Cd40-Cd40 Ligand System and P-selectin as Early Markers for Detection of Pre-eclampsia

SA Khodeer[1], AM Abdu-Allah[2], WG El-Damaty[3]

ABSTRACT

Objectives: Pre-eclampsia (PE) is a principal cause of maternal and fetal mortality and morbidity. The aim of this work was to assess the presence of inflammatory and thrombotic response during pre-eclampsia by demonstrating CD40-CD40 ligand and P-selectin in pre-eclamptic pregnant women.

Methods: The study included 81 pregnant women divided into three groups, Group I: 39 pregnant women with mild pre-eclampsia, Group II: 22 women with severe pre-eclampsia and Group III: 20 apparently healthy pregnant women. All Groups were subjected to complete clinical examination; prothrombin time and concentration, urea, creatinine, urinary protein, CBC, expression of CD40 on monocytes, sCD40L and P-selectin.

*Results: There was a statistically significant higher expression of CD40 on monocytes, sCD40lL and P-selectin in the pregnant women with mild and severe pre-eclampsia compared with normotensive pregnant women (*p < 0.001*). The most sensitive marker was sCD40L while the highest specificity was the expression of CD40 on monocytes.*

Conclusion: The study shows presence of activation of the CD40–CD40L system and elevated levels of P-selectin in women with pre-eclampsia – whether mild or severe types. Using of such markers may be essential for developing better prediction polices as well as for affecting patient treatment decisions forward and improving patient outcome for both babies and mothers suffering from pre-eclampsia.

Keywords: Inflammatory, preeclampsia, pregnant, p-selectin, mortality

El Sistema Ligando Cd40-Cd40 y la Selectina P como Marcadores Tempranos para la Detección de la Pre-eclampsia

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RESUMEN

Objetivos: La pre-eclampsia es la principal causa de mortalidad y morbilidad materna y fetal. El objetivo de este trabajo fue evaluar la presencia de una respuesta inflamatoria y trombótica durante la pre-eclampsia mediante la demostración del ligando o mecanismo de acoplamiento CD40-CD40 y la selectina P en las mujeres embarazadas pre-eclámpticas.

Métodos: El estudio incluyó 81 mujeres embarazadas divididas en tres grupos: Grupo I: 39 mujeres embarazadas con pre-eclampsia leve; Grupo II: 22 mujeres con pre-eclampsia grave; y Grupo III: 20 embarazadas evidentemente sanas. Todos los grupos fueron sometidos a examen clínico completo, tiempo y concentración de protrombina, urea, creatinina, proteínas urinarias, CBC, expresión de CD40 en monocitos, sCD40L y selectina P fueron evaluados.

*Resultados: Hubo una expresión mayor estadísticamente significativa de CD40 en monocitos, sCD40lL y selectina P en las mujeres embarazadas con pre-eclampsia leve y grave, en comparación con las mujeres embarazadas normotensas (*p < 0.001*). El marcador más sensible fue sCD40L, mientras que la especificidad más alta fue la expresión de CD40 en monocitos.*

Conclusión: El estudio muestra la presencia de activación del sistema CD40-CD40L y el nivel elevado de selectina P en las mujeres con pre-eclampsia, ya sean los tipos leves o severos. El uso de estos mar-

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cadores puede ser esencial para desarrollar mejores políticas predicción, modificar la decisión de tratamiento del paciente a la hora de seguir adelante, y mejorar los resultados de los pacientes, tanto para los bebés como para las madres que sufren de preeclampsia.

Palabras claves: Inflamatoria, pre-eclampsia, embarazada, selectina P, mortalidad

INTRODUCTION

Pre-eclampsia is a principal cause of maternal and fetal mortality and morbidity. It is associated with an increase in maternal blood pressure and deterioration of renal function, intrauterine growth retardation, persistent immune activation and multi-organ dysfunction (1, 2). The prevalence of pre-eclampsia ranges between 2–10% of pregnancies so, its incidence varies worldwide. World health organization estimates the occurrence of pre-eclampsia to be seven times higher in developing countries (2.8% of live births) than in developed countries [0.4%], (3).

Early-onset pre-eclampsia occurs within 34-weeks of pregnancy. Despite ensuring maternal safety, the only treatment for PE is early pregnancy termination, as PE often results in serious neonatal developmental complications. For that reason, prevention and early discovery from PE is crucial (4). The principal mechanisms responsible for the pathogenesis of pre-eclampsia are inadequately understood (5). Even though the cause of pre-eclampsia is unknown, endothelial cell dysfunction plays an important part in its pathogenesis (4).

The CD40L is a trimeric, trans-membrane protein of the tumour necrosis factor family and, together with its receptor CD40, plays an important role in the inflammatory processes that cause atherosclerosis and thrombosis (6). The CD40–CD40L system is extensively distributed in a variety of cells, including leukocytic, and non-leukocytic, endothelial and smooth muscle cells (7) and on activated platelets (6). It also occurs in a soluble form that is fully active biologically, termed soluble CD40 ligand (sCD40L), which is shed from stimulated lymphocytes and is actively released after platelet stimulation (8). Soluble sCD40L is pro-inflammatory for endothelial cells and provoke coagulation by stimulating expression of tissue factor on monocytes.

P-selectin, which is a 140kD protein is present in the alpha granules of platelets and the Weibel-Palade bodies of endothelial cells. It is one of the selectin family of adhesion molecules, which take part in reproduction and haemostasis (10–12). During pregnancy, maternal P-selectin expression occurs entirely at the implantation site and permit the mechanism for maternal and fetal cell interaction to give a chance to the trophoblast to implant itself within the uteroplacental vessel lumen (13). P-selectin is secreted from the cell surface and circulates as a soluble molecule in the plasma (14). Both the membrane and the soluble form of P-selectin are agonists of the processes of thrombosis and inflammation (15). Moreover, P-selectin could support platelet-platelet interactions and play an important role in the early stages of inflammation, throm-bosis and atherosclerosis by promoting plaque formation and progression (15).

This work was done to assess the presence of inflammatory and thrombotic response during pre-eclampsia by demonstrating CD40-CD40 Ligand and P-selectin in pre-eclamptic pregnancies compared with normal pregnancies and how it could be used as markers for early detection of pre-eclampsia.

SUBJECTS AND METHODS

This study was carried out at the Clinical Pathology Department in collaboration with Medical Biochemistry and Obstetrics and Gynecology Departments, Menoufiya University Hospitals, Faculty of Medicine, in the duration between March 2010 and December 2013.

Subjects: the study included 81 pregnant women from those attending the Outpatient Clinic and Inpatient Department of Menoufiya University Hospitals. According to the clinical examination, laboratory findings, subjects sharing in this study were classified into three groups as follows: **Group I:** Consisted of 39 pregnant females suffering from mild pre-eclampsia at gestational age after 20 weeks of pregnancy whose age-range from 25–33 years old: **Group II:** Consisted of 22 pregnant females suffering from severe pre-eclampsia at gestational age after 20 weeks of pregnancy and whose ages ranged from 25–33 years old and **Group III:** Consisted of 20 apparently healthy pregnant females, at gestational age after 20 weeks of pregnancy whose ages ranged from 25–30 years old.

*Exclusion criteria: Women with and without pre-eclampsia with a history of hypertension, hepatic or renal disease, cardiac disease, diabetes mellitus, established atherosclerosis, malignancy, autoimmune disease, systemic infection, recent major surgery or trauma, alcohol consumption and cigarette smoking.

*Inclusion criteria: patients with pre-eclampsia in Groups I and II and normal pregnant women in Group III; gestational age corroborated by ultrasound after 20 weeks; a singleton fetus and no fetal structural anomaly.

Blood samples were collected before the administration of any ante-partum treatment agents.

The study design was accepted by the local Ethics Committee. Informed consents were obtained from the shared women.

Methods: All subjects included in this study were subjected to the following: Thorough history taking and complete clinical examination, prothrombin time and concentration, urea, crea-

tinine, urinary protein, CBC, expression of CD40 on monocytes, sCD40L and P-selectin.

A-Sample collection

Blood samples: Venous blood samples were collected from each woman under complete aseptic condition by sterile vein puncture without venous stasis. Each sample was divided as follows: 4 mL of blood were allowed to clot at 37 °C, serum was separated by centrifugation and used for immediate assay of kidney and liver function tests. The remaining portion was divided into two aliquots and kept at -80 °C until testing for assaying of sCD40L and P-selectin. One mL of blood was added to a EDTA-contained sterile tube for platelet count, 1.8 mL of blood was added to a citrated tube for estimation of prothrombin time and concentration and 2 mL of blood were added to a citrated tube, the citrated blood was fixed immediately by adding 1% formaldehyde (1:1) for determination of CD40 expression on monocytes .

     Urine sample: Twenty-four-hour urine was collected from each woman for estimation of total protein in urine.

B) Analytical Methods

1. **Biochemical tests** for detection of urea, creatinine, ALT, AST were done on SYNCHIRON CX9 auto analyser using a kit supplied by Beckman (BacKman, Instrument. Inc., Scientific Instrument Division, Fullerton, CA92634-3100. USA).

2. **Determination of sCD40L concentrations:** A sandwich enzyme immunoassay test kit (Quantikine, Human soluble CD40 Ligand Immunoassay R&D system, USA) was used for the quantitative determination of soluble CD40L. A polyclonal antibody specific for CD40 L was pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CD40 Ligand present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for CD40 Ligand was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and colour changes developed in proportion to the amount of CD40 Ligand bound in the initial step. The colour development is stopped and the intensity of the colour was measured at 450nm using an ELISA reader. The concentrations of sCD40L were read from the standard calibration curve.

3. **Soluble P-selectin:** This was determined using a sandwich ELISA assay (human sP-selectin sandwich ELISA kit, Invitrogen Corporation Camarillo. www.invitrogen.com). An anti-sP-selectin monoclonal coating antibody was adsorbed onto microwells. sP-selectin present in the sample or standard, bound to antibodies adsorbed to the microwells; an HRP-conjugated monoclonal anti-sP-selectin antibody was added and bound to sP-selectin captured by the first antibody. Following incubation, unbound enzyme conjugated with anti-sP-selectin was removed during a wash step and substrate solution reactive with HRP was added to the wells. A coloured product was formed in proportion to the amount of soluble sP-selectin

present in the sample or standard. The reaction is terminated by addition of acid and absorbance was measured at 450 nm.

4. **Flow cytometric analysis for determination of CD40 on monocytes:** (FACS Calibur cytometer; Becton Dickinson, USA): fixed blood was diluted 1:5 and incubated with FITC-conjugated anti-CD40 and anti-CD14 phycoerythrin for 30 minutes at room temperature. Erythrocytes were removed by adding 2 mL of FACS lysis solution for 10 minutes at room temperature. Leucocytes were washed twice with PBS and fixed in 1% formaldehyde in PBs. Monocytes were identified by gating for CD14+ cells. The results were presented as mean fluorescent intensity (MFI).

Statistical analysis

The statistical analysis was undertaken using SPSS software (version 17; SPSS Inc, Chicago, IL, USA). Descriptive statistics in the form of mean and standard deviation for parametric data were used. Analysis of variance test for comparison between the three groups having quantitative variables normally distributed and Kruskal-Wallis test for comparison between three groups not normally distributed having quantitative variables followed by least significant difference (LSD). The significant level was set at 0.05 or less. Spearman correlation test was used to measure the association between two parametric quantitative variables. The receiver operating characteristic curve is a graphic representation of the relationship between sensitivity and specificity at different cut-off points for a diagnostic test.

RESULTS

The details of clinical characteristics and laboratory findings in patients' groups and normal pregnant women are listed in (Table1). There were no statistical significant differences between the women with mild and severe pre-eclampsia and the normotensive pregnant women regarding maternal age, or gestational age at blood sampling. Blood pressure was significantly higher in the women with mild and severe pre-eclampsia compared with the normotensive pregnant women. There were significant decrease in platelet count and albumin concentration and significant increase in creatinine and urea in women with mild and severe pre-eclampsia compared with the normal pregnancy group. The expression of CD40 on monocytes and the serum concentration of sCD40L showed statistically significant increase in both groups of pre-eclampsia patients compared with normal pregnant women. Meanwhile, no statistically significant difference was observed between the studied groups of mild and severe pre-eclampsia. Regarding P-selectin, the highest level was recorded by the group of severe pre-eclampsia followed by group of mild pre-eclampsia and lastly the group of normal pregnant women.

     There were statistically significant differences between both groups of women with pre-eclampsia and normal pregnant women, but no statistically significant difference was found between women in the mild and severe pre-eclamptic groups. Spearman correlation showed positive significant

Table 1:   Statistical comparison of the clinical and laboratory characteristics of the studied groups

Variable	Normal pregnant n = 20	Mild pre-eclampsia n = 39	Severe pre-eclampsia n = 22	F test	p-value
Age (years) X ± SD	27.55 ± 1.70	27.43 ± 1.76	27.59 ± 1.7	0.06	> 0.05
Gestational age at sampling (weeks) X ± SD	31.20 ± 1.28	33.97 ± 0.90	34.0 ± 0.93	56.8	< 0.001
Systole (mm/Hg)				22.3	< 0.001[1]
X ± SD	115.0 ± 4.59	145.13 ± 5.06	167.95 ± 5.04	35.5	< 0.001[2]
				16.9	
Diastole (mm/Hg)				19.58	< 0.001[1]
	74.0 ± 5.02	99.49 ± 4.10	105.0 ± 5.12	19.8	< 0.001[2]
				4.3	
SGOT (U/L)				0.44	0.66[1]
X ± SD	30.2 ± 3.29	29.82 ± 3.04	30.0 ± 2.81	0.21	0.83[2]
				0.23	0.82[3]
SGPT (U/L)				0.001	0.99[1]
X ± SD	30.05 ± 3.36	30.05 ± 3.40	29.64 ± 3.44	0.39	0.70[2]
				0.45	0.65[3]
Albumin (gm/dL)				14.05	< 0.001[1]
X ± SD	3.85 ± 0.22	2.79 ± 0.29	2.83 ± 0.31	12.3	< 0.001[2]
Range				0.40	0.69[3]
Prothrombin conc. (%)				4.68	< 0.001[1]
X ± S	92.4 ± 5.9	85.82 ± 2.	85.68 ± 3	4.5	< 0.001[2]
				0.17	0.86[3]
Urea (mg/dL)				7.36	< 0.001[1]
X ± SD	38.15 ± 1.81	42.23 ± 2.11	42.5 ± 2.15	7.0	< 0.001[2]
				0.47	0.64[3]
Creatinine (mg/dL)				5.66	< 0.001[1]
X ± SD	0.79 ± 0.19	1.14 ± 0.29	1.16 ± 0.28	5.10	< 0.001[2]
				0.24	0.81[3]
Protein in 24-hour urine (mg/24h)				18.01	< 0.001[1]
X ± SD				13.7	< 0.001[2]
	120.5 ± 40.06	606.92 ± 159.1	610.45 ± 162.49	0.08	0.93[3]
Platelets (x10^{3})				2.72	0.009[1]
X ± SD	180.0 ± 16.22	161.79 ± 35.1	167.05 ± 34.25	1.59	0.12[2]
				0.57	0.57[3]

Table 2: Comparison between the studied groups as regard sCD40L, CD40 on monocytes and P-selectin

Variable	Studied groups Normal pregnant n = 20	Mild pre-eclampsia n = 39	Severe pre-eclampsia n = 22	Mann Whitney	p value
sCD 40L (ng/mL)				6.2	
X ± SD	0.33 ± 0.11	1.71 ± 1.88	1.75 ± 0.89	5.5	< 0.001
				1.26	< 0.002
					0.213
CD 40 expression on				6.3	
monocytes (FMI)	5.92 ± 2.14	22.42 ± 4.99	20.43 ± 6.95	4.4	< 0.001[1]
X ± SD				0.80	< 0.051[1]
					0.43[3]
P-selectin (ng/mL)				4.2	0.001[1]
X ± SD	137.65 ± 54.29	251.18 ± 79.76	284.56 ± 46.02	6.0	0.001[2]
				1.19	0.23[3]

1 = Comparison between group of pregnant women with mild pre-eclampsia and group of women with normal pregnancy.
2 = Comparison between group of pregnant women with mild pre-eclampsia and group of women with normal pregnancy.
3 = -comparison between groups of pregnant women of mild and severe pre-eclampsia

Table 3: Presentation of data extracted from the ROC curve of sCD40L in serum, CD40 expression in monocytes and P-selectin for detection of preeclampsia

	CD40 ligand in serum	CD40 in monocytes	P selectin
AUC	0.998	0.97	0.94
P value	< 0.001	< 0.001	< 0.001
95% CI	0.99 – 1	0.93 – 1.01	0.89 – 0.99
Cutoff point	0.575	12	203
Sensitivity	100	96.7	88.5
Specificity	95	100	85
PPV	98.4	100	95.2
NPV	100	90.9	89.5

r = + 0.59   P value <0.001
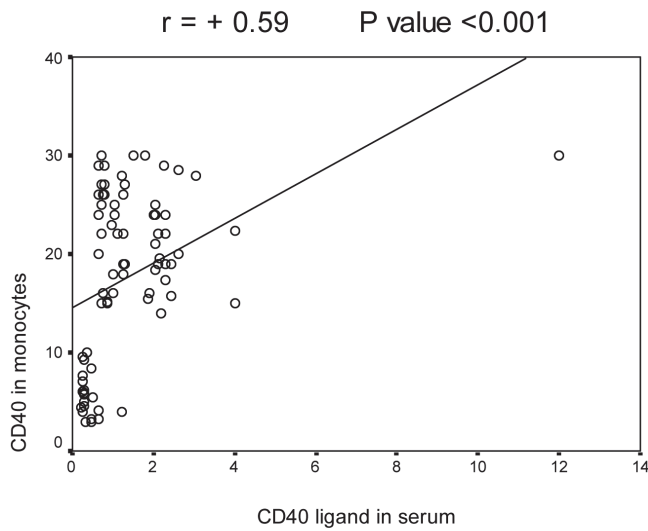
Fig. 1: Spearman correlation between Scd40L and CD40 expression on monocytes in the studied group.

r = + 0.46   P value <0.001
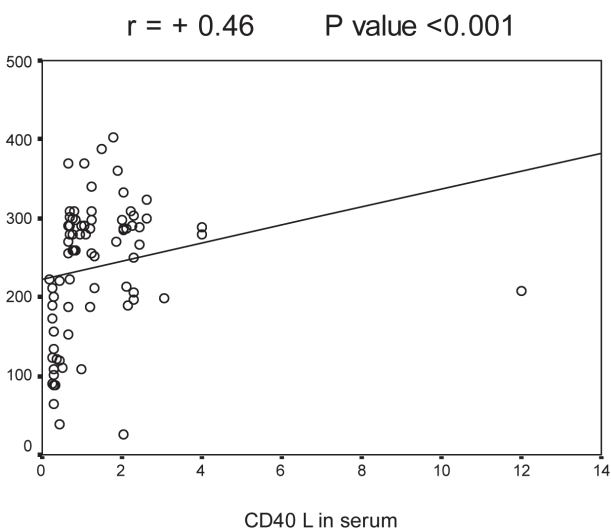
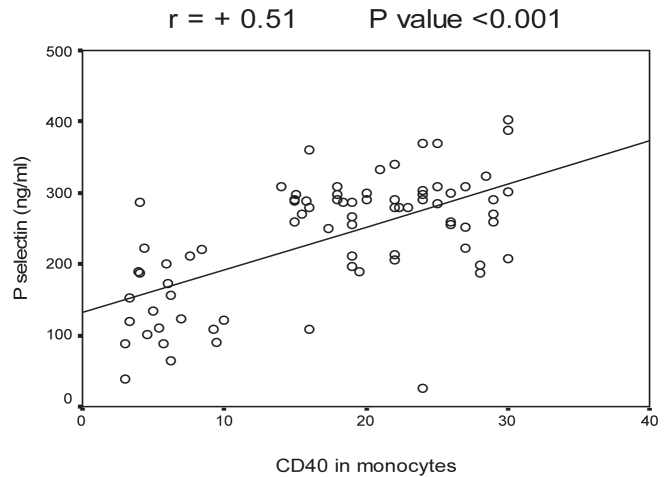Fig. 2: Spearman correlation between sCD40L and P-selectin in the studied group.

r = + 0.51   P value <0.001

Fig. 3: Spearman correlation between P-selectin and CD40 expression on monocytes in the studied groups.

ROC Curve

Source of the Curve
Reference Line
P selectin
CD40 in monocytes
CD40 L in serum
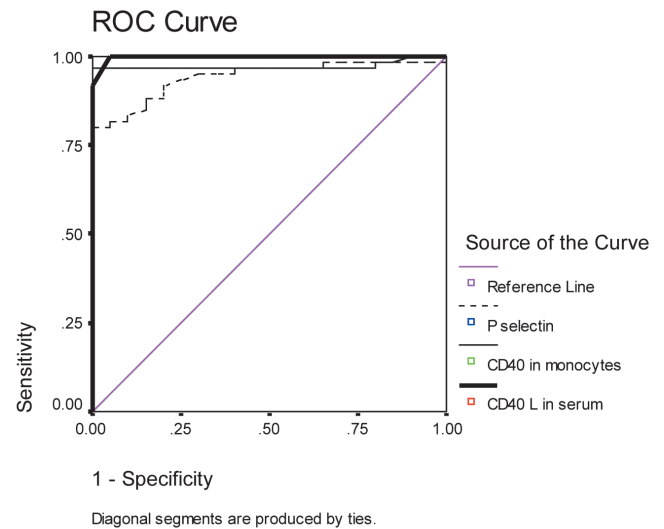
Diagonal segments are produced by ties.

Fig. 4: The ROC curve of sCD40L in serum, CD40 expression in monocytes and P-selectin for detection of pre-eclampsia.

correlation between sCD40L in serum, CD40 expression on monocytes and P-selectin in the whole studied groups. By using ROC curve for CD40 expression on monocytes, the best cutoff level was 12 foundation medicine reports with a sensitivity of 96.7%, specificity 100%, PPV 100%, NPV 90.9%. Meanwhile, the best cutoff level for sCD40L was 0.57 ng/mL with a sensitivity of 100%, specificity 95%, PPV 98.4%, NPV 100%. Moreover, the best cutoff level of P-selectin was 203 ng/mL with a sensitivity of 88.5%, specificity 85%, PPV 95.2%, NPV 89.5.

DISCUSSION

Pre-eclampsia has been studied extensively because of its involvement with high maternal and fetal morbidity. The pathogenesis of pre-eclampsia is not obviously understood. Roberts *et al* (16), reported maternal endothelial dysfunction with pre-

eclampsia. Also, Bar *et al* (17), found that platelet activation, platelet aggregation and progressive damage to endothelial cells were all characteristics of pre-eclampsia. The relationship between the exaggerated platelet activation and inflammation leading to endothelial damage in pre-eclampsia can be explained by the described CD40–CD40L system (18).

In the current work, the mean level of P-selectin was found to be statistically higher not only in women with mild pre-eclampsia compared with the control group, but also in women with severe pre-eclampsia compared with women with normal pregnancy. In addition, the mean level of p-selectin is higher in women with severe pre-eclampsia than in mild pre-eclamsia but this difference did not reach a statistically significant level. Yoneyama *et al* (19), presented a significantly higher expression of P-selectin in pre-eclampsia than in apparently healthy pregnant women.

Similar results of increased levels of sP-selectin in pre-eclamptic pregnancies were obtained by Halim *et al* (20) and Chaiworapongsa *et al* (21). Furthermore, Bosio *et al* (22) found higher levels of sP-selectin in pre-eclampsia but levels of soluble P-selectin were not significantly increased in women with pregnancy induced hypertension without proteinuria. Also, they proposed that their findings support an inflammatory model for pre-eclampsia, while the endothelial cell activation might be secondary to a primary inflammatory response. However, Karalis (23) observed significantly increased levels of both P-selectin and its soluble form in women with pregnancy-induced hypertension in comparison with pregnant women with normal blood pressure and normal pregnancy is connected with increased platelet activation followed by further alteration in these parameters in pregnancies complicated by hypertension. In contrast to our results, Heyl *et al* (24), found levels of P-selectin in hypertensive patients similar to those found in healthy controls.

Also, our findings were in accordance with the results reported by Laskowska *et al* (25), who suggest that the elevated level of the soluble P-selectin is associated with pre-eclampsia. sP-selectin acts as a marker for platelet and endothelial activation and as an initiator of the procoagulant state, as well as playing an important role in atherosclerosis. Our results of elevated sP-selectin in pre-eclampsia seem to confirm that these processes occur in pre-eclamptic pregnant women and the excess circulating sP-selectin aid in the pathogenesis of this pregnancy-specific disorder.

In the present study, the expression of CD40 on monocytes and sCD40L were significantly higher in women with mild and severe pre-eclampsia compared with normal pregnant women. Meanwhile, no statistically significant differences were found between both groups of women with pre-eclampsia regarding the expression of CD40 on monocytes and sCD40L. These findings were unexpected but this may be explained by the smaller number of women with severe pre-eclampsia

Engagement of CD40 on monocytes induces many procoagulant and pro-inflammatory effects (26). CD40 on mono-

cytes might be upregulated by pro-inflammatory mediators, such as C-reactive protein (CRP) (27). CRP has been found to be elevated in pre-eclampsia (28). CD40 engagement on monocytes might also occur through enhanced interaction between platelets and monocytes, because monocytes rapidly adhere for prolonged periods to activated platelets that display p-selectin (29). This enhanced interaction between platelets and monocytes, through the engagement of CD40, increases the release of pro-inflammatory and pro-thrombotic factors. Thus, the link between the exaggerated platelet activation and inflammation leading to endothelial dysfunction and damage in pre-eclampsia may be explained by the persistent upregulation of the CD40–CD40L system and the mediatory role of CD40L.

In accordance with our results, Mellembakken *et al* (30) observed increasing the concentrations of sCD40L in plasma of patients with pre-eclampsia containing platelets in comparison to plasma deprived of thrombocytes. They suggested that enhanced platelet activation during preeclampsia might result in increased secretion of inflammatory factors, possibly leading to inflammation, leukocyte stimulation and endothelial malfunction.

Also, our results agreed with the results of a study done by Oron *et al* (31) who reported that the mean levels of sCD40L were significantly different among the studied groups. The highest levels were detected in the non-pregnant women and the pre-eclamptic women and the lowest level was found in the normal pregnant women.

Moreover, the obtained results agreed with those done by Darmochwal-Kolarz *et al* (32), who studied the concentration of the inflammatory markers CD40L and sCD40L in normal pregnancy and pre-eclampsia. In the group of patients with pre-eclampsia, they observed significantly higher serum concentrations of sCD40L than in the normal third trimester. They found significantly lower concentrations of sCD40L all over the trimesters of normal pregnancy than in healthy non-pregnant women. Lower concentrations of sCD40L may suggest decreased CD40-CD40L interactions of CD4$^+$ tlymphocytes with antigen-presenting cells and decreased activation of innate immunity during normal pregnancy. This might be one of the mechanisms protecting the developing fetus against the activation of the maternal immune system. A study done by Lukanov *et al* (33) reported that there was a significantly higher expression of CD40 and CD40L on platelets and CD40 on monocytes in the women with pre-eclampsia compared with normotensive pregnant women. The serum concentration of sCD40L was significantly higher in women with pre-eclampsia compared with normotensive pregnant women. In a study done by Lukanov *et al* (34) it was reported that the preeclamptic women showed a significant increase in CD40L and in CD40 on monocytes when compared with normotensive pregnant women and non-pregnant women.

Recently, a study done by Azam *et al* (35) demonstrated that expression of CD40L was significantly higher in women with mild and severe pre-eclampsia compared with the normal pregnancy group ($p = 0.001$; $p \leq 0.001$; $p = 0.003$, respec-

tively). However, whether sCD40L can elicit an inflammatory response remains controversial. Henn *et al* (8) and Laskowska *et al* (36) found that even high-levels of sCD40L failed to induce an inflammatory reaction. They concluded that the CD40–CD40 L interaction leads, over time, to the transformation of CD40L to the biologically inactive sCD40L, thereby effectively limiting the inflammatory process in the vascular system, and protecting maternal immune cells bearing CD40 receptors from Fas-mediated apoptosis.

By using the ROC curve for comparison between the three studied markers, the highest sensitivity belonged to sCD40L, followed by CD40 expression on monocytes and lastly P-selectin regarding the specificity, the highest specificity was a share of CD40 expression on monocytes then sCD4 40L then P-selectin. It could be concluded that the present study has shown activation of the CD40–CD40L system and elevated level of P-selectin in women with pre-eclampsia – whether mild or severe types – compared with normal pregnant women. This may indicate an exaggerated activation of platelets and endothelial cells in the disorder which could be used as early markers for detecting pre-eclampsia. Using of such markers may be essential for developing better prediction polices influencing patient treatment decision forward and improving patient outcome for both babies and mothers suffering from pre-eclampsia.

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