Oxidative Stress and Antioxidant Status in Neonates Born to Pre-eclamptic Mother

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Summary

Objective: Pre-eclampsia is a significant health problem and is the leading cause of maternal and perinatal mortality and morbidity. Low birth weight and prematurity are very common in pre-eclamptic mothers. Pre-eclampsia is associated with oxidative stress in the maternal circulation. To observe the effect of pre-eclampsia on neonates, this study was designed to explore oxidative stress and anti-oxidant status in the fetal circulation in pre-eclampsia.
Materials and Methods: For this purpose, we collected cord bloods during delivery from Bangabandhu Sheikh Mujib Medical University. Twenty samples were collected from uncomplicated (normotensive) mothers and 15 samples were collected from pre-eclamptic mothers (maternal age matched). Thiobarbituric acid reactive substances (TBARS), lipid hydroperoxide, protein carbonyl value, lipid profile, total anti-oxidant status (TAS), vitamin C, serum total protein and albumin were measured.
Results: It was observed that TBARS and lipid hydroperoxide were significantly ($P < 0.001$) increased, protein carbonyl content were also significantly ($P < 0.001$) increased but total anti-oxidant status ($P < 0.001$) and vitamin C level were significantly ($P < 0.05$) decreased in cord blood from pre-eclamptic mother compared to control group. Cholesterol, TG, LDL level was elevated and HDL were lowered in cord blood in pre-eclamptic group compared to normotensive group. In pre-eclamptic group, cord blood total protein, albumin and globulin level were significantly decreased compared to control group.
Conclusions: As pre-eclampsia is associated with increased oxidative stress and decreased anti-oxidant status, the results of these investigations suggest that oxidative stress and antioxidant status are altered towards proatherogenic level in cord blood of pre-eclamptic women which may ultimately be responsible for different complications of newborn babies of pre-eclamptic mothers.

Key words: pre-eclampsia, cord blood, oxidative stress, neonates.

Introduction

World Health Organization (WHO) reported that pre-eclampsia affects 2–3% of all pregnancies and is responsible for about 60 000 deaths worldwide every year, mostly in developing countries [1]. Pre-eclampsia is a significant health problem that affects ~6–8% of all gestations and is the leading cause of fetal growth retardation, infant morbidity and mortality, premature birth and maternal death [2]. In severe pre-eclampsia, women are at risk of multi organ failure and death and their babies are at risk of intrauterine growth restriction, intrauterine death and complications of prematurity. In most cases, the interests of mother and baby coincide when delivery is indicated but tragically it is sometimes necessary to deliver a woman for her own safety at the expense of a baby who is too premature to survive delivery [3].

Abnormal placentation seems to be involved in the etiology of pre-eclampsia [4]. Maternal endothelial cell dysfunction is the key event resulting in the diverse clinical manifestations of pre-eclampsia and evidence has since accumulated [5]. There is increased attention for the hypothesis that placental and maternal free radical reactions promote a cycle of events that comprise the defensive functioning of the vascular endothelium in pre-eclampsia. Lipid peroxides, the highly reactive products of lipid peroxidation, are formed when free radicals attack polyunsaturated fatty acids in membranes or lipoproteins. They are also formed by cyclooxygenase or lipoxygenase [6]. Uncontrolled lipid peroxidation can result in cellular dysfunction and damage.

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Many endothelial changes of potential relevance to pre-eclampsia can be induced by lipid peroxidation [7]. ROS can arise from various sources, including (placental) mitochondria [8], stimulated phagocytes, oxidation of catecholamines and metabolism of arachidonic acid [9].

In the newborn infant, infection and inflammation cause activation of polymorphonuclear leucocytes, which in turn can increase oxidative stress by generating ROS. The anti-oxidant defense mechanisms are not fully developed in preterm infants, which further increases their vulnerability to oxidative stress. Although pre-eclampsia is associated with oxidative stress in maternal circulation. To observe the effect of pre-eclampsia on neonates, this study was designed to explore oxidative stress and anti-oxidant status in the fetal circulation in pre-eclampsia.

Methods and Materials

Subjects and sample collection

The study was conducted with cord blood from two groups of women [normotensive (NT) without any complication ($n = 20$) and pre-eclamptic pregnant (PE) ($n = 15$)] during delivery. The subjects were recruited from Department of Obstetrics and Gynecology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Bangladesh. The study was approved by the local Ethics Committee, and all participants gave informed consent.

Pre-eclamtic patients met the following inclusion criteria: systolic blood pressure >140 mmHg or a rise of at least 30 mmHg; diastolic blood pressure >90 mmHg or a rise of at least 15 mmH; proteinuria of 300 mg/24 h urine. The exclusion criteria of pre-eclamsia patients were: diagnosis of other complications for example HBV infection, tumor and cancer. The NT subjects were healthy pregnant women of similar age and socio-economic status as PE pregnant subjects. Cord blood samples were collected from umbilical cord of all of the subjects during delivery at a single time point and analyzed in Biochemistry and Molecular Biology Department, Dhaka University.

Analytical methods

Serum total cholesterol, HDL-cholesterol and TG, were determined by commercially available kits by Biolabo, France. LDL-cholesterol was calculated from Friedewald Formula [10]. Serum total protein, albumin, glucose, uric acid, urea and creatinine were determined by the commercially available kit (Randox, UK). Thiobarbituric acid reactive substances (TBARS) value was determined according to the method of Yagi [11]. Lipid hydroperoxide value was determined by colorimetric method based on the oxidation of ferrous to ferric ion in the presence of xylenol orange [12]. Serum carbonyl contents were measured using reaction with dinitrophenylhydrazine (DNPH), leading to the formation of stable hydrazone products [13]. Serum ascorbic acid was measured by dinitrophenyl hydrazine method with modification according to Lowry *et al*. [14]. Total anti-oxidant status was determined by using a kit by Randox, UK [15].

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) (version 11.0 for Windows, SPSS Inc., Chicago, USA). The statistical method used was student's *t*-test (two-tailed). Differences were considered significant at $P < 0.05$.

Results

Table 1 shows the baseline characteristics of the study subjects. Age was matched but gestational age was ($P < 0.05$) significantly different between groups. As expected, both the systolic and diastolic blood pressures were significantly increased ($P < 0.001$) in PE group than control group (NT group). Offspring from women with PE group had significantly lower birth weights ($P < 0.01$) compared to NT group.

Table 2 shows lipid profile of the study subjects. Cord blood total cholesterol (TC), TG and LDL levels of PE group were significantly higher than the NT group but HDL level was significantly lower than the NT group. Table 3 shows the protein status of the study subjects. Cord blood total protein, albumin and globulin level of PE group is significantly decreased ($P < 0.01$) compared to NT group.

Table 4 shows the markers of oxidative stress. The mean TBARS value in cord blood are 1.94 ± 0.13 and 3.33 ± 0.18 (nmol MDA eq ml^{-1}) in NT and PE group, respectively. TBARS value in cord blood of PE group was significantly higher ($P < 0.001$) than the NT group. Lipid hydroperoxides in cord blood, the major initial reaction products of lipid peroxidation, were also significantly higher in PE ($P < 0.001$) than the NT group. The mean protein carbonyl value in cord blood are 0.38 ± 0.01 and 0.62 ± 0.02 (nmol mg^{-1} of protein) in NT and PE group, respectively. Cord blood protein carbonyl value of PE group was significantly higher ($P < 0.01$) compared to control group.

Table 5 shows the anti-oxidant status in different study subjects. The mean vitamin C value in cord blood are 0.51 ± 0.02 and 0.34 ± 0.03 (mg dl^{-1}) in NT and PE group, respectively. Cord blood Vitamin C level of PE group was significantly decreased ($P < 0.05$) compared to NT group. Total anti-oxidant status was found to significantly decreased in cord blood of PE mother (0.72 ± 0.02 nmol l^{-1}) compared to control (1.22 ± 0.05 nmol l^{-1}).

TABLE 1
Baseline characteristics of study subjects

Test parameter	Control group ($n=20$)	Pre-eclampsia group ($n=15$)
Maternal age (years)	27.6 ± 1.43	25.9 ± 2.0^{NS}
Gestational age (weeks)	36.9 ± 0.40	$33.25 \pm 0.82^{*}$
Systolic blood pressure (mmHg)	107 ± 3.34	$157 \pm 6.83^{***}$
Diastolic blood pressure (mmHg)	69 ± 2.33	$102.5 \pm 3.09^{***}$
Fetal Weight (kg)	2.57 ± 0.10	$1.75 \pm 0.14^{**}$

Data are presented as mean ± SE. Student's *t*-test was performed to analyze data.
$^{*}P < 0.05$, $^{**}P < 0.001$, NS, not significant.

TABLE 2
Lipid profile status in different study groups

Test parameter	Cord blood (control group, $n=20$)	Cord blood (Pre-eclampsia group, $n=15$)
Total Cholesterol (mg dl^{-1})	48.15 ± 2.85	$70.94 \pm 8.49^{**}$
Triglycerides (mg dl^{-1})	31.65 ± 3.39	$52.40 \pm 10.94^{*}$
HDL (mg dl^{-1})	13.34 ± 0.58	$10.33 \pm 0.33^{**}$
LDL (mg dl^{-1})	28.79 ± 2.59	$65.61 \pm 7.29^{**}$

Data are presented as mean ± SEM (standard error of mean). Student's *t*-test was performed to analyze data.
n, number of subjects; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

TABLE 3
Protein status in different study groups

Test parameter	Cord blood (control group, $n=20$)	Cord blood (Pre-eclampsia group, $n=15$)
Total protein (g l^{-1})	61.20 ± 2.25	$46.16 \pm 3.44^{**}$
Albumin (g l^{-1})	36.9 ± 1.55	$27.5 \pm 2.12^{**}$
Globulin (g l^{-1})	24.26 ± 0.14	$18.05 \pm 0.335^{**}$

Data are presented as mean ± SEM (standard error of mean). Student's *t*-test was performed to analyze data.
n, number of subjects.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

TABLE 4
Oxidative stress in different study groups

Test parameter	Cord blood control group ($n=20$)	Cord blood Pre-eclampsia group ($n=15$)
TBARS value (nmol MDA eq ml^{-1})	1.94 ± 0.13	$3.33 \pm 0.18^{***}$
Lipid hydroperoxide (nmol ml^{-1})	2.28 ± 0.07	$3.96 \pm 0.08^{***}$
Protein carbonyl value (nmol mg^{-1} of protein)	0.38 ± 0.01	$0.62 \pm 0.02^{**}$

Data are presented as mean ± SEM (standard error of mean). Student's *t*-test was performed to analyze data.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Discussion

In this study, systolic and diastolic blood pressures were normal in NT group but systolic and diastolic blood pressure were very high in pre-eclampsia group that is one of the symptoms of pre-eclampsia (Table 1). It has been implied that in pre-eclampsia there could be an increase in the production of superoxide anion which may inactivate nitric oxide

TABLE 5
Anti-oxidant status in different study groups

Test parameter	Cord blood (Control group, $n=20$)	Cord blood (Pre-eclampsia, $n=15$)
Total anti-oxidant status (mmol l^{-1})	1.22 ± 0.05	$0.72 \pm 0.02^{**}$
Serum ascorbic acid (mg dl^{-1})	0.51 ± 0.02	$0.34 \pm 0.03^{*}$

Data are presented as mean ± SEM (standard error of mean). Student's *t*-test was performed to analyze data.
$^{*}P < 0.05$, $^{**}P < 0.01$.

(NO) leading to reduced relaxation and increased vasoconstriction [16].

In the pre-eclampsia group, total protein, albumin and globulin was lower than in the uncomplicated pregnancy group. It may be due to loss of protein in the urine. Lower protein and albumin levels in pre-eclampsia subjects were also found by Anne Barden *et al*. [17].The result of our present study also found that total protein, albumin and globulin levels in fetal circulation in pre-eclampsia were lower than the control subjects.

The alterations in the fetal lipid profile observed may be due to changes in transplacental transport. Dyslipidemia is common in PE and their newborn [18] and possibly via oxidation of susceptible lipids, may contribute to endothelial activation. The human placenta is known to express lipoprotein receptors in high amounts [19] and the LDL receptor plays an important role in the uptake of maternal plasma lipoproteins for placental steroid metabolism [20]. The presence of lipoprotein receptors in the placenta also indicates the potential for fetal tissues to take up cholesterol from maternal lipoproteins.

Maternal triglyceride does not cross the placental barrier, but TG in maternal lipoproteins can be hydrolyzed by placental lipase and the resulting fatty acids transferred across the placenta by fatty acid binding proteins. The up-regulation of these mechanisms might increase fatty acid transport across the placenta, thus supplying the fetal liver with substrate for TG synthesis. On reaching the fetal circulation the non-esterified fatty acids can be transported to the fetal liver for triglyceride synthesis [21].

Many studies showed that in patients with pre-eclampsia the plasma levels of lipid peroxidation products were significantly increased, while antioxidants were significantly decreased compared to healthy pregnant woman [22, 23]. Veronica M Chamy *et al*. [24] hypothesizes that the same significant biochemical changes observed in pre-eclampsia women were seen in their newborns. The results of the present study also suggest that in fetal circulation of pre-eclampsia group there is an increase in free radical/ROS generation (as indicated by an increase in the levels of lipid peroxides) and a decrease in the concentration of anti-oxidants like total anti-oxidant status and vitamin C. Our findings do not comply with the findings of Tastekin *et al*. [25]. They reported that cord plasma MDA are similar in the cord blood from PE and control mother. In our study, two products of lipid peroxidation such as thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxide increase in the similar way. These data indicate that in pre-eclampsia there is an increase in oxidative stress and that, possibly, radical-scavenging anti-oxidants are consumed by the enhanced level of free radicals/ROS.

Proteins, which constitute major components of living cells, are among the main targets of ROS-induced oxidation that provokes specific chemical oxidative modifications. Zusterzeel *et al*. [26] showed higher plasma protein carbonyl levels that are markers of oxidative protein damage in PE pregnant women than those in healthy pregnant women. The findings of our present study demonstrate that the alterations of protein carbonyl level in fetal circulation in pre-eclampsia where protein carbonyl levels were significantly higher in neonates of pre-eclampsia group compared to control subjects. This is an indication of oxidative stress.

Shaarawy *et al*. [27] observed that serum total antioxidant status in mild and severe pre-eclampsia and eclampsia was significantly lower than that of healthy pregnant women. Veronica M Chamy *et al*. [24] hypothesizes that anti-oxidant status changes observed in PE mother was in similar fashion as it was in their newborns. Our findings also suggested that anti-oxidant levels in fetal circulation in pre-eclampsia is lower than that of control subject as it was reported in case of PE mother. On the basis of the present study, it is clear that infants born to PE mother are associated with increased oxidative stress, low activities of anti-oxidant activity and increased lipid peroxidation and protein oxidation. Although the long term effect of these are not clear but babies born to PE mothers deserve a closer clinical follow up later in life.

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