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Oxidative Stress and Preeclampsia: During Pregnancy and After Delivery

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ABSTRACT

Preeclampsia (PE) is a leading cause of perinatal morbidity and mortality. Our aim of the study was to evaluate the association of oxidative stress and antioxidant defence systems with preeclampsia during pregnancy and after delivery. The study comprises a total of 52 subjects including PE women (n=27) and uncomplicated pregnant women (n=25) matched by age. Serummalondialdehyde (MDA), phospholipid hydroperoxide (PHP), superoxide dismutase (SOD), vitamin C and total antioxidant status (TAS) were estimated by spectrophotometric methods. Serum levels of MDA and PHP were significantly higher ($p<0.001$, respectively) while SOD, vitamin C and TAS were significantly lower ($p<0.001$, respectively) in patients both during and after delivery. The systolic and diastolic blood pressure significantly decreased among patients after delivery. On the other hand, the MDA and PHP concentrations non-significantly decreased in PE women after delivery whereas the antioxidant system non-significantly increased. Our results suggested that the raise in oxidative stress and reduction in antioxidant defence systems persisted after delivery for 1 to 3 days. Therefore, evaluation of oxidative status and antioxidant defence systems may be a useful tool for diagnosis and treatment of preeclampsia during and after pregnancy.

Keywords: Preeclampsia, Perinatal morbidity, Oxidative stress, Antioxidant system.

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INTRODUCTION

A life threatening disorder during pregnancy and postpartum period is preeclampsia (PE). It is a triad of oedema, hypertension and proteinuria occurring primarily after the 20th gestational week and most frequently near term.[1] Intrauterine growth retardation (IUGR), pre-term delivery, low birth weight, fetal death and neo-natal death due to complications of pre-term delivery are common perinatal outcomes associated with preeclampsia.[2] Preeclampsia affects between 0.4% and 2.8% of all pregnancies in developed countries and many more in developing countries, leading to as many as 8 370 000 cases worldwide per year.[3] In developing nations, the incidence of the disease is reported to be 4-18%.[4] Though PE is a serious problem its etiology is still poorly understood. Oxidative stress (OS) of the placenta is considered to be a key intermediary step in the pathogenesis of preeclampsia. It has recently been reported that oxidative stress and inflammatory mechanisms are involved in endothelial cell activation and dysfunction in preeclampsia.[5] Oxidative stress is the result of an imbalance between the intracellular production of reactive oxygen species (ROS) and the cellular defence mechanisms.[6] Sharma et al. reported the association of oxidative stress with preeclampsia.[6] Elevated levels of oxidative stress status in pregnancy were shown in many other studies observed impaired antioxidant activity in women with preeclampsia.[7,8]Oxidative stress can trigger a number of potentially damaging biochemical reactions.[9] Oxidative stress may be responsible not only for preeclampsia but also for other endothelial cell generated dysfunctions such as atherosclerosis and cardiovascular diseases.[10-12] The fetus as well as the mother may therefore develop cardiovascular problems after pregnancies with elevated oxidative stress, especially if oxidative stress persists post-partum.[13] In a study measuring lipid peroxidation products elevated levels have been found before and also after delivery in maternal serum from women with PE compared to controls.[14] Kressig et al. reported elevated malondialdehyde (MDA) a biomarker of OS in PE patients during and after pregnancy.[15] Mutlu-Tu et al. also showed increased MDA levels in maternal serum before and after delivery in women with PE compared to normal pregnancy[14]. Preeclampsia is also a common problem in Bangladesh. Though we showed the association of oxidative stress with preeclampsia in our previous study the overall effects of the oxidative stress markers during and after pregnancy were not clear.[16] We hypothesized that patients with preeclampsia may also suffer from oxidative stress after delivery. If untreated various complications may develop in patients. The objective of this study was to measure oxidative stress markers in PE patients before and after delivery. We measured serum MDA, phospholipid hydroperoxide (PHP) and

superoxide dismutase (SOD) activity as markers of oxidative stress in study subjects. Vitamin C and total antioxidant status (TAS) were also measured as the ability to protect from oxidative stress. All the parameters were compared with healthy control group with uncomplicated pregnancy.

MATERIALS AND METHOD

Study Subjects

The study was conducted on 52 subjects (27 preeclamptic pregnant women and 25 healthy uncomplicated pregnant women) matched by age. Preeclamptic pregnant women were recruited from Dhaka Medical College Hospital and uncomplicated pregnant women were recruited from Azimpur Maternity Hospital, Dhaka, Bangladesh.

Subjects were selected based on following criteria:

- Systolic blood pressure greater than 140 mmHg or a raise of at least 30 mmHg.
- Diastolic blood pressure greater than 90 mmHg or a raise of at least 15 mmHg.
- Proteinurea of 300 mg in a 24 hours urine collection.
- Antepartum and postpartum Preeclampsia.

Subjects with uncomplicated pregnancies were normotensive throughout gestation and had no proteinurea.

Sample Collection

Blood samples were obtained from all subjects during February 2012 through June 2012. Blood samples were taken two times from each subject, 1st in 1 to 3 days before delivery and 2nd in 1 to 3 days after delivery. About 5.0 mL of peripheral blood was drawn from each individual with the help of an expert. Then the blood was transferred to a sterile glass tube without any disturbance. Blood samples were kept in an ice chamber following collection and during transportation to the laboratory. After centrifugation of the clotted blood, serum samples were collected in eppendorf tubes and the oxidative stress markers were measured immediately.

Assay of oxidative stress markers

In this study we measured the values of malondialdehyde (MDA), phospholipid hydroperoxide (PHP) and superoxide dismutase (SOD) as oxidative stress markers. We also measured vitamin C and total antioxidant status (TAS) levels as the ability to protect from oxidative stress. The MDA value was measured according to the method of Yagi.[17] At first the lipoprotein portion was precipitated by trichloroacetic acid. Then the solution was boiled with thiobarbituric acid, which reacted with malondialdehyde to form a pink color. The absorbance of the color was measured at

the wavelength of 535 nm. Phospholipid hydroperoxide value was determined by the method of Miyazawa based on the oxidation of ferrous to ferric ion in the presence of xylenol orange.[18] The color formed in this reaction was measured at 520 nm. The determined values for MDA and PHP were expressed as nmol/mL. Superoxide dismutase value was assayed by commercial reagent kit (Sigma-Aldrich, USA).[19] The kit contained xanthine oxidase enzyme that produced superoxide anion which reacted with Dojindo's highly water-soluble tetrazolium salt, WST-1{2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt} that produces a water-soluble formazan dye. Superoxide dismutase inhibits the formation of the dye. Thus SOD activity is inversely proportional to the dye. The absorbance of the dye was measured at 450 nm by ELISA reader. The SOD activity was expressed as percentage. Serum Ascorbic acid (Vitamin C) levels were estimated calorimetrically using a DTCS reagent prepared by mixing dinitrophenyl hydrazine, thiourea, and copper sulfate in a 1:1:20 ratio, according to the method developed by Teitzwith few mobifications.[20] Ascorbic acid in serum is oxidized by Cu^{2+} to form dehydroascorbic acid, which reacts with acidic 2,4-dinitrophenylhydrazine to form a red bis-hydrazone, which was measured at 520 nm. Ascorbic acid concentration was expressed as mg/dL. Total Antioxidant Status was also measured by Antioxidant Assay Kit (Sigma-Aldrich, USA).[21] Metmyoglobin and hydrogen peroxide produce feryl myoglobin radical which oxidizes the ABTS (2,2'-azino-bis(3- ethylbenzthiazoline-6-sulfonic acid) to produce a radical cation, $ABTS^{\cdot+}$, a soluble chromogen that is green in color. Antioxidants suppress the production of the radical cation and the color intensity decreases proportionally. The color intensity was read at 405 nm by ELISA reader. The determined value for TAS was expressed as mM.

Statistical Analysis

All the results were expressed as mean ± SEM. The statistical analysis of the data was carried out with Statistical Package of Social Science (SPSS), version 17 and Graph pad Prism version-5. The comparisons between two groups were tested by unpaired t-test. A 95% confidence interval was used. *P* values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Oxidative stress is a potential indicator in preeclampsia. We have studied the malondialdehyde (MDA), phospholipid hydroperoxide (PHP), level of superoxide dismutase (SOD), vitamin C and total antioxidant status (TAS) which indicate the severity of preeclampsia. Other related parameters like Blood pressure (BP) and Birth weight also studied.

Clinical and laboratory data

These are shown in Table 1 and in Figure 1. The maternal age of study subjects was not significantly different. On the other hand the gestational age was significantly decreased in preeclampsia as compared with normal pregnancy ($p<0.001$) (Table 1).

Table 1: Baseline characteristics of the study subjects

Parameters	Mean ± SEM		*p value*
	Control (n=25)	PE Patient (n=27)	
Maternal ages (years)	26±0.1	25.04±0.1	ns
Gest. ages (weeks)	38.36±0.7	34.11±0.5	< 0.001
Birth weight (kg)	2.9±0.1	2.2±0.1	< 0.001

Unpaired t-test was done as the test of significant. $p<0.05$ was taken as level of significance. PE; Preeclampsia.

The fetal weight was also significantly lower in preeclampsia as compared with normal pregnancy ($p<0.001$) (Table 1). As shown in Figure 1 the systolic and diastolic blood pressure levels were significantly lower in normal pregnancy as compared with preeclampsia ($p<0.001$, respectively). In preeclampsia oxidative stress increase and potential free radicals damage the vasospasm which in turn increases the peripheral resistance, hence BP increases.[22]

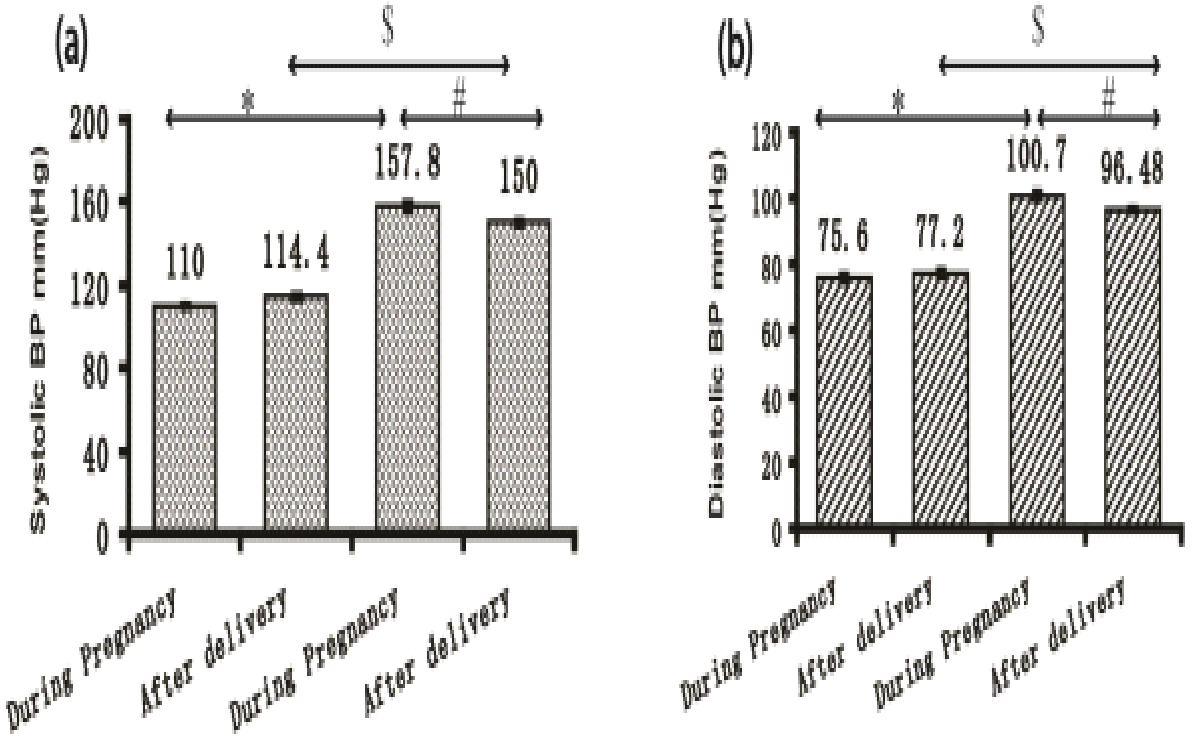

Figure 1: (a) Systolic and (b) diastolic blood pressure of study subjects at different period.

Unpaired t-test was done as the test of significant. $p<0.05$ was taken as level of significance. [*, $ = $p<0.001$, # = $p<0.05$, *= p<0.01].

Analysis of different parameters during pregnancy and after delivery

MDA is generally used as a marker of oxidative stress and in accordance with some studies our study showed higher degree of serum oxidative stress in the form of MDA and PHP ($p<0.001$, respectively) in PE patients compared with normal pregnant women (Table 2).[6, 23] Other studies also reported elevated serum MDA concentration in patients with preeclampsia.[24] SOD and vitamin C are important antioxidant systems. Several investigators have reported reduction of SOD and vitamin C level in PE patients[25]. In this study, we found serum SOD, vitamin C and TAS levels in PE women were significantly ($p<0.001$, respectively) than in normal pregnancy (Table 2). Kharb et al. and Sharma et al. also reported decreased antioxidant levels in complicated pregnancy[6, 26]. The higher levels of oxidative stress markers and reduced levels of antioxidants may persist after delivery. Ozan et al. investigated total plasma antioxidant status, plasma lipid profile, and uterine artery doppler velocity waveform in non-pregnant women with a history of preeclampsia, and observed that the mean total plasma antioxidant status was subnormal in 72% of the formerly preeclamptic group, in contrast to 35% in the control group.[27] In our present study, after the delivery of child, the levels of MDA and PHP were significantly ($p< 0.001$, respectively) low in normal mothers compared to complicated mothers (Table 2).

Table 2: Levels of different parameters in study subjects at different periods.

Parameters	During pregnancy			After delivery		
	Control (n=25)	Patients (n=27)	P value	Control (n=25)	Patients (n=27)	P value
MDA (nmol/mL)	5.25 ± 0.17	8.45 ± 0.11	<0.001	4.89 ± 0.14	8.12 ± 0.14*	<0.001
PHP (nmol/mL)	6.38 ± 0.17	8.56 ± 0.23	<0.001	5.64 ± 0.12	8.34 ± 0.22*	<0.001
SOD (U/mL)	5.18 ± 0.12	1.64 ± 0.06	<0.001	5.36 ± 0.12	1.80 ± 0.06*	<0.001
TAS (nM)	1.51 ± 0.06	0.84 ± 0.04	<0.001	1.61 ± 0.05	0.96 ± 0.06*	<0.001
Vitamin C (mg/dL)	1.45 ± 0.06	0.67 ± 0.08	<0.001	1.56 ± 0.06	0.82 ± 0.07*	<0.001

Results are expressed as Mean ± SEM. Unpaired t-test was done as the test of statistical significant. $p<0.05$ was taken as level of significant. *; $p>0.05$ (Comparison among patients during pregnancy and after delivery).

In this study we compared the oxidative status and antioxidant system among the PE patients during pregnancy and after delivery. We found significant ($p<0.05$, $p<0.01$, respectively) reduction in systolic and diastolic blood pressure after delivery (Figure 1). On the other hand, the MDA and PHP concentrations non-significantly decreased in PE women after delivery whereas the antioxidant system increased though statistically non-significant (Table 2). This result suggested that the oxidative stress caused in preeclampsia persists even after delivery for 3 days.

Kressig et al. (2008) showed that elevated MDA concentration persisted 24 hours in PE patients after delivery[15]. The imbalance between lipid peroxidation and antioxidant defences in preeclampsia, leads to endothelial dysfunction and free radical-mediated endothelial cell injury.[15, 26] Oxidative-stress-induced damage to DNA and macromolecules is associated with the onset and development of many other diseases including cardiovascular disease, neurological degenerations (e.g., Alzheimer's disease, ischemic stroke), and cancer, as well as the normal ageing processes. Thus the oxidative stress in preeclampsia may cause serious problems in mothers after delivery if untreated.

CONCLUSION

The results of our present study suggested that circulating levels of oxidative stress markers (MDA and PHP) were statistically elevated and antioxidant defence systems (SOD, vitamin C and TAS) were significantly reduced in women with preeclampsia and persisted for 3 days after delivery. This study will help to take preventive care for mothers. So treatment should persist even after delivery of child for few days. Further follow up study is needed for accurate findings.

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