Original Research Article

Placental ischemia modified albumin may be a marker of oxidative stress in pre-eclampsia

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Abstract: Preeclampsia is a known ischemic condition. It increases the maternal oxidative stress. Babies born to the preeclamptic mothers may have appropriate birth weight and gestational age. Studies about the oxidative stress in those apparently healthy babies are scarce. 45 preeclamptic and 40 age matched control mothers were selected for the study. Ischemia modified albumin [albumin cobalt binding assay] and protein carbonyl [DNPH method] were estimated in them. Significant rise of Ischemia modified albumin was found in cord blood of babies born to preeclamptic mothers.(p<0.0001 ). No significant alteration was found in cord blood protein carbonyl levels between case and control groups. Babies born to preeclamptic mothers were suffering from oxidative stress. Those babies should be kept under observation and should be supplemented by antioxidants.

Keywords: Cord blood, Ischemia modified albumin, protein carbonyl, Preeclampsia

INTRODUCTION:

Normal foetal development depends on placental growth. Problem like obesity and delayed pregnancy may cause placenta related disorders. Oxidative stress in placental tissues plays a significant role in the progress of foetal abnormalities and major causes of maternal as well as peri-natal mortalities [1]. Preeclampsia is associated with uterine hypoxia and inadequate uterine blood supply [2].

Ischemia modified albumin (IMA) is a recently identified biomarker, extensively studied in acute coronary syndrome [3]. Metals like cobalt, copper can bind with the amino terminal end of serum albumin that is specifically susceptible to damage by ischemia [4]. Ischemia reperfusion injury generates Reactive Oxygen Species (ROS) that modifies N terminal region of human serum albumin. This modified albumin has reduced binding capacity to transitional metals, and called IMA [5, 6, 7].

As placental hypoxia is the hallmark of etiopathogenesis of preeclampsia, cord blood IMA can be a potential biomarker of foetal hypoxia or foetal oxidative stress among preeclamptic mothers. However, the ROS generation when exceeds antioxidant capacity, they stimulate lipid peroxidation and protein inactivation, the resulting damage leads to oxidative stress. Protein oxidation can be assessed by protein carbonyl [8].

The increment of those plasma biomarkers may also cause perinatal asphyxia and hypoxic ischemic encephalopathy in term infants [9].

Increased level of IMA and protein carbonyl have been observed in blood of PE mothers [10, 11, 12] but a very few studies were found regarding them in cord blood of preeclamptic mothers. In our study, the potential of ACB and protein carbonyl assay in cord blood remains to be explored, to assess the oxidative stress in foetus of preeclamptic mothers.

MATERIALS AND METHODS:

The present study was undertaken in the neonatal care unit (Department of Pediatric Medicine), labour room (Department of Obstetrics), and Department of Biochemistry, Calcutta National Medical College, and Kolkata. It was an observational analytical study of case-control design and the study period extended from 01.06.2013 to 31.05.2014.

Selection of cases and controls:

45 term newborns born to preeclamptic mothers were recruited into the study. These babies were delivered through spontaneous vertex delivery.
They were appropriate birth weight and gestational age. 40 newborn babies born to normal mothers of same criteria were also selected as controls. The cases and control group were age and gestational age matched.

**Exclusion criteria:**
Each mother was certified of not being a known hypertensive, diabetic, and active smokers. Babies of women with prolong labour were also excluded. None of the babies was among a set of multiple gestations and those with obvious congenital malformation were excluded. Low-birth weight, premature and babies born of caesarean section were also excluded from the study.

**Sample collection and processing:**
A volume of 5 ml of cord blood was collected before cord is clamped and kept in ice-filled containers and brought to the Biochemistry Laboratory of Medical College within ½ h then serum is separated and stored at −20°C for IMA estimation. Baby was weighed in kilogram.

**ACB Assay for IMA**

**Principle**
ACB assay for determination of the level of IMA in serum is done by addition of a known amount of cobalt (II) to a serum specimen and measurement of the unbound cobalt (II) from the absorbance of the coloured complex between dithiotreitol (DTT) and free cobalt by spectrophotometer which is indicative of the level of IMA [13]. Intensity of the coloured complex varies inversely with the ACB.

**Assay protocol**
A volume of 200 μL of serum was mixed with 50 μL of 1 g/l cobalt chloride (CoCl2) solution. Vigorous mixing was done followed by incubation for 10 min. Then 50 μL of 1.5g/l solution of DTT was added and mixed following which an incubation for 2 min. Finally, 1 ml of 9 g/l of Nacl was added, and absorbance was read at 470 nm in a spectrophotometer [13]. The blank was prepared similarly with the exclusion of DTT. Standard curve was prepared using different concentrations of CoCl2. There is a considerable degree of variation among the units of expression.

Measurement of protein oxidation was assayed for protein carbonyl content using the 2,4-dinitrophenyl hydrazine reaction. Serum samples were divided into two equal aliquots containing approximately 1.0 mg of protein each. Both aliquots were precipitated with 10% trichloroacetic acid (w/v, final concentration). One sample was treated with 2N HCl, and the other sample was treated with an equal volume of 0.2% (w/v) dinitrophenylhydrazine (DNPH) in 2 N HCl. Both samples were incubated at 25°C in 15-mL conical glass centrifuge tubes and stirred at 5-minutes intervals. The samples were then re-precipitated with 10% trichloroacetic acid (final concentration) and subsequently extracted with ethanol/ethyl acetate (1: 1, v/v) and then re-precipitated at 10% trichloroacetic acid. The pellets were carefully drained and dissolved in 6M guanidine HCl in 20 mM sodium phosphate buffer, pH 6.5. Insoluble debris was removed by centrifugation at 6000 ×g at 4°C. The difference between the spectra of the DNPH-treated sample versus the HCl control was determined and the results are expressed as nmol of DNPH incorporated/mg of protein based on an average absorptivity of 21.0 mM-1 cm-1 for most aliphatic hydrazones [14].

**Ethical clearance:**
The study was approved by the Institutional Ethical Committee of the said institutions. All mothers included in the study provided signed, informed consent before participation.

**Statistical analysis**
Statistical analysis has been done by unpaired t test (Graph pad software).

**RESULTS:**
Table 1 and 2 shows the profile and parameters of cases and controls; table 3 compares the means of cases and controls, as well as significance. Mean maternal age was found to be 23.83± 0.47 years in controls and 24.55± 0.58 years in cases. No significant difference was observed. Mean gestational age was 38.5±0.19 weeks in controls and 38.2±0.16 weeks in cases. No significant difference was found. Mean birth weight of the babies was 2.72± 0.17 kgs in controls and 2.87± 0.14 in cases. No significant alteration was found. Mean protein carbonyl level was 5.48±0.10 in controls, 5.53±0.14 in case group, so significant difference was found. Mean IMA in controls was 31.55± 1.15 U/ML in controls whereas the same in cases are 45.66±1.6 UNIT/ML. We observed the significant difference of mean IMA between cases and controls (p<0.0001).
Table 1: showing demographic profile and Biochemical parameters of controls (n= 40)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MEAN</th>
<th>SD</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>23.83</td>
<td>0.47</td>
<td>0.67</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>38.5</td>
<td>0.19</td>
<td>0.16</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>2.72</td>
<td>0.17</td>
<td>0.036</td>
</tr>
<tr>
<td>Ima (units/ml)</td>
<td>31.55</td>
<td>1.15</td>
<td>0.18</td>
</tr>
<tr>
<td>Protein carbonyl (nmol/mg)</td>
<td>5.48</td>
<td>0.10</td>
<td>0.0158</td>
</tr>
</tbody>
</table>

Table 2: showing demographic profile and Biochemical parameters of cases (n= 45)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MEAN</th>
<th>SD</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>24.55</td>
<td>0.58</td>
<td>0.47</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>38.2</td>
<td>0.16</td>
<td>0.14</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>2.87</td>
<td>0.14</td>
<td>0.031</td>
</tr>
<tr>
<td>Ima (units/ml)</td>
<td>45.66</td>
<td>1.6</td>
<td>0.23</td>
</tr>
<tr>
<td>Protein carbonyl (nmol/mg)</td>
<td>5.53</td>
<td>0.14</td>
<td>0.0209</td>
</tr>
</tbody>
</table>

Table 3: Showing comparison of means of different variables between cases and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean of cases</th>
<th>Mean of controls</th>
<th>95 % Cl of Difference</th>
<th>t value</th>
<th>Significance p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age in years</td>
<td>24.55</td>
<td>23.83</td>
<td>38.11-38.84</td>
<td>2.17</td>
<td>0.66*</td>
</tr>
<tr>
<td>Gestational age in weeks</td>
<td>38.2</td>
<td>38.5</td>
<td>21.87-24.92</td>
<td>0.52</td>
<td>0.85*</td>
</tr>
<tr>
<td>Birth weight in kgs</td>
<td>2.87</td>
<td>2.72</td>
<td>2.70-2.84</td>
<td>1.47</td>
<td>0.92*</td>
</tr>
<tr>
<td>Ima in units/ml</td>
<td>45.66</td>
<td>31.55</td>
<td>-14.71 to 13.50</td>
<td>46.16</td>
<td>&lt;0.0001**</td>
</tr>
<tr>
<td>Protein carbonyl in nmol/mg</td>
<td>5.53</td>
<td>5.48</td>
<td>-0.1031 to 0.0031</td>
<td>1.8731</td>
<td>0.0646*</td>
</tr>
</tbody>
</table>

* NS (not significant)    ** S (significant)

DISCUSSION:

Of all pregnancies the prevalence of preeclampsia and eclampsia are 2%-8% and overall 10%-15% of maternal deaths are associated with them [15].

Preeclampsia is marked by ischemic reperfusion injury leading to oxidative stress and generation of Reactive Oxygen Species (ROS), failure to control the condition complicates to eclampsia.

Different biochemical markers have been tried in maternal and cord blood in oxidative stress related conditions [16]. But in preeclampsia, results are inconsistent, particularly in those, where the outcome is apparently normal. (Spontaneous vertex delivery with appropriate birth weight and gestational age).

Obviously, preeclampsia is marked by placental hypoxia; furthermore it causes ischemic
reperfusion injury and generation of free radicals which alters the amino terminal of serum albumin resulting in reduced binding of albumin to metal compared to normal pregnant mothers. Roy et al found that the ROS can modify the N terminus of albumin, resulting reduced affinity to cobalt. This test has shown increased sensitivity and specificity as compared to more conventional cardiac enzymes in diagnosis of acute coronary syndrome.[3] and myocardial ischemia [17]. The scope of IMA assay was extended to find out intrauterine hypoxia. IMA levels were found to be significantly higher in cord blood of normal term delivery newborns compared to complicated deliveries [18]. Elevated levels of IMA were found in blood in mothers with recurrent first-trimester abortions [19] and preeclampsia [11, 20]. The above mentioned studies, has established the position of IMA in ischemic or hypoxic conditions.

Results of our study shows significant increase of IMA in cord blood of preeclamptic mothers(p<0.0001), but no significant difference are found in maternal age, gestational weeks, birth weights and mean protein carbonyl levels between cases and controls. Mean maternal age, gestational age, and birth weights were kept as close as possible, all the newborns were delivered under spontaneous vaginal delivery so that the effect of following factors cannot alter the oxidative stress parameters like IMA and protein carbonyl levels. From the above findings it is obvious that, the newborns of preeclamptic mothers are having some amount of oxidative stress than the newborns of control mothers. Our study is similar with the study of Mehmetoglu I, et al.; who observed that significant rise of cord blood oxidized LDL and IMA levels 7 days after birth in neonates born to preeclamptic mothers, which might be a reflection of increased oxidative stress of preeclampsia [21].

We didn’t found the significant increase of protein carbonyl in cord blood of preeclamptic mothers when compared to controls. This corroborates the study of Noh EJ [22] et al.; who observed the similar findings. As the deliveries are apparently healthy in respect of maternal and gestational age, as well as birth weights, we can hypothesize that the oxidative stress is not sufficient to alter protein carbonyl levels in case group.

Ischemia poses a risk in infants by altering cerebral blood flow [23]. The chances of brain damage being amplified many times in reperfusion injury. Furthermore it leads the generation of free radicals that damages cell membrane by lipid peroxidation, inactivation of enzymes, DNA damage and degradation of structural lipids:IMA and protein carbonyl reflect the protein damage [24].

Oxidative stress in newborns develops in a same way like adults. When the ROS exceeds the counteraction of available antioxidants, the oxidative stress takes place. All the oxidants and antioxidants are associated with placenta and birth weight. Oparinde DP [16] suggested that the remnant oxidative stress of pregnant women has been observed in newborns. This observations were further strengthened [25, 26, 27]. Furthermore, antioxidant capacity may not have been fully developed in newborns [16].

From the results of our study we can assume that some amount of oxidative stress is still persisting in the cord blood of preeclamptic mothers.IMA is a simple and cost effective biochemical parameter which can be assayed in cord blood of preeclamptic mothers.

**CONCLUSION:**

Oxidative stress is still persisting in babies of preeclamptic mothers with spontaneous vaginal delivery and vertex presentations, with appropriate placental and birth weight. The babies should be in observation and antioxidant supplementation.

**REFERENCES:**