

## ORIGINAL ARTICLE

# Antioxidant Activity and Lipid Peroxidation in Preeclampsia

Usha Adiga<sup>1\*</sup>, Vivian D'souza<sup>2</sup>, Asha Kamath<sup>3</sup>, Nandini Mangalore<sup>2</sup>

*Departments of <sup>1</sup>Biochemistry and <sup>3</sup>Community Medicine, Kasturba Medical College, Manipal, and*

*<sup>2</sup>Department of Biochemistry, Kasturba Medical College, Mangalore, India.*

**Background:** Epidemiologic studies demonstrate a relation between preeclampsia and an increased risk of future maternal coronary heart disease. The pathophysiology of the underlying mechanism is unknown. Disorders of lipoprotein metabolism may contribute to endothelial dysfunction. Oxidative stress and decreased antioxidant defense enhances free radical-mediated membrane lipid peroxidation and possibly vascular endothelial damage. The aim of this study was to elucidate the possible relation between lipidemic status, lipid peroxidation and albumin with total antioxidant activity (AOA) that may contribute to atherogenicity in preeclamptic women.

**Methods:** Twenty-five women with preeclampsia and 25 normal pregnant women who were matched for maternal and gestational age were selected for the study. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), atherogenic index (AI), malondialdehyde (MDA), a marker of lipid peroxidation, AOA and albumin levels were measured.

**Results:** MDA, TC and AI were significantly elevated ( $p < 0.001$ ), and HDL-C, AOA and albumin levels were significantly decreased ( $p < 0.001$ ) in preeclamptic patients compared to the control group.

**Conclusion:** We conclude that hypercholesterolemia leads to excessive lipid peroxidation. Coexistent diminution in antioxidant activity leads to an imbalance between prooxidants and antioxidants, resulting in oxidative stress. Oxidative stress and elevated AI may contribute to atherogenicity in preeclampsia. [*J Chin Med Assoc* 2007;70(10):435–438]

**Key Words:** antioxidant activity, atherogenic index, lipid peroxidation, preeclampsia

## Introduction

Preeclampsia is a multisystem disorder that is characterized by hypertension, edema and proteinuria, which is induced by pregnancy after the 20<sup>th</sup> week and is a leading cause of maternal morbidity and mortality. Endothelial dysfunction may play a pivotal role in the genesis of the multisystem disorder that develops in preeclampsia. The mechanisms involved in the induction of endothelial cell dysfunction are poorly understood. Multiple circulating factors may provoke the spectrum of endothelial changes, including altered lipoproteins. Various studies have reported elevated lipid levels in preeclamptics.<sup>1,2</sup> The changes in the lipid profile are physiologic and are mostly due to the hormonal variations during pregnancy.<sup>3</sup> Normally, the lipid levels come down to those of the prepregnant state in 6–8 weeks in the postpartum period.<sup>1,3</sup> Though the

cholesterol levels normalize, the reduction in the risk of coronary heart disease (CHD) is only 50%.<sup>4</sup> Thus, the risk of CHD in preeclampsia is much higher and of concern.<sup>5</sup>

Elevated levels of oxidative lipid derivatives and reduced antioxidants in the circulation of preeclamptics may contribute to endothelial damage. There is good evidence for significant increase in the levels of plasma and erythrocytic malondialdehyde (MDA), a marker of lipid peroxidation in normotensive pregnant women, which were further increased in women with pregnancy-induced hypertension.<sup>6</sup> Evidence of increased oxidative lipid derivatives in the decidual placental tissues was observed in women with established preeclampsia.<sup>7</sup> Elevated levels of oxidative lipid derivatives, conjugated dienes and reduced antioxidative capacity in the maternal circulation have also been reported.<sup>8</sup>

\*Correspondence to: Dr Usha Adiga, Department of Biochemistry, Kasturba Medical College, Manipal, Karnataka, India.

E-mail: [ushachidu@yahoo.com](mailto:ushachidu@yahoo.com) • Received: December 8, 2006 • Accepted: August 17, 2007

The aim of this study was to elucidate the possible relation of lipidemic status, lipid peroxidation and albumin with total antioxidant activity (AOA) in preeclampsia.

## Methods

### Study design

The study was conducted in the city of Mangalore. Twenty-five preeclamptic patients and 25 normotensive pregnant women, within the age range of 18–40 years, admitted to Lady Goshen Hospital and Chirashree Hospital, in their third trimester, were selected for the study. Subjects with blood pressure > 140/90 mmHg, proteinuria and edema were included and eclamptic patients were excluded from the study. Normal pregnant women, diagnosed on clinical and ultrasonography findings were taken as controls. Patients and controls were matched for gestational age, maternal age and parity. Prepregnancy body mass index was in the range of 19.8–24.4 kg/m<sup>2</sup>. Elderly primigravid subjects, gestational diabetics, chronic hypertensives, multiple gestations and those with a family history of preeclampsia were excluded from the study. Subjects also had to be nonsmokers, nonalcoholics, and not suffering from any acute infections or chronic illnesses. The demographic profile of the subjects is shown in Table 1.

All subjects were informed about the objectives of the study and informed consent was obtained. The study was approved by the institutional ethics committee.

### Sample collection

Five mL of venous blood was collected in EDTA bottles using disposable syringes, after an overnight fast of 12 hours. Plasma was separated and analyzed for MDA, AOA, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and albumin. Atherogenic index (AI) was also calculated.

The oxidant status was studied by estimating MDA levels.<sup>9,10</sup> MDA, a product of lipid peroxidation, reacts

with thiobarbituric acid (TBA) in acidic medium to give a pink pigment at 97°C and pH 2–3. The pink-colored complex was extracted with butanol and absorbance read at 535 nm.

The total AOA was determined by Koracevic et al's method.<sup>11</sup> The assay measures the capacity of the serum to inhibit the production of TBA reactive substances (TBARS) from sodium benzoate, under the influence of the oxygen free radicals derived from Fenton's reaction. The reaction was measured spectrophotometrically at 532 nm. Antioxidants from the added sample cause suppression of the production of TBARS, and the inhibition of color development is defined as AOA.

TC, HDL-C and albumin were measured by enzymatic and colorimetric methods.

### Statistical analysis

Statistical analysis was performed using the Mann-Whitney *U* test. Correlations between the variables were estimated by Spearman's rank correlation coefficients.

## Results

Blood pressure, a diagnostic criterion for preeclampsia, was analyzed in both groups. A significant increase in blood pressure was observed in preeclamptics compared to controls (Table 2).

A significant elevation in TC, MDA and AI, and a marked diminution in HDL-C, AOA and albumin levels were observed in preeclamptics (Table 3).

**Table 1.** Demographic profile of subjects\*

	Control subjects (n = 25)	Preeclampsia subjects (n = 25)
Age (yr)	25.5 ± 3.3	27.5 ± 5.3
Gestational age at sampling (wk)	34.3 ± 0.8	33.3 ± 3.6
Parity	2 ± 1	2 ± 1
BMI at sampling (kg/m <sup>2</sup> )	20.3 ± 1.9	24.8 ± 2.6

\*Data are presented as mean ± standard deviation. BMI = body mass index.

**Table 2.** Blood pressure (BP) of subjects\*

	Control subjects (n = 25)	Preeclampsia subjects (n = 25)
Systolic BP (mmHg)	120.32 ± 10.04	181.42 ± 12.94 <sup>†</sup>
Diastolic BP (mmHg)	71.04 ± 6.54	105.2 ± 8.72 <sup>†</sup>

\*Data are presented as mean ± standard deviation; <sup>†</sup>p < 0.001.

**Table 3.** Oxidant status and lipid profile\*

	Control subjects (n = 25)	Preeclampsia subjects (n = 25)
MDA (μmol/L)	6.56 ± 0.73	7.64 ± 0.58 <sup>†</sup>
AOA (mmol/L)	1.40 ± 0.25	1.08 ± 0.15 <sup>†</sup>
TC (mg/dL)	203.0 ± 20.37	246.43 ± 23.87 <sup>†</sup>
HDL-C (mg/dL)	66.33 ± 4.02	48.02 ± 7.40 <sup>†</sup>
Atherogenic index	3.07 ± 0.36	5.25 ± 0.95 <sup>†</sup>
Albumin (g/dL)	3.32 ± 0.35	2.80 ± 0.43 <sup>†</sup>

\*Data are presented as mean ± standard deviation; <sup>†</sup>p < 0.001. MDA = malondialdehyde; AOA = antioxidant activity; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol.

A significant positive association of MDA with both systolic and diastolic blood pressures was found in preeclamptics ( $r=0.657$  for systolic and  $r=0.583$  for diastolic blood pressure). MDA also bore a significant positive correlation with TC in controls ( $r=0.432$ ).

A strong and statistically significant negative correlation was found between AOA and albumin only in controls ( $r=-0.502$ ).

Positive correlation between AOA and TC was observed in both groups ( $r=0.114$  for controls;  $r=0.158$  for preeclamptic patients). Similarly, HDL-C also showed a weak positive correlation with AOA ( $r=0.092$  for controls;  $r=0.040$  for patients). However, these correlations were statistically insignificant.

## Discussion

The present study revealed a 20% increase in TC and 27% decrease in HDL-C levels in preeclamptics. The rise in AI was 70%. Our results are in accordance with various studies, such as those of Cong et al<sup>12</sup> and Kokia et al.<sup>13</sup> Kinnunen et al<sup>14</sup> and Alvarez et al<sup>15</sup> have reported decreased lipoprotein lipase and hepatic lipase activities as possible causes for the lipid changes during gestation, which in turn could be attributed to the heightened insulin resistance and raised estrogen levels, respectively. Ramsay et al reported that an exaggerated insulin resistance observed in preeclampsia caused further suppression of lipoprotein lipase.<sup>16</sup> The raised estrogen levels coupled with suppression of lipoprotein lipase may be responsible for the dyslipidemia observed in our study. Genetic factors (apoE polymorphism) have also been found to be related to lipoprotein lipase activity.<sup>17</sup>

MDA is significantly elevated in preeclamptics compared to healthy pregnant women ( $p<0.001$ ). The values obtained are in close agreement with those reported by Aydin et al.<sup>18</sup> Excessive lipid peroxidation occurring in preeclampsia can be attributed to hypercholesterolemia. Hypercholesterolemia promotes the formation of free radicals. Increased oxygen demand to meet the bodily functions in pregnancy is also a contributory factor for the oxidative stress that results in the formation of free radicals. Thus, lipid alterations observed may promote oxidative stress, leading to endothelial dysfunction in preeclampsia. Highly significant reduction in AOA was observed in the preeclamptic patients in this study. We have not encountered any study concerning AOA and preeclampsia. The total antioxidative serum capacity is not a simple sum of the activities of the various antioxidative substances but the

cooperation of the antioxidants in human serum that provides greater protection against attacks by free radicals. Decreased AOA is indicative of a disturbance in the antioxidant system which could be due to diminished individual antioxidants. A decrease in essential antioxidants, vitamins A and E and carotene, have been reported in preeclampsia.<sup>8,19</sup> The concentration of albumin, one of the major plasma proteins that contributes significantly to AOA, was decreased significantly in preeclamptic subjects.

Wayner et al showed that the free radical trapping capacity of albumin in plasma is 10–50%.<sup>20</sup> The observed decrease in plasma albumin can be attributed to the proteinuria that occurs in preeclampsia. The negative correlation between albumin and AOA in controls indicates that whenever AOA decreases, albumin increases as a compensatory mechanism. But in preeclamptics, due to proteinuria, albumin level decreases and cannot compensate for the diminished AOA. These factors associated with the reduced intake of antioxidants could be the possible reason for reduced AOA in preeclampsia. Coexistent diminution of AOA along with hyperlipidemia and excessive lipid peroxidation contributes to the imbalance between prooxidants and antioxidants, leading to oxidative stress and probably permanent endothelial dysfunction in preeclampsia. Impairment of endothelial function observed even after 3 years in previously preeclamptic women was shown to be reversed by ascorbic acid supplementation.<sup>21</sup> Balanced amounts of essential and endogenous antioxidants may reduce the severity of preeclampsia and decrease the risk of future CHD.

Thus, we conclude that the hypercholesterolemia observed in our study resulted in excessive lipid peroxidation and generation of free radicals. Diminution in AOA adds to the imbalance between prooxidants and antioxidants, resulting in oxidative stress, which in turn may cause endothelial damage. Raised AI suggests increased susceptibility to atherogenicity in preeclamptics. Dyslipidemia appears to be the starting point of this chain of events. Further studies on the role of genetic factors (apoE polymorphism) in causing dyslipidemia may contribute to the understanding of the mechanism underlying endothelial dysfunction in preeclampsia.

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## References

- Potter JM, Nestel PJ. The hyperlipidemia of pregnancy in normal and complicated pregnancies. *Am J Obstet Gynecol* 1979; 133:165-70.
- Franz H, Wendler D. A controlled study of maternal serum concentrations of lipoproteins in pregnancy-induced hypertension. *Arch Gynecol Obstet* 1992;252:81-6.
- Desoye G, Schweditsch MO, Pfeiffer KP, Zechner R, Kostner GM. Correlations of hormones with lipid and lipoprotein levels during normal pregnancy and post partum. *J Clin Endocrinol Metab* 1987;64:704-12.
- Law MR, Wald NJ, Thompson SG. By how much and how quickly does reduction in serum cholesterol concentration lower risk of ischemic heart disease? *BMJ* 1994;308:367-72.
- Wolf M, Hubel CA, Lam C, Sampson M, Ecker JL, Ness RB, Rajakumar A, et al. Preeclampsia and future cardiovascular disease: potential role of altered angiogenesis and insulin resistance. *J Clin Endocrinol Metab* 2004;89:6239-43.
- Kabi BC, Goel N, Rao YN, Tripathy R, Tempe A, Thakur AS. Levels of erythrocytic malondialdehyde, vitamin E, reduced glutathione, G6PD activity and plasma urate in patient of pregnancy induced hypertension. *Indian J Med Res* 1994;100:23-5.
- Henriksen T. The role of lipid oxidation and oxidative lipid derivatives in the development of preeclampsia. *Semin Perinatol* 2000;24:29-32.
- Uotila J, Tuimala R, Pyykko K, Ahotupa M. Pregnancy induced hypertension associated with changes in maternal and umbilical blood antioxidants. *Gynecol Obstet Invest* 1993;36: 153-7.
- Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malondialdehyde and 4-hydroxynonenal. *Methods Enzymol* 1990;186:407-21.
- Wasowicz W, Neve J, Peretz A. Optimized steps in fluorometric determination of thiobarbituric acid reactive substances in serum: importance of extraction pH and influence of sample preservation and storage. *Clin Chem* 1993;39:2522-6.
- Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. *J Clin Pathol* 2001;54:356-61.
- Cong KJ, Wang TT, Liu GR. Lipid metabolism and pregnancy induced hypertension. *Zonghua Fu Chan ke Za Zhi* 1994;29: 651-8.
- Kokia E, Barkai G, Reichman B, Segal P, Goldman B, Mashiach S. Maternal serum lipid profile in pregnancies complicated by hypertensive disorders. *J Perinat Med* 1990;18: 473-8.
- Kinnunen PK, Unnerus HA, Ranta T, Ehnholm C, Nikkila E, Seppala M. Activities of post-heparin plasma lipoprotein lipase and hepatic lipase during pregnancy and lactation. *Eur J Clin Invest* 1980;10:469-74.
- Alvarez JJ, Montelongo A, Iglesias A, Lasuncion MA, Herrera E. Longitudinal study on lipoprotein profile, high-density lipoprotein subclass, and postheparin lipases during gestation in women. *J Lipid Res* 1996;37:299-308.
- Ramsay JE, Jamieson N, Greer IA, Sattar N. Paradoxical elevation in adiponectin concentrations in women with preeclampsia. *Hypertension* 2003;42:891-4.
- McGladdery SH, Frohlich JJ. Lipoprotein lipase and apoE polymorphism: relationship to hypertriglyceridemia during pregnancy. *J Lipid Res* 2001;42:1905-12.
- Aydin S, Benian A, Madazli R, Uludag S, Uzun H, Kaya S. Plasma malondialdehyde, superoxide dismutase, sE-selectin, fibronectin, endothelin-1 and nitric oxide levels in women with preeclampsia. *Eur J Obstet Gynecol Reprod Biol* 2004;113:21-5.
- Mohindra A, Kabi BC, Kaul N, Trivedi SS. Vitamin E and carotene status in preeclamptic pregnant women from India. *Panminerva Med* 2002;44:261-4.
- Wayner DD, Burton GW, Ingold KU, Barclay LR, Locke SJ. The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxy radical trapping antioxidant in human plasma. *Biochim Biophys Acta* 1987;924:408-19.
- Chambers JC, Fusi L, Malik IS, Haskard DO, De SM, Kooner JS. Association of maternal endothelial dysfunction with preeclampsia. *JAMA* 2001;285:1607-12.