Significance of changes in lipid peroxides and antioxidant enzyme activities in pregnant women with preeclampsia and eclampsia

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Summary

This review addresses the general hypothesis that the pathogenesis of preeclampsia and enclampsia are related to an imbalance of increased oxidative stress and lipid peroxidation coupled with a deficiency of antioxidant protection. Accordingly, this study was initiated to assess total antioxidant status and free-radical activity in preeclampsia and eclampsia. The patients studied were 44 healthy pregnant women and 45 women with hypertension classified as having preeclampsia (n=27), and eclampsia (n=18).

The serum levels of lipid peroxide were significantly increased ($p<0.0001$) and antioxidant enzyme activities (superoxide dismutase and gluthathione levels) in erythrocytes were significantly decreased ($p<0.0001$) in women with preeclampsia and eclampsia compared with the controls. The groups of preeclampsia and eclampsia had similar values of catalase activities as the controls ($p>0.05$).

There were no correlations between serum levels of lipid peroxide and antioxidant enzyme activities or systolic-diastolic blood pressure of pregnant women with preeclampsia and eclampsia. The mean systolic and diastolic blood pressure, the serum lactate dehydrogenase (LDH) and aspartate transaminase (AST) levels of preeclamptic and eclamptic women were high, whereas haemoglobin (Hb), Hematocrit (Htc) and platelet levels were lower than those of the control subjects ($p<0.0001$). There were no differences in mean gestational week, whereas the mean age of eclamptic women was lower than that of the other two groups ($p<0.001$). The serum levels of Alanin-transaminasis (ALT) and urea in eclamptic women were significantly higher compared with the other two groups ($p<0.0001$), whereas creatinin levels were lower than those of the other two groups ($p<0.05$).

Our findings give support to those few studies considering lipid peroxidation as an important factor in the pathogenesis of preeclampsia and eclampsia. Further studies are needed to clarify the relations between lipid peroxidation and antioxidative function and their pathophysiological significance in preeclampsia and eclampsia.

Key words: Lipid peroxide; Glutathione; Superoxide dismutase; Catalase; Preeclampsia; Eclampsia.

Introduction

Preeclampsia and eclampsia are still a major cause of obstetrical and perinatal morbidity and mortality and no breakthrough has yet been achieved in the understanding of its pathophysiology [1-3]. Lipid peroxidation (LPO) has been suggested as a causative factor in preeclampsia and eclampsia. The serum levels of lipid peroxidation products have been found to be higher in preeclampsia and eclampsia patients. The serum levels of lipid peroxidation products have been found to be higher in preeclamptic patients than in normal pregnant women [1, 2, 4-6]. Under normal conditions there are varieties of antioxidant mechanisms that serve to control LPO; however the balance of oxidant-antioxidant systems may be impaired in women with preeclampsia. In addition, the pathophysiologic characteristics of preeclampsia have been attributed to endothelial cell dysfunction. To date, the effect of elevated lipid peroxides on vascular endothelial cell function during pregnancy has been investigated. Elevated lipid peroxides could lead to endothelial cell dysfunction by altering important biochemical reactions in the cell. The action of lipid peroxides may involve a modulation of prostaglandin synthesis [7]. The walls of the umbical arteries in preeclamptic women produce less prostacyclin than those in normal pregnants and the capacity for endothelial-dependent relaxation is impaired [8]. Lipid peroxides are also known to inhibit prostacyclin synthase [7].

LPO is a process, which is determined by the extent of peroxide-forming free radical mechanisms and the peroxide-removing antioxidative system. The source of the lipid peroxides in preeclampsia is unknown, but it has been suggested that poorly perfused placental tissue may evoke the free radical process and the inception of generalized lipid peroxidation [4].

In normal pregnancy, the levels of LPO products have been found to be low in cord blood [8]. Certain antioxidants are found to be decreased in preeclampsia and may be reflective of increased reactive oxygen species activity. Therefore, oxidative stress is likely an important feature of preeclampsia [1]. Free radical production in cells is relatively low in normal conditions due to the various and very active defence systems, including chemical scavengers or antioxidant molecules and the three enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) [9, 10]. SOD is an important enzyme capable of inactivating free radicals in vivo and in vitro. It is well known that SOD is connected with body defence against the superoxide anion O_2 that damages tissue in vivo and in vitro [9]. Plasma thiol and GSH are general scavengers whereas SOD specifically

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detoxifies superoxide anion radicals. The decrease in these three antioxidant markers suggests that there is an increased oxidant stress in patients with preeclampsia and eclampsia [11].

Lipid peroxides are involved in endothelial cell injury, vasoconstriction, and in the imbalance between thromboxane and prostacyclin that is associated with preeclampsia and eclampsia. Deficiency of antioxidants could also increase LPO in the maternal circulation, because antioxidants reduce the levels of lipid peroxides by limiting their generation or by inactivating them once they are formed. In preeclampsia and eclampsia, antioxidant activities of erythrocytes and placenta are significantly reduced in comparison with activity levels in normal pregnancies, thus contributing to the increase of placental and circulating levels of lipid peroxides [10].

In light of this knowledge, in this study we have investigated the differences in serum lipid peroxide and antioxidant enzyme activities and those of clinical significance in pregnant women with eclampsia and preeclampsia.

Material and Methods

The study was conducted between April 1997 and February 1998 in the Department of Gynecology and Obstetrics, Dicle University Faculty of Medicine. Twenty-seven patients with preeclampsia, 18 patients with eclampsia and 44 healthy women as a control group were included in the study. The gestational age of the study and control groups was between 24-40 weeks and their age ranged from 20 to 40 years.

Patients with preeclampsia were defined on the basis of the following clinical and laboratory criteria. Systolic blood pressure >140 mmHg and diastolic blood pressure >90 mmHg noted on at least two occasions 6 h apart, no fundoscopic findings with hypertensive retinopathy, and proteinuria levels >300 mg/dl found in at least two random specimens 6 h apart. Eclampsia was diagnosed on the development of convulsion with the clinical signs and symptoms consistent with preeclampsia. Forty-four patients in the second and third trimester without maternal and fetal compliations during the pregnancy period were selected as the control group. Gestational age was defined based on the last menstrual period and ultrasonography. Furthermore, all patients were consulted by the internal medicine department.

None of the women had received antihypertensive medication until the study samples were taken.

Heparinized blood and whole blood samples were taken from each patient fasting overnight. Blood samples were centrifuged. The erythrocyte package was prepared by washing the erythrocytes fractioned blood plasma taken by heparin with 0.15 mol/L NaCl solution at a rate of 1:5 three times, and by centrifuging them at 3,000 rpm for ten minutes each time. The measurements of SOD, catalase and GSH were conducted in erythrocyte serum LPO by the TBA method of Asakawa [12]. In lipid peroxide measurement, malondialdehyde released was used as an index. The basic principle of the method is based upon the fact that MDA, a product of lipid peroxidation, produces a complex giving maximum absorbance at 532 nm by reacting with thiobarbituric acid (TBA). Erythrocyte GSH was measured by the 2 nitrobenzoic acid (DTNB) method from Beutler [13] and erythrocyte SOD was measured by the modified Winterbourn and Hawkins method. Catalase levels were determined by Aebi's modified colorimetric method [15]. The method is based upon alteration of H_2O_2 optic density, depending upon enzymatic decomposition of H_2O_2 (by the effect of catalase in the sample). Data were changed to k/g Hb after "k" value was determined, taking suitable absorbance for each analysis according to calculated regression. Drabkin's method was used to determine erythrocyte hemoglobin.

ALT, AST, LDH and urea and creatinin levels activities were measured in an Abbott spectrum autoanalyzer by the enzymatic-colorimetric method. Hematocrit and thrombocyt levels were measured in an Abbott Cell Dyn 3500 blood counter analyzer.

In the statistical evaluation of the results, one way variance analysis was used and the Tukey HSD was used as a post hoc. Analyses were performed using SPSS software (Statistical Package for the Social Sciences, version 6.0). Correlations between different markers were determined.

Results

The clinical and laboratory findings of the preeclamptic, eclamptic and control groups are shown in Table 1.

There was no statistical difference between mean gestational weeks and age in all three groups ($p<0.05$). The mean systolic and diastolic blood pressure of the two study groups were higher compared to the control group ($p<0.001$).

The level of LPO and antioxidant enzyme activities and systolic-diastolic blood pressure of pregnant women with preeclampsia and eclampsia showed no correlation.

Table 1. — *Clinical and laboratory characteristics of the preeclampsia, eclampsia and control groups.*

Variables	Control group (n=44)	Preeclamptic group (n=27)	Eclamptic group (n=18)
Age	29.95±5.75	29.25±7.09	23.66±6.46
Gestational age at delivery (weeks)	35.84±4.45	35.51±5.14	34.94±3.81
Systolic blood pressure (mmHg)	119.11±8.208	150.74±10.35	147.77±14.37
Diastolic blood pressure (mmHg)	82.66±7.804	103.33±10.37	96.11±8.49
ALT (IU/L)	38.84±12.47	57.00±63.57	120.05±146.73
AST (IU/L)	25.31±9.16	87.44±150.81	118.50±118±36
LDH (IU/L)	201.34±23.03	427.29±183.30	473.16±188.69
Platelets (/mm^3)	298909.09±62915.16	233629.62±138454.42	175177.77±111626.80
Hb (gr)	11.22±2.22	12.06±2.39	14.21±1.24
Htc (%)	34.00±5.98	36.01±5.52	44.65±3.32
Urea (mg/dl)	23.64±6.43	25.62±9.68	30.94±14.25
Creatinin (mg/dl)	1.009±0.32	0.82±0.30	1.04±0.36

Table 2. — *Lipid peroxides and antioxidant enzyme activities of pregnant women with eclampsia and preeclampsia*

Variables	Groups	X±SD	p
Serum LPO nmol/ml	Control group	1.306±0.213	p<0.0001
	Preeclamptic group	4.836±0.904	
	Eclamptic group	4.756±0.539	
Erythrocyte GSH mg/100 ml	Control group	76.39±2.11	p<0.0001
	Preeclamptic group	61.65±3.84	
	Eclamptic group	61.43±3.68	
Erythrocyte SOD u/g Hb	Control group	2295.22±115.104	p<0.0001
	Preeclamptic group	1646.88±169.47	
	Eclamptic group	1565.33±164.30	
Erythrocyte CATALASE k/g Hb	Control group	1458.75±21.78	p>0.05
	Preeclamptic group	1466.74±6.57	
	Eclamptic group	1467.44±14.15	

Hb, Htc, HDH and AST platelet, levels of preeclamptic and eclamptic women were higher than those of control subjects. The differences between them were statistically significant (p<0.0001).

Creatinin levels of pregnant women with preeclampsia were lower than those of women with eclampsia and the control group (p<0.001). The higher serum levels of ALT in eclamptic women relative to the other two groups were statistically significant (p<0.0001).

The difference in serum urea levels in eclamptic women and the other two groups were statistically significant (p<0.002).

LPO levels in the eclamptic and preeclamptic groups were found to be higher than those of the control group (p<0.0001) (Table 2, Figure 1).

The activities of SOD and GSH in pregnant women with preeclampsia and eclampsia were lower than those of the control group (p<0.0001) (Table 2, Figures 2, 3).

The difference in erythrocyte catalase activities among the three groups was not significant (p>0.005) (Table 2, Figure 4).

Discussion

Preeclampsia, one of the most critical health problems in human pregnancies complicating approximately 6-8% of all gestations, is the leading cause of fetal growth retardation, infant morbidity and mortality, premature birth and maternal death [16]. Several authors have suggested that oxidative stress has had an influence on the pathogenesis of preeclampsia [17, 18, 19].

It has been suggested that increased lipid peroxidation could cause degeneration of organs or tissues, and that lipid peroxides formed at the primary site could be transferred

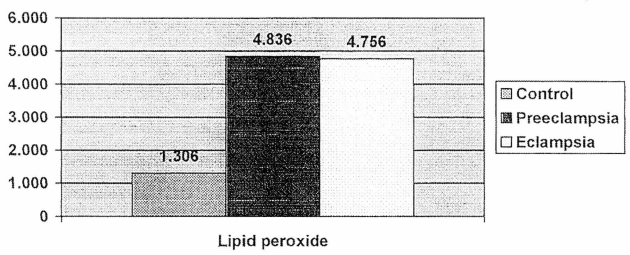

Figure 1. — Changes of lipid peroxides in preeclamptic, eclamptic and control groups.

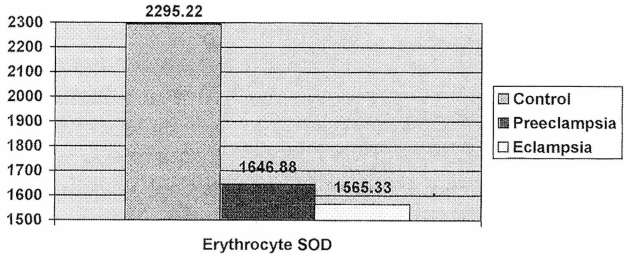

Figure 3. — Erythrocyte SOD levels of preeclamptic, eclamptic and control groups.

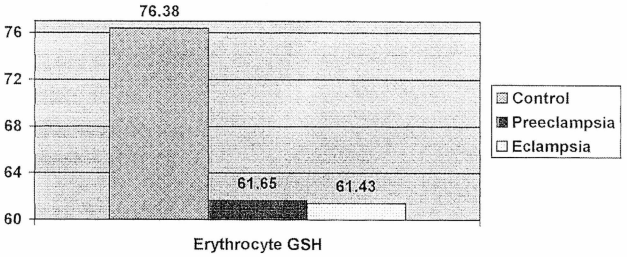

Figure 2. — Erythrocyte GSH levels of preeclamptic, eclamptic and control groups.

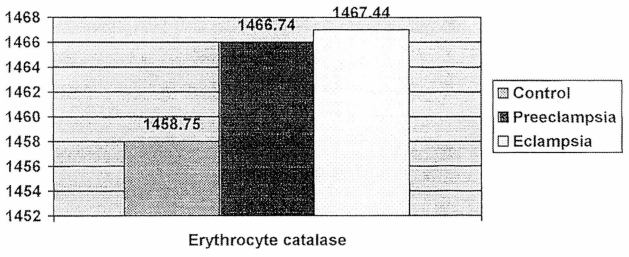

Figure 4. — Erythrocyte catalase levels of preeclamptic, eclamptic and control groups.

through the circulation to other organs or tissues and cause damage by propagating LPO. Ishihara *et al.* [21] reported that a remarkable increase in serum lipid peroxide levels was observed in pregnant subjects when compared with non-pregnant subjects, and that the levels in preeclamptic subjects and that the levels in preeclamptic subjects were higher than those in normal pregnant subjects [20].

As lipid peroxidation is a rapid and complex process, the accurate estimation of the extent of LPO is problematic. Direct measurement of LPO in vivo is difficult and may be misleading because of the diversity of peroxide compounds and the very short half-life of some lipid peroxides. Hence, in lipid peroxide measurement, malondialdehyde release was used as an index. Consistent with previous reports [1, 2, 6, 16, 17, 22] we also noted an increase in the appearance of LPO in preeclamptic and eclamptic pregnancies. Among speculations regarding the etiology of enhanced lipid peroxidation in preeclampsia, some authors have indicated a possible deficiency in antioxidants [1, 2, 4, 22]. Our study supports such a conception.

Liang *et al.* found that LPO and SOD levels were significantly high in a study of pregnant women with hypertension [23]. They reported that oxidation and antioxidation imbalance and endothelial cell damage might play an important role in the pathogenesis of hypertension. Some other researchers also found that SOD levels were high in pregnant women with hypertension [8, 9]. Jendryczko *et al.* observed that LPO levels were high, but erythrocyte, SOD and GSH-Px levels were significantly low in a hypertensive group [24]. They suggested this derangement of the antioxidant enzyme system was important in the pathogenesis of hypertension and might affect the antioxidant function of the fetus.

Many *et al.*and Shaarawy *et al.* noted high LPO and low antioxidant enzyme activities in pregnant women with preeclampsia [1, 17].

We found significantly decreased levels of some antioxidant enzymes (SOD and GSH) in patients with preeclampsia and eclampsia. GSH is a general scavenger whereas SOD specifically detoxifies superoxide anion radicals. The decrease in these two antioxidant markers suggests that there is an increased oxidant stress in patients with preeclampsia and eclampsia. The low SOD levels may therefore be the result of an intracellular attack. There is evidence to suggest that the increased activity of ROS may play a role in this process. However, other factors may work in combination with ROS to contribute to such changes [11].

On the other hand, erythrocyte catalase activities in the eclamptic and preeclamptic groups did not differ from those observed in the control group. Wang *et al.* found placenta catalase activities in pregnant women with preeclampsia to be high - different ext from our study. We could not find any study about erythrocyte catalase activities in pregnant women with preeclampsia and eclampsia.

In general, while authors were in agreement with the high LPO levels in pregnant women with preeclampsia and eclampsia [7, 16, 18, 19], they found different results in antioxidant enzyme activities [6, 8, 11, 16, 18, 25].

Uotila *et al.* reported a positive correlation between systolic-diastolic blood pressure, mean arterial blood pressure and MDA levels [4]. However, we did not find any correlation between the same parameters.

Lipid peroxides and blood oxidative imbalance could be part of the cytotoxic mechanisms leanding to endothelial cell injury [2]. The deficiency of antioxidant enzymes can cause accumulation of LPO products, which, in turn can induce vasoconstriction.

Obviously, further and more comprehensive studies are needed to clarify the mechanism of abnormal LPO production and decreased antioxidant enzyme activities in preeclamptic and eclamptic pregnancies and the present study should be regarded as preliminary.

References

[1] Many A., Hubel C. A., Roberts J. M.: "Hyperuricemia and xanthine oxidase in preeclampsia, revisited". *Am. J. Obstet. Gynecol.*, 1996, *174* (1 Pt 1), 288.

[2] Gratacos E., Casals E., Deulofeu R., Cararach V., Alonso P., Fortuny A.: "Lipid peroxide and vitamin E patterns in pregnant women with different types of hypertension in pregnancy". *Am. J. Obstet. Gynecol.*, 1998, *178* (5), 1072.

[3] Atasü T., Gezer A.: "Prevention and treatment of eclampsia". *Journal of Gynecol and Obstet*, 1997, *11*, 132.

[4] Uotila J. T., Tuimala R. J., Aarnio T. M.: "Findings on lipid peroxidations of pregnancy". *British J. Obstet. and Gynaecol.*, 1993, *100*, 270.

[5] Davidge S. T.: "Oxidative stress and altered endothelial cell function in preeclampsia". *Semin Reprod. Endocrinol.*, 1998, *16* (1), 65.

[6] Lefevre G., Berkane N., Uzan S., Etienne J.: "Preeclampsia and oxygenated free radicals". *Ann. Biol. Clin.*, 1997, *55* (5), 443.

[7] Davidge S. T., Hubel C. A., McLaughlin M. K.: "Lipid peroxidation increases arterial cyclooxygenase activity during pregnancy". *Am. J. Obstet. Gynecol.*, 1994, *170* (1 Pt 1), 215.

[8] Uotila J., Tuimala R., Pyko K., Ahotupa M.: "Pregnancy induced hypertension is associated with changes in maternal and umbilical blood antioxidants". *Gynecol. Obstet. Invest.*, 1993, *36*, 153.

[9] Zhang L. C., Liang G. D., Yang M. G., Zhang Y. H., Shi F. T.: "Significance of changes in serum superoxide dismutase level in hypertensive syndrome of pregnancy". *Chin. Med. J. (Eng.)*, 1991, *104*, 472.

[10] Tabacova S., Balabaeva L., Little R. E.: "Maternal exposure to exogenous nitrogen compounds and complications of pregnancy". *Archives of Environmental Health*, 1997, *52* (5), 341.

[11] Chen G., Wilson R., Cumming G., Walker J. J., Smith W. E., McKillop J. H.: "Prostacyclin, trombaxane and antioxidant levels in pregnancy-induced hypertension". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 1993, *50* (3), 243.

[12] Asakawa T., Matsushita S.: "Coloring conditions of thiobarbituric acid test for detecting lipid hydroperoxides". *Lipids*, 1980, *15*, 137.

[13] Beutler E., Duran O., Kelly B. M.: "Improved method for the determination of blood glutathione". *J. Lab. Clin. Med.*, 1963, *61* (5), 882.

[14] Winterbourn C. C., Hawkins R. E., Brian M., Earrell R. W.: "The estimation of red cell superoxide dismutase activity". *J. Lab. Clin. Med.*, 1975, *85* (2), 337.

[15] Aebi H.: "Catalase in virtro". *Methods in enzmology*, 1984, *105*, 21.

[16] Buhimschi I. A., Saade G. R., Chwalisz K., Garfield R. E.: "The nitric oxide pathway in preeclampsia: pathophysiological implications". *Hum. Reprod. Update*, 1998, *4* (1), 25.

[17] Shaarawy M., Aref A., Salem M. E., Sheiba M.: "Radical-scavenging antioxidants in preeclampsia and eclampsia". *Int. J. Gynecol. Obstet.*, 1998, *60* (2), 123.

[18] Walsh S. W., Wang Y.: "Deficient glutathione peroxidase activity in preeclampsia is associated with increased placental production of thromboxane and lipid peroxides". *Am. J. Obstet. Gynecol.*, 1993, *169* (6), 1456.

[19] Walsh S. W., Wang Y., Jesse R.: "Peroxide induces vasocontriction in the human placenta by stimulating thromboxane". *Am. J. Obstet. Gyencol.*, 1993, *169 (4)*, 1007.
[20] Ishihara M.: "Studies on lipoperoxide of normal pregnant women and of patients with toxemia of pregnancy". *Clin. Chim. Acta.*, 1978, *84*, 1.
[21] Walker J. J.: "Antioxidants and inflammatory cell response in preeclampsia". *Semin Reprod. Endocrinol.*, 1998, *16* (1), 47.
[22] Hubel C. A., Roberts J. M., Taylor R. N.: "Lipid peroxidation in pregnancy. New perspectives on preeclampsia". *Am. J. Obstet. Gynecol.*, 1989, *161*, 1025.
[23] Liang X, Lin Y, Cheng Y.: "Changes in plasma endothelin-1 and lipid peroxidate levels and amount of superoxide dismutase in red blood cell in patients with pregnancy-induced hypertension". *Chung-Hua-Fu-Chan Ko-Tsa-Chih*, 1996, *31* (4), 220.
[24] Jendryczko A., Tomala J.: "Decreased activity of oxideroreductases in erythrocytes and blood platelets from venous and umbilical blood of women with pregnancy-induced hypertension". *Ginekol-Pol*, 1995, *66* (12), 652.
[25] Wang Y., Walsh S. W.: "Antioxidant activities and mRNA expression of superoxide dismutase, catalase and glutathione peroxidase in normal and preeclamptic placentas". *J. Soc. Gynecol. Investig.*, 1996, *3* (4), 174.

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