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Original Research Article

Cord Blood Ischemia Modified Albumin May Predict the Oxidative Stress In Newborns, a Prospective Study in Kolkata

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ABSTRACT

Background- Preeclampsia is an ischemic condition which increases the maternal oxidative stress. Babies may born to the preeclamptic mothers with appropriate birth weight and gestational age. Studies about the oxidative stress in those babies are scrace.
Materials and methods- 45 preeclamptic and 40 normal mothers were selected for the study. Cases and controls were age matched. Ischemia modified albumin (IMA) was estimated in them by albumin cobalt binding assay.
Results-Significant increase of IMA was found in cord blood of babies born to preeclamptic mothers ($p<0.0001$).
Conclusion: Babies born to preeclamptic mothers were suffering from oxidative stress. Those babies should be kept under observation and should be supplemented by antioxidants.

Key Words: Cord blood, Ischemia modified albumin, Preeclampsia.

INTRODUCTION

Fetal growth and well-being depends on placental growth. Recently the problem like obesity and delayed pregnancy have increased the placenta related disorders. Oxidative stress in utero placental tissues plays a significant role in the progress of placental abnormalities. Placental diseases are major causes of maternal and perinatal mortality. [1] Preeclampsia is associated with uterine hypoxic environment characterized by inadequate uterine blood supply. [2]

Ischemia modified albumin (IMA) is a recently developed biomarker extensively studied and used in acute coronary syndrome. [3] Transitional metals like cobalt, copper can bind with the amino terminal end of (S) albumin, which is particularly susceptible to damage by ischemia. [4] Ischemia reperfusion injury generates Reactive Oxygen Species (ROS) which modifies N terminal region of human serum albumin. This modified albumin reduces the capacity to bind transitional

metals, and called IMA. [5-7]

Binding site can be altered within minutes of the ischemic event via induced endothelial and extracellular hypoxia, acidosis, free radical injury, and sodium and calcium pump disruptions. [8]

Decreased affinity of albumin to cobalt binding has been utilized to produce a rapid automated test, called as albumin cobalt binding (ACB) assay. [9]

As placental hypoxia is the hallmark of etiopathogenesis of preeclampsia, cord blood IMA can be a potential biomarker of fetal hypoxia or fetal oxidative stress among preeclamptic mothers.

Increased level of IMA has been observed in blood of preeclamptic (PE) mothers; [10,11] but a very few studs were found regarding ACB assay in cord blood of preeclamptic mothers.

In our study, the potential of ACB assay in cord blood remains to be explored, to assess the oxidative stress in fetus of preeclamptic mothers.

MATERIALS AND METHODS:

The present study was undertaken in the neonatal care unit (Department of Pediatric Medicine), labor room (Department of Obstetrics), and Department of Biochemistry, Calcutta National Medical College, 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 newborn babies 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 colored complex between dithiothreitol (DTT) and free cobalt by spectrophotometer which is indicative of the level of IMA. [12] Intensity of the colored 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 ($CoCl_2$) solution. Vigorous mixing was done followed by incubation for 10 min. Then 50 μL of 1.5 g/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 $CoCl_2$. There is

a considerable degree of variation among the units of expression.

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 showed the profile and parameters of cases and controls, table 3 compared 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 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 cases (n=45)

Parameter	MEAN	SD	SEM
Maternal age (years)	23. 83	0.47	0.67
Gestational age (weeks)	38.5	0.19	0.16
Birth weight(kg)	2.72	0.17	0.036
Ima (units/ml)	31.55	1.15	0.18

SD- Standard Deviation, SEM- Standard error of Mean

Table 2: Showing Demographic profile and Biochemical parameters of controls (n=40)

Parameter	MEAN	SD	SEM
Maternal age (years)	24.55	0.58	0.47
Gestational age (weeks)	38.2	0.16	0.14
Birth weight(kg)	2.87	0.14	0.031
Ima (units/ml)	45.66	1.6	0.23

SD- Standard Deviation, SEM- Standard error of Mean

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

Variables	Mean of cases	Mean of controls	95% CI of Difference	t value	Significance
Maternal age in years	24.55	23.83	38.11-38.84	2.17	p = 0.66 *
Gestational age in weeks	38.2	38.5	21.87-24.92	0.52	P = 0.85 *
Birth weight in kgs	2.87	2.72	2.70-2.84	1.47	P = 0.92 *
Ima in units/ml	45.66	31.55	-14.71to -13.50	46.16	P<0.0001 **

*NS (Not Significant) **S (Significant)

DISCUSSION

Prevalence of preeclampsia and eclampsia are 2%-8% of all pregnancies and overall 10%-15% of direct maternal deaths are associated with them. [14]

Preeclampsia is characterized by failure of conversion of small diameter high resistance vessels to large diameter low resistance vessels hence leading to ischemic reperfusion injury leading to oxidative stress and generation of Reactive Oxygen Species (ROS), failure to control the condition complicates to eclampsia characterized by generalized seizures.

Different biochemical markers have been investigated in maternal and cord blood in oxidative stress related conditions. [15] 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).

Preeclampsia is characterized by placental hypoxia; furthermore it causes ischemic reperfusion injury results in generation of free radicals which alters the NH2 terminal of serum albumin resulting in reduced binding of albumin to cobalt compared to normal pregnant mothers. Roy et al reported that the ROS can modify the N terminus of albumin, resulting decreased 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. [16] The scope of ACB assay was further extended to find out intrauterine hypoxia. IMA levels were significantly higher in cord blood of newborns from normal term delivery compared to newborns from complicated deliveries. [17] In mothers with recurrent first-trimester abortions, [18] and preeclampsia cases elevated levels of IMA were found in blood. [11,19] 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 and birth weights between cases and controls.

All subjects were born at term. This has helped to remove the effect gestational age has on oxidative stress.

The age range of mothers of recruited babies was also considered. The effect of ageing on our selected biochemical parameter was also removed. All babies were born through spontaneous vertex delivery bearing in mind the effect that different mode of deliveries could have on oxidative stress. Birth weights of the babies selected for the study are also identical. The presence of oxidative stress may have some effects on birth weight. [15] The effect of oxidative stress on birth weight was also removed.

From the above findings it is obvious that, the newborns of preeclamptic mothers are having some amount of oxidative stress than the newborns of controls.

Our study is similar with the study of Mehmetoglu I, et al who has found that significant increase of cord blood oxidized LDL and IMA levels 7 days after birth in neonates born to preeclamptic mothers, which might be an indicator of increased oxidative stress in preeclampsia. [20]

Ischemia poses a risk in infants by altering cerebral blood flow regulating mechanism. [21] Reperfusion is a critical event, since the chances of brain damage being amplified many times. Reperfusion injury leads to the generation of free radicals which setup a chain of reactions that damages cell membrane by lipid peroxidation, inactivation of enzymes, DNA damage and degradation of structural lipids.IMA itself reflects the protein damage. [22]

Oxidative stress in babies develops in a same way it develops in adults. When the ROS exceeds the counteraction of available antioxidants, the oxidative stress takes place. All the oxidants and antioxidants are correlated with placenta and birth weight. According to Oparinde DP, the antioxidants are positively correlated with placenta and birth weights of newborns, as well as the oxidants are negatively correlated with them. [15]

They also suggested that the remnant oxidative stress of pregnant women has been found in the new born. It has been reported that oxidative stress in pregnant women is well correlated with that of the newborn (Arikan *et al.*, 2001; Erdem *et al.*, 2012; Şahinli *et al.*, 2012). [23-25] Furthermore, antioxidant capacity may not have been fully developed in them. [15]

From the results of our study we can assume that some amount of oxidative stress still exists 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.

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