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Original Research

GESDAV

Study of cord blood ischemia modified albumin levels in the evaluation of birth asphyxia

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ABSTRACT

Objective: In the birth asphyxia several new biochemical markers are now investigated. Recent studies indicate the role of ischemia modified albumin (IMA), a biomarker determined by assay of cobalt binding activity of albumin. The aim of this study was to evaluate the role of IMA in birth asphyxia. **Materials and Methods:** Forty newborns with Apgar score <7 were selected at random. Low-birth weight, premature and newborns from caesarian section were excluded. IMA was determined by assay of its cobalt binding activity with spectrophotometer. Mothers with a history of preeclampsia and pre-existing renal disease were kept out of the study. Similar observations were made in 40 normal newborns taken as control. **Results:** Cord blood IMA levels were higher in asphyxiated newborns compared to controls (55.7 ± 1.7 U/ml compared with 32.44 ± 1.15 U/ml). Raised levels of IMA in those subjects had shown very significant correlations with the Apgar score of 5 min, a finding also observed in the case of controls. **Conclusion:** The findings of the current study indicate that IMA can be a very useful marker in the evaluation of birth asphyxia.

KEY WORDS: Albumin cobalt binding assay, birth asphyxia, ischemia modified albumin, 5 min Apgar score

INTRODUCTION

Each year globally about 4 million babies die in the first 4 weeks of life [1]. Birth asphyxia is one of the leading (23%) causes of neonatal mortality [1]. Between 4 and 9 million neonates develop birth asphyxia out of which about 1.2 million die and at least the same number develop severe neurological consequences [2]. In India, the commonest primary cause of neonatal death is perinatal asphyxia (54.9%) [3]. So far all the recommended definitions of birth asphyxia make use of Apgar score [3,4]. It has been established that an Apgar score at 5 min is a better tool than 1 min score [5,6] for the assessment of newborn. However, the Apgar score is an expression of the infant's physiologic conditions and includes subjective components. Multiple studies have also established the relation between an Apgar score <7 at 5 min with increased risks of neurologic complications including cognitive impairment, epilepsy and cerebral palsy [7-9]. Still, using it to predict long-term outcome is not considered appropriate [9,10].

The limitations of Apgar score have prompted researchers to explore into other molecular markers of organ damage. Recent periodicals suggest role of protein markers like activin A [11,12], adrenomedullin [11,12], neuronal specific enolase [11], S100B [11,14], glial fibrillary acidic protein [11], matrix metalloproteinase-9 [13] and interleukin-6 [13] in birth asphyxia. Markers of lipid peroxidation like malondialdehyde [14,15], protein carbonyl [14], and nitric oxide [16] were also reported. However, the predictive value of these markers in perinatal asphyxia is limited [17]. Ischemia modified albumin (IMA) is a recently developed biomarker that is being extensively studied and used in the diagnosis of acute coronary syndromes (ACS) [18]. Transition metals like cobalt, copper and nickel bind to NH_2 terminus of serum albumin. Using the techniques of high-performance liquid chromatography, liquid chromatography-mass spectrometry and nuclear magnetic resonance it could be seen that the N-terminus of albumin is particularly susceptible to degeneration by ischemia [19].The binding site can be altered within minutes of an ischemic event via induced endothelial and extracellular hypoxia, acidosis, free radical injury, and sodium and calcium pump disruptions [20]. Roy *et al.* [21] had reported that reactive oxygen species including hydroxyl (OH) can modify the N-terminus of albumin, which can result in decreased affinity to cobalt. The resulting molecule, IMA and its reduced binding of cobalt, have been exploited to produce a rapid automated test, the albumin cobalt binding (ACB) assay [22]. This test

has shown increased sensitivity and specificity as compared to more conventional cardiac enzymes in diagnosis of ACS [18]. Measuring IMA levels using the ACB assay has been approved only as a rule-out marker for cardiac ischemia [23].

The scope of ACB assay was further extended to rule out intrauterine hypoxia. IMA levels were significantly higher in cord blood of newborns from normal term delivery compared to newborns from complicated deliveries [24]. In mothers with recurrent first-trimester abortions [25] and preeclampsia cases elevated levels of IMA were found in blood [26,27]. However, no significant changes in cord blood IMA levels were observed in babies of intrauterine growth retardation. Perinatal asphyxia is a state of hypoxia resulting in oxidative stress and generation of oxygen free radicals. There is also reperfusion injury to many organs of the body. Like other newer biomarkers mentioned earlier, the potential of ACB assay in these cases remains to be explored. Only few initial reports are coming [28,29]. The objective of the current study was to evaluate the role of IMA levels determined by ACB assay in birth asphyxia.

MATERIALS

The Study

The present study was undertaken in the neonatal care unit (Department of Pediatric Medicine), labor room (Department of Obstetrics), R G Kar Medical College and Hospital, Kolkata and Department of Biochemistry, Medical College, Kolkata. It was an observational analytical study of case-control design and the study period extended from 01.06.2011 to 31.05.2012.

Subjects

40 cases of birth asphyxia delivered at R G Kar Medical College and Hospital, Kolkata between 9 am to 3 pm during the study period were selected at random. Babies were of normal birth weight, did not establish the respiration at birth and having Apgar score ≤6 at 1 min. 40 normal babies who are or normal birth weight, established respiration at birth and Apgar score ≥7 at 1 min were chosen as a control. 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.

Exclusion Criteria

Low-birth weight, premature and babies born of caesarian section were excluded. Mothers with a history of preeclampsia were kept out of the study. To keep mothers with kidney disease out from the study serum creatinine level of >3.0 mg/dL was used as the exclusion criteria [18].

METHODS

Collection of Clinical Data and Cord Blood Sample

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. Respiratory status at birth, Apgar score at 1 min and 5 min and type of resuscitation required were noted in predesigned proforma. Clinical case records which also included gender of the baby, birth weight, age and parity of the mother, presence or absence of any obstetric complications like preeclampsia/eclampsia, presence or absence of chorioamnionitis, prelabor rupture of membranes, duration of labor, and detailed clinical examination of the baby.

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 dithiotreitol (DTT) and free cobalt by spectrophotometer which is indicative of the level of IMA [30]. 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 [31]. 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 of both ACB and IMA in different clinical studies [19]. In the current study values of IMA were expressed in units/ml.

Statistical Analysis

Analysis of data was done after computing the data for graphical and statistical analysis in SPSS Inc. Released 2007. SPSS 16.0 for Windows Version 16.0, Chicago SPSS Inc. Mean, standard error of the mean of different variables for each group was calculated. Independent sample *t*-tests were done for group comparison. Pearson's product moment correlation was computed for cases and control groups separately.

RESULTS

Results of the present study have been summarized in Tables 1-5. The mean birth weight of the cases was normal and within 95% confidence limit. It did not differ significantly with the control subjects. Mean of maternal age of the study population was slightly lower than controls (22.95 ± 0.48 compared to 24.67 ± 0.58 years). Mean gestational age was very similar among case and control group (38.4 ± 16 and 38.5 ± 0.19 weeks, respectively). The Apgar scoring of the cases and control subjects however showed very significant ($P < 0.01$) differences at both

1 min and 5 min. The mean Apgar score of the 40 cases was 4.75 ± 0.16 which indicates a moderate degree of asphyxia. The mean of 5 min Apgar score however was 7.35 ± 0.25 which indicates recovery. The mean Apgar score both at 1 and 5 min were in the nonhypoxic range in the control group. The IMA levels measured by ACB assay had shown very significant difference among the two groups [Table 3]. In Tables 4 and 5, the degree of correlation among the different parameters of the cases and controls, respectively, are shown.

Table 1: Descriptive statistics of case subjects (*n*=40)

Variables	Minimum	Maximum	Mean	SEM	95% CI of the difference
Birth weight	2.50	3.4	2.79	0.036	2.72-2.87
Maternal age (years)	19.00	29.0	22.95	0.48	38.06-38.73
Gestational age (weeks)	37.00	41.0	38.4	0.16	21.97-23.92
Apgar score at 1 min	3.00	6.0	4.75	0.16	4.42-5.08
Apgar score at 5 min	4.00	9.0	7.35	0.25	6.85-7.85
IMA (units/ml)	32.6	84.7	55.7	1.7	52.26-59.15

IMA: Ischemia modified albumin, CI: Confidence interval, SEM: Standard error of mean

Table 2: Descriptive statistics of control subjects (*n*=40)

Variables	Minimum	Maximum	Mean	SEM	95% CI of the difference
Birth weight (kg)	2.50	3.34	2.72	0.031	2.66-2.78
Maternal age (years)	19.00	34.0	24.67	0.65	38.15-38.94
Gestational age (weeks)	37.00	41.0	38.5	0.19	23.35-25.99
Apgar score at 1 min	3.00	9.0	7.9	0.17	7.55-8.25
Apgar score at 5 min	4.00	10.0	9.05	0.17	8.75-9.39
IMA (units/ml)	21.2	60.8	32.44	1.15	30.10-34.78

IMA: Ischemia modified albumin, CI: Confidence interval, SEM: Standard error of mean

Table 3: Comparison of means of the different variables among cases and controls

Variables	Mean of cases	Mean of controls	*t* value	Significance
Birth weight (kg)	2.79	2.72	1.50	Not significant
Maternal age (years)	22.95	24.67	2.13	$P<0.05$
Gestational age (weeks)	38.4	38.5	0.58	Not significant
Apgar score at 1 min	4.75	7.9	13.18	$P<0.01$
Apgar score at 5 min	7.35	9.05	5.66	$P<0.01$
IMA (units/ml)	55.7	32.44	11.29	$P<0.01$

IMA: Ischemia modified albumin

DISCUSSION

Perinatal asphyxia and the resultant hypoxic ischemic encephalopathy (HIE) remain the leading cause of neonatal mortality and morbidity particularly in developing countries. Hypoxia-ischemia poses a risk in infants by altering cerebral blood flow regulatory mechanisms. Hypotension, cerebral ischemia and reperfusion set a cascade of events leading to catastrophic consequences [32]. Biochemical events, such as energy failure, membrane depolarization, brain edema, production of oxygen-free radicals, and lipid peroxidation may lead to brain dysfunction and neuronal death. Reperfusion particularly is a critical event, because of the chances of brain damage being amplified many times during this period. Reperfusion injury leads to the generation of free radicals including the dangerous OH radical which setup a chain of reactions that injure membranes of lipid peroxidation, inactivation of enzymes, and damage of DNA and degradation of structural lipids. Generation of nitric oxide is a significant finding in cases of HIE.

A series of events take place as a consequence of ischemia including hypoxia, acidosis and free radical generation. As a result of these, there is a conformational change of the N-terminus of albumin and reduction in the binding with transition metals such as copper, nickel, and cobalt. The resulting molecule, IMA and its reduced binding of cobalt, has been exploited to produce a rapid automated test, the ACB assay [17].

IMA is a biomarker which can identify myocardial ischemia in advance or in the absence of myocardial necrosis. It is also

Table 4: Values of Pearson's coefficient of correlation (*r*) among the different parameters of the case subjects (*n*=40)

Parameters	Birth weight	Gestational age	Maternal age	Apgar score at 1 min	Apgar score at 5 min	IMA (units/ml)
Birth weight	-					
Gestational age	0.343*	-				
Maternal age	0.045	0.086	-			
Apgar score at 1 min	−0.199	0.047	0.330*	-		
Apgar score at 5 min	−0.003	0.068	0.127	0.820†	-	
IMA (units/ml)	0.331*	−0.020	−0.034	−0.488†	−0.322*	-

Significance of r values are expressed as follows: *$P<0.05$, †$P<0.01$. IMA: Ischemia modified albumin

Table 5: Values of Pearson's coefficient of correlation (*r*) among the different parameters of the control subjects (*n*=40)

Parameters	Birth weight	Gestational age	Maternal age	Apgar score at 1 min	Apgar score at 5 min	IMA (units/ml)
Birth weight	-					
Gestational age	0.252	-				
Maternal age	0.213	−0.120	-			
Apgar score at 1 min	0.196	−0.127	−0.210	-		
Apgar score at 5 min	−0.073	−0.269	−0.272	0.603†	-	
IMA (units/ml)	−0.274	0.050	−0.089	−0.447†	−0.398*	-

Significance of r values are expressed as follows: *$P<0.05$, †$P<0.01$. IMA: Ischemia modified albumin

established that IMA rises within minutes from the onset of an ischemic event and remains elevated for several hours after cessation of ischemia. Elevated IMA levels may result from increased oxidative stress, caused by ischemia reperfusion injury or other mechanisms linked to primary reduction of coronary blood flow or muscle damage. There have been few studies relating to IMA levels in birth asphyxia [17,29]. Increased levels of IMA in cord blood of newborns suffering from asphyxia have been reported.

Hegyi *et al*. [33] had shown that in low-birth weight and premature babies Apgar score was unreliable for assessment of asphyxia. So we preferred not to include those babies. Caesarian section can increase IMA levels [29]. Elevated levels of IMA were reported in mothers with pre-eclampsia [26,27]. Hence pre-eclamptic and cesarian mothers were excluded from the study. Since serum albumin levels might be altered in a patient with kidney disease, all the mothers with renal disease were excluded from the study.

In the current study, the cord blood IMA levels were significantly elevated compared to the control group (55.7 ± 1.7 U/ml compared to 32.44 ± 1.15 U/ml). Table 4 shows that low-birth weight was strongly correlated with IMA levels ($r = -0.331$, $P < 0.05$), a finding which was not seen in cases of controls [Table 5]. Although there was a significant difference in the age of mothers of two groups [Table 3], maternal age seemed not related to the IMA levels [Table 4]. The IMA levels were very significantly correlated with both 1 min and 5 min Apgar score, the fact that establishes its value as a marker of birth asphyxia. In the control group [Table 5] similar correlations were found. The findings of the study were similar with the findings of other studies done recently.

There were few limitations of the current study. Maternal serum IMA levels were not studied. The assay technique used was a simple one. Mothers with gestational diabetes were not eliminated, although elevated IMA levels were reported [34] later on. No follow-up asphyxiated babies could be done. In neonates with low-birth weight the validity of cord blood, IMA levels as a marker of asphyxia was not established.

Apgar score is used worldwide for immediate assessment of newborn, to determine the need of resuscitation and to evaluate the effectiveness of such resuscitation. A low 1 min Apgar score alone does not correlate with the infant's future outcome. Although the 5 min Apgar score is a valid predictor of neonatal mortality, considering the possible confounding factors, the usefulness of the score in birth asphyxia is questioned [9,10]. In the immediate minutes after the delivery of asphyxiated newborn, the chance of brain damage is more. The therapeutic window for pharmacological intervention (6-12 h) is short, and reperfusion injury takes place during in full swing. At this point IMA can be estimated from the cord blood. The assay is inexpensive and can be performed using automated instruments also.

Cord blood IMA levels were higher in asphyxiated newborns compared to controls. Raised levels of IMA in those subjects had shown very significant correlations with the Apgar score of 5 min, a finding also observed in the case of controls.

From the findings of the current study, one can conclude that IMA levels in cord blood of newborns is a biochemical marker which can be a very useful in the diagnosis and interpretation of birth asphyxia if used along with the Apgar score.

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