Relationship Between Oxidative Stress Parameters and Cystatin C Levels in Patients With Severe Preeclampsia

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Key Words: preeclampsia; total antioxidant status; total oxidant status; oxidative stress index; cystatin C.

Summary. Background and Objective. Oxidative stress is believed to play a role in the development of preeclampsia (PE). It is known that an increased cystatin C level is also associated with PE. The aim of this study was to investigate the relationship between oxidative stress parameters and cystatin C levels in patients with severe PE.

Material and Methods. Forty-four patients with severe PE and 40 healthy pregnant women were recruited for the study. All study subjects were divided into 2 groups: group 1 (n=44) consisted of patients with severe PE, and group 2 (n=40) included healthy pregnant subjects. Blood samples were obtained from all subjects in order to measure the cystatin C level, total antioxidant status, and total oxidant status. The oxidative stress index was calculated.

Results. The group 1 had significantly higher cystatin C, total oxidant status, oxidative stress index levels and lower total antioxidant status level as compared with the group 2 (P=0.001, P<0.001, P<0.001, P=0.036, respectively). The serum cystatin C level was significantly correlated with the oxidative stress index (r=0.609, P<0.001).

Conclusions. The present study demonstrated that both oxidative stress and cystatin C levels were increased in patients with PE, and the increased cystatin C levels seem to be a consequence of oxidative stress.

Introduction

Preeclampsia (PE) is a potentially severe complication affecting 5%–7% of all pregnancies and is the leading cause of maternal and perinatal mortality and morbidity worldwide. The pathophysiological mechanisms of PE are still unclear, but are considered to result from an insufficient function of the placenta (1–3). The role of oxidative stress in the pathophysiology of PE has increasingly been postulated; reactive oxygen species produced by the relatively hypoxic placenta are transferred to the maternal circulation and subsequently cause endothelial dysfunction (4, 5). Besides, cystatin C as a sensitive marker of renal function is increased in women with PE secondary to renal impairment, and studies have demonstrated that the cystatin C level is a reliable diagnostic marker for PE (6–8).

Despite all these putative relationships between PE, oxidative stress, and cystatin C mentioned above, no study has been performed yet to evaluate both oxidative stress and cystatin C levels in patients with PE. Therefore, this study was carried out to investigate whether cystatin C levels increased or not due to oxidative stress in patients with severe PE.

Material and Methods

Study Design and Population. This cross-sectional study was conducted at the Harran University School of Medicine, Sanliurfa, Turkey. Prior to subject recruitment, the study protocol was reviewed and approved by the local ethics committee in accordance with the ethical principles for human investigations as outlined by the Second Declaration of Helsinki. Written informed consents were obtained from all the patients or their proxies. From February 2010 to March 2011, 44 consecutive patients with severe PE from the Intensive Care Unit and 40 healthy pregnant women matched for age and gestational week were recruited for the study.

All study subjects were divided into 2 groups: group 1 (n=44) consisted of patients with severe PE, and group 2 (n=40) consisted of healthy pregnant subjects. The control group comprised consecutive pregnant women followed up at our institution, who underwent routine third-trimester blood analysis and did not meet any exclusion criteria. All the patients in the control group were followed up till delivery, and none of them developed preeclampsia. The exclusion criteria were as follows: acute infectious diseases (pneumonia, pyelonephritis, urinary tract infection, etc.) within the last 4 weeks; or infiltrative disorders (tuberculosis, sarcoidosis, pul-
Oxidative Stress, Cystatin C, and Preeclampsia

monary fibrosis, etc.) or autoimmune diseases; any evidence of liver, kidney, or respiratory disease; diabetes mellitus; essential hypertension or use of antihypertensive drugs before the diagnosis of preeclampsia; heart failure; regular alcohol consumption; smoking during pregnancy; malignancy; HELLP syndrome; multiple gestation; intrauterine growth restriction (9) (as a fetus with an estimated weight below the 10th percentile); oligohydramnios (10) (amniotic fluid index less than 5 cm); magnesium prophylaxis; and labor with ruptured membranes. None of the study participants had a history of PE in previous pregnancies, and none received multivitamin and iron supplementation. Blood samples were obtained at the same time when PE diagnosis was established.

Baseline Definitions and Measurements. According to the International Society for the Study of Hypertension in Pregnancy (ISSHSP), PE can be defined as de novo hypertension occurring after 20 weeks of pregnancy together with proteinuria (11). Severe PE was defined if one or more of the following criteria was present: blood pressure greater than or equal to 160 mm Hg/110 mm Hg on two occasions at least 6 hours apart at rest, proteinuria greater than 5.0 g during a 24-hour period, increased serum creatinine level (10 minutes at 10°C to 18°C. Serum samples were transferred immediately to polypropylene tubes, which were then centrifuged at 3000 rpm for 10 minutes at 10°C to 18°C. Serum samples were stored in plastic tubes at –80°C until analysis. The cystatin C level, total antioxidant status (TAS), and total oxidant status (TOS) were measured from the blood samples, and the oxidative stress index (OSI) was calculated. The total antioxidant status (TAS) was evaluated as an indicator of antioxidant status.

Measurement of Cystatin C Levels. The cystatin C level was determined in serum using an enzyme linked immunosorbent assay (Human Cystatin C ELISA assay, BioVendor Research and Diagnostic Products, CTPark Modrice, Czech Republic). The intra- and interassay cross reactivity for the assay standards were less than 10%, and the results were expressed in ng/mL.

Measurement of Total Antioxidant Status. Serum TAS was evaluated using a novel automated method developed by Erel (13). In this method, hydroxyl radicals, the most potent biological radical, are produced. In the assay, ferrous ion solution in reagent 1 is mixed with hydrogen peroxide present in reagent 2. Sequentially produced radicals, such as the brown-colored dianisidinyl radical cation produced by the hydroxyl radical, are also potent radicals. This method allows measuring the antioxidative effect of the sample against potent free-radical reactions that are initiated by the hydroxyl radical. The assay has excellent precision values of more than 97%. The results are expressed in mmol Trolox equiv/L.

Measurement of Total Oxidant Status. Serum TOS was evaluated using a novel automated method developed by Erel (14). Oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundant in the reaction medium. Ferric ions generate a colored complex with xylenol orange in an acidic medium. Color intensity, which can be measured spectrophotometrically (V-530; Jasco®), is related to the quantity of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in micromolar hydrogen peroxide equivalents per liter (μmol H₂O₂ equiv/L).

Oxidative Stress Index. The OSI was defined as the ratio of TOS to TAS levels. For calculations, TAS units were changed to mmol/L, and the OSI was calculated according to the following formula (13, 14):

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\text{OSI (arbitrary units)} = \frac{\text{TOS (μmol H}_2\text{O}_2 \text{equiv/L)}}{\text{TAS (mmol Trolox equiv/L)}}
\]

Other Parameters. Serum urea, creatinine, blood glucose, aspartate aminotransferase, alanine aminotransferase levels were determined using commercially available assay kits (Abbott®, Abbott Park, North Chicago, Illinois, USA) with an autoanalyzer (Abbott®, Abbott Park, North Chicago, Illinois, USA).
Statistical Analysis. Statistical analysis was performed using SPSS for Windows version 17.0 (SPSS, Chicago, IL, USA). The Kolmogorov-Smirnov test was used to test the normality of data distribution. The data were expressed as arithmetic means and standard deviations. The independent sample t test was used to compare continuous variables between groups. Pearson’s correlation analysis was used to examine the association between oxidative stress parameters and cystatin C levels in patients with severe PE. A two-sided P value of <0.05 was considered statistically significant.

Results
Table summarizes the demographic, clinical, and biochemical characteristics of all patients. Urine output was less than 500 mL per 24 hour in 5 patients. There were no significant differences in age and gestational age between the groups (P>0.05). The group 1 had significantly higher cystatin C, TOS, and OSI levels and the lower TAS level as compared with the group 2 (P=0.001, P<0.001, P<0.001, and P=0.036, respectively) (Table, Fig. 1). In bivariate analysis, serum cystatin C levels were significantly correlated with OSI (r=0.609, P<0.001) (Fig. 2).

Discussion
To the best of our knowledge, this is the first study to evaluate the relationship between oxidative stress and cystatin C levels in patients with PE. The findings of the present study have shown that both

| Characteristic                  | Group 1 (n=44)        | Group 2 (n=40)        | P*     |
|--------------------------------|-----------------------|-----------------------|--------|
| Maternal age, years            | 27.27 (3.30)          | 27.45 (3.02)          | 0.799  |
| Gestational age, weeks         | 30.90 (2.94)          | 31.07 (3.08)          | 0.802  |
| BMI, kg/m²                     | 27.46 (3.44)          | 27.74 (3.08)          | 0.692  |
| Systolic BP, mm Hg             | 169.43 (12.63)        | 134.25 (8.36)         | <0.001 |
| Diastolic BP, mm Hg            | 97.50 (6.86)          | 79.00 (7.26)          | <0.001 |
| Glucose, mmol/L                | 4.85 (0.45)           | 4.86 (7.54)           | 0.919  |
| Urea, mmol/L                   | 19.91 (5.28)          | 10.29 (2.64)          | <0.001 |
| Creatinine, µmol/L             | 82.21 (19.44)         | 60.99 (12.37)         | <0.001 |
| AST, U/L                       | 48.90 (18.91)         | 26.37 (10.16)         | <0.001 |
| ALT, U/L                       | 47.90 (17.56)         | 24.92 (10.28)         | <0.001 |
| Hemoglobin, g/L                | 134.11 (13.23)        | 134.28 (20.26)        | 0.975  |
| Platelet count, ×10⁹/L          | 242.47 (61.94)        | 272.42 (69.07)        | 0.040  |
| Proteinuria, g/L per 24 hours  | 2.67 (1919)           | 0.151 (0.062)         | <0.001 |
| TAS, mmol Trolox equiv/L       | 0.91 (0.15)           | 1.06 (0.23)           | 0.036  |
| TOS, µmol H₂O₂ equiv/L         | 19.93 (5.36)          | 13.83 (2.91)          | <0.001 |
| OSI, arbitrary units           | 2.05 (0.72)           | 1.55 (0.30)           | <0.001 |
| Cystatin C, ng/mL              | 1192.93 (330.90)      | 980.60 (210.14)       | 0.001  |

Values are mean (standard deviation). BMI, body mass index, BP, blood pressure, ALT, alanine aminotransferase, AST, aspartate aminotransferase, TAS, total antioxidant status, TOS, total oxidant status, OSI, oxidative stress index.

*Independent sample t test.

Fig. 1. Differences in total antioxidant status (A), total oxidant status (B), oxidative stress index (C), and cystatin C levels (D) between groups.
studies have demonstrated increased maternal cir-
in PE (15). Vasospasm increases vascular resistance with hypoperfusion in the uteroplacental microcirculation, predisposes to placental hypoxia and ischemia, and leads to dysfunction of the maternal vascular endothelium that are a major contributor to endothelial dysfunction in PE (15). In contrast to normal pregnancy, several studies have demonstrated increased maternal circulating levels, placental tissue levels, and production rate of lipid peroxides, and the levels of several antioxidants are markedly decreased in PE (16–18). However, some other studies have failed to provide the evidence of increased circulating secondary products of lipid peroxidation in PE (19, 20).

However, data are still controversial; it is unclear if antioxidant levels increase, decrease, or remain unchanged in preeclamptic women (21). Knapen et al. showed the increased placental and decidual levels of antioxidant enzymes glutathione and glutathione peroxidase in patients with severe PE compared with healthy pregnant women (22). Myatt et al. indicated that increased oxidative stress in late gestation was documented in pregnancies complicated by PE in association with increased trophoblast apoptosis and deformation and altered placental vascular reactivity (23). Moreover, in many studies, the malondialdehyde (MDA) level has been shown to be increased in serum, plasma, and placental tissue samples of preeclamptic women (18, 24, 25). Anastakis et al. observed that MDA levels in preeclamptic women who had abnormal Doppler find-
ings of the uterine artery at 20–23 weeks’ gestation were significantly higher (18). Rudra et al. showed that a high plasma MDA concentration at 20 weeks’ gestation was associated with PE (25). On the other hand, Diedrich et al. and Bowen et al. suggested that oxidative stress increased with the severity of eclampsia. In less severe forms of the disease, the antioxidants and the placenta may be able to scavenge pro-oxidants (19, 26). The study performed by Bowen et al. found no significant differences in the MDA levels in the umbilical vein between patients with PE and healthy control subjects (26). Further, Llurba et al. found similar TAS levels comparing women with PE and those with normal pregnancy (17). Their results demonstrated that the levels of oxidative stress biomarkers were mildly elevated in PE and that increased lipid peroxidation and protein oxidation might be limited to the placental compartment (17). In our study, TAS and OSI, which are considered as oxidative stress parameters, were found to be increased in patients with severe PE compared with healthy pregnant women.

It is known that the serum cystatin C level is increased in pregnancy and are higher in women with PE. Studies have demonstrated that the cystatin C level is a reliable diagnostic marker for the transition from normal pregnancy to PE and shows the severity of PE (7, 8). Although increased cystatin C levels were suggested to be caused by impaired renal function and were found to be significantly correlated with glomerular filtration rate, recent evidence has also demonstrated that PE results in the increased placental production of cystatin C (27, 28). Similarly in our study, cystatin C levels were found to be increased in the patients with severe PE as compared with the healthy pregnant women.

Even though preeclampsia is commonly identified in the second half of pregnancy, damage usually occurs in the first half of pregnancy. Early prediction of PE is imperative for improving the maternal and fetal consequences. A possible predictive value of cystatin C in preeclampsia was highlighted in a recent study by Farag et al. (29). The authors showed the significantly elevated levels of cystatin C and beta 2 microglobulin in women who were in the second trimester of pregnancy and subsequently developed preeclampsia compared with healthy pregnant women (29). Oxidative stress and maternal endothelial dysfunction due to placental ischemia might be detected before the onset of PE; thus, combining oxidative stress markers and cystatin C with other markers in early periods of pregnancy to evaluate the predictive value in preeclampsia might be in scope of future studies.

In literature, little is known about the relationship between oxidative stress and cystatin C levels, and these markers have never been investigated together in PE. The oxidative stress-stimulated up-
regulation of cystatin C expression may be mediated by reactive oxygen species including superoxide anion, hydrogen peroxide, hydroxyl radical, and peroxynitrite, although it remains unclear how reactive oxygen species trigger the cystatin C expression (30, 31). It has been reported that oxidative stress rapidly initiates the translocation of cathepsins B and L from lysosomes to the cytosol due to the lysosomal membrane injury or rupture, and cystatin C may also be released from lysosomes into the cytoplasm where neuroglobin exists under oxidative stress conditions (32). Additionally, it has been previously reported that cystatin C expression is up-regulated in cultured neurons exposed to oxidative stress (29). Savas et al. demonstrated that the serum cystatin C levels were significantly elevated in rats after renal ischemia-reperfusion injury (33). In our study, the cystatin C levels were increased and were significantly correlated with increased oxidative stress index parameters in patients with severe PE. According to the data of this study, renal function impairment seemed to be moderate in the preeclampsia group; we believe that further studies focusing on the severity of renal function impairment and oxidative stress and/or cystatin C might help clinicians identify the clinical etiopathogenesis of preeclampsia. On the other hand, studies evaluating the predictive value of oxidative stress and/or cystatin C or other biochemical markers in the early pregnancy period in which renal impairment is not observed might be valuable for the early diagnosis and close follow-up.

Conclusions
The findings of the present study demonstrate that both oxidative stress and cystatin C levels increased in patients with severe preeclampsia. Increased cystatin C levels seem to be a consequence depending on the hazardous effects of oxidative stress on the renal parenchyma. As a relatively small sample size and a cross-sectional design were limitations of this study, large-scale prospective studies are needed to address this issue.

Statement of Conflict of Interest
The authors state no conflict of interest.

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