnancies.

Healthy human pregnancy requires appropriate adaptation of the maternal immune system. In doing so, it should be kept at the optimum level to protect the body on one hand and provide a slight tolerance in order to be able to accept the semiallogenic fetus on the other hand. This adjustment is made by the shifts and balances between the adaptive and congenital immune systems. Therefore, increases in acute phase reactants and cytokine levels mediate this interaction [7].

PE is regarded as an exaggerated maternal inflammatory response to placental stimuli, which is completely different from the controlled response seen in normal pregnancy. It is currently a worldwide cause of maternal, fetal, and neonatal morbidity and mortality with a prevalence of 2-8% [8]. Definitive pathophysiology and pathogenesis are not yet clear despite some progress [9]. The IUGR refers to an increased desidual cellular immunity that limits trophoblastic invasion and leads to inadequate placentation. For this reason, the hypothesis that placental insufficiency in IUGR cases is an immunological phenomenon has been investigated in recent years as in PE [10]. Some mediators released from the placenta are thought to result in inflammation on the maternal side. Increased maternal inflammation and released cytokines lead to endothelial damage and impaired placental circulation.

CRP, a sensitive marker of nonspecific tissue damage and inflammation, is an acute phase reactant primarily produced by hepatocytes, which is proposed to play a role in revealing the characteristic inflammatory response seen in preeclampsia [11]. Similarly, Garcia and colleagues showed an increase in CRP levels in PE and stated that this increase from the first trimester could be used as a predictor for PE [12]. But in a different study by Stefanovič and colleagues they observed that CRP as the marker of inflammation was not increased in their preeclamptic patients [13]. In our study, when we compared PE and IUGR with normal healthy control group pregnancies, we did not find any significant difference among the groups in terms of CRP levels. Auer et al in their study reported that the fetus could reduce the inflammation in mother to some extent in order to present itself with a more nutritional factor in developing cases of preeclampsia and that CRP could decrease as a result. Again, in the same study, they found that 166 protein was modified in preeclampsia and IUGR cases. From this point of view, it is possible to indicate that markers associated with preeclampsia and IUGR are not composed of acute phase reactants and there are too many unknowns on this subject [14].

Von Versen- Hoeynck FM and colleagues compared CRP levels between pregnant and nonpregnant women and observed significant difference between pregnant and nonpregnant women [15]. In fact, this increase in CRP levels may mask the potential differences in CRP concentration in PE and IUGR complicated pregnancies. This result may also partly explain why in our study, there was no association in terms of CRP levels between PE and IUGR.

SA, similar to CRP, is an acute phase reactant and is a strong predictor of coronary heart disease and cardiovascular mortality. Women with preeclamptic stories have increased CVD risk. More than one study emphasize that accelerated atherosclerosis makes this increased risk of CVD more apparent. It also suggests that endothelial dysfunction and inflammation play an important role in the risk of CVD in preeclamptic women [16]. The change in CRP levels has been shown

Table 2
Comparison of some inflammatory markers between the groups.

	Preeclampsia (*n*: 20)	**Control (*n*: 36)**	**IUGR (*n*: 24)**	***P*-value**
Hs-CRP (mg/L)	5.1±6.2	4.7±5.0	4.6±7.9	0.990
IL-6 (pg/mL)	3.7±20.1	4.2±16.3	2.3±8.9	0.132
Sialic (mg/dL) Asid	23.1±24.2	19.5±13.1	18.1±68.9	0.116
White blood cell count (/mm^3)	10300±2361	11950±4345	9500±1856	0.194

to be more significant in women with lower BMI in a study. However, as the CRP levels were not adjusted for BMI, reliability of CRP levels was shown suspicious in this study [17].

Von Versen-Hoeynck et al. observed that SA increased significantly during pregnancy in their study similar to CRP. However, they did not observe any difference in SA concentrations when comparing preeclamptic patients with uncomplicated pregnancies, transient hypertension, or SGA infants [15].

Alvi and her colleagues reported an increase in pregnancy levels consistent with the data of Goni and colleagues [18, 19]. But Sydow et al. reported that there was no significant increase in serum SA levels in pregnancy [20]. As can be seen, there are contradictory publications about sialic acid. This may be due to the fact that sialic acid can be greatly affected by the dietary. Because, in the studies conducted, the short-term application of sodium benzoate, which is used as a preservative in food, decreased the amount of sialic acid in the serum and tissues significantly [21]. Considering the fact that fast foods are consumed more and more nowadays, it can be thought that sialic acid levels in pregnancy may be less than that.

Similarly, in our study we could not detect a significant difference in SA levels between PE and IUGR pregnancies and healthy control pregnancies. Such differences in the results of these studies may be due to heterogeneity between working populations in developed and developing countries, differences between laboratories and differences in chronic subclinical infection levels [19].

IL-6 is the most important cytokine responsible for the synthesis of acute phase reactants and causes apoptosis in endothelial cells. Therefore, this may cause endothelial dysfunction. In a study by Sapmaz and his colleagues, 20 mild and 20 severe PE patients were compared with 20 normal healthy pregnant women [22]. In preeclamptic patients they found that IL-6, CRP and neutrophil values increased and platelet values decreased according to the severity of the disease. Similarly, in a study conducted by Stallmach et al., IL-6 and IL-8 levels in venous blood samples of preeclamptic pregnant women were significantly higher than those in healthy pregnancies [23]. But in another study however, Obsjon et al. compared preeclamptic patients with healthy group patients and found lower IL-6 levels in preeclamptic patients [24].

In a study comparing 14 IUGR pregnancies with 28 healthy control group pregnancies, Bartha et al. found significantly higher levels of IL-6 and TNF-a in the IUGR group [10]. In such studies, an important point to consider is the use of anti-inflammatory drugs. The use of anti-inflammatory drugs may cause low inflammatory markers. Lindsey A et al. in their study found lower inflammatory cytokine and acute phase protein levels in patients using aspirin [25].

We have accepted the use of any anti-inflammatory drugs as an exclusion criterion. Despite this exclusion criterion, IL-6 levels did not differ significantly between PE, IUGR, and healthy control group pregnant patients in our study.

Our study is the first study of all these markers investigated together. The fact that patients did not take any medication, that our study was prospective, and that we received serum samples at the time of initial diagnosis were powerful parts of our study. Moreover, our results were not correlated with the severity of preeclampsia and IUGR as we did not group our patients as severe, moderate, and mild IUGR or preeclampsia. However, as it can be understood from the findings, most of our patients are in the mild group, which may have caused the inflammatory markers to become insignificant. Few number of patients included in our study is the another limited aspect of our study.

As a result, if we make an assessment in terms of IL-6 or other investigated markers, we could not find meaningful differences in our study because the possibility that the pregnancy itself is a process with inflammation and that the increase of these markers is masked by the inflammation itself. Therefore, there is a need for more comprehensive and homogeneous studies to clarify the role of inflammation in the pathogenesis of the PE and IUGR [26].

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REFERENCES

1. Redman CWG, Sargent IL. Preeclampsia and the Systemic Inflammatory Response. *Semin Nephrol* 2004; 24(6):565-70.
2. Tokmak A, Güney G, Aksoy RT, *et al*. May maternal antimullerian hormone levels predict adverse maternal and perinatal outcomes in PE? *J Matern Fetal Neonatal Med* 2015; 28 (12):1451-6.
3. American College of Obstetricians Gynecologists. ACOG Practice bulletin no. 134 fetal growth restriction. *Obstet Gynecol* 2013; 121(5):1122-33.
4. Radulescu L, Ferechide D, Popa F. The importance of fetal gender in intrauterine growth restriction. *J Med Life* 2013; 6 (1):38-9.
5. Monte S. Biochemical markers for prediction of preclampsia: review of the literature. *J Prenat Med* 2011; 5(3):69-77.
6. Albu AR, Anca AF, Horhoianu VV, Horhoian IA. Predictive factors for intrauterine growth restriction. *J Med Life* 2014; 7 (2):165-71.
7. Moffett King A. Natural killer cells and pregnancy. *Nat Rev Immunol* 2002; 2(9):656-63.
8. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet* 2010; 376(9741):631-44.
9. Al-Jameil N, Aziz Khan F, Fareed Khan M, Tabassum H. A brief overview of preeclampsia. *J Clin Med Res* 2014; 6(1):1-7.
10. Bartha JL, Romero-Carmona R, Comino-Delgado R. Inflammatory cytokines in intrauterine growth retardation. *Acta Obstet Gynecol Scand* 2003; 82(12):1099-102.
11. Ustun Y, Engin-Ustun Y, Kamaci M. Association of fibrinogen and C-reactive protein with severity of pre-eclampsia. *Eur J Obstet Gynecol Reprod Biol* 2005; 121(2):154-8.
12. Garcia RG, Celedon J, Sierra Laguado J, *et al*. Raised CRP and impaired flow mediated vasodilatation precede the development of preeclampsia. *Am J Hypertension* 2007; 20(1):98-103.
13. Stefanović M1, Vukomanović P, Milosavljević M, *et al*. Insulin resistance and C-reactive protein in preeclampsia. *Bosnian J Bas Med Sci* 2009; 9(3):236-8.

14. Auer J, Camoin L, Guillonneau F, *et al*. J Serum profile in preeclampsia and intra-uterine growth restriction revealed by iTRAQ technology. *Proteomics* 2010; 73(5):1004-17.

15. Von Versen-Hoeynck FM, Hubel CA, Gallaher MJ, Gammill HS, Powers RW. Plasma levels of inflammatory markers neopterin, sialic acid and c-reactive protein in pregnancy and preeclampsia. *Am J Hypertens* 2009; 22(6):687-92.

16. De Jager SCA, Meeuwsen JAL, van Pijpen FM, *et al*. Preeclampsia and coronary plaque erosion: Manifestations of endothelial dysfunction resulting in cardiovascular events in women. *Eur J Pharmacol* 2017; 816 : 129-37.

17. Wolf M, Kettyle E, Sandler L, Ecker JL, Roberts J, Thadhani R. Obesity and preeclampsia: the potential role of inflammation. *Obstet Gynecol* 2001; 98 : 757-62.

18. Alvi MH1, Amer NA, Sumerin I. Serum 5-nucleotidase and serum sialic acid in pregnancy. *Obstet Gynecol* 1988; 72(2):171-4.

19. Goni M, Sayeed M, Shah GM, Hussain T. Serum sialic acid levels in normal pregnant and non-pregnant women. *Indian J Physiol Pharmacol* 1981; 25(4):356-60.

20. Sydow G, Morack G, Jung U, Semmler K, Christ S. Serum sialic acid levels in cancer, pregnancy and upper respiratory infections. *Arch Geschwulstforsch* 1986; 56(6):413-7.

21. Bakar E. Besin koruyucularřin šiçan dokularřinda sialik asit düzeyleri ve membran glikozaminoglikanlarři üzerine etkileri. *Trakya Üniversitesi Fen Bilimleri Enstitüsü* 2008. http://dspace.trakya.edu.tr/xmlui/handle/1/534.

22. Sapmaz E, Çelik A, Bulut V, Ilhan F, Hanay F. Examination of interleukin-6, crp, neutrophil and platelet levels in preeclampsia cases. *Gynecol Obst* 2006; 16 : 218-23.

23. Stallmach T, Hebisch G, Joller H, Kolditz P, Engelmann M. Expression pattern of cytokines in the different compartments of the feto-maternal unit under various conditions. *Reprod Fertil Dev* 1995; 7(6):1573-80.

24. Opsjon SL, Austgulen R, Waage A. Interleukin-1, interleukin-6 and tumor necrosis factor at delivery in preeclamptic disorders. *Acta Obstet Gynecol Scand* 1995; 74(1):19-26.

25. Sjaarda LA, Radin RG, Silver RM, *et al*. Preconception low-dose aspirin restores diminished pregnancy and live birth rates in women with low-grade inflammation: a secondary analysis of a randomized trial. *J Clin Endocrinol Metab* 2017; 102(5):1495-504.

26. Chouman K, Korioth-Schmitz B, Sack M, *et al*. Characterization of new anti-IL-6 antibodies revealed high potency candidates for intracellular cytokine detection and specific targeting of IL-6 receptor binding sites. *Eur Cytokine Netw* 2018; 29(2):59-72.