

## **Studies on Oxidative stress in Preeclampsia**

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### **Abstract**

**The aetio-pathogenesis of preeclampsia, a complication of pregnancy, is poorly understood. Oxidative stress has been shown to be the causative factor for hypertension as well as for target tissue damage in hypertension. Studies that explain the role of oxidative stress in preeclampsia have been few in number. The present study was aimed at (1) determining the extent of oxidative stress in preeclampsia and measurement of lipid peroxidation as a marker of oxidative stress, (2) correlation of lipid peroxidation to ceruloplasmin levels and (3) correlation of lipid peroxidation to blood pressure changes and urinary protein excretion during the antenatal period as well as the post-partum period.**

**Thirty women with preeclampsia, thirty normotensive pregnant women (who were matched for maternal and gestational age) and thirty normotensive, non-pregnant women were selected for the study. Lipid peroxidation was measured as malonaldehyde (MDA) levels, ceruloplasmin was quantitated by its oxidase activity and urinary protein excretion was determined by turbidometry.**

**Lipid peroxidation was significantly increased in preeclampsia when compared to normotensive pregnant women. Malonaldehyde levels were moderately increased in normotensive pregnant controls ( $1.3\text{nM/mL} \pm 0.15$ ) and markedly increased in preeclamptic pregnant women ( $2.52\text{nM/mL} \pm 0.22$ ) when compared to non pregnant normotensive women ( $0.91\text{nM/mL} \pm 0.17$ ). Oxidase activity of ceruloplasmin was significantly increased in preeclamptic women compared to normal pregnant women. A significant correlation was found between the increase in uric acid levels and ceruloplasmin in preeclamptic women. The elevated blood pressure returned to normal levels after delivery, but lipid peroxidation and urine protein excretion remained higher than normal, suggesting that factors other than oxidative stress may contribute to the development of hypertension in preeclamptic women. Oxidative stress, however, plays a very important role in the causation of target tissue damage such as renal injury.**

**Key Words:** Preeclampsia, oxidative stress, ceruloplasmin, malonaldehyde

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### **Introduction**

Preeclampsia is a progressive, unpredictable and potentially dangerous human pregnancy specific disorder characterised by the onset of hypertension ( $>140/90$  mmHg) and proteinuria after 20 weeks of gestation in previously normotensive, non-proteinuric women. It is one of the

most commonly occurring complications of pregnancy, leading to fetal growth retardation, pre-term delivery and maternal and fetal morbidity and mortality [1,2]. The aetiology and pathogenesis of preeclampsia remain poorly understood. Increasing evidence indicates that dysfunction of the vascular endothelium accounts for the altered vascular reactivity, activation of the coagulation cascade

and loss of vascular integrity that accompanies preeclampsia [3,4,5].

Recent reports suggest that free radical induced endothelial cell injury might be an etiologic factor [6]. An imbalance of increased oxidant status and decreased antioxidant status in women with preeclampsia has also been noted. Low levels of antioxidants such as the catalase, vitamins A, and E, and glutathione are seen in women with preeclampsia [7]. Lipid peroxidation seems to play a role in the pathogenesis of preeclampsia, but, the results are conflicting. Few studies do not support the role of oxidative stress in the genesis of preeclampsia [8]. In addition, the role of oxidative stress in the progression of preeclampsia and its associated complications has not been well addressed. Ceruloplasmin is a free radical scavenger as well as a late acute phase reactant protein. It has been shown to be an important antioxidant implicated in the modulation of oxidative stress responses in patho-physiological states such as diabetes mellitus [9]. However, its role in preeclampsia has not been explained.

The present study was aimed at (1) determining the extent of oxidative stress in preeclampsia and measurement of lipid peroxidation as a marker of oxidative stress (2) correlation of lipid peroxidation to ceruloplasmin levels and (3) correlation of lipid peroxidation to blood pressure changes and urinary protein excretion during the antenatal period as well as post partum period

## Methods

### Study Design

A Cross Sectional Study was carried out on 30 normotensive non-pregnant women and 30 normotensive pregnant women as controls and 30 patients of preeclampsia as cases, attending the outpatient department and wards of Obstetrics and Gynaecology, Kempegowda Institute of Medical Sciences, Bangalore during the year 2003-2004. The diagnosis of preeclampsia was established (blood pressure > 140/90mmHg, proteinuria and edema) in accordance with the definitions of the report of the Working group members of National High Blood Pressure Education program (NHBPEP) [10]. The inclusion criteria for the subjects were age between 18 to 35 years and gestational age between 28 to 40 weeks, singleton pregnancy and absence of any other medical complication concurrent with preeclampsia for the pregnant women. Patients and controls were matched for gestational age, maternal age and parity. Elderly primigravid subjects, gestational diabetics, chronic hypertensives, multiple gestations and those with a family history of preeclampsia were excluded from the study. Subjects included were also non-smokers, non-alcoholics, and not suffering from any acute infections or chronic illnesses. A history of cardiovascular

disease, renal disease or any underlying medical disorders (determined by a comprehensive physical examination and routine laboratory tests) or concurrent intake of any drug were the exclusion criteria in the study. The Institutional Ethical Committee approved the study protocol.

History and personal physical data was obtained from both cases and controls. After informed written consent, 5ml of venous blood was collected from the subjects. The serum was separated and analysed for malonaldehyde (MDA), ceruloplasmin, glucose, urea, creatinine and uric acid. A random urine sample was collected for urine protein (expressed as protein to creatinine ratio), urine glucose detection and urine creatinine tests. Fifteen women were randomly chosen from among the preeclamptic group. Post partum blood and urine samples were collected from these patients within 5days after delivery; these samples were analyzed for the same parameters as the antenatal samples.

Lipid peroxidation was measured by estimating malonaldehyde (MDA). MDA, a product of lipid peroxidation, reacts with thiobarbituric acid (TBA) to form a pink colored TBA-MDA adduct. This color is measured at 532nm [11]. Ceruloplasmin was quantitated by its oxidase activity. It was measured by its property to catalyze the oxidation of colorless para-phenylene-di-amine to blue or violet colored compounds [12]. Urinary protein excretion was determined by turbidimetric method using sulphosalicylic acid to precipitate the protein [13]. The estimation of serum glucose, serum creatinine, urine creatinine, serum urea and serum uric acid were carried out on Technicon RA-XT autoanalyser using diagnostic reagent kits obtained from Bayer Diagnostics India Ltd.

### Statistical analysis

The analysis of variance (ANOVA) was carried out to find the significance of mean values between the three groups. Once significance was noticed, the post hoc test of Tukey and the Chi-square test [14] were used.

## Results

The systolic blood pressure values, diastolic blood pressure values, mean values of urine protein to creatinine (PC) ratio, random serum glucose, serum urea and serum creatinine of the study subjects are presented in Table 1. A significant increase in blood pressure was observed in preeclamptic patients compared to pregnant and non-pregnant controls. The urine PC ratio was elevated significantly among the preeclamptics ( $p < 0.001$ ) when compared with both non-pregnant controls and pregnant controls. There were no significant differences in the values of the urine PC ratio among the two control groups.

**Biomarkers of oxidative stress**

Serum MDA (malonaldehyde), serum ceruloplasmin and serum uric acid levels expressed as mean  $\pm$  S.D are presented in Table 2. Serum MDA levels were moderately increased in pregnant controls (1.3nM/mL  $\pm$  0.15  $p$  < 0.001 for non-pregnant controls vs pregnant controls). In preeclamptic women (2.52 nM/mL  $\pm$  0.22), the rise in MDA levels was more marked. In this group, there was

no significant correlation between serum MDA and serum ceruloplasmin levels. However, a highly significant correlation was seen to exist on comparing serum MDA levels to the systolic blood pressure values ( $p$  < 0.05) (Fig 1) and serum MDA levels to urine protein to creatinine ratios ( $p$  < 0.05) (Fig 2) in preeclamptic women. No significant correlation was noted between serum MDA and other parameters in control groups.

**Table 1: Patient characteristics and basic biochemical data**

Parameters	Non-pregnant controls (n=30)	Pregnant controls (n=30)	Cases (n=30)
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Age (years)	21.17 $\pm$ 2.45	22.80 $\pm$ 2.66	23.70 $\pm$ 4.21
Primigravida	-	16	18
Multigravida	-	14	12
Gestational age (weeks)	-	35.40 $\pm$ 3.92	35.33 $\pm$ 3.73
Systolic blood pressure (mm Hg)	115.37 $\pm$ 4.94	115.87 $\pm$ 6.19	147.40 $\pm$ 7.54 <sup>b, c</sup>
Diastolic blood pressure (mm Hg)	76.47 $\pm$ 4.60	77.47 $\pm$ 5.53	96.53 $\pm$ 6.54 <sup>b, c</sup>
Random serum glucose (mg/dL)	73.70 $\pm$ 9.71	82.40 $\pm$ 17.05	87.33 $\pm$ 18.54 <sup>d</sup>
Serum urea (mg/dL)	23.40 $\pm$ 6.64	17.97 $\pm$ 4.23 <sup>a</sup>	19.60 $\pm$ 5.54
Serum creatinine (mg/dL)	0.84 $\pm$ 0.14	0.85 $\pm$ 0.18	0.88 $\pm$ 0.19
Urine protein to creatinine ratio	0.117 $\pm$ 0.013	0.17 $\pm$ 0.02	1.21 $\pm$ 0.42 <sup>b, c</sup>

a: non-pregnant controls vs pregnant controls  $p$  < 0.001

b: non-pregnant controls vs cases  $p$  < 0.001

c: pregnant controls vs cases  $p$  < 0.001

d: non-pregnant controls vs cases  $p$  < 0.003

**Table 2: Biomarkers of oxidative stress**

Parameters	Non-pregnant controls (n=30)	Pregnant controls (n=30)	Cases (n=30)
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Serum malonaldehyde (nM/mL)	0.91 $\pm$ 0.17	1.35 $\pm$ 0.15 <sup>a</sup>	2.52 $\pm$ 0.22 <sup>b, c</sup>
Serum ceruloplasmin (mg/dL)	20.27 $\pm$ 2.39	53.94 $\pm$ 2.26 <sup>a</sup>	66.72 $\pm$ 2.21 <sup>b, c</sup>
Serum uric acid (mg/dL)	3.03 $\pm$ 0.53	2.97 $\pm$ 0.55	4.68 $\pm$ 0.96 <sup>b, c</sup>

a: non-pregnant controls vs pregnant controls  $p$  < 0.001,

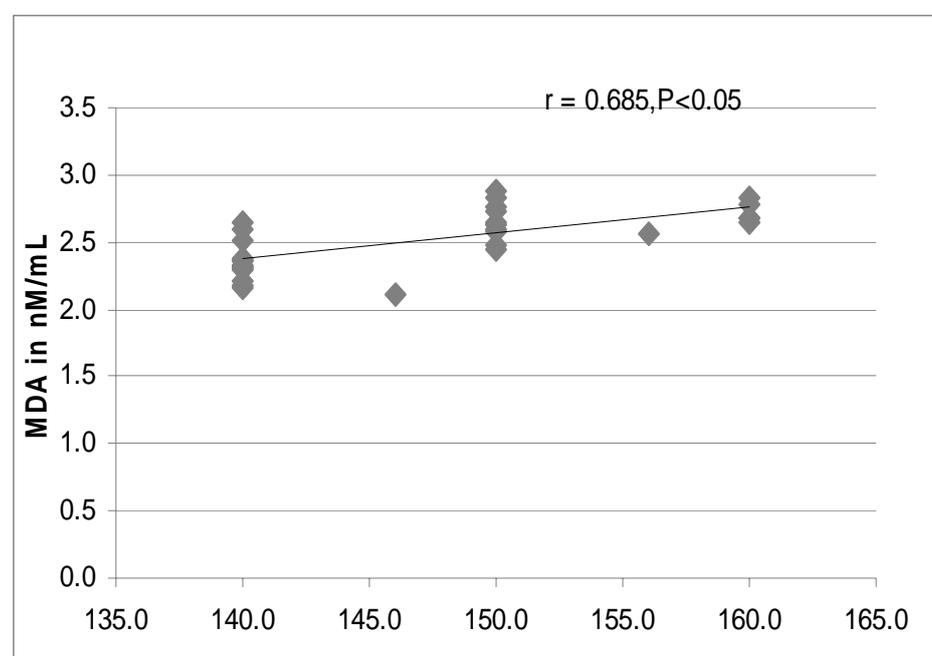
b: non-pregnant controls vs cases  $p$  < 0.001

c: pregnant controls vs cases  $p$  < 0.001

**Table 3: Comparison of biochemical data of antepartum and postpartum samples of preeclamptic cases**

Parameters	Antepartum sample (n=15)	Postpartum sample (n=15)
	Mean $\pm$ SD	Mean $\pm$ SD
Systolic blood pressure (mm Hg)	145.07 $\pm$ 6.27	118.0 $\pm$ 6.76 <sup>a</sup>
Diastolic blood pressure (mm Hg)	94.93 $\pm$ 4.89	76.67 $\pm$ 4.88 <sup>a</sup>
Random serum glucose (mg/dL)	86.53 $\pm$ 15.99	79.73 $\pm$ 10.89
Serum urea (mg/dL)	20.87 $\pm$ 3.64	17.27 $\pm$ 3.84 <sup>b</sup>
Serum creatinine (mg/dL)	0.89 $\pm$ 0.19	0.79 $\pm$ 0.21
Serum malonaldehyde (nM/mL)	2.44 $\pm$ 0.23	1.14 $\pm$ 0.15 <sup>a</sup>
Serum ceruloplasmin (mg/dL)	67.15 $\pm$ 2.64	41.71 $\pm$ 2.16 <sup>a</sup>
Serum uric acid (mg/dL)	4.71 $\pm$ 1.07	2.75 $\pm$ 0.48 <sup>a</sup>
Urine protein to creatinine ratio	1.05 $\pm$ 0.33	0.33 $\pm$ 0.39 <sup>a</sup>

a:  $p < 0.001$   
b:  $p < 0.014$

**Figure 1: Correlation of serum malonaldehyde (MDA) to systolic blood pressure in Preeclamptic cases**

Serum ceruloplasmin concentrations of  $20.27 \text{ mg/dL} \pm 2.39$  were found in the non-pregnant control group. In the pregnant controls, there was a 2.6 fold increase in serum ceruloplasmin values over those observed in non-pregnant controls. In preeclamptic women a 3.2 fold increase in ceruloplasmin concentration was observed in comparison to the non-pregnant group ( $p=0.001$ ). The rise in ceruloplasmin levels was significant in the preeclamptic cases when compared to pregnant controls ( $p<0.001$ ). Serum ceruloplasmin levels also correlated significantly with serum uric acid levels in the preeclamptic women ( $p<0.05$ ). No significant correlation was noted between serum ceruloplasmin and other parameters in other study groups. The preeclamptic group showed a marked in-

crease (1.5 fold) in serum uric acid levels when compared with both non-pregnant controls ( $p < 0.001$ ) and pregnant controls ( $p < 0.001$ ). The serum uric acid concentrations in both the control groups were not different from each other.

The data showing the differences in the parameters of preeclamptic antenatal and postpartum samples of 15 women are presented in Table 3. Systolic and diastolic blood pressure readings showed a significant reduction in postpartum period compared to antenatal states ( $p < 0.001$  for both systolic and diastolic blood pressure). The systolic and diastolic blood pressure levels reached values that are observed in normal pregnant women. The

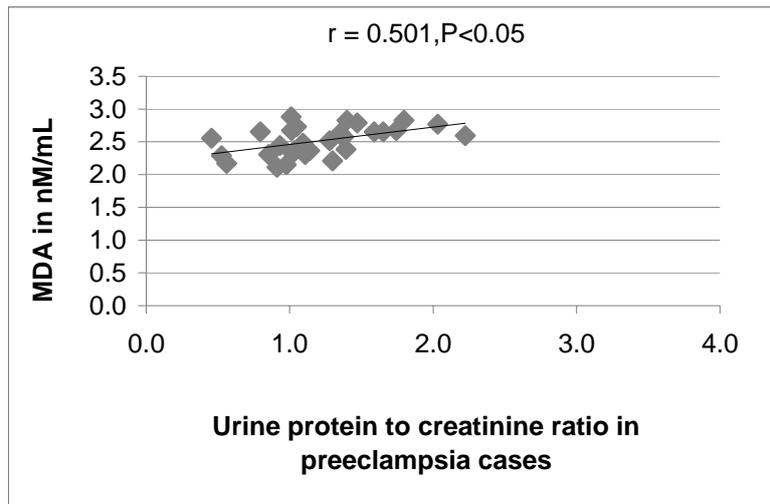


Figure 2: Correlation of serum malonaldehyde (MDA) to urine protein to creatinine ratio in preeclamptic cases

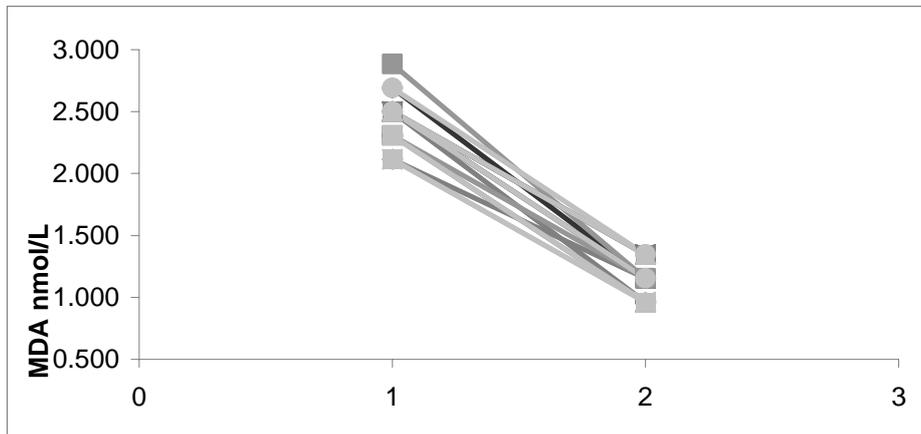


Figure 3: Individual comparison of serum malonaldehyde of preeclamptic antenatal sample and postpartum samples

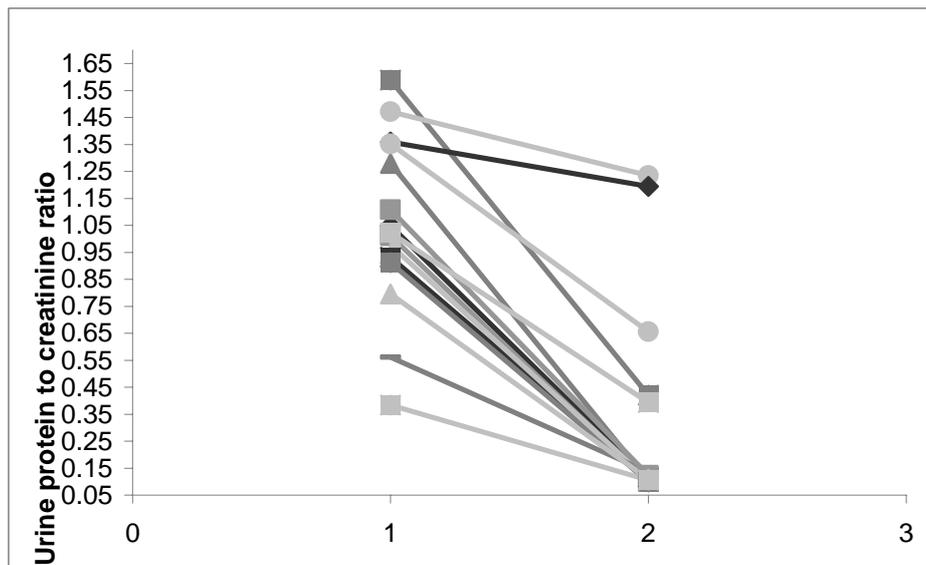


Figure 4: Individual comparison of urine protein to creatinine ratio of preeclamptic antenatal sample and postpartum samples

random serum glucose, serum urea and serum creatinine concentrations did not differ significantly between the two groups of samples.

#### *Markers of oxidative stress during the postpartum period*

The serum malonaldehyde, (Fig.3) showed a 2.1 fold decrease in the postpartum samples when compared to the antenatal samples ( $p=0.001$ ). However, the serum MDA did not decrease to the levels seen in non-pregnant women. A 1.6 fold decrease in serum ceruloplasmin levels was observed in the postpartum samples compared to the antenatal samples ( $p < 0.001$ ). Serum uric acid levels in the postpartum samples also fell significantly ( $p < 0.001$ ), reaching values similar to those in the antenatal pregnant controls and non-pregnant control groups. The fall in the urine protein to creatinine ratios in the postpartum samples are depicted in Fig.4. The decrease in urine protein to creatinine ratios was significant compared to the antenatal values ( $p < 0.001$ ), though not reaching the values observed in the pregnant controls.

## **Discussion**

Oxidative stress has been proposed as one of the underlying mechanisms contributing to the pathophysiology of preeclampsia. Most studies on oxidative stress in preeclampsia have reported increased levels of MDA (malonaldehyde) in serum from preeclamptic women compared to pregnant women [15, 16]. In the present study, lipid peroxidation is significantly increased in preeclampsia when compared to pregnant controls. This is similar to the results of previous studies [15,16].

*Oxidative stress as a cause of hypertension:* Hypertension is associated with an elevation of reactive oxygen species (ROS) and frequently also with an impairment of endogenous antioxidant mechanisms. Experimental manipulation of the redox state in vivo shows that reactive oxygen species (ROS) can be a cause of hypertension [17]. Exposure of cells or tissues to exogenous oxidants mimics events that contribute to the development of hypertension. Generators of superoxide abolish endothelium-dependent relaxation of aortic rings from spontaneously hypertensive rats (SHR), induce contraction of mesenteric beds, and increase chloride reabsorption by isolated thick ascending limbs of the loop of Henle [18, 19]. In whole rats, infusion of  $H_2O_2$  in the renal medulla increases blood pressure (BP) and decreases blood and urine flows, as well as sodium excretion [20]. In both animal models and humans, increased blood pressure has been associated with oxidative stress in the vasculature. This excessive endothelial production of reactive oxygen species (ROS) may be both a cause and an effect of hypertension. Apart from NADPH oxidase, nitric oxide synthase, lipoxigenases, cyclo-oxygenases, xanthine oxidase

and cytochrome P450 enzymes, mitochondria also could be considered a major source of reactive oxygen species (ROS): in situations of metabolic perturbation, increased mitochondrial ROS generation might trigger endothelial dysfunction, possibly contributing to the development of hypertension. However, the use of antioxidants in the clinical setting induced only limited effects on human hypertension or cardiovascular endpoints [21]. Antihypertensive treatment aims to reduce not only blood pressure values but also hypertension-induced organ damage and as a consequence morbidity and mortality. Reports have demonstrated that lowering blood pressure leads to reduction of oxidative stress [22, 23]. In a study conducted by Saez et al. [24], antihypertensive treatment for three months significantly reduced oxidized/reduced glutathione ratio and malonaldehyde, significantly increased the activity of superoxide dismutase and catalase. The changes were independent of the kind of antihypertensive treatment (non-pharmacologic,  $\beta$  blockers or angiotensin receptor blockers) and the beneficial effect of treatment increased over time [24]. However, in a study conducted recently, antioxidant supplementation (vitamin C and vitamin E) in a group of preeclamptic women did not result in a reduction in blood pressure [25]. In the present study, the elevated blood pressure returned to normal levels after delivery, but lipid peroxidation was still higher than normal levels. This suggests that factors other than oxidative stress may contribute to the development of hypertension in preeclampsia.

*Oxidative stress and progression of renal injury:* Preeclampsia and eclampsia are associated with impaired renal function, reduced renal perfusion and glomerular filtration [26]. This may be attributed to intrinsic renal changes caused by severe vasospasm. There is considerable evidence suggesting that reactive oxygen species (ROS) are implicated in the pathogenesis of ischemic, toxic and immunologically mediated renal injury [26]. In experimental immune glomerulonephritis, reactive oxygen species (ROS) are generated by polymorphonuclear leucocytes, monocytes and glomerular mesangial cells. Their formation results in morphologic lesions and in modification of glomerular permeability to proteins through activation of proteases and reduction of proteoglycan synthesis [27]. In the present study, both lipid peroxidation and protein excretion remained elevated after delivery in preeclamptic women compared to pregnant antenatal cases. Recently, administration of antioxidants to preeclamptic women resulted in the decrease in magnitude of proteinuria [28]. These findings favor the role for oxidative stress in the causation of target tissue damage such as renal injury.

*Ceruloplasmin and oxidative stress and inflammatory response:* Ceruloplasmin is a major contributor to the antioxidant defense system of human serum. Ceruloplasmin acts as an antioxidant by several mechanisms like

inhibiting iron-dependent lipid peroxidation and hydroxyl radical (HO•) formation from hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) via its ferroxidase activity, reacting with and scavenging H<sub>2</sub>O<sub>2</sub> and superoxide anion and by inhibiting copper-induced lipid peroxidation by binding copper ions [29]. Ceruloplasmin can also function as a pro-oxidant and can oxidatively modify low density lipoproteins (LDL) in vitro [30].

There are conflicting results for ceruloplasmin levels and its ferroxidase activity in preeclampsia. Recently, elevated ceruloplasmin levels were found in normotensive pregnant women as well as preeclamptic women [31]. Women with preeclampsia showed significantly higher levels of serum ceruloplasmin. Nevertheless, the ferroxidase activity is not significantly higher when compared to that of women with normal pregnancy. This suggests that the ferroxidase activity of ceruloplasmin may be dysfunctional in preeclampsia [32]. In another study, the ferroxidase activity of ceruloplasmin was significantly lower in women with normal pregnancy compared to non-pregnant women [33]. Both these studies have limited number of cases compared to the present study. In our study, oxidase activity is significantly increased in preeclamptic women compared to pregnant women. The oxidase activity is moderately increased in pregnant women with concomitant moderate increase in lipid peroxidation in pregnant women in the present study. Thus, dysfunction of antioxidant role of ceruloplasmin may not be the underlying mechanisms for enhanced oxidative stress in preeclampsia.

Normal pregnancy is characterized by a mild systemic inflammatory response, especially in the second half of pregnancy. Redman et al. [34], propose that preeclampsia develops when the systemic inflammatory process, common to all women in the second half of their pregnancies, causes one or other maternal system to decompensate [34]. Preeclampsia may not be a separate condition but simply the extreme end of a range of maternal systemic inflammatory responses engendered by pregnancy itself [34]. As ceruloplasmin is an acute-phase protein, a high serum ceruloplasmin level can be a reaction to systemic inflammation associated with preeclampsia. In the present study, significant correlation was found between the increase in uric acid levels and ceruloplasmin in preeclamptic women. Since uric acid has been shown to induce inflammatory response [35] and ceruloplasmin is also considered as acute phase inflammatory response marker [36], increased ceruloplasmin levels are suggestive of exaggerated inflammatory response in preeclampsia.

To conclude, normal pregnancy is associated with a moderate increase of oxidative stress whereas the increase is marked in preeclampsia. The increase in lipid peroxidation is not significantly correlated to increase in the acute phase reactant ceruloplasmin levels but is significantly correlated to the systolic blood pressure and urine protein

excretion in preeclampsia. Oxidative stress has been shown to be the causative factor for hypertension as well as causative factor for target tissue damage in hypertension. A concomitant increase of uric acid and ceruloplasmin in the sera of patients is suggestive of exaggerated inflammatory response in preeclampsia. In the present study, the blood pressure returned to normal levels during the post partum period but lipid peroxidation and protein excretion did not return to normal levels. This may be due to factors other than oxidative stress that are responsible for causation of hypertension in preeclampsia. It also implies that oxidative stress plays a very important role in the causation of target tissue damage such as renal injury. Further studies may clearly demonstrate the role played by oxidative stress in preeclampsia.

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