

Hatice PAŞAOĞLU¹
Neslihan BUKAN¹
Gülşen BULDUK²
Şevki ÇELEN³

Lipid Peroxidation, Nitrate and Nitrite Levels in Eclamptic and Intrauterine Growth Retarded Pregnancies

Received: October 22, 2002

¹Department of Biochemistry, Faculty of Medicine, Gazi University, Ankara,
²Department of Biochemistry, Ankara Training and Research Hospital, Ankara,
³Department of Obstetrics and Gynecology, Zekai Tahir Burak Women's Health Training and Research Hospital, Ankara - TURKEY

Abstract: In this study we investigated nitric oxide metabolites nitrate+nitrite and lipid peroxidation product (malonyldialdehyde: MDA) related to the pathogenesis of eclampsia and intrauterine growth retarded (IUGR) pregnancy.

Plasma nitrate+nitrite levels were higher in the eclamptic ($31.6 \pm 16.7 \mu\text{mol/L}$ $p < 0.001$) and IUGR ($24.3 \pm 9.5 \mu\text{mol/L}$ $p < 0.005$) groups than in the control group ($18.8 \pm 6.9 \mu\text{mol/L}$). Plasma MDA levels were higher in the eclamptic group ($5.5 \pm 2.5 \text{ nmol/ml}$ $p < 0.05$) but not significantly different in the

IUGR ($3.4 \pm 2.4 \text{ nmol/ml}$ $p > 0.05$) group than control group ($3.0 \pm 1.3 \text{ nmol/ml}$). In addition, the plasma nitrite+nitrate and MDA levels in the eclamptic group were higher than in the IUGR group ($p < 0.05$).

These variations could play different roles in the pathogenesis of eclampsia and IUGR pregnancy.

Key Words: Eclampsia, IUGR, nitric oxide, lipid peroxidation

Introduction

Eclampsia is a multisystemic disorder the aetiology of which is still unknown. There is increasing evidence that altered endothelial cell functions play an important role in the pathogenesis of pre-eclampsia and eclampsia (1).

An intrauterine growth retarded (IUGR) infant is commonly defined as one weighting less than the 10th percentile in birth weight for its gestational age. Such infants are also at a higher risk of perinatal morbidity and mortality, the risk rising with the severity of the growth restriction (2). Evaluations of blood flow velocity waveforms by Doppler ultrasound have shown that 20% to 88% of cases of IUGR are associated with increased vascular resistance in the foetoplacental circulation (3).

Nitric oxide (NO) is a potent vasodilator agent which modulates pulmonary and systemic vascular tone (4). In our previous study, we found low NO levels in patients with pulmonary hypertension and congenital heart defects (5). Altered production of NO by the vascular endothelium may influence the pathogenesis of pre-eclampsia (6). Relative to normal pregnancy there have been reports of either a decrease (7), an increase (8,9) or no change (10,11) in NO levels in pre-eclampsia.

Oxygen free radicals are extremely reactive molecules and might form the link between the injury of the

endovascular trophoblast and endothelial cells (12). Some reports indicate that blood levels of lipid peroxidation products (Malonyldialdehyde: MDA) are elevated in pre-eclamptic pregnancy and it has been suggested that these play a role in the aetiology of the disease (12,13).

We have encountered no studies concerning NO metabolites nitrite+nitrate and lipid peroxidation product in the same cases with eclamptic and IUGR pregnancy.

The aim of this study was to investigate MDA and nitrite+nitrate concentrations in the plasma in normal, eclamptic and IUGR pregnancies and to determine the parameters which may contribute to the pathophysiology of these cases.

Materials and Methods

With the approval of the local ethics committee patients and controls in the third trimester were selected at the Zekai Tahir Burak Women's Health Training and Research Hospital. Peripheral venous blood specimens were collected into tubes with EDTA. Samples were centrifuged at 3000 rpm for 15 min and the plasma removed from each sample.

Nitrite+nitrate levels were determined by the Griess reaction, which relies on a colorimetric reaction between

nitrite, sulphanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride to produce a pink Azo product. Prior to the Griess reaction all nitrate was converted to nitrite using bacterial enzyme nitrate reductase. Concentrations were determined by comparison to a standard solution of sodium nitrite (14).

Plasma MDA assays were performed according to Hunter et al. (15).

All data were expressed as the mean value \pm SD. Statistical analysis was performed with a determination of the correlation coefficient and comparison between groups by Student's t-test and the Kruskal Wallis analysis of variance test. Differences of $p < 0.05$ were considered significant.

Results

Table 1 summarises the clinical parameters of the groups included in this study.

Plasma nitrite+nitrate and MDA levels of the patient and control groups are given in Table 2 and the Figure.

Eclamptic patients had higher plasma nitrite+nitrate ($p < 0.001$) and MDA levels ($p < 0.005$) than the controls. Only the plasma nitrite+nitrate levels were high in IUGR patients ($p < 0.05$), and the MDA levels were not significantly different to the control group.

Furthermore, plasma nitrite+nitrate levels in eclamptic patients were higher than in IUGR patients ($p < 0.05$). In our study there was no correlation between the parameters of the groups.

Discussion

In this study we found higher plasma MDA levels in the eclamptic group than in the control group and IUGR group ($p < 0.05$). There were no significant differences between the IUGR and control groups.

The results of Wang et al. (16) showed that pregnancy creates oxidative stress and increased stress levels in pre-eclampsia. Vasoconstriction was the main pathophysiological defect in pre-eclampsia. In addition, endothelial cell damage and related protein increased sensitivity to vasopressor substances. Functional defects in the cell membrane are other pathophysiological changes caused by lipid free radicals and lipid peroxides. It has been claimed that oxygen free radicals and lipid peroxides disturb NO formation in endothelial cells (17).

We have encountered no studies concerning plasma nitrite+nitrate levels in eclampsia. There have been studies which suggested that endothelial changes in pre-eclampsia pathophysiology might be related to either an increase or a decrease in the synthesis of NO. Some researchers found high nitrite+nitrate levels of plasma in

Table 1. Clinical and chemical parameters of the groups.

	Eclampsia (n = 15)	IUGR (n = 17)	Control (n = 31)
Primigravida	75%	50%	48%
Age (years)	24.5 \pm 5.0	24.9 \pm 5.2	24.8 \pm 4.23
Gestation at sampling (weeks)	34 \pm 1.7	36 \pm 1.9	36.6 \pm 1.5
Gestation at delivery (weeks)	34 \pm 1.9	37 \pm 1.6	38 \pm 1.76
Infant birthweight (g)	2064 \pm 395	2190 \pm 167	3354 \pm 320
Systolic blood pressure (mmHg)	162 \pm 25	135 \pm 25	109 \pm 8
Diastolic blood pressure (mmHg)	108 \pm 20	90 \pm 19	67 \pm 9
Haemoglobin (g/dl)	12.7 \pm 1.2	12.3 \pm 1.0	12.4 \pm 1.4
Urea (mg/dl)	31 \pm 12	21 \pm 11	20 \pm 4
Creatinine (mg/dl)	1.0 \pm 0.2	0.7 \pm 0.2	0.8 \pm 0.1
Proteinuria (mg/dl)	930 \pm 56	232 \pm 99	----

The data are shown as mean \pm SD values or as percentages.

Table 2. Plasma nitrite+nitrate, MDA levels of patients and controls.

	Eclampsia (n = 15)	IUGR (n = 17)	Control (n = 31)
Nitrite + nitrate ($\mu\text{mol/L}$)	31.6 \pm 16.7 ^a	24.3 \pm 9.5 ^{b,c}	18.8 \pm 6.9
MDA (nmol/ml)	5.5 \pm 2.5 ^d	3.4 \pm 2.4 ^c	3.0 \pm 1.3

The data are shown as mean \pm SD values. ^a $p < 0.001$ compared to control group, ^b $p < 0.05$ compared to control group, ^c $p < 0.05$ compared to eclamptic group, ^d $p < 0.05$ compared to control group.

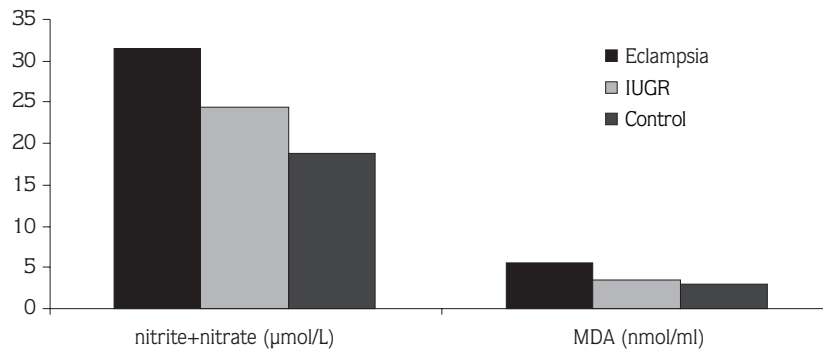


Figure. Plasma nitrite+nitrate, MDA levels in patients and controls.

pre-eclamptic patients compared to normal pregnancies (8,18).

Norris et al. (19) found high NO levels in the uteroplacental, foetoplacental and peripheral circulations in pre-eclamptic pregnancies. Zeeman et al. (20) suggested that high lipid peroxides lowered vascular prostacycline and NO release in uteroplacental vascular region. Pinto et al. (17) found low NO release in umbilical vessel endothelial cells in pre-eclamptic patients. On the other hand, some researchers have not detected any difference in plasma NO levels between pre-eclamptic and normal pregnancies (9,14). This finding could have several explanations. Nitrite+nitrate levels are altered by renal function. However, our results showed that serum creatinine was not high in the patient groups and did not correlate with nitrite+nitrate levels. Furthermore, gestational ages at sampling and the severity of the disease may be another factor in pre-eclamptic and eclamptic studies. The authors observed some alterations in NO levels between different gestational ages in normal pregnancy (20). Our significantly high levels of plasma nitrite+nitrate might be due to the clinical progress of eclampsia being more severe than pre-eclampsia.

Furthermore we thought that the factors which maintain endothelial function might be kept in balance in the early stages of the disease, but that this balance might be damaged in the further stages of eclampsia.

IUGR, because of the variety of its mechanisms, is a complicated clinical occurrence, which requires new developments and research in diagnosis and therapy (21).

Although placental pathology as a factor in IUGR remains to be fully elucidated, there is agreement that altered uteroplacental haemodynamics are important in the pathophysiology of the problem. Due to the vasodilator effects of NO on the placental vasculature, an impairment in NO production has been proposed as a possible mechanism for reducing foetoplacental circulation in pregnancy complications associated with vasospasm, such as pre-eclampsia and/or IUGR (6,19).

In our study, plasma nitrite+nitrate levels were significantly higher in the IUGR group than in the control group ($p < 0.05$). We found a significant difference between eclampsia and IUGR ($p < 0.05$) for plasma nitrite+nitrate levels. We found no marked differences in plasma MDA levels in the IUGR group compared to the control group ($p > 0.05$).

These results show that plasma NO and MDA levels may possibly be related to the pathogenesis of eclampsia. However, IUGR pathogenesis may be related to NO, not to lipid peroxidation, in contrast to the pathology of eclampsia.

The evidence indicates that NO can have either a pro-oxidant or an anti-oxidant effect on lipid peroxidation, depending on a variety of contingent factors (22). At relatively high concentrations, NO can attenuate membrane dysfunction and tissue injury, while acting as a reactive oxygen metabolite (23). On the other hand, when generated at lower concentrations in the presence of oxygen, superoxide and other reactive oxygen species, NO can be converted into a range of potent oxidants (such as nitrogen dioxide and peroxynitrite) which might amplify and exacerbate the harmful effects of lipid peroxidation (24).

Van Buren et al. (25) claimed that, NO might play a role in uteroplacental blood circulation during pregnancy. They said that treating sheep in the final stage of

pregnancy with NO synthetase inhibitors might cause a decrease in uterine blood flow. Yallampalli and Garfield (26), in their experiment, gave NO inhibitor to pregnant rats, thus decreasing uterine blood flow, and investigated foetal birth weights, finding significant growth retardation. They claimed that this was due to vasoconstriction in the placental vessels caused by a decrease in NO release. Moreover, Di Iorio et al. (27) suggested that in IUGR pregnancies a compensatory increase in the synthesis of NO within the placenta might occur in an attempt to maintain an adequate blood flow through the placenta.

It seems that further experimental and clinical studies are necessary to clarify the role of NO in IUGR pregnancy.

Correspondence author:

Hatice PAŞAOĞLU

Department of Biochemistry,

Faculty of Medicine, Gazi University,

Beşevler, Ankara - TURKEY

References

- Cunningham FG, MacDonald PC, Gant NF et al. Hypertensive disorders of pregnancy. Williams' Obstetrics (Eds. Cunningham FG, MacDonald PC, Gant NF et al.) 19th ed. Norwalk, Connecticut: Appleton & Lange. 1993, pp: 763-817.
- Goldenberg RL, Cutter GR, Hoffman HJ et al. Intrauterine growth retardation; Standards for diagnosis. Am J Obstet Gynecol 161: 271-277, 1989.
- Bates JA, Evans JA, Mason G. Differentiation of growth retarded from normally grown fetuses and prediction of intrauterine growth retardation using Doppler ultrasound. Br J Obstet Gynecol 103: 670-675, 1996.
- Singh S, Evans TW. Nitric oxide the biological mediator of the decade: Fact or fiction? Eur Respir J 10: 699-707, 1997.
- Narin F, Narin N, Paşaoğlu H et al. Nitric oxide level in children with pulmonary hypertension. Ind J Physiol Pharmacol 44: 335-339, 2000.
- Sladek SM, Magness RR, Conrad KP. Nitric oxide and pregnancy. Am J Physiol 272: 441-463, 1997.
- Seligman SP, Buyon JP, Clancy RM et al. The role of nitric oxide in the pathogenesis of preeclampsia. Am J Obstet Gynecol 171: 944-948, 1994.
- Smarason AK, Allman KG, Young O et al. Elevated levels of serum nitrate, a stable end product of nitric oxide, in women with preeclampsia. Br J Obstet Gynecol 104: 538-543, 1997.
- Ranta V, Vionikka L, Halmesmaki E et al. Nitric oxide production with preeclampsia. Obstet & Gynecol 93: 442-445, 1999.
- Davidge ST, Stranko CP, Roberts JM. Urine but not plasma nitric oxide metabolites are decreased in women with preeclampsia. Am J Obstet Gynecol 174: 1008-1013, 1996.
- Silver RK, Kupfermine MJ, Russell TL et al. Evaluation of nitric oxide as a mediator of severe preeclampsia. Am J Obstet Gynecol 175: 1013-1017, 1996.
- Maseki M, Nishigaki I, Hagihara M et al. Lipid peroxide levels and lipid serum content of serum lipoprotein fractions of pregnant subjects with and without preeclampsia. Clin Chim Acta 155: 155-161, 1981.
- McLaughlin MK: Lipid peroxidation in pregnancy: New perspectives on preeclampsia. Am J Obstet Gynecol 161: 1025-1034, 1989.
- Curtis NE, Gudi NM, King RG et al. Nitric oxide metabolites in normal human pregnancy and preeclampsia. Hypertens Pregnancy 14: 339-349, 1995.
- Hunter MIS, Nlemadim BC, Davidson DLW. Lipid peroxidation products and antioxidant proteins in plasma and cerebrospinal fluids from multiple sclerosis patients. Neurochem Res 10: 1645-1652, 1985.
- Wang Y, Walsh SW, Guo J et al. The imbalance between thromboxane and prostacyclin in preeclampsia is associated with an imbalance between lipid peroxides and vitamin E in maternal blood. Am J Obstet Gynecol 165: 1695-1700, 1991.

17. Pinto A, Sorrentino R, Sorrentino P et al. Endothelial-derived relaxing factor released by endothelial cells of human umbilical vessels and its impairment in pregnancy-induced hypertension. *Am J Obstet Gynecol* 164: 507-513, 1991.
18. Davidge ST, Billiar TR, Roberts JM. Plasma nitrites and nitrates, stable end products of nitric oxide, are elevated in women with preeclampsia. In: *Proceedings of the ninth international congress of the International Society for the Study of Hypertension in Pregnancy*. Sydney; Australia, March 15-18, 1994.
19. Norris LA, Higgins JR, Darling MRN et al. Nitric oxide in the uteroplacental, fetoplacental and peripheral circulations in preeclampsia. *Obs & Gyn* 93: 958-963, 1999.
20. Zeeman CG, Dekkar GA, Van Geijn HP et al. Endothelial function in normal and preeclamptic pregnancy: A hypothesis. *Eur J Obstet Gynecol Reprod Biol* 43: 113-122, 1992.
21. Creasy RK, Resnik R. Intrauterine growth restriction, Creasy & Resnik *Maternal Fetal Medicine: Principles and practice* (Eds. RK. Creasy, R. Resnik) W.B. Saunders Company, Philadelphia 1989, pp: 547-564.
22. Paternoster DM, Stella A, Mussap M et al. Predictive markers of preeclampsia in hypertensive disorders in pregnancy. *Int J Gynecol Obstet* 66: 232-243, 1999.
23. D'Ischia M, Palumbo A, Buzzo F. Interactions of nitric oxide with lipid peroxidation products under aerobic conditions: inhibitory effects on the formation of malondialdehyde and related thiobarbituric acid-reactive substances. *Nitric Oxide: Biology and Chemistry* 4: 4-14, 2000.
24. O'Donnell VB, Chumley PH, Hogg N et al. Nitric oxide inhibition of lipid peroxidation: Kinetics of reaction with lipid peroxy radicals and comparison with α -tocopherol. *Biochem* 36: 15216-15523, 1997.
25. Van Buren GA, Yang D, Clark KE. Estrogen-induced uterine vasodilatation is antagonized by L- nitroarginine methyl ester, an inhibitor of nitric oxide synthesis. *Am J Obstet Gynecol* 167: 828-833, 1992.
26. Yallampalli C, Garfield RE. Inhibition of nitric oxide synthesis in rats during pregnancy produces signs similar to those of preeclampsia. *Am J Obstet Gynecol* 169: 1316-1320, 1993.
27. Di Iorio R, Marinoni E, Coacci F et al. Amniotic fluid nitric oxide and uteroplacental blood flow in pregnancy complicated by intrauterine growth retardation. *Br J Obstet Gynecol* 104: 1134-1139, 1997.