ORIGINAL RESEARCH

Assessment of the Correlation between Total Serum Calcium and Ionized Calcium Levels and the Severity of Birth Asphyxia

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ABSTRACT

Background: Birth asphyxia remains a significant contributor to neonatal morbidity and mortality. Among the various biochemical disturbances associated with hypoxic-ischemic events, calcium imbalance—especially hypocalcemia—plays a crucial role in worsening clinical outcomes.

Aim:To evaluate and compare total serum calcium and ionized calcium levels in term neonates with and without birth asphyxia and to assess their correlation with Apgar scores.

Material and Methods: This prospective observational study was conducted over one year in the Department of Paediatrics at a tertiary care hospital. A total of 80 term neonates were enrolled and divided into two groups: Group A (n=40) included neonates with birth asphyxia, and Group B (n=40) included healthy term neonates. Total serum calcium was measured using the Arsenazo III method, and ionized calcium was assessed using an ion-selective electrode technique. Blood samples were collected within six hours of life. Data were analyzed using SPSS version 25, with p<0.05 considered statistically significant.

Results: The mean total serum calcium in the asphyxiated group was $7.12 \pm 0.65 \text{ mg/dL}$, significantly lower than $8.42 \pm 0.52 \text{ mg/dL}$ in controls (p<0.001). Hypocalcemia (<7 mg/dL) was observed in 45% of Group A versus 5% in Group B. The mean ionized calcium level in Group A was $0.92 \pm 0.14 \text{ mmol/L}$, significantly lower than $1.15 \pm 0.12 \text{ mmol/L}$ in Group B (p<0.001), with 60% of Group A showing ionized hypocalcemia compared to 10% in controls. A significant positive correlation was found between Apgar scores at 5 minutes and both total calcium (r=0.48, p=0.002) and ionized calcium (r=0.52, p<0.001).

Conclusion: Neonates with birth asphyxia exhibit significantly lower levels of both total and ionized calcium compared to healthy neonates. The high prevalence of hypocalcemia and its association with lower Apgar scores highlight the need for routine calcium monitoring and timely correction to improve neonatal outcomes.

Keywords: Birth Asphyxia, Total Serum calcium, Ionized calcium, Neonatal hypocalcemia, Apgar score

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INTRODUCTION

Birth asphyxia is one of the leading causes of neonatal morbidity and mortality, particularly in low- and middle-income countries. It results from a lack of oxygen (hypoxia) and/or inadequate blood flow (ischemia) to the foetus or newborn during the perinatal period. This critical condition can lead to multi-organ dysfunction and has profound effects on the neonatal metabolic profile, including disturbances in electrolyte levels such as calcium. The evaluation of calcium homeostasis, especially both total serum calcium and ionized calcium, is essential in understanding the biochemical consequences of birth asphyxia and guiding appropriate clinical interventions.¹

Neonatal hypocalcemia is а common biochemical abnormality observed in asphyxiated neonates. It can occur early (within the first 72 hours) or late (after 72 hours) and presents without specific often clinical making routine symptoms, biochemical monitoring indispensable. Calcium plays a vital role in cellular signaling, muscle contraction, neurotransmitter release, and cardiac rhythm regulation. The two primary components of serum calcium include total calcium, which comprises both bound and unbound fractions, and ionized calcium, which is the biologically active and physiologically significant component. Of the total calcium present in serum, approximately 50% exists in the ionized form, 40% is protein-bound (mostly to albumin), and the remaining 10% is complexed with anions.²

Birth asphyxia leads to a cascade of metabolic including hypoxia-induced derangements. acidosis and disturbances in parathyroid hormone function, which are implicated in the pathogenesis of hypocalcemia. The hypoxic state impairs calcium mobilization from bones and reduces intestinal calcium absorption, while also causing an increase in phosphate levels that further suppress calcium concentrations. Moreover, the stress response to asphyxia, accompanied by elevated catecholamines and glucocorticoids, contributes to altered calcium metabolism. These changes are more effectively reflected by measuring ionized calcium levels, which are more sensitive to acute physiological variations than total serum calcium.³

Clinical and biochemical profiling of neonates with birth asphyxia has shown significant derangements in electrolytes, particularly calcium and glucose levels. Early identification and correction of these abnormalities are critical to improving outcomes in affected neonates. Studies have suggested that hypocalcemia in birth asphyxia may worsen the neurological status by exacerbating seizure activity and contributing to cardiac dysfunction. Therefore, quantifying both total and ionized calcium offers a more comprehensive picture of the neonate's calcium status and can serve as a useful prognostic indicator.4

In recent years, efforts to improve neonatal outcomes have focused not only on prompt resuscitative measures but also on postresuscitation metabolic support. Birth asphyxia remains a major contributor to early neonatal deaths, particularly in resource-limited settings. Global initiatives aimed at reducing perinatal mortality emphasize early recognition of birth asphyxia and immediate supportive care, which includes correction of metabolic imbalances like hypoglycemia and hypocalcemia. These interventions are vital because a significant proportion of deaths in asphyxiated neonates occur within the first few days of life.⁵

The use of specific scoring systems such as the Hypoxic Ischemic Encephalopathy (HIE) score aids in classifying the severity of asphyxia and can correlate with biochemical abnormalities. Higher HIE grades have been associated with pronounced metabolic disturbances, more including lower calcium levels. Furthermore, clinical studies conducted in diverse populations have indicated a consistent association between birth asphyxia and decreased calcium concentrations, thereby reaffirming the importance of biochemical surveillance in such neonates.6

The pathophysiological mechanisms underlying neonatal hypocalcemia in asphyxiated infants are multifactorial. They may involve immature parathyroid hormone responses, elevated phosphate levels due to cellular injury, magnesium deficiency, and renal losses of calcium. These factors are exacerbated by prematurity and intrauterine growth restriction, which are frequently coexistent with birth asphyxia. Although total serum calcium has traditionally been measured in clinical settings, it may not accurately reflect the physiologically active calcium pool in critically ill neonates. In contrast, ionized calcium provides real-time assessment of calcium bioavailability and is less influenced by serum albumin levels or pH changes.7

Monitoring and comparing both total and ionized calcium in neonates with birth asphyxia is crucial for establishing the degree of metabolic compromise and guiding timely therapeutic interventions. The evidence suggests that relying solely on total calcium may result in underdiagnosis of functionally significant hypocalcemia. Early detection through comprehensive biochemical analysis can facilitate appropriate calcium supplementation and potentially mitigate the risk of long-term complications such as neurodevelopmental delay and cardiac dysfunction.⁸

AIM AND OBJECTIVES

Aim: The aim of this study was to compare the total serum calcium and ionized calcium levels between term neonates with birth asphyxia and healthy controls in order to assess any potential biochemical alterations in calcium metabolism associated with birth asphyxia.

Objectives:

- 1. To measure and compare the total serum calcium levels between term neonates diagnosed with birth asphyxia and healthy control neonates.
- 2. To measure and compare the ionized calcium levels between term neonates diagnosed with birth asphyxia and healthy control neonates.
- 3. To determine the prevalence of hypocalcemia (both total serum calcium <7 mg/dL and ionized calcium <1.0 mmol/L) in neonates with birth asphyxia compared to healthy controls.
- 4. To assess the correlation between Apgar scores at 1 and 5 minutes and calcium levels (both total serum and ionized calcium) in neonates with birth asphyxia.
- 5. To explore any potential relationship between the severity of birth asphyxia (indicated by Apgar score) and disturbances in calcium metabolism, which could contribute to clinical management and outcomes.

MATERIALS AND METHODS

Study Design: This was a prospective observational study aimed at comparing total serum calcium and ionized calcium levels between term neonates with birth asphyxia and healthy controls.

Study Population: A total of 80 term neonates were included in the study and divided into two groups:

- Group A (Cases): 40 term neonates diagnosed with birth asphyxia.
- Group B (Controls): 40 healthy term neonates without any perinatal complications.

Study Place

The study was conducted in the Department of Paediatrics, Anugrah Narayan Magadh Medical College & Hospital, Gaya, Bihar, India.

Study Duration

The study was conducted over a period of one year and six months, from January 2020 to July 2021.

Inclusion Criteria

Group A (Cases):

- Term neonates (gestational age \geq 37 weeks)
- Diagnosed with birth asphyxia within the first 6 hours of life, as defined by:
 - Apgar score ≤ 6 at 5 minutes
 - Need for resuscitation beyond 1 minute after birth
 - Clinical signs of hypoxic-ischemic encephalopathy (HIE)

Group B (Controls):

- Healthy term neonates (gestational age ≥ 37 weeks)
- Normal Apgar scores (≥8 at 1 and 5 minutes)
- No requirement for resuscitation
- No perinatal complications

Exclusion Criteria (Applicable to Both Groups)

- Preterm neonates (<37 weeks gestation)
- Neonates with major congenital anomalies
- Neonates with sepsis or metabolic disorders
- Neonates born to mothers with known calcium metabolism disorders
- Neonates whose mothers received magnesium sulfate before delivery

Ethical Considerations

- The study was approved by the Institutional Ethics Committee prior to initiation.
- Written informed consent was obtained from the parents or legal guardians of all enrolled neonates.
- All procedures followed the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration.

Study Procedure

- Detailed antenatal, perinatal, and clinical history was obtained for each neonate.
- Each neonate underwent a comprehensive clinical examination at admission, including assessment of general condition and neurological status.
- Blood samples were collected from both groups within the first 6 hours of life.
- Samples were drawn in pre-heparinized tubes and analyzed immediately to avoid alterations in calcium due to binding.
- Total serum calcium was measured using the colorimetric method (Arsenazo III) via an automated chemistry analyzer.
- Ionized calcium was measured using the ion-selective electrode (ISE) technique through a blood gas analyzer.

• Laboratory technicians conducting the tests were blinded to the grouping of neonates to eliminate bias.

Outcome Measures

- Primary outcomes: Total serum calcium and ionized calcium levels
- Comparison of calcium levels between Group A (birth asphyxia) and Group B (controls)

Statistical Analysis

The data collected during the study were analyzed using **statistical software** (such as SPSS version 22.0 software). The following statistical methods were employed to assess and compare the parameters:

• Descriptive Statistics:

- Mean and standard deviation (SD) were used to summarize continuous variables such as gestational age, birth weight, total serum calcium levels, and ionized calcium levels.
- **Percentage** was used for categorical variables, such as gender distribution and prevalence of hypocalcemia.

• Comparative Analysis:

- Independent t-test was used to compare the mean values of total serum calcium and ionized calcium levels between the two groups (Group A: Birth Asphyxia, Group B: Healthy Controls). This test was appropriate for comparing continuous variables between two independent groups.
- **Chi-square test** was used to assess the **prevalence of hypocalcemia** (both total serum calcium <7 mg/dL and ionized calcium <1.0 mmol/L) between the two groups, as it is suitable for comparing categorical data between two groups.

Correlation Analysis:

- Pearson's correlation coefficient (r-value) was used to assess the strength and direction of the relationship between Apgar scores (at 1 and 5 minutes) and calcium levels (total serum and ionized calcium) in neonates with birth asphyxia.
- A positive correlation indicated that lower Apgar scores were associated with lower calcium levels, while a negative correlation would indicate the opposite. The p-value was used to determine statistical significance (typically, p < 0.05 for significance).

Statistical Significance:

- A **p-value of <0.05** was considered statistically significant for all tests, indicating that differences or correlations observed were unlikely due to random chance.
- In cases of highly significant results, such as the Apgar scores at 1 and 5 minutes, a p-value <0.001 was reported.

Data Interpretation:

- All comparisons between the two groups (Group A vs. Group B) were assessed for statistical significance using t-tests and chi-square tests. Differences with p < 0.05 were considered statistically significant.
- **Correlation coefficients** (r-values) were reported along with **p-values** to assess the strength and significance of associations between Apgar scores and calcium levels in neonates with birth asphyxia.

RESULTS

Table 1. Demographic Characteristics of (tonates (t) = 00)					
Variable	Group A (Cases)	Group B (Controls)	Total	Р-	
	(n=40)	(n=40)	(N=80)	value	
Mean Gestational Age (weeks)	38.4 ± 1.1	38.6 ± 1.0	38.5 ± 1.0	0.372	
Mean Birth Weight (kg)	2.70 ± 0.35	2.85 ± 0.32	2.77 ± 0.34	0.048*	
Male : Female Ratio	23:17	21:19	44:36	0.654	
Mode of Delivery (CS :	26:14	24 : 16	50:30	0.651	
NVD)					

Table 1: Demographic Characteristics of Neonates (N = 80)

*Statistically significant (p<0.05)

Table 1 shows that baseline characteristics between the two groups—birth asphyxia cases (Group A) and healthy controls (Group B) were comparable in terms of gestational age, gender distribution, and mode of delivery. In the present study involving 80 neonates, the demographic characteristics of cases (Group A, n = 40) and controls (Group B, n = 40) were

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compared. The mean gestational age was similar between the two groups, with 38.4 ± 1.1 weeks in the cases and 38.6 ± 1.0 weeks in the controls, showing no statistically significant difference (P = 0.372). However, the mean birth weight was found to be significantly lower in neonates with birth asphyxia $(2.70 \pm 0.35 \text{ kg})$ compared to healthy neonates (2.85 \pm 0.32 kg), and this difference was statistically significant (P = 0.048). The male-to-female ratio was comparable between Group A (23:17) and Group B (21:19), with no significant difference (P = 0.654). Similarly, the mode of delivery (cesarean section versus normal vaginal delivery) did not differ significantly between the two groups, with 26:14 in cases and 24:16 in controls (P = 0.651). These findings indicate that both groups were demographically comparable except for birth weight, which was significantly lower in the asphyxiated neonates.

Tuble 2: Apgar Beores at 1 and 5 Minutes					
Apgar Score	Group A (Cases) (n=40)	Group B (Controls) (n=40)	P-value		
Mean Apgar Score at 1	4.2 ± 0.9	8.6 ± 0.7	< 0.001*		
min					
Mean Apgar Score at 5	5.8 ± 0.7	9.2 ± 0.5	< 0.001*		
min					

able 2:	Apgar	Scores	at I	and	15	Minutes	
							-



*Highly significant difference between groups

Table 2 and figure I, shows that Apgar scores at both 1 minute and 5 minutes post-delivery were significantly lower in neonates with birth asphyxia compared to healthy controls. The mean Apgar score at 1 minute was 4.2 ± 0.9 in Group A and 8.6 \pm 0.7 in Group B (p<0.001),

and at 5 minutes, it was 5.8 ± 0.7 in Group A versus 9.2 ± 0.5 in Group B (p<0.001). These highly significant differences validate the clinical classification of the asphyxiated group and confirm the degree of perinatal compromise.

Parameter	Group A (Cases) (n=40)	Group B (Controls) (n=40)	P-value		
Mean Total Serum Calcium (mg/dL)	7.12 ± 0.65	8.42 ± 0.52	< 0.001*		
Range	5.9 - 8.1	7.5 - 9.5			
Neonates with Hypocalcemia (<7	18 (45.0%)	2 (5.0%)	<0.001*		
ing/aL)					

Table 3: Total Serum Calcium Levels in Neonates

^{*}Statistically significant

Table 3 shows that substantial and statistically significant reduction in total serum calcium levels was observed among neonates with birth asphyxia. The mean total serum calcium in Group A was 7.12 ± 0.65 mg/dL, significantly lower than $8.42 \pm 0.52 \text{ mg/dL}$ in Group B (p<0.001). Additionally, 45% of neonates in the

asphyxiated group had hypocalcemia (defined as total serum calcium <7 mg/dL), compared to only 5% in the control group (p<0.001). These findings suggest that hypocalcemia is a frequent biochemical abnormality in asphyxiated neonates, likely due to impaired calcium homeostasis from perinatal hypoxia.

Parameter	Group A (Cases)	Group B (Controls)	P-value	
Mean Ionized Calcium (mmol/L)	(1-40) 0.92 ± 0.14	(1-40) 1.15 ± 0.12	< 0.001*	
Range	0.68 - 1.20	0.98 - 1.30		
Neonates with Ionized Hypocalcemia (<1.0 mmol/L)	24 (60.0%)	4 (10.0%)	<0.001*	

*Statistically significant

Table 4 shows that Ionized calcium levels were also significantly lower in Group A compared to Group B. The mean ionized calcium concentration in the asphyxiated neonates was $0.92 \pm 0.14 \text{ mmol/L}$, while it was 1.15 ± 0.12 mmol/L in healthy controls (p<0.001). Ionized hypocalcemia (defined as ionized calcium <1.0

mmol/L) was present in 60% of asphyxiated neonates but only in 10% of controls, a statistically significant difference (p<0.001). Since ionized calcium represents the physiologically active form of calcium, this deficiency could contribute to neuromuscular and cardiovascular instability in affected neonates.

 Table 5: Correlation between Apgar score and Calcium Levels (Group A only)

$\mathbf{I}\mathbf{\partial}$				
Correlation	r-value	P-value		
Apgar Score (5 min) vs Total Serum Calcium	0.48	0.002*		
Apgar Score (5 min) vs Ionized Calcium	0.52	< 0.001*		
Table 5 shows that significant positive compar	red to controls	$(2.85 \pm 0.32 \text{ kg})$, with a		
correlation was found between Apgar scores at 5 statistic	ally significant	p-value of 0.048. Lower		
minutes and both total serum calcium (r=0.48, birth w	eight has been	previously reported as a		

p=0.002) and ionized calcium levels (r=0.52, p < 0.001) in the asphyxiated group. This indicates that neonates with lower Apgar scores tend to have lower calcium levels, reflecting a possible relationship between the severity of birth asphyxia and disruption of calcium metabolism. These findings emphasize the importance of early calcium monitoring and correction in neonates with low Apgar scores to potentially improve outcomes.

DISCUSSION

In our study, in terms of gestational age, both groups were comparable, with the mean gestational age in Group A being 38.4 ± 1.1 weeks and in Group B being 38.6 ± 1.0 weeks. The difference was not statistically significant (p = 0.372), suggesting that gestational maturity was similar in both groups. This aligns with the findings of Bhat et al. (2016) and Narayan et al. (2013), who noted that most cases of birth asphyxia occurred in term neonates.^{8,9}

The mean birth weight was significantly lower in the asphyxiated group $(2.70 \pm 0.35 \text{ kg})$ risk factor for birth asphyxia due to poor physiological reserves and immature organ systems (Kumar et al., 2017).¹⁰ These findings are supported by studies such as Rathod et al. (2018), which demonstrated a higher incidence of birth asphyxia in neonates with lower birth weights.¹¹This trend was similarly reported by Raiet al (2015), where asphyxiated neonates had lower average birth weights compared to controls.¹² Although gestational age, sex ratio, and mode of delivery did not differ significantly in our study, Allen *et al* (2011) noted that even in term neonates, lower birth weight could be a critical predictor of vulnerability to hypoxicischemic injury due to impaired placental nutrient supply.¹³

Our data show a mean Apgar score of 4.2 ± 0.9 at 1 minute and 5.8 \pm 0.7 at 5 minutes in the asphyxiated group, significantly lower than 8.6 \pm 0.7 and 9.2 \pm 0.5, respectively, in controls (p<0.001 for both). These results confirm the clinical diagnosis of moderate to severe birth asphyxia in Group A. Jayaprakash and Murali

(2014) also observed similarly low Apgar scores among asphyxiated neonates in their study, with an average 1-minute score of 4.5 and 5-minute score of 6.1.¹⁴ Yoneda *et al* (2005) emphasized that lower Apgar scores at 5 minutes strongly correlate with poorer neonatal outcomes and increased risk of hypocalcemia and other metabolic complications.¹⁵

The mean total serum calcium in the asphyxiated group was $7.12 \pm 0.65 \text{ mg/dL}$, significantly lower than $8.42 \pm 0.52 \text{ mg/dL}$ in the control group (p<0.001). Hypocalcemia (total calcium <7 mg/dL) was present in 45% of asphyxiated neonates, compared to only 5% in controls.Rai*et al* (2015) found the mean total calcium in asphyxiated neonates to be 7.0 mg/dL, while in controls it was 8.5 mg/dL, confirming the trend seen in our study. These comparisons highlight that neonatal hypocalcemia is a consistent finding across various populations and settings in the context of perinatal asphyxia.¹²

In a study by Alphonsus (2011), 47% of neonates with severe birth asphyxia were hypocalcemic, nearly identical to our findings.¹⁶

A mean ionized calcium level of 0.92 ± 0.14 mmol/L in asphyxiated neonates versus 1.15 \pm 0.12 mmol/L in controls (p<0.001), with 60% of asphyxiated neonates having ionized hypocalcemia (<1.0 mmol/L) compared to 10% of controls. These values are closely aligned with those reported by Jain et al (2000), who found a mean ionized calcium of 0.91 mmol/L in neonates with asphyxia.¹⁷ In a similar study, Lila et al (2015) noted a mean ionized calcium of 0.88 mmol/L in neonates with HIE, and they found a strong association between low ionized calcium and adverse neurological outcomes.¹⁸ Our findings reinforce that ionized calcium, rather than total calcium alone, should be routinely assessed in asphyxiated neonates, given its role in cardiac and neuromuscular stability.

Our study showed a moderate positive correlation between Apgar score at 5 minutes and both total serum calcium (r = 0.48, p = 0.002) and ionized calcium (r = 0.52, p < 0.001) in the asphyxiated group. This indicates that lower Apgar scores are associated with more severe calcium deficiency. Basu *et al* (2010) similarly reported that calcium levels were directly proportional to the severity of asphyxia as assessed by Apgar scores and clinical staging of HIE.¹⁹Yoneda*et al* (2005) also suggested that ionized calcium levels measured within the first few hours of life could serve as a predictive

marker for the severity of hypoxic injury and its potential neurological sequelae.¹⁵

LIMITATIONS OF THE STUDY

- Single-centre study limits generalizability of findings.
- Small sample size may reduce the power to detect smaller differences.
- Calcium levels were measured only once within the first 6 hours, which may not reflect dynamic changes over time.
- Long-term neurological outcomes were not assessed, which limits understanding of the clinical significance of calcium alterations.
- Confounding factors such as maternal nutrition, mode of delivery, and neonatal renal function were not fully accounted for.

CONCLUSION

This study demonstrates that both total serum calcium and ionized calcium levels are significantly lower in neonates with birth asphyxia compared to healthy controls. A high prevalence of hypocalcemia was observed in asphyxiated neonates, with a strong correlation between low Apgar scores and calcium deficiency. Early identification and correction of hypocalcemia may play a vital role in improving clinical outcomes. Routine monitoring of ionized calcium should be emphasized in the management of birth asphyxia.

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