CEOG Clinical and Experimental Obstetrics & Gynecology

Maternal serum soluble CD40 ligand concentration as a predictor of preeclampsia at first trimester

F. Hatiboğlu[1], S. Kumbasar[2], B.A. Şık[3], E. Sever[2], M. Temur[2], S. Salman[4], Ö. Çot[2], A. Özcan[5], F. Yazıcıoğlu[5]

[1] Department of Obstetrics and Gynecology, Adıyaman University School of Medicine, Adıyaman
[2] Department of Obstetrics and Gynecology, Sakarya Research and Education Hospital, Sakarya
[3] Department of Obstetrics and Gynecology, Istanbul Aydın University School of Medicine, Istanbul
[4] Department of Obstetrics and Gynecology, Gaziosmanpaşa Taksim Research and Education Hospital, Istanbul
[5] Department of Obstetrics and Gynecology, Süleymaniye Research and Education Hospital, Istanbul (Turkey)

Summary

Aim: The aim of this study was to investigate the use of serum soluble CD40 ligand (sCD40L) concentration values, measured between 11+0 and 13+6 weeks of pregnancy, in the prediction of preeclampsia development and to determine the presence of a statistically significant difference. *Materials and Methods:* sCD40L concentrations of 202 cases who were admitted to the present hospital for routine control between 11+0 and 13+6 gestational weeks were measured and antenatal follow up was performed until delivery. *Results:* Among 202 patients who completed gestational period, 172 subjects developed no preeclampsia, while two cases had severe and 28 cases had mild preeclampsia (30 subjects in total). sCD40L level was detected as 4212.35 ± 3366.46 pg/ml in normotensive pregnant cases, while it was 5244.63 ± 3633.27 pg/ml in the patients with preeclampsia. There was no statistically significant difference between preeclamptic and normotensive patient group in terms of sCD40L concentrations ($p < 0.05$). *Conclusion:* The authors revealed that the mean sCD40L did not significantly increase during first trimester in the patients with preeclampsia, while it showed a tendency to increase in these cases. They believe that other than sCD40L concentration values, consideration of other patient-related factors such as some parameters including S endoglin, and uterine artery pulsatility may provide more successful results in the prediction of preeclampsia. Therefore, prospective, randomized, and controlled studies are required to investigate the importance of sCD40L concentrations in the prediction of preeclampsia during first trimester.

Key words: sCD40L, Preeclampsia, First-trimester screening.

Introduction

Preeclampsia is one of the leading causes of maternal and fetal morbidity and mortality in underdeveloped and developing countries has a prevalence of 3-4% [1]. Preeclampsia and gestational hypertension (GH) are seen in about 8-10% of primigravidas. The etiopathology of preeclampsia is not fully understood despite extensive investigations [2]. In the studies on the etiopathogenesis of preeclampsia, different mechanisms such as endothelial dysfunction, inflammatory processes, oxidative stress, and derangement in renin-angiotensin system, prostaglandins, nitric oxide, endothelins, genetic predisposition, and immunological factors have been proposed [3]. All of these cause vasoconstriction and blood pressure increases [3]. The main pathology underlying preeclampsia is decreased or absent trophoblastic invasion from maternal spiral arteries causing endothelial damage in uteroplacental and systemic circulation, which ends up with abnormal placentation [3]. Although abnormalities of placentation occur during 10^{th} -16^{th} gestational weeks, the clinical signs and symptoms appear in the second and third trimester [1].

The main pathology in preeclampsia is endothelial cell damage caused by increased inflammatory response beginning in the early stages of pregnancy. In the pathogenesis of preeclampsia, various molecules such as vascular endothelial growth factor (VEGF), placental growth factor (PIGF), soluble VEGF-receptor1, tumor necrosis factor (TNF), and serum soluble CD40 ligand (sCD40L) have been proposed to play role in endothelial cell dysfunction and inflammatory response [4]. Flow cytometric studies involving the patients with preeclampsia showed that thrombocytes were over activated [5]. Increased thrombocyte activation during first and second trimester indicates increased risk for preeclampsia [5]. It was shown that increase in CD63, which indicates increased thrombocyte activation in the first trimester, was an independent risk factor in the development of preeclampsia [5]. Activated thrombocytes induce the release of mediators triggering inflammatory response in leukocytes and endothelial cells. sCD40L produced by activated thrombocytes will bind to CD40 on endothelial cells inducing expression of tissue factor that plays an important role in the inflammatory response [6]. When the present authors examined the studies on thrombocyte activation, they noticed that the main molecule responsible for the development of

Revised manuscript accepted for publication February 10, 2016

inflammation and initiating thrombocyte activation was sCD40L. For this reason, based on the fact that endothelial cell damage begins in the first trimester, they measured the concentration of sCD40L, which is synthesized as a result of increased thrombocyte function, as a factor triggering endothelial cell damage. The measurements were performed during the first trimester with the combined test.The aim of this study was to investigate whether serum sCD40L measurement in the first trimester could be a novel determinant in prediction of preeclampsia.

Materials and Methods

The study included antenatal follow up of 480 subjects who were admitted to Perinatology Department of Istanbul Süleymaniye Gynecology and Obstetrics Education and Research Hospital for the first trimester ultrasound scans. The subjects were informed about the study and informed consent form was obtained. The authors planned a retrospective-cohort study here.

Initial admission evaluation was performed between 11+0 and 13+6 gestational weeks. A detailed history including subjects' age, BMI, parity, past medical history (such as diabetes mellitus, chronic hypertension, thrombophilia, antiphospholipid syndrome), drugs used (antihypertensive drugs, steroids, insulin, betamimetics, aspirin, anticoagulants, antiepileptics, antidepressants, antithyroids, thyroxin, anti-inflammatory drugs), and conception method (spontaneous, ovulation induction, IVF) were taken. The enrollment criteria were intact pregnancy in 11^{th} -14^{th} gestational week and presence of no detected anomaly in 11^{th} -14^{th} gestational week. The cases with multiple pregnancy, chronic hypertension, diabetes mellitus, thrombophilia, molar pregnancy, and history of drug use were excluded. Antenatal follow up of the cases was made by perinatology outpatient clinic until delivery. The data on pregnancy outcomes were obtained from the records of the present hospital. All reported obstetrical outcomes or pregnancy related hypertension cases were assessed to determine whether the condition was preeclampsia.

Following the collection of blood samples from 480 pregnant women for the first trimester screening test during 11^{th} -14^{th} week first trimester ultrasound scan, they were centrifuged at 1,000xg cycle for 30 minutes to separate serum and then stored in sealed tubes at -20°C freezer. sCD40L levels were measured in all samples by using enzyme-linked immunosorbent assay (ELISA) method.

The mean systolic and diastolic blood pressure values during pregnancy, urinary protein excretion in 24-hour urine, total weight gain, time of delivery, mode of delivery and birth weight, antihypertensive drugs used during pregnancy, history of hospitalization due to hypertension, history of severe headache and abdominal pain in the hospital, and need for magnesium sulfate antepartum treatment, were examined.

For all subjects enrolled in the study, gestational week at delivery, mode of delivery, birth weight, and presence of preeclampsia (classified as mild and severe according to Sibai criteria) were determined.

SPSS 10.0 package software was used for the assessment of the data. Normal distribution of numerical data was evaluated by Student's *t*-test, Fisher exact test, and chi-square tests were used for the comparison of the groups and $p < 0.05$ was accepted as statistically significant.

Table 1. — *Demographical features, serum biochemistry, and ultrasonography results of the patients.*

Features	Normotensive pregnant subjects (n=172) mean±ss /n (%)	Preeclamptic subjects (n=30) mean±ss /n (%)	p^* value
Age (years)	28.3±5.9	29.1±6.3	0.48
Gravida	1.9±1.1	2.1±1.0	0.55
Parity	1.2 ± 0.9	1.5 ± 1.4	0.60
BMI (kg/m^2)	28.3 ±3.3	29.3± 6.7	0.59
Gestational week according to CRL	12.3 ± 0.8	12.1 ± 0.7	0.22
Gestational age at delivery	38.0±2.8	38.4±1.8	0.53
Cesarean delivery	98(%57)	19(%63)	0.63**
Birth weight of newborn (grams)	3209.77± 594.55	3310.17± 674.95	0.40
Mean sCD40L (pg/ml)	4212.35 ± 3366.46	5244.63 ± 3633.27	0.11

*t-test; **chi-square, CRL: crown rump length,
Values are mean standard deviation or n (%).

Results

First-trimester screening test and sCD40L concentration measurement of 480 subjects were performed and the cases were retrospectively evaluated. Cases whose pregnancy outcomes could not be obtained (273 patients), were excluded from the study. Also five cases were excluded due to fetal death or abortus before 24^{th} week of gestation. A total of 278 patients were excluded. Among 202 cases with known results of 11^{th} -14^{th} week screening, sCD40L concentration and pregnancy outcome, 172 (85%) cases had no preeclampsia, while two patients developed severe and 28 cases had mild preeclampsia, 30 cases (14.9%) in total. Table 1 shows demographical features and pregnancy outcomes of the patients. There was no statistically significant difference between preeclamptic and normotensive pregnant cases with regard to their gestational week, gravida status, number of parity, body mass index, gestational age at delivery, birth weight ($p > 0.05$) (Table 1). None of the patients enrolled in the study were smokers and had pregnancy via assisted reproductive technique (IVF). sCD40L level was detected to be 4212.35 ± 3366.46 pg/ml in normotensive pregnant subjects, while it was 5244.63 ± 3633.27 pg/ml in preeclamptic cases. There was no statistically significant difference between preeclamptic and normotensive subjects in terms of serum soluble CD40L concentrations ($p > 0.05$) (Table 1).

Among 30 preeclamptic cases, only two received MgSO4 treatment. None of the subjects had seizure or visual findings. Two subjects were hospitalized due to preeclampsia. One preeclamptic patient received alfamed in the last month, five subjects received nidilat, while one patient received nidilat plus alfamed in the last month of pregnancy, and one subject received nidilat from the 20^{th} week (Table 2).

Table 2. — *Clinical characteristics of the women with preeclampsia.*

	Preeclamptic pregnant woman (n=30) mean ±ss
Systolic blood pressure (mm Hg)	148±7.7
Diastolic blood pressure (mm Hg)	101 ± 5.4
Proteinuria in 24 hours (mg/day)	564± 382
Clinical presentation	n (%)
Severe hypertension (>160/110 mm Hg)	2 (6)
Symptoms of end-organ involvement	1 (3)
Intrauterine growth restriction	1 (3)
Proteinuria >5 g / 24 hours	2 (6)
Eclampsia	-
Headache	6 (20)
Changes in vision	-
Magnesium sulfate antepartum treatment	2 (6)
Edema	16 (53)
Outpatient treatment (alfametildopa, nifedipin)	8 (26)
- Alfametildopa (in the last month)	1
- Nifedipin	5
- Alfametildopa + nifedipin (in the last month)	1
- Nifedipin (from the 20th week)	1

Table 3. — *Comparison of pathological serum sCD40L concentrations of preeclamptic and normotensive subjects.*

	Normal sCD 40L		Pathological sCD 40L		Fisher Exact *p* value
	n	%	n	%	
Normotensive pregnant woman	167	97.1	5	2.9	
Preeclamptic pregnant woman	27	90.0	3	10	0.099

Cut-off pathological value of sCD40L was 11,000 pg/ml obtained by adding +2 SD to the mean value of normal pregnancy. There was no statistically significant difference between normal and preeclamptic groups with regards to number of cases with values above cut-off pathological value ($p > 0.05$) (Table 3).

However, the mean sCD40L concentration was found higher in preeclamptic patients (5244.63 ± 3633.27 pg/ml) when compared to normotensive cases (4212.35 ± 3366.46 pg/ml) (Figure 1). In normotensive group, minimum level of sCD40L concentration was found to be 846 pg/ml and maximum level was 7,578 pg/ml. However, in preeclamptic group, minimum level of CD40 concentration was 1,611 pg/ml and the maximum level was 8,877 pg/ml. Figure 1 shows sCD40L concentration in preeclamptic and normotensive pregnant cases.

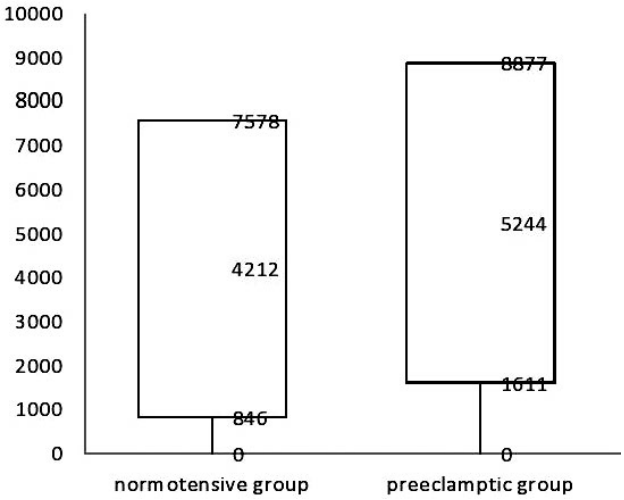

Figure 1. — Distribution of sCD40L concentration in preeclamptic and normotensive subjects.

Discussion

Since maternal and fetal morbidity of preeclampsia is high, many studies have been conducted on this issue. Early identification and close follow up of high-risk patients play an important role in preventing complications. The pathogenesis of preeclampsia is still unclear. The cause of maternal clinical symptoms seen in preeclampsia is systemic endothelial cell dysfunction, while the causes of endothelial cell damage are unknown. There are many studies showing that maternal and fetal inflammatory response play role in the pathogenesis of preeclampsia [5]. Increased inflammatory markers in the serum during first and second trimester indicate increased risk for preeclampsia. It was shown that TNF, soluble TNF-alpha receptor, TNF-alpha receptor 1 and 2, interleukin 2 (IL-2), sCD40L, and leukocyte activation were increased in preeclampsia [7, 8]. CD40L is a trimeric transmembrane protein of tumor necrosis factor family. CD40-CD40L is present in leukocytes, endothelial cells, smooth muscle cells, and activated thrombocytes [9]. The CD40-CD40L was first identified on activated T cells. In addition to its expression on T cells, CD40 is expressed by macrophages, dentritic cells, and monocytes and its interaction with CD40L leads to the synthesis of pro-inflammatory cytokines, such as TNF-α ,interleukin (IL)-1, and IL-6. Furthermore, CD40L expression has been reported in mast cells, eosinophils, and basophils; mast cells and basophils induce IgE production by B cells through the activation of the CD40 receptor by CD40. Commonly, these reports support the important role of CD40-CD40L system in allergic reactions, inflammation, and humoral immunity [10]. Ninety percent of circulating sCD40L originates from activated thrombocytes. sCD40L

induces coagulation by increasing the synthesis and expression of tissue factor from endothelial cells and monocytes [11]. Studies showed that thrombocyte activation occurred during first trimester and the clinical picture of preeclampsia appeared weeks and months later [12, 13]. Activated thrombocytes play a role in acute and chronic inflammation by degranulating and activating monocytes [14, 15]. Thrombocyte activation and aggregation take place after release of some mediators such as thromboxane A2 and sCD40L [16]. Thrombocytes express CD40L in their membranes during activation. Afterwards, CD40L leaves the membrane and switches to soluble form. sCD40L can be measured in the blood [17, 18]. sCD40L plays role in thrombocyte activation and stabilization of arterial thrombus. sCD40L concentration was found to be increased in chronic inflammatory diseases such as cystic fibrosis, inflammatory bowel diseases, and systemic lupus erythematosus [19]. Thromboxane A2 increases inflammation and endothelial damage by causing vasoconstriction and thrombocyte aggregation. Resultant endothelial damage plays an important role in the pathogenesis of preeclampsia. Thrombocyte activation was found to be more prominent in the patients with pregnancy-related complications such as preeclampsia and intrauterine growth retardation when compared to normal pregnancies and non-pregnant individuals [20]. Harlow *et al.* measured sCD40L concentration in preeclamptic individuals, pregnant subjects with gestational and essential hypertension, normotensive pregnant subjects, and non-pregnant individuals and revealed that it was significantly higher only in the subjects with preeclampsia [21]. The results of other groups were similar [20]. Lukanov *et al.* measured the concentrations of CD40L and CD62P located on thrombocyte surface and CD40L on monocyte surface in non-pregnant, preeclamptic, and normotensive pregnant subjects and detected significantly higher levels only in preeclamptic patients when compared to other groups [22]. In the study by Alacacıoğlu *et al.*, sCD40L concentration was found to be higher in preeclamptic subjects than that of normotensive pregnant subjects [23].

A study by Erez *et al.* showed higher concentrations of sCD40L in pregnant women when compared to non-pregnant subjects. In this study, sCD40L concentration was found to be higher in the subjects who were in labour when compared to women who were not in labour [24].

Inwald *et al.* suggested that sCD40L induces leukocyte activation by causing CD62P expression and the release of alpha granule and dense granules [25]. As a result, CD40-CD40L interactions and sCD40L are regarded as responsible for increased thrombocyte activation and inflammation seen in preeclampsia [26].

Azzam *et al.* analyzed 11 women with mild preeclampsia, 11 women with severe preeclampsia, and six women with HELLP syndrome and compared with 13 normotensive pregnant women as a control group. They found that sCD40L concentration and the platelet surface expression of CD40L was significantly higher in women with mild and severe preeclampsia and HELLP syndrome compared with normal pregnancy group [27].

Wu *et al.* investigated the role of CD40/CD40L in the pathogenesis of preeclampsia. Maternal serum was obtained from 20 patients with preeclampsia (PE group) as well as 20 healthy pregnant women (control group). The human umbilical endothelial cell line was cultured in the presence of maternal serum, after which cell growth and apoptosis were assessed by MTT and flow cytometry analysis. As compared to human umbilical endothelial cell line cells treated with control sera, those treated with preeclampsia sera had altered morphology, decreased cell growth, increased apoptosis, and greater CD40/CD40L protein and mRNA expression. They asserted that PE sera may induce endothelial cell damage possibly through increased CD40/CD40L expression [28].

sCD40L concentration has been measured in non-pregnant individuals, preeclamptic, and in normotensive pregnant subjects in many studies [29]. However, the present authors did not find any study in the literature in which sCD40L concentration was measured in the first trimester. In the present study, the authors measured sCD40L , one of the most important markers of thrombocyte activation, during the first trimester double screening test of 202 subjects, and investigated the place of mean sCD40L concentration in the prediction of preeclampsia. In the literature, there are some studies in which first trimester thrombocyte activation has been measured by using flow cytometry and various indicators. A study in which CD63 was used, CD63 increase in the first trimester was found to be an independent risk factor for the development of preeclampsia [26].

Conclusion

In the present study, the authors revealed that the mean sCD40L was not significantly increased in preeclamptic cases during first trimester, while it showed a tendency to increase in these cases No statistically significant difference was detected between preeclamptic and normotensive pregnant subjects in terms of sCD40L concentration. Other than sCD40L concentration, addition of other patient-related factors may be beneficial in obtaining more successful results in the prediction of preeclampsia.

References

[1] Papageorghio A.T., Yu CK., Bindra R., Pandis G., Nicholaides K.H., Fetal Medicine Foundation Second Trimester Screening Group: "Multicenter screening for preeclampsia and fetal growth restriction by transvaginal uterine artery Doopler at 23 weeks of gestation". *Ultrasound. Obstet. Gynecol.*, 2001, *18*, 441.

[2] Bronses I.A.: "Morphological changes in uteroplacental bed, in pregnancy hypertension". *Clin. Obstet. Gynaecol.*,1977, *4*, 573.

[3] Sibai M.D.: "Diagnosis, prevention, and management of eclampsia". *Obstet. Gynecol.*, 2005, *105*, 402

[4] Romero R., Nien J.K., Espinoza J., Todem D., Fu W., Chung H., *et*

al.: "A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor -1) factors in normal pregnancy and patients destined to gestational age neonate". *J. Matern. Fetal. Neonatal. Med.*, 2008, *21*, 9.

[5] Konijnenberg A., Stokkers E.W., Van der Post J.A., Schaap M.C., Boer K., Bleker O.P., *et al.*: "Extensive platelet activation in preeclampsia compared with normal pregnancy: Enhanced expression of cell adhesion molecules". *Am. J. Obstet. Gynecol.*, 1997, *176*, 461.

[6] Mellembakken J.R., Solum N.O., Ueland T., Videm V., Aukrust P.: "Increased concentrations of soluble CD 40 ligand, RANTES and GRO-alpha in preeclampsia – possible role of platelet activation". *Thromb. Haemost.*, 2001, *86*, 1272.

[7] Carr D.B., McDonald G.B.,Brateng D., Desai M., Thach C.T., Easterling T.R.: "The relationship between hemodynamics and inflammatory activation in vomen at risk for preeclampsia". *Obstet. Gynecol.*, 2001, *98*, 1109.

[8] Del C.I., Cruz M.A., Zhang H., Lopez J., Afshar-Kharghan V.: "Platelet activation leads to activation and propagation of the complement system". *J. Exp. Med.*, 2005, *201*, 871.

[9] Aruffo A., Hollenbaugh D., Wu L.H., Ochs H.D.: "The molecular basis of X-linked agammaglobulinemi,hyper-IgM syndrome, and severe combined immunodeficiency in humans". *Curr. Opin. Hematol.*, 1994, *1*, 12.

[10] Van Kooten C., Banchereau J.: "CD40-CD40 ligand''. *J. Leukoc. Biol.*, 2000, *67*, 2.

[11] Cipollone F., Feri C., Desideri G., Paloscia L.,Materazzo G., Mascellanti M. "Preprocedural level of soluble CD40L is predictive of enhanced inflammatory response and restenosis after coronary angioplasty". *Circulation*, 2003, *108*, 2776.

[12] Bar J., Ben –Haroush A., Lahav J., Sullivan M.: "Interaction between platelets and cytokines-a possible role in the pathogenesis of preeclampsia". *Vasc. Dis. Prev.*, 2004, *1*, 101.

[13] Janes S.L., Kyle P.M., Redman C., Goodall A.H.: "Flow Cytometric detection of activated platelets in pregnant women prior to the development of pre-eclampsia". *Throm. Haemost.*,1995, *74*, 1059.

[14] Mause S.F., Von H.P., Zernecke A.,Koenen R.R., Weber C.: "Platelet microparticles:a transcellular delivery system for rantes promoting monocyte recruitment on endothelium. "*Arterioscler. Thromb. Vasc. Biol.*, 2005, *25*, 1512.

[15] Nijm J., Wikby A., Tompa A., Olsson A.G., Jonasson L.: "Circulating levels of proinflammatory cytokines and neutrofil-platelet aggregates in patients with coronary arter disease". *Am. J. Cardiol.*, 2005, *95*, 452.

[16] Mulvihill N.T., Foley J.B.: "Inflammation in acute coronary syndromes". *Heart*, 2002, *87*, 201.

[17] Henn V., Slupsky J.R., Grafe M.,Anagnostopoulos I., Forster R.,Muller –Berghaus G.: "CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells". *Nature*, 1998, *391*, 591.

[18] Henn V.,Steinbach S., Buchner K., Presek P.,Kroczek R.A: "The inflammatory action of CD40 ligand (CD154) expressed on activated human platelets is temporally limited by coexpressed CD40". *Blood*, 2001, *98*, 1047.

[19] Goules A, Tzzzioufas A.G., Manousakis M.N., Kirou K.A.,Crow M.K., Routsias J.G.: "Elevated levels of soluble CD40 ligand (sCD40L) in serum of patients with systemic autoimmune disease". *J. Autoimmun.*, 2006, *26*, 11165.

[20] Ahlawat S., Pati H.P., Bhaatla N., Fatima L., Mittal S.: "Plasma platelet aggregating factor and platelet aggregation studies in preeclampsia". *Acta. Obstet. Gynecol. Scand.*,1996, *75*, 428.

[21] Harlow F.H., Brown M.A., Brighton T.A., Smith S.L., Trickett A.E., Kwan Y.L., Davis G.K.: "Platelet activation in the hypertensive disorders of pregnancy". *Am. J. Obstet. Gynecol.*, 2002, *187*, 688.

[22] Lukanov T.H., Bojinova S.I., Popova V.S., Emin A.L., Veleva G.L., Gecheva S.P., Konova E.I.: "Flow cytometric investigation of CD40-CD40 ligand system in preeclampsia and normal pregnancy". *Clin. Appl. Thromb. Hemost.*, 2010, *16*, 306.

[23] Alacacioglu I., Ozcan M.A., Piskin O., Yuksel F., Alacacioglu A., Demirkan F., *et al.*: "Increased concentration of soluble CD40 ligand in preeclampsia". *Clin. Appl. Thromb. Hemost.*, 2007, *13*, 201.

[24] Erez O., Romero R., Hoppensteadt D., Fareed J., Chaiworapongsa T., Kusanovic J.P., *et al.*: " Premature labor: a state of platelet activation? " *J. Perinat.Med.*, 2008, *36*, 377.

[25] Inwald D.P., McDowall A., Peters M.J., Callard R.E., Klein N.J.: "CD40 Is constitutively expressed on platelets and provides a novel mechanism for platelet activation". *Circ. Res.,* 2003, *92*, 1041

[26] Konijnenberg A., Van der Post J.A., Mol B.W., Schaap M.C., Lazarov R., Bleker O.P.: "Can flow cytometric detection of platelet activation early in pregnancy predict the occurence of preeclampsia.Aprospective study". *Am. J. Obstet. Gynecol.*, 1997, *177*, 434

[27] Azzam H.A., Abousamra N.K., Goda H., El-Shouky R., El-Gilany A.H.: "The expression and concentration of CD40 ligand in normal pregnancy, preeclampsia, and hemolytic anemia, elevated liver enzymes and low platelet count (HELLP) syndrome". *Blood Coagul. Fibrinolysis*, 2013, *24*, 71.

[28] Wu C.F., Huang F.D., Sui R.F., Sun J.X.: "Preeclampsia serum upregulates CD40/CD40L expression and induces apoptosis in human umbilical cord endothelial cells". *Reprod. Biol. Endocrinol.*, 2012, *10*, 28

[29] Qın W., Lı C.P., Zhan Y., Ye Y.H., Cuı X.N.: "The correlation between soluble plasma cd40-cd40 ligand and morbidıty of preeclampsia". *Acta Academiae. Medicinae. Qingdao. Universitatis*, 2011, *5*, 396.

Corresponding Author:
S. KUMBASAR, M.D.
Department of Obstetrics and Gynecology
Sakarya University School of Medicine
Sakarya Research and Education Hospital
Sakarya (Turkey)
e-mail: doktor1977@hotmail.com