A Study on Serum Calcium Level in Birth Asphyxia

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ABSTRACT

Introduction: Birth asphyxia continues to form one of the five leading cause of perinatal death all over and the most important single contributor to various forms of physical or mental retardation.

Materials & methods: 75 newborn were enrolled for the present study. Out of which 50 neonates asphyxiated full term served as cases and 25 were healthy neonates served as controls. To study Serum Calcium level in neonates at the age of 24 h, 72 h. & 120h Serum calcium was estimated by OCPC method.

Result: There is a highly significant Hypocalcaemia in Hypoxic children from day of birth which persisted up to the study period 1st, 3rd and 5th day. There are abnormal Calcium metabolism percentage increases with severity of asphyxia and this abnormality persists up to 5th day in Stage III but it gets improved in Stage I and II by 5th day of life.

Conclusion: We found factors like Birth Weight, Stages of HIE to be associated with Birth asphyxiated newborn.

Key words: Serum calcium, Birth asphyxia.

INTRODUCTION

Birth asphyxia or asphyxia neonatorum is an insult to the foetus or newborn due to lack of oxygen and lack of perfusion to various organs. [1] The effect of hypoxia and ischemia may not be identical, but they are difficult to separate clinically. Both factors probably contribute to asphyxia injury.

Birth asphyxia continue to form one of the five leading cause of perinatal death all over and the most important single contributor to various forms of physical or mental retardation and less well appreciated behavioral problems and learning disabilities in later childhood. Since the condition could have often been preventable, an urgent need to develop a clearer understanding of pathophysiology has been widely recognized.

Perinatal mortality rate in India has remained almost static at appealingly high figure of around 65 per 1000 for nearly three decades. [2] Therefore, it has also been appreciated that investigations and management based on less sophisticated and financially more feasible technology has perhaps greater relevance in the Indian
context than those based on expensive electronic equipments.

Over past many years, various biochemical parameters including electrolye and blood urea, serum calcium, serum magnesium, serum glucose, serum creatinine have been extensively evaluated with regard to several correlates like birth weight, gestational age, assessment of intrauterine growth and well being of foetus and newborn infants etc. The influence of various biochemical parameter of numerous confounding variables like immaturity of organs especially the brain, lungs and kidneys, the fact that the disturbances due to asphyxia at birth to multisystem involvement have all been recognized.

However, considering the incidence of the problem, etiological factors implicating in birth asphyxia and magnitude of its immediate and long term effects, it is rather surprising to note that there is relative paucity of literature on incidence of birth asphyxia, etiological factors implicating in birth asphyxia and simple biochemical parameters especially change in Calcium level which may occur following birth asphyxia among various categories of newborn infants.

With this background, it was deemed worthwhile to investigate the incidence of birth asphyxia in the hospital delivery, and biochemical changes especially serum calcium during blood asphyxia.

**MATERIALS AND METHODS**

The present study was carried out in neonatal intensive care unit of Department of Pediatrics, in action collaboration with Department of Biochemistry, Index Medical College, and Hospital & Research Center.

75 Newborns admitted to department of Pediatrics and its neonatal unit was enrolled for the present study. The enrolled babies were further divided into study group A (50 neonates) and control group B (25 neonates).

**Study Group:**

The study group comprised of 50 asphyxiated term neonates. A baby was considered full term, the gestational age 37 weeks, completed of gestation. The assessment of gestational age was done by the Modified Dubowitz criteria and the staging of asphyxia was done by Sarnat and Sarnat staging.

**Inclusion criteria:**

1. The documentation of intrapartum fetal distress through recognition of abnormal fetal heart rate patterns with or without passage of meconium.
2. The presence of immediate neonatal distress as evidenced by a low minute (< 7) Apgar score.
3. The need for immediate neonatal resuscitation including bag and mask ventilation.
4. An abnormal neurologic examination during the first 24 hours of life as judged by application of Sarnat and Sarnat staging.

A full term newborn fulfilling any two of the above mentioned criteria was included in the study.

**Exclusion criteria:**

All neonates with evidence of septicemia, intrauterine infection, congenital anomalies, necrotizing enterocolitis, and marked respiratory distress were excluded from the study.

**Control Group:**

The control group had 25 healthy term neonates delivered in the department of Gynecology and obstetrics, Index Medical College. The control were selected by random sampling and screened for any health problem. The control group comprised of healthy and gestational age matched neonates only.

**Collection of Blood Samples:**

3 ml of blood was collected from peripheral vein within 24 hours, 72 hours
and 120 hours in a clean sterilized vial with due precautions to avoid contamination. Serum was separated using a pipette and centrifuged at 3000-4000 rpm from 5-10 minutes. After centrifugation samples were analyzed on the same day. Hemolysed samples were discarded.

**Estimation of Serum Calcium:**
Method: Serum calcium is estimated by OCPC method.\(^{[3,4]}\)

Principle- Calcium in an alkaline medium combines with O-Cresolphthalein complexone to form a purple coloured complex. Intensity of colour formed is directly proportional to the amount of calcium present in the sample.

\[
\text{Calcium} + \text{OCPC} \rightarrow \text{Purple Coloured Complex.}
\]

**Normal Reference Values**
Serum/plasma: 8.7-11.0 mg/dl

**Materials provided:**
Test contents
- L1: Buffer reagent
- L2: Colour reagent
- S: Serum calcium standard(10mg/dl)

**Reagent Preparation** –
For convenience a single working reagent may be prepared by mixing equal parts of the buffer Reagent and color Reagent. This combined reagent is stable for 7 days at 2-8°C.

**Calcium Standard:**

**Procedure:**

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer reagent(L1)</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Colour reagent (L2)</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>20 µl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Calcium standard(s)</td>
<td>-</td>
<td>20 µl</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix well and incubate at R.T (25°C) for 5 min. (Measure the absorbance of standard and test sample, against the Blank, within 60 min.)

**Calculation:**
\[
\text{Calcium in mg/dl} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 10
\]

**RESULTS**

**Table No 1: Distribution of Cases and Controls as per Sex.**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>32(64%)</td>
<td>15(60%)</td>
</tr>
<tr>
<td>Female</td>
<td>18(36%)</td>
<td>10(40%)</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>25</td>
</tr>
</tbody>
</table>

The table I depicts, total number of cases and controls, of these 47(63%) were male and 28(37%) were female.

**Table 2: Weight wise distribution of cases and controls.**

<table>
<thead>
<tr>
<th>Birth weight (gms)</th>
<th>Cases (n=50)</th>
<th>Controls (n=25)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000-2249</td>
<td>4(8%)</td>
<td>1(4%)</td>
<td></td>
</tr>
<tr>
<td>2250-2499</td>
<td>10(20%)</td>
<td>4(16%)</td>
<td></td>
</tr>
<tr>
<td>≥2500</td>
<td>36(72%)</td>
<td>20(80%)</td>
<td></td>
</tr>
</tbody>
</table>

Above table shows that the maximum number of cases and controls (72% & 80%) were of birth weight ≥ 2500 gms, (20% &16%) cases and controls were of birth weight between 2250-2499 gms and 8% & 4% were of birth weight between 2000-2249 gms.

**Table 3: Serum calcium level (mean ± SD) on day 1st, 3rd & 5th in Study and Control.**

<table>
<thead>
<tr>
<th>Days</th>
<th>Cases (n=50)</th>
<th>Controls (n=25)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>7.00±±0.691</td>
<td>8.78±±0.399</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3rd</td>
<td>7.48±±0.760</td>
<td>9.47±±0.250</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5th</td>
<td>8.18±±0.811</td>
<td>9.99±±0.277</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

It is evident from the above table that there is a very significant (p<0.001) Hypocalcaemia in Hypoxic children from day of birth which persisted up to the study period (p<0.01) 1st, 3rd and 5th day.

The observation depicts that abnormal calcium metabolism percentage increases with severity of asphyxia (46.6% abnormal in Stage I while 71.4% abnormal in Stage III) and this abnormality persists up to 5th day in Stage III but it get improved in Stage I and II by 5th day of life.
### DISCUSSION

The present was carried out in Index Medical College, hospital & research Center over a period of one year. It included 50 asphyxiated neonates and 25 healthy neonates.

Even though birth asphyxia have been extensively investigated by clinical, hematological, electrophysiological and more recently by sophisticated imaging technique, it appears that certain simple biochemical parameters received relative scant attention of perinatologists. As birth asphyxia is common and more over carries extremely serious immediate and long term adverse effects, the present study was planned to evaluate incidence & effect of birth asphyxia on serum calcium.

Out of the total 50 asphyxiated newborns, according to the Sarnat and Sarnat stages of Hypoxic Ischemic Encephalopathy 15 (30%) were in stage I, 28 (56%) were in stage II and 7 (14%) were in stage III.

The mean value of serum calcium (Table-3) on day 1st, 3rd and 5th (study and control) were - 7.004±0.691, 8.788±0.399, 7.482±0.760, 9.476±0.250, 8.184±0.811, 9.992±0.277 respectively. It shows a very significant (p≤0.001) Hypocalcaemia in Hypoxic children from day of birth which persisted up to the study period (p≤0.01) 1st, 3rd and 5th day.

When the serum calcium were compare according to Stages of HIE. Abnormal calcium metabolism percentage increases with severity of asphyxia (46.6% abnormal in Stage I while 71.4% abnormal in Stage III) and this abnormality persists up to 5th day in Stage III but it get improved in Stage I and II by 5th day of life (Table-4.). But when serum calcium level according to birth weight was measured, the values were found to be non significant.

Stoliar et al (1971) in their study on serum calcium levels in birth anoxic neonates found 26.76% incidence in of hypocalcaemia in full term infants and 58.3% in premature infants. Hypocalcaemia was associated with acidosis in the first 24 hours of life. [8]

Tsang et al (1973) in their study found that 37.6% of premature infants had serum calcium of <7 mg% they also found that Apgar scores was associated with low serum calcium values from 12-72 hours of age (p <0.01). [5] Birth asphyxia therefore appears to play a separate role in neonatal calcium homeostasis apart from role of gestational age.

P Ilves (2000) studied on 46 asphyxiated and 35 healthy term infants .At the age of 24 - 48 h hypocalcaemia was discovered in 23% of asphyxiated infants. [6]

Jajoo D (1995) studied on 35 term infants with asphyxia. Asphyxiated infants had significantly lower serum calcium levels than control infant during each of time period studied. [7]

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Table 4: Serum calcium in different stages of HIE on day 1st, 3rd and 5th.

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>Day I</td>
<td>8(53.3%)</td>
<td>7(46.6%)</td>
</tr>
<tr>
<td>Day III</td>
<td>9(67.85%)</td>
<td>10(32.14%)</td>
</tr>
<tr>
<td>Day V</td>
<td>10(66.6%)</td>
<td>5(33.3%)</td>
</tr>
<tr>
<td>Stage II</td>
<td>Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>Day I</td>
<td>2(28.57%)</td>
<td>5(71.42%)</td>
</tr>
<tr>
<td>Day III</td>
<td>3(42.85%)</td>
<td>4(57.14%)</td>
</tr>
<tr>
<td>Stage III</td>
<td>Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>Day I</td>
<td>19(38%)</td>
<td>31(62%)</td>
</tr>
<tr>
<td>Day III</td>
<td>22(44%)</td>
<td>28(56%)</td>
</tr>
<tr>
<td>Total</td>
<td>24(48%)</td>
<td>26(52%)</td>
</tr>
</tbody>
</table>
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