A Comparative Study on the Association of Labour process with Oxidative stress in Normal and Preeclamptic mothers

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Abstract
Oxidative stress plays an important role in the pathophysiology of preeclampsia. An increase in lipid peroxidation and decrease in antioxidant activity in preeclamptic women with hypertension have been reported. The present contribution aimed to understand the effect of labour on the levels of free radicals in normotensive pregnant mothers (control group) and hypertensive preeclamptic mothers. Serum MDA (Malondialdehyde) is used as a marker of oxidative stress mediated changes in the study subjects and the levels of enzymatic antioxidants viz SOD, GRx, GPx in the arterial blood (before delivery) and in cord blood are estimated. The results indicate that in hypertensive preeclamptic mothers, there is a significant (p<0.001) increase in the levels of serum MDA and a significant (p<0.001) decrease in the levels of antioxidant enzymes in cord blood when compared to control groups. This is attributed to the oxidative stress during labour pain. Hence it is inferred that antioxidant supplementation during pregnancy could prevent oxidative stress mediated changes to cell and biomolecules during the gestational period.

Key words: Oxidative stress, hypertension, pregnancy, lipid peroxidation, antioxidant enzymes

Introduction
Preeclampsia is a specific disorder of pregnancy, characterized by hypertension, proteinuria and oedema developing in the second half and, leading to maternal and neonatal morbidity as well as mortality. It is characterized by endothelial cell dysfunction, lipid peroxidation, decreased antioxidants and alteration in immune responses (1-3). The endothelial changes are more appropriately described as dysfunction or activation of an altered state of endothelial cell differentiation in response to sublethal injury or cytokine stimulation (4), rather than damage to endothelium (5). The pathologic changes in the endothelial cells that line the renal glomerular capillaries (glomerular endotheliosis) are a consistent feature in the histopathology of women with pre-eclampsia (6, 7). Much evidence points to the role of Reactive Oxygen Species (ROS) in the etiology of hypertension and oxidative stress in preeclamptic mothers. ROS usually accompany increased peroxidation of lipids and is evidenced in the blood samples in the form of MDA. Free oxygen free radicals are highly reactive and are potentially damaging to most macromolecules like polyunsaturated fatty acids, proteins, carbohydrates and nucleic acids. Peroxidation of polyunsaturated fatty acids produces malondialdehyde. The presence of this oxidation product can be measured with thiobarbituric acid and its levels correlate with the extent of lipid peroxidation (8-11). Elevated level of lipid peroxide by-products like malondialdehyde is an indicator of oxidative
stress mediated changes in vivo (12). In the present study, we have investigated pair matched biochemical profile of oxidative stress indices and the levels of antioxidant defense enzymes. We evaluated in a comparative study of these enzymes in blood samples from maternal and cord blood of preeclamptic mothers and compared these data with those of normotensive mothers. We also studied the levels of these enzymes before and during the labour process, so as to estimate the role of labour as a stress factor.

The association between pregnancy-related hypertension and severity of hypertension (stage 2 according to Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC VII)) and end-organ damage was assessed in a logistic regression model. Gestational hypertension contribute to obstetrical complications and maternal mortality making it as an important health issue globally with a systolic blood pressure =140 mmHg and/or a diastolic blood pressure =90 mmHg, in the absence of proteinuria, in a previously normotensive pregnant woman at or after 20 weeks of gestation (13).

The objectives of this study were to investigate the levels of reactive oxygen species and those of antioxidant enzymes in maternal and cord blood samples of normotensive and preeclamptic mothers and their fetuses. The presence of pregnancy related hypertension is established by questionnaire and clinical examination.

**Materials and Methods**

**Collection of Samples**

Studies were conducted on blood samples from 40 patients falling in the age group of 25 to 35 years, who attended the Out Patient Department of Meenakshi Medical College and Research Institute, Chennai-600 078, Tamil Nadu. The study is cleared by Ethical Committee.

**Experimental Setup**

The patients were divided into four groups of twenty patients each. Group I consisted of maternal blood samples of normotensive patients and served as a control. Cord blood samples from fetuses of normotensive mothers served as Group II. Maternal blood samples of preeclamptic mothers formed Group III and cord blood samples of fetuses of preeclamptic mothers served as Group IV. Smoker, alcohol consumers and other drug abusers were excluded from the study. Demographic data pertaining to clinical characteristics of control and experimental subjects were documented. Blood Pressure at the time of delivery, pulse rate was documented using standardized procedures. Urine samples were analysed for protein. Clinical examination for pedal edema is documented in case report forms. Informed consent of the participants and clearance of the ethical committee was obtained prior to the onset of study. Maternal blood samples were collected at the time of delivery and cord blood samples from umbilical cord were collected immediately after delivery from the study participants (control and preeclamptic mothers). The samples were processed for the separation of plasma, red blood cells and haemolysate. Lipid peroxidation assay was estimated using plasma samples and haemolysate was used for the enzymatic antioxidants and the results were documented.

**Lipid peroxidation**

Lipid peroxidation in the plasma was estimated using TBA (Thiobarbituric acid) reaction method (14). Standard MDA 50mM solution of malondialdehyde was prepared in distilled water using 1,1,3,3 tetramethoxypropane. This was stored in 4OC and diluted just before use such that working standard contains 50 nM/ml. Plasma TBARS values were expressed in moles of MDA/L (15).
Assay of antioxidant enzymes
Preparation of hemolysate was done (16, 17). Blood collected with EDTA was centrifuged at 2000×g for 20 minutes at 4°C. The packed cells were washed with saline to remove the buffy coat. An aliquot of packed cells was washed with isotonic Tris – HCl buffer. 1.0 ml of washed cells was lysed using 9.0ml of hypotonic Tris – HCl buffer pH 7.2. The lysed cells were centrifuged at 15000 ×g for 30 minutes. The supernatant fraction (hemolysate) was used for the assay of antioxidant enzymes. The enzyme superoxide dismutase (SOD) was measured by the method of Hartz et al (18). The enzyme glutathione peroxidase (GPx) catalyses the reaction of ROS – like H2O2 leading to elevated LPO. GPx activity was measured spectrophotometrically following the method of Paglia and Valentine (19). Activity of glutathione reductase (GRx) was measured as described by Goldberg and Spooner (20).

Statistical analysis
Values are expressed as mean ± SD. Mean and Standard Deviation (SD) were estimated for different variables in each group. Mean values were compared between different study groups by using One way ANOVA. Values of p<0.05 were considered to be statistically significant.

Results
Table 1 depicts the demography, physiological status and clinical characteristics of normotensive and preeclamptic study participants. The mean maternal age was 29.1 ±6.9 in normotensive group while it was 26.8 ± 7.2 in preeclamptic subjects which is significant at ( p<0.275) .Gestational age was 33.8 ±3.9 weeks in normotensive group while it was 32.2 ± 4.8 weeks in preeclamptic study participants which is significant (p<0.221). The systolic Blood Pressure at delivery is 109.8 ± 12.9 mm Hg in normotensive group and it was 164.5 ± 13.6 mm Hg in preeclamptic group which is significant (p<0.0001). The diastolic Blood pressure at delivery is 65.6 ± 11.4 mm Hg in normotensive group and it was 111.4 ± 14.2 mm Hg in preeclamptic group which is also significant (p<0.0001). Ischemia, reperfusion injury is known to be a potent source of free radicals like superoxide anion (O2-), Hydroxyl radicals (OH), Hydrogen peroxide H2O2 which again cause oxidative damage to cell membrane and vascular endothelium setting up a viscous cycle.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normotensive control group I</th>
<th>Preeclamptic group III</th>
<th>P*-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of maternal/neonatal pairs</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>29.1 ± 6.9</td>
<td>26.8 ± 7.2</td>
<td>0.275</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>33.8 ± 3.9</td>
<td>32.2 ± 4.8</td>
<td>0.221</td>
</tr>
<tr>
<td>BP at delivery systolic (mm/Hg)</td>
<td>109.8 ± 12.9</td>
<td>164.5 ± 13.6</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Table 1. Demographic and clinical characteristics of normotensive (Control) and severe preeclamptic subjects
Endothelial dysfunction is considered to be a main cause of classical clinical features of pre-eclampsia (21). Values are expressed as mean ± SD; BP- blood pressure, P*= Two-Samples t-test probability.

Table 2 depicts the concentrations of malondialdehyde and activities of various antioxidant enzymes in pair matched normotensive (control) group and preeclamptic maternal and cord blood. The MDA level in preeclamptic maternal blood was significantly high (p<0.001) compared to that of control. However its content in pre ec-

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normotensive maternal blood (Group I) (n = 20)</th>
<th>Normotensive cord blood (Group II) (n = 20)</th>
<th>Preeclamptic maternal blood (Group III) (n = 20)</th>
<th>Preeclamptic cord blood (Group IV) (n = 20)</th>
<th>P*-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondialdehyde (MDA) (mmol/gHb)</td>
<td>2.77 ± 0.361</td>
<td>1.716 ± 0.415</td>
<td>3.7879 ± 0.371</td>
<td>2.394 ± 0.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Superoxide dismutase (SOD) (IU/gHb)</td>
<td>587.8 ± 44.42</td>
<td>608.82 ± 40.88</td>
<td>497.17 ± 25.07</td>
<td>452.56 ± 24.68</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Glutathione peroxidase (GPx) (IU/gHb)</td>
<td>25.87 ± 1.653</td>
<td>27.53 ± 1.40</td>
<td>22.37 ± 1.03</td>
<td>21.19 ±1.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glutathione reductase (GR) (IU/gHb)</td>
<td>8.94 ± 0.86</td>
<td>8.25 ± 0.78</td>
<td>7.35 ± 0.86</td>
<td>6.68±0.62</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
lamptic cord blood compared to their pair matched blood was significantly low (p<0.001). Values are expressed as mean ± SD. Mean and Standard Deviation were estimated for different variables in each study group. Mean values were compared between different study groups by using One way ANOVA

Several defense enzymes have been adopted by the erythrocyte to protect itself against the aggressive oxygen species by utilizing enzymatic and non enzymatic anti oxidants. Preeclamptic patients showed a decrease in activities of enzymatic antioxidants when compared to normotensive group. (Group I vs group III).

The levels of enzymatic antioxidants in pair matched normotensive (control) group and pre eclamptic maternal and cord blood are depicted in Figure 2.

Preeclamptic patients had low levels of enzymatic antioxidants. The activity of Superoxide radical was significantly increased in cord blood (p<0.05) of normotensive as compared to pair-matched maternal blood whereas in preeclamptic cord the activity of Superoxide radical was significantly decreased (p<0.05) in comparison to pair-matched preeclamptic maternal blood. Levels of Glutathione peroxidase and Glutathione Reductase were significantly increased (p<0.001) in normotensive cord blood and significantly decreased (p<0.001) in preeclamptic cord blood compared to pair matched maternal blood. Figure 3 illustrated that Comparison of balance in biologic actions of antioxidants and lipid peroxides in normal pregnancy with imbalance of increased lipid peroxides and decreased vita-

Figure 1. MDA concentrations in pair matched normotensive and preeclamptic maternal and cord blood

The figure-1 depicts, MDA concentrations in pair-matched normotensive and preeclamptic
Discussion

There is an increasing evidence that oxidative stress may be an important contributing factor to the pathogenesis of pre-eclampsia (23-27). The pregnant women affected by pre-eclampsia may have abnormal ROS production, particularly NO and O$_2^\cdot$ and abnormal levels of antioxidant defenses and increased placental lipid peroxidation (28). An imbalance between the pro–oxidants and the antioxidants has been defined as ‘oxidative stress’. In severe pre-eclampsia, the balance between ROS and antioxidants is disturbed due to an increase in oxidants and compromise of antioxidants which is depicted in Figure 3.

Lipid peroxidation is a process that occurs normally at low levels in all cells and tissues. It involves conversion of unsaturated fatty acids to lipid hydroperoxides. This process can be initiated by free radicals, which are unstable molecules that possess an unpaired electron in their outer orbital. The organism normally has anti-oxidative mechanisms that limit this process. Moreover, low concentrations of lipid peroxides are essential and may act endogenously as intracellular messengers. Oxygen derived free radicals are produced as a result of metabolism of oxygen biradical during reduction reactions (29).

Superoxide radicals are unique in that they can lead to the formation of many other reactive oxygen species including hydroxyl radicals. Superoxide radicals also reacts with Hydrogen peroxide to generate the singlet oxygen molecule (30). It is difficult to block the oxidative stress – induced injury to cells or tissues because ROS are continuously produced by cellular aerobic metabolism (31). Oxidative stress may be limited by using chain – breaking antioxidants such as vitamin E which neutralize hydroxyl, superoxide, and hydrogen peroxide radicals and prevents
oxidative stress (32). During normal human pregnancy, serum lipid peroxidation products are elevated (33) but are counterbalanced by and increased activity of the antioxidant system (34-36). Several studies suggest that pre-eclampsia is associated with increased circulating lipid peroxides compared to normal pregnancy (37-38).

The most in vivo source of oxygen derived free radical is molecular oxygen itself. The metabolism of oxygen (i.e reduction) generates ROS (39). Imbalance between the production of ROS and in vivo availability of antioxidants might play an indirect role in the etiology and complications of the disease (40). LPO formed at the primary site could be transferred through circulation and could provoke damage to internal organs (41). Hence LPO is a Biomarker for oxidative stress.

Antioxidant enzymes like SOD and glutathione peroxidase form the first line defense against ROS and decrease in their activities contributes to the oxidant assault on cells. Erythrocyte SOD is an important enzyme that specifically scavenges ROS – like superoxide radicals produced in the cells. GPx has been considered as a major protective enzyme against the accumulating organic peroxides (ROOH), which are potential radical forming species within the cell. GPx catalyzes the conversion of oxidized glutathione to reduced glutathione. Endogenous protective mechanisms against reactive oxygen species are enzymatic superoxide scavengers such as superoxide dismutase and glutathione peroxidase. In addition, there is an extensive non – enzymatic anti – oxidant network of different lipid – and water – soluble molecules that have in common the ability to scavenge free radicals. α – Carotene and vitamin E are two of the lipid – soluble substances and ascorbic acid, uric acid and glutathione are some of the water – soluble free radical scavengers.

Lipid peroxidation products are candidate factors that may mediate disturbance of the maternal vascular endothelium (42). Glutathione peroxidase, an enzyme that removes hydrogen peroxide and converts lipid hydroperoxides to less reactive alcohols, may be deficient in placental tissue from preeclamptic women. This is seen in conjuction with increased in vitro placental production of lipid hydroperoxides and thromboxane A₂ (TXA₂) (43). TXA₂ is a vasoconstrictive and pro – aggregatory prostaglandin normally counterregulated by prostacyclin (PGI₂). Chemical inhibition of placental glutathione peroxidase resulted in increased production of lipid hydroperoxides and an increase in the placental TXA₂ to PGI₂ output ratio (44). Lipid hydroperoxides can inhibit PGI₂ synthase enzyme activity and simultaneously stimulate the cyclooxygenase component of PGH synthase. (45) Whereas TXA₂ synthase activity is unchanged or even stimulated (43, 46). Since expression of the synthases is not altered in the uteroplacental unit (47), these effects of lipid hydroperoxides could be the source of the decreased placental PGI₂ to TXA₂ production ratio in preeclampsia. The altered prostaglandin ratio might provoke vasospasm with exacerbation of placental ischemia, increased cell damage, and increased lipid peroxidation (amplification of oxidative stress) (48). The pathophysiological changes that occur in normal pregnancy compared with preeclampsia is depicted in Figure 4.

The present study investigated maternal plasma enzymatic antioxidant levels during normal and complicated preeclamptic pregnancies and their relationships with neonatal cord blood antioxidant levels. Cumulative evidences in recent years show that a biochemical imbalance in preeclampsia occurs with an increase of oxidative stress and a

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deficient antioxidant protection (49) Indeed, free radicals released from the poorly perfused fetoplacental unit initiate lipid peroxidation by attacking polyunsaturated fatty acids in cell membranes, converting them to lipid peroxides and to a variety of antioxidant enzymes in preeclamptic cord blood which subsequently stimulate membrane phospholipid peroxidation by alkoxy radicals (50). This study offers an opportunity to observe that oxidative stress is increased as the severity of the disease increases. From the findings of significantly low MDA contents in the pair-matched cord blood, it is hypothesized that antioxidant capacity of cord blood is sufficient and placental barrier is adequate, to shield the fetus from the oxidative injury.

Conclusion
To conclude, the oxidative stress status is low in the blood of neonates compared to its level in the pair-matched preeclamptic mothers, and oxidative stress status is increased in preeclamptic mothers compared to normotensive mothers. Further studies are needed to explore strategies so that the normal levels of antioxidant vitamins are maintained to combat preeclampsia in women at high risk. Hence it is inferred that antioxidant supplementation could prevent oxidative stress mediated changes to cell and biomolecules during the gestational period and further work is in progress in this direction.

References

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