Tohoku J. Exp. Med., 2004, **202**, 87-92

Nitric Oxide, Lipid Peroxides, and Uric Acid Levels in Pre-Eclampsia and Eclampsia

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Department of Biochemistry, Gazi University, Medical Faculty, Ankara Turkey, [1]Department of Biochemistry, Ankara Training and Research Hospital, Ankara, Turkey, [2]Division of Obstetrics and Gynecology, Gazi University Mediko Sosyal Center, Ankara, Turkey and [3]Department of Obstetrics and Gynecology, Ankara Training and Research Hospital, Ankara, Turkey

PAŞAOĞLU, H., BULDUK, G., ÖĞÜŞ, E., PAŞAOĞLU, A. and ÖNALAN, G. *Nitric Oxide, Lipid Peroxides, and Uric Acid Levels in Pre-Eclampsia and Eclampsia*. Tohoku J. Exp. Med., 2004, **202** (2), 87-92 —— The aim was to study the role of nitric oxide (NO), lipid peroxides (LPX), and uric acid in pre-eclampsia and eclampsia. Plasma levels of NO metabolites (nitrite+nitrate), malonyldialdehyde (MDA), and uric acid and erythrocyte MDA levels were compared between normal pregnant, pre-eclamptic, and eclamptic pregnant women in third trimester. Student's *t*-test was used for statistical evaluation. Plasma NO metabolites levels were higher in eclamptic group (35.7±16.5 μmol/liter, $p<0.05$) but not in pre-eclamptic group (22.1 ±10.8 μmol/liter) than control group (18.8±6.9 μmol/liter). Plasma MDA and uric acid concentrations were higher in preeclamptic (4.4±1.7 nmol/ ml, $p<0.05$; 0.45±0.11 mmol/liter, $p<0.05$, respectively) and eclamptic (5.8±1.9 nmol/ ml, $p<0.05$; 0.47±0.12 mmol/liter, $p<0.05$) groups compared with control group (3.0±1.3 nmol/ml; 0.35± 0.06 mmol/liter). Erythrocytes MDA concentrations were higher only in eclamptic group (174.4±62 nmol/gHb, $p<0.05$) than control group (139.2±49.5 nmol/gHb). These results suggest that NO, LPX, and uric acid are important factors in the pathogenesis of pre-eclampsia and eclampsia, and that NO production and LPX are directly related to the severity of disease. ——— Pre-eclampsia; eclampsia; nitric oxide; lipid peroxidation; uric acid

Received October 1, 2003; revision accepted for publication December 15, 2003.

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In hypertensive disorders of pregnancy, pre-eclampsia is a common and major complication causing significant morbidity and mortality in the fetus. Pre-eclampsia is a multisystemic disorder and clinical condition characterized by hypertension, proteinuria, edema. Eclampsia is characterized by generalized tonic-clonic convulsions in additon to the pre-eclamptic findings (Cunningham et al.1993). Yet the etiology of this disease is still unknown. There is increasing evidence that endothelial cell injury and altered endothelial cell function play an important role in the pathogenesis of preeclampsia (Rappaport et al. 1990; Rodgers et al. 1998). Dysfunction of endothelial cells can contribute to inappropriate vasoconstriction and platelet aggregation which are early signs of atherosclerosis, hypertension and coronary vasospasm (Vane and Botting 1992). Oxygen free radicals and increased lipid peroxidation might form the link between the hypothetical immunologic mechanisms and the injury of endovascular trophoblast and endothelial cells. A number of reports suggested that lipid peroxidation may play a role in the etiology of the disease (Mclaughlin 1989; Gratacos et al. 2001). It has been suggested that oxidative stress may cause endothelial dysfuntion which may lead to hypertension by reduced release of vasodilating agents such as nitric oxide (NO) (Mutlu et al. 1999). NO is a potent vasodilatator and is thought to have a major effect on gestational vasodilatation (Sladek et al. 1997; Narin et al. 2000). Altered production of NO by the vascular endothelium may influence the pathogenesis of pre-eclampsia (Sladek et al. 1997; Norris et al. 1999). Some studies have been conducted to measure blood levels of the NO metabolites (nitrite+nitrate); however the data are conflicting. Relative to normal pregnancy there have been reports of either a decrease (Seligman et al. 1994; Mutlu et al. 1999), an increase (Smarason et al. 1997; Ranta et al. 1999; Shaamash et al. 2000) or no change (Curtis et al. 1995; Dabigge et al. 1996; Silver et al. 1996) of NO metabolites (nitrite+nitrate) in pre-eclampsia. Clinical utility of uric acid values in various hypertensive diseases of pregnancy appears to be limited (Lim et al. 1998). There have been many theories concerning the cause of increased plasma uric acid concentrations in pre-eclampsia (Paternoster et al. 1999). Whether the cause of increased plasma uric acid levels in pre-eclampsia is secondary to a true tubular damage because of renal vasoconstriction and ischemia, or to a pure functional adaptation due to the well known hypovolemia existing in this disease, is not known (Abuja and Albertini 2001). In this work we measured concentrations of LPX in erythrocytes and plasma, and NO metabolites and uric acid levels in plasma of normal, pre-eclamptic, and eclamptic pregnant to evaluate their relations to the severity of pre-eclampsia and the diagnostic significance of these parameters for the prediction of this syndrome.

Material and Methods

With the approval of the local ethics committee, patients in third trimester and controls were studied. Forty pre-eclamptic pregnants were studied and pre-eclampsia was defined as blood pressure constantly greater than 140/90 mmHg and proteinuria above 0.3 g/24 hour with no urinary tract infection and first pregnancy with no previous history of hypertension. Eclamptic pregnant (n=25), have typical eclamptic fits in addition to pre-eclamptic findings. Thirty one pregnant served as controls, no subject was known to have chronic hypertension or renal or metabolic disease. Also having their first pregnancy and were normotensive (Table 1). Peripheral venous blood specimens collected into glass tubes with ethylenediamine tetraacetic acid (EDTA) from all women before any drug was given to patients group. Samples were centrifuged at 3000 rpm for 15 minutes and the packed red blood cells and plasma were carefully removed from each sample. The remaining erythrocytes were washed with 0.9% sodium chloride (saline) solution for 4 times. Plasma and red cells were stored at –70°C until assays. Measurement of plasma nitrite and nitrate (Smarason et al. 1997): To 300 μl of plas-

TABLE 1. *Clinical parameters of the patients and controls*

Parameters	Control *n*=31	Pre-eclampsia *n*=40	Eclampsia *n*=25
Age (years)	24.8±4.2	25.6±4.8	24.5±5.1
Gestation at sampling (weeks)	36.6±1.5	34.1±2.5	34±1.6
Gestation at delivery (weeks)	37.6±1.76	35.1±2.66	34±1.6
Infant birthweight	3354±320	2819±387*	2083±410*, **
Systolic blood pressure (mmHg)	109±8	158±16*	162±23*
Diastolic blood pressure (mmHg)	67±9	100±8*	108±21*

The data are shown as mean±S.D. value.
*$p<0.05$ as compared to normal pregnant (Control).
**$p<0.05$ as compared to pre-eclampsia.

TABLE 2. *Plasma NO metabolites (nitrite+nitrate) MDA, and uric acid levels and erythrocyte MDA levels of patients and controls*

	Control *n*=31	Pre-eclampsia *n*=40	Eclampsia *n*=25
Plasma Nitrite+nitrate (μmol/liter)	18.8±6.9 (9-32)	22.1±10.8 (10-69)	35.7±16.5*, ** (20-84)
Plasma MDA (nmol/ml)	3.0±1.3 (1.1-6.0)	4.4±1.7* (2.2-7.2)	5.8±1.9*, ** (2.7-11.6)
Erythrocyte MDA (nmol/g Hb)	139.2±49.5 (70-277)	154.7±98.7 (82-297)	174.4±62.1* (74-301)
Plasma Uric acid (mmol/liter)	0.35±0.06 (0.25-0.46)	0.45±0.11* (0.29-0.61)	0.47±0.12* (0.34-0.67)

The data are shown as mean±S.D. (range) value.
*$p<0.05$ as compared to normal pregnant (Control).
**$p<0.05$ as compared to pre-eclampsia.

ma was added 300 μl of KH_2PO_4/K_2HPO_4 buffer (pH 7.5), 50 μl of 2 mmol/liter NADPH, 50 μl of 50 μmol/liter FAD and 50 μl of 1 unit/ml aspergillus nitrate reductase (Sigma Chemicals). This was incubated at room temperature for one hour followed by the addition of 500 μl of 0.2 mol/liter $ZnSO_4$ and 70 μl 2 mol/liter NaOH to deproteinate the sample. After centrifugation 0.75 ml of the supernatant was added to 1 ml of 1% sulphanilic acid (in 4 mol/liter HCL). After 10 minutes at room temperature 0.75 ml of freshly prepared 1% N-naphtylethylene diamine (in HPLC grade methanol) was also added. The resultant colour change was measured at 548 nm using a spectrophotometer. Nitrite concentration calculated from nitrite standarts. Erythrocytes MDA levels were determined using technique of Ohkawa et al. (1979). Plasma MDA assays were performed according to Hunter et al. (1985). MDA, the product of lipid peroxidation, reacts with thiobarbituric acid under acidic conditions at 95°C to form a pink coloured complex with an absorbance at 532 nm. 1,1,3,3-Tetraethoxypropane was used as the standard. The results were expressed as nmol MDA/g of hemoglobin for erythrocytes and nmol/ml for plasma. Hemoglobin (Hb) and uric acid levels were determined using routine laboratory methods with Abbott Systems.

All data were expressed as the mean± S.D. MDA, uric acid, and NO metabolites were compared between groups with two-tailed Student's *t*-test for unpaired data. A *p*-value less than 0.05 was considered significant.

Results

Clinical parameters of the groups are shown in Table 1. Plasma nitrite and nitrate, plasma and erythrocyte MDA levels and plasma uric acid levels of patient and control groups are shown in Table 2.

Plasma nitrite and nitrate levels in pre-eclamptic patients were not significantly different from controls but plasma MDA and uric acid levels were higher than controls. Eclamptic patients have elevated plasma nitrite and nitrate, MDA, and uric acid concentrations compared to controls. Erythrocytes MDA concentrations were not significantly different between the control and pre-eclamptic groups, but higher in eclamptic group than controls. Furthermore plasma nitrite and nitrate levels in eclamptic patients were higher than pre-eclamptic patients.

Discussion

In pre-eclampsia the pathophysiologic changes, which include increase sensivity to pressors, activation of the coagulation cascade, and increased vascular permeability, suggest that vascular endothelial dysfunction is an important component of this disorder (Pinto et al. 1991; Seligman et al. 1994). Pregnancy creates oxidative stress (Wang et al. 1991) and stress level increase in pre-eclampsia (Kumar and Das 2000). It has been implied that in pre-eclampsia there could be an increased vasoconstriction (Davidge et al.1998). Also some results showed that pre-eclampsia is associated with an imbalance between lipid peroxides and the anti- oxidant system (Paronen et al. 1996; Kumar and Das 2002). Kumar et al. (2000) suggested that in patients with pre-eclampsia there was an increase in free radical generation as indicated by an increase in the levels of lipid peroxides and a decrease in the concentrations of anti-oxidants such as superoxide dismutase, catalase and glutathione peroxidase. In a recent study (Bowen et al. 2001) plasma concentrations of uric acid, MDA, ascorbic acid, and vitamin E were not significantly different in pre-eclampsia as compared with normal pregnancy. But uric acid concentrations were significantly increased in eclampsia as compared with normal pregnancy and pre-eclampsia. They suggested that the antioxidant uric acid has a protective role. In our study we found plasma MDA levels significantly higher in pre-eclamptic and eclamptic groups than control group. Our findings support the notion that lipid peroxidation is an important factor in the pathogenesis of preeclampsia and eclampsia. Furthermore, plasma MDA levels were higher in eclamptic group than pre-eclamptic group, and erythrocytes MDA levels were higher in eclamptic group than control group. These results suggest that lipid peroxidation is related to the severity of this disorder.

It has been suggested that the increase of uric acid level was important in the evaluation of prognosis (Lim et al. 1998; Paternoster et al. 1999). Lim et al. (1998) observed that high uric acid level could identify women with an increased likelihood of having superimposed pre-eclampsia. Our results showed that uric acid was important in pre-eclampsia and eclampsia, but not related to the severity of pre-eclampsia since plasma uric acid levels were not significantly different between eclamptic and pre-eclamptic groups.

The present study also showed slightly higher plasma nitrite and nitrate levels in pre-eclampsia compared with control group but the difference was not statistically significant. In eclamptic group, however we measured higher nitrite and nitrate levels than normal pregnant and pre-eclamptic groups. Seligman et al. (1994) suggested that plasma nitrites levels were lower in pre-eclampsia. Davigge et al. (1996) found no difference in the plasma nitrites levels in this syndrome. Mutlu et al. (1999) showed that in patients with pre-eclampsia the plasma levels of nitrite and nitrate were decreased, while lipid per-

oxide levels were increased compared to healthy pregnant women. They suggested that high levels of lipid peroxide in the circulation may be caused by lowered NO synthesis. In contrast, Silver et al. (1996) showed that circulating nitrite and nitrate levels are not reduced in patients with severe pre-eclampsia compared with normotensive controls, and sera from these women do not supress endothelial cell NO synthesis in vitro. Ranta et al. (1999) suggested that NO production was increased with pre-eclampsia. The biologic significance of increased production is unknown, but it might be compensation for the vasoconstriction of pre-eclampsia. Nishikawa et al. (2000) support this results. They found a significant correlation between nitrite and nitrate level and endothelin-1 (ET-1, a vasoconstrictor) levels in sera from pre-eclamptic group and suggested that increased production of nitrite and nitrate in pre-eclampsia may contribute to homeostatic vasodilatation against vasoconstriction caused by a higher ET-1 concentrations. Conflicting results about pre-eclampsia and eclampsia may be explained by the gestational ages at sampling and a more severe disease process may be another factors in pre-eclamptic eclamptic studies. Our significantly high levels of plasma nitrite and nitrate might be due to clinical progress of eclampsia being more severe compared to pre-eclampsia. Shaamash et al. (2000) found increased nitrite and nitrate concentrations in sera of pre-eclamptic and eclamptic women compared with normal pregnant. Consistent with the present study Pathak et al. (1999) showed that plasma nitrite and nitrate levels of severe pre-eclamptic patiens were higher than mild pre-eclamptic patiens. Recently Shaamash et al. (2001) showed placental nitric oxide synthase activity and NO production were significantly increased in pre-eclampsia and eclampsia. This increase was directly related to the severity of the disorder. They concluded that such increase possibly represents a physiologic adaptive response to overcome the increased placental vascular resistance and to minimize platelet and leucocyte adhesion to the surface of placental villi or within the intervillous spaces.

In conclusion, plasma NO, LPX, and uric acid levels are related to pathogenesis of pre-eclampsia and eclampsia, and the increase in NO production and LPX are directly related to the severity of the disease. This might have diagnostic significance for the prediction of severity of the disease. Further experimental and clinical studies are necessary to clarify the pathogenesis of pre-eclampsia and eclampsia.

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