Increased Concentration of Soluble CD40 Ligand in Preeclampsia

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Summary: Preeclampsia has been associated with increased platelet activation detected before disease onset. Platelets are involved in hemostasis and also directly initiate an inflammatory response of the vessel wall. Inappropriate activation of platelets may be involved in pathogenesis in preeclampsia by promoting coagulation and thrombosis, and also as a mediator of inflammation. Platelets may release inflammatory mediators such as soluble CD40 ligand. The plasma level of soluble CD40 ligand was investigated during preeclamptic (n =20) and normal pregnancies (n = 20) to emphasize inflammatory response in preeclampsia. The mean soluble CD40 ligand levels were 1.08 ± 0.43 ng/mL in patients with preeclampsia and 0.76 ± 0.24 ng/mL in healthy pregnant women, which was statistically significant ($P = .01$). To clarify whether inflammation may cause inappropriate endothelial cell activation or inappropriate endothelial cell activation may start this inflammatory response, future studies are needed in a larger study population.

Key Words: preeclampsia—inflammation—soluble CD40 ligand

Preeclampsia is a clinical condition induced by pregnancy after 20 weeks of gestation that is characterized by the development of hypertension with proteinuria or edema in previously healthy women. It has been described by Chesley as a "disease of theories" because its cause is still unknown.[1] Uteroplacental circulation defect owing to increased microthrombogenesis,[2] or abnormal cytotrophoblast infiltration,[3] may cause preeclampsia. In this view, enhanced inflammation[4] and endothelial cell activation[5] appear to have a major role in the pathogenesis of this disorder.

Preeclampsia has also been associated with increased platelet activation detected even before the onset of the disease.[6] Platelets are not only involved in hemostasis but they also directly initiate an inflammatory response of the vessel wall.[7] Inappropriate activation of platelets may be involved in pathogenesis in preeclampsia by promoting coagulation and thrombosis and also as an important mediator of inflammation in these women.[8] Platelets may release inflammatory mediators such as soluble CD40 ligand (sCD40L). In a previous study, Mellembakken et al[8] showed raised plasma levels of this ligand and enhanced platelet activation in preeclampsia. We also investigated the plasma level of sCD40L during preeclamptic and normal pregnancies.

MATERIALS AND METHODS

Patients

The study included 20 patients with preeclampsia aged (mean ± SD) 29.5 ± 1.22 years (range, 22 to 38 years) and 20 women with a normal pregnancy aged 25.7 ± 0.82 years (range, 20 to 33 years). Informed consent was obtained from every patient.

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TABLE 1. Patient Characteristics and Laboratory Findings

Findings[a]	Preeclampsia (n = 20)	Normal Pregnancy (n = 20)	*P*
Blood pressure (mm Hg)			
Systolic	154 ± 9.9	116.5 ± 1.3	
Diastolic	96 ± 5.9	74.5 ± 9.9	
Urinary protein (mg/d)	853.5 ± 481.9	—	
Hemoglobin (g/dL)	10.82 ± 1.74	11.26 ± 0.98	.583
WBC (10^9/L)	10.80 ± 2.37	11.16 ± 2.47	.414
Platelets (10^9/L)	236.85 ± 63.35	223.65 ± 67.51	.602
BUN (mg/dL)	20.05 ± 5.46	20.25 ± 8.93	.738
Creatinine (mg/dL)	0.58 ± 0.20	0.57 ± 0.18	.718
AST (U/L)	21.65 ± 7.96	20.10 ± 7.37	.547
ALT (U/L)	22.75 ± 7.12	17.2 ± 5.37	.012
Total Protein (g/dL)	5.39 ± 0.41	5.51 ± 0.39	.301
Albumin (g/dL)	2.36 ± 0.38	2.60 ± 0.21	.035

WBC = white blood count; BUN = blood urea nitrogen; AST = aspartate aminotransferase; ALT = alanine aminotransferase.
a. Values are shown as mean ± standard deviation.

TABLE 2. Coagulation Parameters of the Patients

Variable[a]	Preeclampsia (n = 20)	Normal Pregnancy (n = 20)	*P*
PT (sn)	12.61 ± 4.93	11.97 ± 0.86	.174
INR	1.01 ± 0.45	0.950 ± 0.07	.183
APTT (sn)	30.05 ± 4.83	28.12 ± 3.01	.134
D-dimer (ng/mL)	0.5	0.5	—

PT = prothrombin time, APTT = activated partial thromboplastin time; INR = international normalize ratio.
a. Values are shown as mean ± standard deviation.

No patients in either group had received any medications such as aspirin, nonsteroidal antiinflammatory drugs, anticoagulants, antibiotics, vasodilators, steroids, antihypertension drugs, or lipid-lowering drugs. No patients had arterial or venous disease, acute infection, renal or liver disease, diabetes mellitus, coronary artery disease, or chronic inflammatory disease.

Patient data recorded included age, proteinuria levels, intensity of edema, blood pressures, gestation week, and number of gestations. Patients were classified according to the Committee on Terminology of the American College of Obstetricians and Gynecologists criteria for determining the severity of preeclampsia.[9]

Blood Collection

Blood samples were taken from the antecubital vein in the third trimester in the normal pregnancy group and after the 20th week of gestation in the preeclamptic group. Samples were collected in citrated tubes (trisodium citrate, 0.129 mol/L; whole blood ratio, 1:9) and centrifuged at 2000*g* for 15 minutes. All plasma samples were divided into aliquots and preserved at –70°C until testing.

Other studies done at the same time the blood samples were obtained were whole blood count, liver function tests, renal function tests, and the coagulation parameters of prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), and D-dimer.

Detection of Soluble CD40 Ligand

The concentrations of sCD40L were measured by enzyme immunoassay (Bender Medsystems, Vienna, Austria).

Statistical Analysis

Statistical analysis was performed by using SPSS 10.0 (SPSS, Chicago, Ill) for Windows (Microsoft, Redmond, Wash). For comparison of the means of the 2 groups, the Mann-Whitney *U* test was used. A $P < .05$ was considered significant.

RESULTS

The mean gestation was 34.5 ± 3.33 weeks (range, 30 to 40 weeks) for patients with preeclampsia

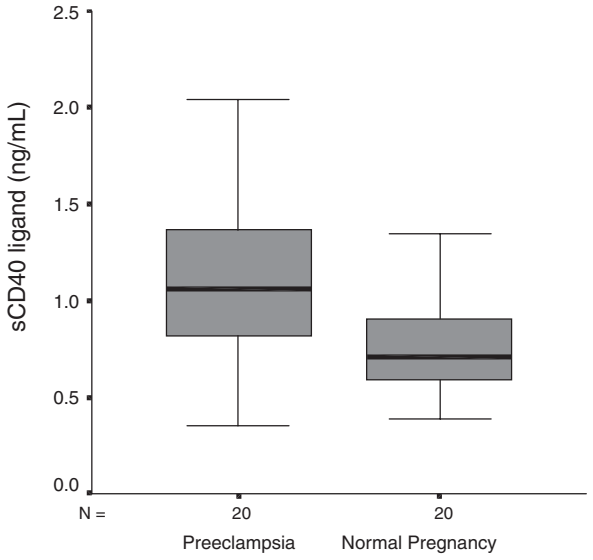


FIG. 1. Solulable CD40 ligand (sCD40) levels. Data are presented as means ± standard deviation.

and 38.0 ± 2.60 weeks (range, 32 to 40 weeks) for the normal pregnancy group. All of the preeclampsia patients had mild-type disease.

The systolic and diastolic blood pressures and urinary protein levels are summarized in Table 1. The urinary protein levels were 853.5 ± 481.9 mg/d in the preeclamptic group (range, 450 to 2400 mg/d). There were no statistically significant differences between the 2 groups for levels of hemoglobin, white blood cell counts, platelet counts, blood urea nitrogen (BUN), aspartate aminotransferase (AST), creatinine, and total protein. As summarized in Table 1, however, a statistically significant difference was found between the 2 groups for the values of alanine aminotransferase (ALT) and albumin:

- ALT was 22.75 ± 7.12 U/L (range, 12 to 40 U/L) in the preeclamptic group compared with 17.2 ± 5.37 U/L (range, 10 to 27 U/L) in the normal pregnancy group ($P = .012$).
- Albumin was 2.36 ± 0.38 g/dL in the preeclamptic group compared with 2.60 ± 0.21 g/dL in the women with a normal pregnancy ($P = .035$).

Although this difference was statistically significant according to ALT levels, these values all were within the normal reference range, so we evaluated this result as there being no hepatic dysfunction in the 2 groups.

In the case of coagulation variables, there were no statistically significant differences between PT, INR, and aPTT values for the 2 groups ($P > .05$). D-dimer levels were within normal ranges in all individuals (Table 2).

The mean sCD40L levels were 1.08 ± 0.43 ng/ mL in patients with preeclampsia and 0.76 ± 0.24 ng/mL in the women with a normal pregnancy. A statistically significant difference was found between sCD40L levels of the 2 groups ($P = .01$; Figure 1).

DISCUSSION

Because of its association with high maternal and fetal morbidity, preeclampsia has been studied widely. The pathogenesis of preeclampsia still is not clearly understood. To explain this with coagulation induction or defective fibrinolysis, various hemostatic parameters were studied.[10-13] Roberts et al[5] also reported maternal endothelial dysfunction with preeclampsia.

The factors that underlie endothelial dysfunction are still not clear. A number of observations also have revealed that women with preeclampsia have a deranged fetal-maternal inflammatory response. During the first and second trimesters, the risk of preeclampsia has been correlated with increased serum levels of inflammatory markers, including tumor necrosis factor-α (TNF-α), soluble TNF-α receptor p55, TNF-α receptors 1 and 2, interleukin 2 (IL-2) receptor, and activation of leukocytes.[4,14-19]

The present study supports the presence of an inflammatory response during preeclampsia by demonstrating raised plasma levels of sCD40L in preeclamptic pregnancies compared with normal pregnancies.

The CD40 ligand (also called CD154) is a trimeric, transmembrane protein of the TNF family and, together with its receptor CD40, is an important contributor to the inflammatory processes that lead to atherosclerosis and thrombosis.[7] The CD40-CD40L system is widely distributed in a variety of leukocytic and nonleukocytic cells, including endothelial and smooth muscle cells[20] and on activated platelets.[7] It also occurs in a soluble form that is fully active biologically, termed soluble CD40 ligand, which is shed from stimulated lymphocytes and is actively released after platelet stimulation.[21,22] Soluble CD40L is proinflammatory for endothelial cells and promotes coagulation by inducing expression of tissue factor on monocytes[23] and endothelial cells.[24] So, enhanced CD40-CD40L interaction has been shown in the pathogenesis of various inflammatory diseases such as autoimmune diseases, allograft rejection, and acute coronary syndromes.[25-27]

In the light of all these reports, we also studied sCD40L levels in the plasma of preeclamptic women compared with uncomplicated pregnant women as the control. The gestation was 34.5 ± 3.33 weeks (range, 30 to 40 weeks) for patients with preeclampsia and 38.0 ± 2.60 weeks (range, 32 to 40 weeks) for normal pregnant women. The PT, INR, aPTT, and D-dimer levels of all individuals were within normal reference ranges. There was also no significant difference between mean platelet numbers of both groups.

CONCLUSION

When we compared mean sCD40L levels between the 2 groups, we found that the difference was statistically significant (P = .01). These results support the study of Mellembakken et al,[8] but they also studied platelet activation and release of CD40L, as well as the chemokines RANTES (regulated upon activation normal T cell expressed and secreted) and growth related protein-a (GRO-a) from platelets, to show possible contribution of platelets to circulating levels of these inflammatory mediators during pregnancy. They showed enhanced platelet activation also.

Although cells other than platelets may contribute to raised plasma levels of sCD40L, it is estimated that more than 95% of circulating CD40L is derived from platelets.[28] So we wanted to emphasize that there is an inflammatory response in preeclampsia by showing a significant increase in sCD40L level in preeclampsia. We believe that future studies are needed to clarify whether inflammation may cause inappropriate endothelial cell activation or inappropriate endothelial cell activation may start this inflammatory response future.

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