Evaluation of reflection of oxidative stress in cord blood of neonates born to hypertensive mothers

Lekha Tejaswi Y, Preethi B P, Kshiti Mouli Kondajji

Department of Biochemistry, JJM Medical College, Davangere, Karnataka, India

Correspondence to: Lekha Tejaswi Y, E-mail: lekha.tejaswi@gmail.com

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INTRODUCTION

Hypertensive disorders remain the most common cause of complications in pregnancy, leading to increased maternal, perinatal and neonatal morbidity and mortality, especially in developing countries. Pregnancies that are complicated by pregnancy-induced hypertension (PIH) are at higher risk to develop maternal complications such as abruptio placenta, HELLP syndrome, stroke, pulmonary edema, disseminated intravascular coagulation, and fetal outcomes such as low birth weight (LBW), prematurity, and intra-uterine growth retardation.\(^1\)

Background: Hypertensive disorders of pregnancy are a multifactorial disease with oxidative stress also contributing to the etiopathogenesis. Assessment of maternal and neonatal oxidative stress may provide insight into early identification of at-risk mothers and neonates and timely intervention to reduce morbidity and mortality associated with hypertensive disorders. Objective: The objective of the study was to evaluate whether there occurs a reflection of oxidative stress in neonates born to hypertensive mothers. Materials and Methods: A total of 100 pregnant women of which 50 were normotensive and 50 were pre-eclamptic were tested for serum uric acid and serum malondialdehyde (MDA) in maternal blood and cord blood of their newborns. Our observations were subjected to statistical analysis Mean ± standard deviation calculated. Comparison is done by student t-test. Relationship between parameters was evaluated by Pearson’s correlation. Results: Increased levels of serum uric acid and MDA were found in maternal and cord blood in hypertensive pregnancies, indicative of oxidative stress. However, cord blood MDA and uric acid levels were lower than the corresponding maternal blood levels. Conclusion: Our study findings suggest that oxidative stress in hypertensive mothers is reflected in their neonates.

KEY WORDS: Pre-eclampsia; Serum Uric Acid; Serum Malondialdehyde; Oxidative Stress; Maternal and Cord Blood

Approximately 12–22% of all pregnancies are complicated by hypertensive disorders, of which pre-eclampsia accounts for 70% of cases. 5–8% of pregnant women worldwide suffer from pre-eclampsia, of which 3–5% are first pregnancies and 1% is subsequent pregnancies, with 5–10% cases are severe.

PIH disorders are characterized by the new onset of hypertension >140/90 mm of Hg manifesting after the 20th week of pregnancy. Pre-eclampsia most commonly presents as hypertension and significant proteinuria of >300 mg/day, and edema. The condition resolves completely by the 6th postpartum week. Pre-eclamptic patients can further be classified as mild pre-eclamptic and severe pre-eclamptic. Mild pre-eclampsia includes patients with hypertension more than 140/90 but <160/110 mm of Hg, along with proteinuria >300 mg but <3 g/day. Severe pre-eclampsia includes patients with hypertension >160/120 and proteinuria >3 g/day.
Pre-eclampsia is a complex multisystem disorder, the etiology of which is still not completely understood. There is widespread endothelial dysfunction accompanied by vasospasm that involves the arteries of the uterus, renal system and even the brain leading to ischemia, hypoxia, and dysfunction in the tissues. There is a failure of the trophoblastic invasion of myometrial segments of spiral arteries. This interferes with fetal growth and oxygenation.[2]

Without any medical intervention, pre-eclampsia may progress to eclampsia. Eclampsia is characterized by convulsions in a pre-eclamptic pregnant woman. There is a transitional condition called fulminating pre-eclampsia or imminent eclampsia, which is characterized by increasing signs and symptoms. Although presentation can be variable and can involve any organ, the patient usually presents with visual disturbances, headache or epigastric pain and may show signs of facial and peripheral edema, dull aching abdominal pain and hyperreflexia.[3]

Oxidative stress, which is a state caused by an imbalance between reactive oxygen species (ROS) and antioxidants, has been implicated in the multifactorial etiology of pre-eclampsia.[4] In pre-eclamptic patients, the placenta generates an excess of ROS leading to lipid peroxidation. Malondialdehyde (MDA) is the end product of lipid peroxidation. MDA damages the low-density lipoprotein (LDL) molecules leading to the formation of oxidized LDL. The altered LDL molecules are taken up by macrophages and form foam cells, eventually leading to atherogenesis,[5] which damages the maternal vascular endothelium.

Uric acid is an insoluble product of purine metabolism and is a marker of oxidative stress and tissue injury. Hyperuricemia has been associated with severe preeclamptic changes in the uteroplacental vascularity and finally, poor fetal outcome. Redman et al. demonstrated that increased serum uric acid was associated with an increase in perinatal morbidity.[6] The normal serum uric acid level in non-pregnant females is 3.5–7 mg/dl. It is 25–30% lower in pregnancy but reaches normal levels during the third trimester.

There is cumulative evidence that there is increased ROS and oxidative stress in pre-eclampsia. However, some studies have reported raised MDA levels in the blood, whereas others have found decreased MDA or no significant change.[7] There are similar discrepancies in the estimation of antioxidant levels. Further, there is evidence based on renal function studies in PIH patients that some of these biochemical changes occur before the onset of any clinical signs and symptoms.

Hence, this study was attempted to compare oxidant and antioxidant status by estimating the levels of serum MDA and uric acid levels in pair-matched maternal and cord blood in normotensive and hypertensive pregnancies. This study also aims to evaluate whether there occurs reflection of oxidative stress from hypertensive mother to their neonate.

**MATERIALS AND METHODS**

This cross-sectional study was carried out in the Department of Biochemistry, JJM Medical College, Davangere, Karnataka. A total of 100 pregnant women admitted in Chigateri government hospital, teaching hospital attached to JJM Medical College, Davanagere were included in the study, of which 50 normotensive normal-proteinuric pregnant women were chosen as controls. Fifty pregnant women diagnosed with pre-eclampsia were chosen as cases.

Written informed consent was obtained from the study subjects after explaining the details of the study in their local language.

Ethical clearance (No. JMMC/IEC/11-2018, dated 14/03/2018) from the Institutional Ethical Committee was obtained.

**Inclusion Criteria**

Women with pre-eclampsia diagnosed based on the definition of the American College of Obstetricians and Gynaecologists:

1. Systolic blood pressure (SBP) >140 mm of Hg or rise of at least 30 mm of Hg
2. Diastolic blood pressure >90 mm of Hg or rise of at least 15 mm of Hg (manifested on two occasions at least 6 h apart)
3. Proteinuria of 300 mg or greater in 24 h urine collection or protein concentration of 1 g/l (on two occasions at least 6 h apart).

**Exclusion Criteria**

Pregnant women with essential hypertension, ischemic heart disease, and renal insufficiency were excluded from the study.

**Specimen Collection**

About 5 ml venous blood from the mother was withdrawn under sterile conditions from the median cubital vein and 5 ml cord blood was obtained during delivery. 2 ml serum obtained after centrifugation from the collected samples was used for estimation immediately after being separated.

**Analysis**

Estimation of serum uric acid by Caraway method where uricase converts uric acid present in sample to allantoin, carbon monoxide, hydrogen peroxide. Hydrogen peroxide is further acted upon by peroxidase resulting in formation of coloured compound (in presence of phenol derivative) which is measured at 520 nm.

MDA is estimated by by Nadiger et al. method. MDA formed by oxidation of unsaturated fatty acids reacts with Thiobarbituric acid forming a pink crystalline compound measured at 530 nm.
Statistics

Results are expressed as Mean ± standard deviation. The comparison was done by student t-test. Pearson’s correlation coefficient was assessed to evaluate the relationship between SBP and other parameters, namely maternal MDA, maternal uric acid, cord blood MDA, and cord blood uric acid.

RESULTS

There is no difference in mean age between normotensive and hypertensive pregnant women [Table 1].

Mean of SBP is higher in pre-eclamptic women, around 146 mm of Hg when compared to normotensive women, around 112 mm of Hg [Table 1].

Mean of birth weight is low, around 2.67 kg in newborns born to hypertensive mothers in comparison to birth weight of newborns to normotensive mothers, around 3.17 kg [Table 1].

Hypertensive women had higher MDA levels of 49.24 nmol/ml when compared to normotensive women with mean MDA of 28.37 nmol/ml [Table 1].

Mean of serum uric acid 9.05 mg/dl is higher in hypertensive women in contrast to normotensive women where mean serum uric acid is 4.76 mg/dl [Table 1].

Cord blood MDA is increased at 30.15 nmol/ml in neonates born to hypertensive mother, whereas it is lesser at 26.98 nmol/ml in neonates born to normotensive mothers [Table 1].

Serum uric acid levels are raised to a level of 7.32 mg/dl in cord blood of newborns born to hypertensive mothers when compared to 4.29 mg/dl of serum uric acid levels in newborns born to normotensive mothers [Table 1].

A positive correlation is observed between maternal SBP with maternal MDA and maternal serum uric acid level and also with cord blood MDA and cord blood uric acid [Table 2].

Strength of association is very high between SBP with maternal MDA and uric acid. There is a moderate association between maternal SBP and cord blood MDA and cord blood uric acid [Figures 1-4].

DISCUSSION

Pre-eclampsia remains a complex multi-system syndrome that is much more than simply gestational hypertension. Extensive research and availability of better research tools along with a better understanding of the underlying disease pathology have led to the development of better tests to detect and diagnose pre-eclampsia. In our present study, we tried to compare the biochemical imbalance in hypertensive pregnancies and normotensive pregnancies and the effect of oxidative stress on the neonates.

In our study, we found a significant increase in serum MDA level in maternal blood in pre-eclamptic women compared to normotensive women. Increased MDA levels indicate excessive lipid peroxidation in pre-eclampsia MDA oxidizes LDL, which is taken up by macrophages and forms foam cells leading to atherogenesis, playing a role in the pathogenesis of pre-eclampsia. Further raise in MDA also suggests overproduction of ROS, which exceeds the capacity of antioxidant defense mechanism to neutralize.

We observed a significant increase in uric acid in pre-eclamptic women when compared to normotensive mothers. Oxidative stress leads to endothelial damage and tissue ischemia, which manifests as glomerular endotheliosis in the kidneys, which leads to decreased glomerular filtration, increased absorption, and decreased secretion; hence, hyperuricemia is evident. Pre-eclampsia is also a condition of heightened inflammation, and uric acid acts as a potent mediator of inflammation.

A positive correlation was observed between SBP and maternal MDA. Increase in SBP was associated with a concomitant increase in MDA. This oxidant was chelated by

| Table 1: Comparison of age, SBP, birth weight, serum uric acid, and MDA in normotensive and hypertensive mothers and cord blood of neonates born to the mothers |

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normotensive</th>
<th>Hypertensive</th>
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<tbody>
<tr>
<td>Age</td>
<td>24.88±3.35</td>
<td>24.90±3.14</td>
</tr>
<tr>
<td>SBP</td>
<td>112.32±10.36</td>
<td>146.52±8.80</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>3.17±0.32</td>
<td>2.67±0.59</td>
</tr>
<tr>
<td>Maternal blood MDA (nmol/ml)</td>
<td>28.37±7.33</td>
<td>49.24±4.96</td>
</tr>
<tr>
<td>Maternal blood serum uric acid (mg/dl)</td>
<td>4.76±1.25</td>
<td>9.05±2.13</td>
</tr>
<tr>
<td>Cord blood MDA (nmol/ml)</td>
<td>26.98±8.83</td>
<td>30.15±10.16</td>
</tr>
<tr>
<td>Cord blood serum uric acid (mg/dl)</td>
<td>4.29±1.07</td>
<td>7.82±1.88</td>
</tr>
</tbody>
</table>

MDA: Malondialdehyde, SBP: Systolic blood pressure

| Table 2: Pearson’s correlation analysis for SBP, serum uric acid, MDA |

<table>
<thead>
<tr>
<th>SBP</th>
<th>Cord blood serum uric acid (mg/dl)</th>
<th>Maternal blood serum uric acid (mg/dl)</th>
<th>Cord blood MDA (nmol/ml)</th>
<th>Maternal blood MDA (nmol/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.695</td>
<td>0.706</td>
<td>0.216</td>
<td>0.833</td>
</tr>
</tbody>
</table>

MDA: Malondialdehyde, SBP: Systolic blood pressure
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an increase in uric acid in maternal blood depicting a positive correlation between maternal MDA and uric acid.

There was a reflection of oxidative stress from hypertensive mother to their neonates with a concomitant increase in MDA and uric acid in cord blood of neonates. However, the increase in MDA and uric acid in the cord blood of neonates was much lower compared to their hypertensive mothers. The intrauterine period is crucial for the proper growth and development of the fetus. Cord blood antioxidant capacity reflects the adequate placental functioning, which protects the fetus from oxidative injury. Oxidative stress induces hypoxia in fetal tissue triggering xanthene oxidase activity leading to purine catabolism, manifesting as hyperuricemia in cord blood.

Our study results were in accordance with that of Saxena et al.,[10] Hubel et al.,[11] and Freund et al.[12] Who reported a significant increase in MDA in severe pre-eclamptic subjects [Figure 1] when compared to normotensive mothers. In contrast to our study, Aksoy and Baksan[13] and Regan et al.[14] found no evidence of oxidative stress in PH. Uric acid levels were increased in hypertensive mothers to chelate the raised MDA, an oxidant generated during oxidative stress induced by hypertension of pregnancy. Similar results were reported by Thangaratinan et al.,[1] Krishna et al.,[15] Tadi and Mahendran[16] and Vyakaranam et al.[17] Contrary to our study, Jacobson et al.[18] and August et al.[19] concluded uric acid to be a poor predictor of pre-eclampsia.

Reflection of oxidative stress among neonates born to hypertensive mothers [Figure 3] was also reported by Suhail et al.[7] and Bharadwaj et al.,[20] whereas Tastekin et al.[21] and Karsdorp et al.[22] reported no significant change in maternal and cord blood MDA and uric acid.
Strength of Our Study

Not many studies are available to correlate oxidative stress among hypertensive mothers and their neonates. The strength in our study lies in the fact that there was clear cut evidence of reflection of oxidative stress from hypertensive mothers to their neonates that manifest as fetal hypoxia, intrauterine growth restriction, and LBW babies.

Limitation of Our Study

We could not evaluate prospectively the oxidative stress progression in hypertensive mothers. PIH becomes evident as early as the 20th week of gestation, so there is a need to assess the oxidative stress and antioxidant status early in the pregnancy and appropriate intervention to prevent mortality and morbidity associated with pre-eclampsia.

CONCLUSION

We conclude that there occurs a reflection of oxidative stress from hypertensive mothers to the neonates. This calls for early identification of hypertensive mothers and their prompt treatment may reduce the reflection of oxidative stress and prevent the morbidity and mortality associated with hypertensive disorders of pregnancy.

ACKNOWLEDGMENTS

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REFERENCES


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