Gender disparity in Oxidative stress in healthy preterm neonates delivered vaginally

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Abstract:
Oxidative stress occurs as a consequence of imbalance between the formation of oxygen free radicals and inactivation of these species by antioxidant defence system. Birth exposes the neonate to a higher PO2 than experienced in utero. Studies have shown that female neonates have better survival as compared to male babies especially in the preterm group. This study was undertaken to find out early sex differences in oxidative stress. The oxidative stress biomarkers MDA and Nitrites and the antioxidant vitamins C & E were measured in cord plasma of these neonates. It was observed that Males had higher levels of plasma MDA and Nitrites (p< 0.05) than females. No difference was found in the antioxidant vitamin C and E levels (p>0.05) in the males and females respectively. Thus, sex-based differences in oxidant injury vulnerability occurring early in life could represent a biological mechanism contributing to better survival and gender disparity later in life.

Keywords: Oxidative stress, Gender disparity, Preterm neonates

Introduction:
Oxidative stress occurs as a consequence of imbalance between the formation of oxygen free radicals and inactivation of these species by antioxidant defense system. Birth exposes the neonate to a higher PO2 than experienced in utero. The sudden increase in alveolar oxygen concentration and arterial PO2 after delivery increases the formation of reactive oxygen species and reactive nitrogen species (ROS & RNS) in the lungs and other organs. Premature neonates, with their decreased antioxidant defense, are highly susceptible to the deleterious effects of reactive oxygen species (ROS) generated in the fetal to neonatal transition. Impaired antioxidant defenses, occurring at a time when OFR production is both frequent and severe, render the premature neonate extremely susceptible to the development of oxidative stress.

The oxidative stress can be measured by measuring its various parameters. Lipid per oxidation products can be measured as an index of oxygen free radicals. Malonaldehyde is one such product and its measurement can provide a sensitive index of lipid per oxidation and oxidative stress. Nitric oxide (NO) is synthesized in an oxygen dependant reaction catalyzed by nitric oxide synthetase (NOS). Since measurement of nitrous oxide radical itself is difficult because of its poor stability with a very short half life, plasma levels of inorganic nitrites and nitrates, representing the stable and final metabolites of the
NO metabolic pathway, can be measured with colorimetric assays. The human body contains a pool of antioxidant substances which finely balance the oxidants to maintain a state of health. The antioxidant water soluble ascorbic acid (Vitamin C) traps free radicals and reactive oxygen species. Under all types of oxidising conditions ascorbic acid completely protects lipids in plasma and low density lipoproteins against per oxidative damage.

The “male disadvantage” with respect to neonatal mortality has been recognised for more than two decades. The relative vulnerability of boys to perinatal mortality and morbidity has been documented in perinatal studies. Brothwood et al confirmed the “relative vulnerability of boys to perinatal mortality and morbidity” described in earlier reports. They observed a higher mortality and more postnatal complications in very low birth weight preterm boys than in girls.

In general, males have increased morbidity and mortality as compared to females especially in the preterm group. Whether this increased mortality and morbidity is related to oxidative stress was the idea behind this study. With this in mind, this study was undertaken to assess whether gender differences are present in the levels of oxidative stress in the preterm newborns and whether these differences in the early developmental progress can partly explain the differences in perinatal and postnatal morbidity and mortality in the male neonates.

**Material and Methods**

We designed a cross sectional study. The present study was carried out in the department of Physiology during the period from 1st November 2011 to 31st October 2013. Newborns delivered vaginally in the labor room of the obstetric department, were selected as the study population. A total of 45 preterm (GA 34-36 weeks) healthy newborns – 23 male and 18 female were included. The gestational age of the newborns were calculated according to the New Ballard Scoring system for assessment of gestational age. These neonates were born to mothers who didn’t have any obvious medical or obstetric complications. The selected newborns cried immediately after birth and required no resuscitation or immediate NICU admission for any other reasons. The birth weight of all these newborns ranged between 2.2- 2.49 kgs. The cord blood was collected in the EDTA bulb from the newborn’s cord immediately after their delivery in the labor room. The blood was centrifuged at 3000 rpm for 30 min and the plasma was separated and processed within 6 hours of collection of sample.

The four parameters namely Malonaldehyde, nitrates, Vitamin C and Vitamin E levels were measured in the cord blood of these newborns. MDA was measured by action of thiobarbituric acid which together with MDA forms a pink chromogen compound whose absorbance at 530nm was recorded. The concentration of MDA (nmol/dl) was calculated using the standard curve obtained from the reaction between varying MDA concentrations. Nitrites were measured using the Griess reagent assay method. In acid solution, nitrite is converted into nitrous acid (HNO$_2$), which is reacted with sulphonamides. This sulphamidamide-dizonium salt is then reacted with N-(1-Naphthyl)- ethylenediamine (NED) to produce a chromophore, which is measured at 540nm. Concentration of nitrites (µmol/ l) is calculated from the standard curve obtained from the reaction between varying nitrite concentrations. Vitamin C was measured by its reaction with phosphotungstate. The acid phosphotungstate (PTA) used in this method serves as plasma protein...
precipitant as well ascorbic acid extractant and color developing agent, as it gets reduced to tungstate blue by ascorbic acid. The blue colour is measured in a spectrophotometer at 700nm. Vitamin C concentrations (mg/dl) was calculated using standard formula. The estimation of vitamin E levels in the cord blood was done by Emmerie Engel procedure in which tocopherol is oxidized to tocopheryl quinone by ferric chloride and resultant ferrous ion is complexed with 2,2’-dipyridyl to produce a red coloured compound whose absorbance was measured at 520nm. The vitamin E levels (mg/dl) were determined from the standard curves already calculated from different concentrations of vitamin E. The present study was approved by the ethical committee of MGIMS, Sewagram.

Data Analysis:
Statistical software EPI INFO version 7 was used for statistical analysis. All values were expressed as Mean ±SD values in each group. We analyzed each parameter separately. We applied the ‘z’ test for calculating the significance. P < 0.05 was considered statistically significant.

Results:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Male</th>
<th>Female</th>
<th>z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td>8.36±0.07</td>
<td>8.19±0.05</td>
<td>8.14</td>
<td>0.000, S, p&lt;0.05</td>
</tr>
<tr>
<td>Nitrates</td>
<td>30.46±0.43</td>
<td>28.78±2.21</td>
<td>3.64</td>
<td>0.000, S, p&lt;0.05</td>
</tr>
<tr>
<td>Vit C</td>
<td>79.48±2.61</td>
<td>80.12±3.31</td>
<td>0.69</td>
<td>0.49, NS, p&gt;0.05</td>
</tr>
<tr>
<td>Vit E</td>
<td>6.73±0.48</td>
<td>6.58±0.61</td>
<td>0.88</td>
<td>0.38, NS, p&gt;0.05</td>
</tr>
</tbody>
</table>

The present study shows significantly increased levels of MDA (8.36±0.07 vs 8.19±0.05; p<0.05) and Nitrates (30.46±0.43 vs 28.78±2.21; p<0.05) in the preterm males as compared to preterm female babies. No significant differences were noticed in the levels of antioxidant vitamin C & E levels in the preterm males and female newborns (p>0.05).

Discussion:
Oxygen, indespensible for maintaining life, sometimes becomes toxic and results in generation of most aggressive agents such as reactive oxygen species (ROS) also called as free radicals (FR). Oxidative stress (OS) results from imbalance between reducing agents and enzymes involved in the
removal of free radicals (FR) and/or reactive oxygen species (ROS). In the present study we found significantly higher levels of MDA and Nitrites in the preterm males as compared to females. The levels of antioxidant vitamins did not change significantly in the two groups. Maulik et al\textsuperscript{14} in their study has stated that the preterm baby is more susceptible to oxidative damage as compared to full term babies. Premature infants are at particular risk from oxidative stress because both endogenous and passively acquired exogenous antioxidant defense systems do not accelerate in maturation until late in the third trimester. It has been previously reported that Preterm delivery is the most important contributor to the neonatal mortality rate\textsuperscript{15}. Secondly, preterm babies have more incidence of oxygen radical diseases of the newborn like bronchopulmonary dysplasia, hypoxic ischaemic encephalopathy, hyperbilirubenemia, retinopathy of prematurity, periventricular leukomalacia and patent ductus arteriosus\textsuperscript{5}. This increased morbidity and mortality could be related to increased oxidative stress and low levels of antioxidants in the preterm babies. As such preterm newborns have higher incidence of mortality and morbidity as compared to term. Saugstad\textsuperscript{16} coined the phrase “oxygen radical diseases of neonatology”. Oxidative stress has been postulated to be implicated in several newborn conditions and the idea contends that oxidative stress affects different organs, often simultaneously, giving rise to different signs according to the organ most affected. He included bronchopulmonary dysplasia/chronic lung disease, retinopathy of prematurity and necrotising enterocolitis in this category. Later, Haynes RL et al\textsuperscript{17} in their study proved that free radicals are also involved in periventricular leukomalacia as well as in regulating the ductus arteriosus and pulmonary circulation.

Many studies have reported that males have increased incidence of complications and poor survival as compared to female babies. This difference is more pronounced in the preterm group. In particular, a greater incidence of problems in pregnancy and complications of delivery, birth asphyxia and neurological signs in the neonatal period\textsuperscript{18} a higher incidence of infections, including those occurring after prolonged rupture of membranes\textsuperscript{19} and congenital malformations\textsuperscript{20} have been reported in boys. Hyaline membrane disease\textsuperscript{21} and bronchopulmonary dysplasia have been found more often in preterm boys. In general, males have increased morbidity and mortality as compared to females especially in the preterm group. Since these diseases have been linked to toxicity of ROS, the increased oxidative stress in the preterm males as is observed in our study can partly explain the differences in perinatal and postnatal morbidity and mortality in the male neonates. There are some hints that the male gender can be disadvantageous for very low birth weight infants\textsuperscript{22} (Stevenson et al.2000) and even healthy male infants are more prone to lower Apgar score at 5 min and have a higher perinatal morbidity and mortality\textsuperscript{23}. Lavoie and Chessex\textsuperscript{24} showed better viability, higher glutathione reductase activity and higher total intracellular glutathione content in female-derived cells (umbilical endothelial cells, cells from tracheal aspirates). Parmigiani et al\textsuperscript{25} concluded that normal female newborns have lower levels of reactive oxygen metabolites. Thus this study is in accordance with the above mentioned studies and proves that preterm male babies have more oxidative stress as compared to female neonates.

Limitations:
Our study has few limitations. We did not take into consideration the maternal levels of antioxidant vitamins. We considered the mothers to be nutritionally normal on the basis of their normal ranges of hemoglobin, serum albumin levels, post pregnancy weight and no obvious signs of vitamin deficiencies. We analyzed antioxidant levels in relation to birth weight and gestational age but not with other parameters of neonatal anthropometry, which might have yielded important information. We recognize that our study and its implications are limited by the modest size and cross-sectional design of the study. Also it remains difficult to determine the extent to which oxidative stress is a contributory pathogenic mechanism in the etiology of newborn pathologies.

Conclusion:
The findings of our study do substantiate that there are sex-based differences in oxidant injury vulnerability occurring early in life which could represent a biological mechanism contributing to better survival and gender disparity later in life. In spite of the limitations, this study has practical and obvious implications: it offers clinicians more understanding of the major antioxidant systems, and may help in the refinement of management strategies for nutrition and the intensive care of preterm neonates. Whether this gender disparity has a hormonal basis or not needs to be studied in details. Perhaps it is the right time to pursue intensive research into oxidative stress in newborn patients with a wide range of diseases.

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