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SERUM CALCIUM LEVEL AMONG NEONATES WITH HYPOXIC ISCHEMIC ENCEPHALOPATHY (STAGE II & III) IN A TERTIARY CARE HOSPITAL

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Abstract

Background: *Hypoxic-ischemic encephalopathy (HIE) is a major cause of neonatal morbidity and mortality, with serum calcium levels potentially influencing its severity..*

Objective: *To assess the serum calcium levels among neonates with hypoxic-ischemic encephalopathy (stage II & III) in a tertiary care hospital.*

Study Design: *Case-control study*

Study Setting: *This study was conducted at Sughra Shafi Medical Complex/Sahara Hospital, Narowal From 20 February 2023 to 20 August 2023.*

Methodology: *Total of 100 neonates were included in the study, comprising 70 cases diagnosed with HIE (stages II and III) and 30 controls without HIE. The classification of 70 neonates as HIE cases and 30 as controls was determined based on the availability of eligible participants during the study period. Blood samples were collected within the first 24 hours of life to measure serum calcium levels. Hypocalcemia was defined as a serum calcium level of less than 8 mg/dl. The collected data were analyzed using IBM SPSS, version 27.0.*

Results: *Among the cases, 46 (65.7%) were male, and 24 (34.3%) were female, compared to 21 (70.0%) males and 9 (30.0%) females in the control group. The prevalence of hypocalcemia (serum calcium < 8 mg/dl) was significantly higher in the cases, with 30 (42.9%) compared to 2 (6.7%) in the controls ($p < 0.001$). The mean serum calcium level was also significantly lower in the cases (7.14 ± 1.44 mg/dl) compared to the controls (8.43 ± 0.35 mg/dl), with a p-value of < 0.001. However, the mean serum calcium level was significantly lower in HIE-III neonates (6.45 ± 1.78 mg/dl) compared to HIE-II neonates (7.58 ± 0.98 mg/dl), with a p-value of 0.005.*

Conclusion: *Our study highlights a significant association between hypocalcemia and the severity of hypoxic-ischemic encephalopathy (HIE) in neonates, emphasizing the need for early detection and management of calcium imbalances to improve outcomes in affected infants*

INTRODUCTION

Hypoxic-ischemic encephalopathy (HIE) is a significant and potentially devastating neurological condition that occurs due to oxygen deprivation and reduced blood flow to the brain during birth.[1] It is a leading cause of neonatal morbidity and mortality worldwide, the prevalence of neonatal encephalopathy is 3 per 1,000 births, whereas that of hypoxic-ischemic encephalopathy (HIE) is 1.5 per 1,000 births.[2] HIE is classified into three stages based on the severity of the neurological symptoms: mild (stage I), moderate (stage II), and severe (stage III).[2] The severity of HIE correlates with the risk of long-term neurological deficits, with stage II and III often resulting in serious complications such as cerebral palsy, cognitive impairments, and, in the most severe cases, death.[3]

Among the various biochemical disturbances observed in neonates with HIE, abnormalities in serum calcium levels have garnered considerable attention. Calcium plays a crucial role in numerous physiological processes, including muscle contraction, nerve transmission, and intracellular signaling.[4] In the context of neonatal brain injury, calcium homeostasis is particularly important due to its involvement in neuronal function and neuroprotection. Hypocalcemia, or low serum calcium levels, is commonly observed in neonates with HIE and has been associated with increased morbidity and mortality.[5]

The pathophysiology of hypocalcemia in neonates with HIE is multifactorial. Perinatal asphyxia, which is the primary cause of HIE, leads to a cascade of metabolic derangements, including respiratory acidosis, metabolic acidosis, and hypoxia. These conditions can disrupt the delicate balance of calcium regulation, leading to decreased calcium absorption, increased urinary calcium excretion, and impaired parathyroid hormone (PTH) secretion.[6] Neonates with moderate to severe HIE (stages II and III) are particularly vulnerable to these disturbances due to the extent of the brain injury and the associated systemic effects. Hypocalcemia in these infants can contribute to the exacerbation of neurological injury by promoting excitotoxicity, a process where excessive calcium influx into neurons leads to cell death. Additionally, hypocalcemia can impair cardiac function, increase the risk of seizures, and lead to other metabolic complications, further complicating the management of these critically ill infants.[7]

Among neonates with hypoxic-ischemic encephalopathy (HIE), 10-15% will die, 10-15% will develop cerebral palsy, and up to 40% will develop other disabilities, including severe and permanent neuropsychological sequelae such as mental retardation, visual-motor or visual-perceptive dysfunction, increased hyperactivity, cerebral palsy, and epilepsy.[8] A variety of markers have been examined to identify perinatal hypoxia, but studies for early determination of tissue damage due to birth asphyxia are still lacking.[9] N-Methyl-D-aspartic (NMDA) acid channels are normally closed by magnesium ions in a voltage-dependent manner. Hypoxia decreases the blocking effect of the magnesium ion channel, leading to a rapid influx of Ca^{2+} into the cell, which has been reported as a major cause of cell death.[10]

In a tertiary care hospital setting, where advanced diagnostic and therapeutic modalities are available, the management of neonates with HIE often involves a multidisciplinary approach. Given the critical role of calcium in neonatal neuroprotection and the potential for hypocalcemia to exacerbate brain injury, there is a growing interest in understanding the patterns of serum calcium levels in neonates with HIE, particularly in those with moderate to severe disease. This knowledge could inform clinical practices and potentially lead to improved therapeutic strategies aimed at minimizing neurological damage and enhancing recovery. The findings may contribute to a better understanding of the pathophysiology of HIE and inform clinical guidelines for the management of calcium homeostasis in affected neonates. Ultimately, the objective is to improve the care and prognosis of infants with this serious condition through evidence-based interventions that address the metabolic challenges associated with HIE.

METHODS

The study protocol was approved by the hospital's ethical review committee. Informed consent was obtained from the parents or legal guardians of all neonates included in the study. This was a case-

control study conducted at Sughra Shafi Medical Complex/Sahara Hospital, Narowal from 20 February 2023 to 20 August 2023. The study included neonates admitted to the neonatal intensive care unit (NICU) during the study period.

Based on the mean serum calcium level of 7.64±0.59 mg/dl, with a significance level (alpha) of 0.05% and a power of 0.8%, the estimated sample size was 100 patients which was calculates using WHO calculator Total of 100 neonates were included in the study, comprising 70 cases diagnosed with HIE (stages II and III) and 30 controls without HIE. The classification of 70 neonates as HIE cases and 30 as controls was determined based on the availability of eligible participants during the study period. This distribution allowed us to ensure a meaningful comparison while reflecting the clinical prevalence of the condition. The inclusion criteria for cases were neonates with a clinical diagnosis of HIE, confirmed through appropriate neurological assessments and categorized into stages II and III based on standard criteria. Controls were neonates of similar gestational age and sex without any signs of HIE or other significant neonatal complications. However, neonates with evidence of sepsis, infections, and major congenital pathologies were excluded A convenience sampling technique was employed to select the study participants.

Data on demographic characteristics, including gender, age, gestational age, mode of delivery, and APGAR scores at 1 and 5 minutes, were collected for all neonates. For the cases, additional information on the stage of HIE was recorded. Blood samples were collected within the first 24 hours of life to measure serum calcium levels. Hypocalcemia was defined as a serum calcium level of less than 8 mg/dl. Serum calcium levels were measured using an automated analyzer. All samples were processed in the hospital's central laboratory, following standardized procedures to ensure accuracy and reliability of the results.

The collected data were analyzed using IBM SPSS, version 27.0. Categorical variables, such as gender, mode of delivery, and the presence of hypocalcemia, were presented as frequency and percentage. These were compared between cases and controls using the Chi-square test. Continuous variables, including age, gestational age, APGAR scores, and serum calcium levels, were expressed as mean and standard deviation (SD). The Mann-Whitney U test was used to compare serum calcium levels between cases and controls, as well as between HIE-II and HIE-III stages among the cases.

RESULT

A total of 100 neonates were included in the study, with 70 enrolled as cases and 30 as controls. The demographic characteristics of the study participants are detailed in Table 1. Among the cases, 46 (65.7%) were male, and 24 (34.3%) were female, compared to 21 (70.0%) males and 9 (30.0%) females in the control group. The majority of neonates in both groups were aged between 30 minutes and 24 hours, with 46 (65.7%) in the cases and 20 (66.7%) in the controls. The mean age was 17.5 ± 12.39 hours in the cases and 16.5 ± 14.55 hours in the controls. The mean gestational age was 37.4 ± 1.37 weeks in the cases and 39.3 ± 1.64 weeks in the controls.

Regarding the mode of delivery, 47 (67.1%) of the cases were delivered via C-section, compared to 8 (26.7%) in the control group. The mean APGAR scores at 1 minute and 5 minutes were significantly lower in the cases (2.6 ± 1.26 and 5.6 ± 1.19, respectively) compared to the controls (8.4 ± 0.96 and 8.7 ± 1.08, respectively). All cases were diagnosed with hypoxic ischemic encephalopathy (HIE), with 43 (61.4%) in stage HIE-II and 27 (38.6%) in stage HIE-III.

Table 1: Demographic characteristics of the study participants.

	Cases (n=70)		Control (n=30)	
	n	%	**n**	%
Gender				
Male	46	65.7%	21	70.0%
Female	24	34.3%	9	30.0%
Age groups				
< 30 min	6	8.6%	4	13.3%

30 min - 24 hours	46	65.7%	20	66.7%
> 24 hours	18	25.7%	6	20.0%
Age (hours), mean ± SD	17.5 ± 12.39		16.5 ± 14.55	
Gestational age (weeks), mean ± SD	37.4 ± 1.37		39.3 ± 1.64	
Mode of delivery				
NVD	23	32.9%	22	73.3%
C-section	47	67.1%	8	26.7%
APGAR score, mean ± SD				
1 minute	2.6 ± 1.26		8.4 ± 0.96	
5 minutes	5.6 ± 1.19		8.7 ± 1.08	
Hypoxic ischemic encephalopathy (HIE) stage				
HIE-II	43	61.4%	0	0.0%
HIE-III	27	38.6%	0	0.0%

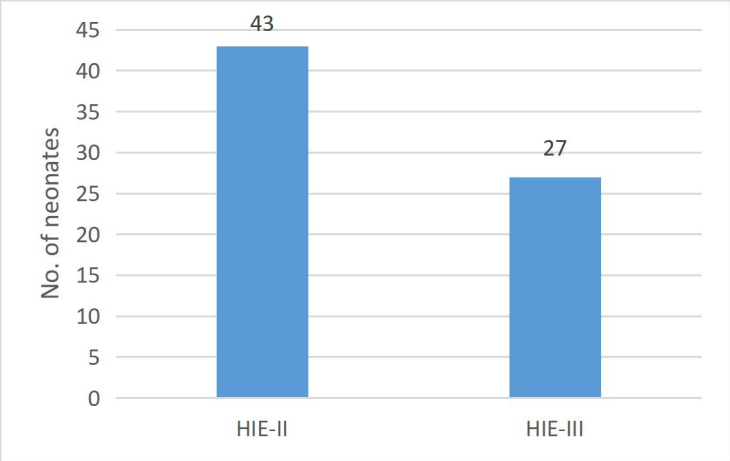

Figure 1: Distribution of neonates based on hypoxic ischemic encephalopathy (HIE) stage.

The prevalence of hypocalcemia (serum calcium < 8 mg/dl) was significantly higher in the cases, with 30 (42.9%) compared to 2 (6.7%) in the controls (p < 0.001) (Table 2). The mean serum calcium level was also significantly lower in the cases (7.14 ± 1.44 mg/dl) compared to the controls (8.43 ± 0.35 mg/dl), with a p-value of < 0.001 (Table 3).

Table 2: Comparison of hypocalcemia in cases and controls.

Hypocalcemia (< 8 mg/dl)	Cases (n=70)		Control (n=30)		p value*
	n	%	n	%	
Yes	30	42.9%	2	6.7%	< 0.001
No	40	57.1%	28	93.3%	

* Chi square test.

Table 3: Comparison of mean serum calcium in cases and controls.

	Cases (n=70)	Control (n=30)	p value*
	Mean ± SD	Mean ± SD	
Serum calcium level (mg/dl)	7.14 ± 1.44	8.43 ± 0.35	< 0.001

*Mann-Whitney U test.

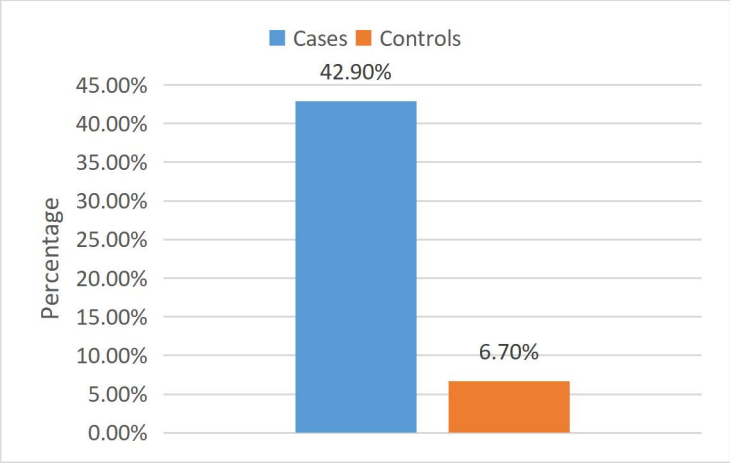

Figure 2: Hypocalcemia between cases and controls.

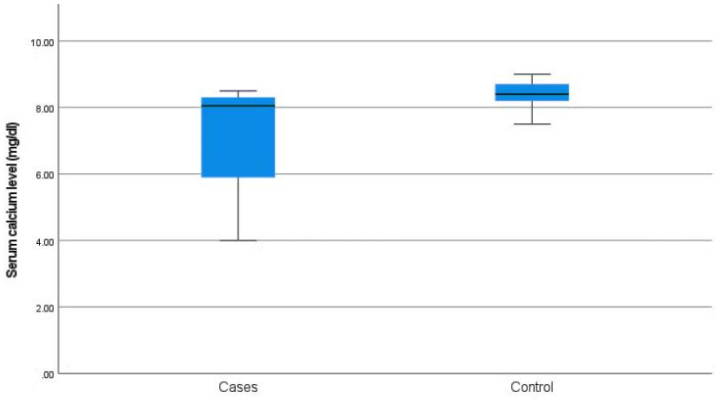

Figure 3: Serum calcium levels between cases and controls.

Among the cases, the prevalence of hypocalcemia was higher in HIE-III neonates (14 [51.9%]) compared to HIE-II neonates (16 [37.2%]), though this difference was not statistically significant (p = 0.228) (Table 4). However, the mean serum calcium level was significantly lower in HIE-III neonates (6.45 ± 1.78 mg/dl) compared to HIE-II neonates (7.58 ± 0.98 mg/dl), with a p-value of 0.005 (Table 5).

Table 4: Comparison of hypocalcemia between HIE-II and HIE-III neonates.

Hypocalcemia (< 8 mg/dl)	HIE-II (n=43)		HIE-III (n=27)		p value*
	n	%	n	%	
Yes	16	37.2%	14	51.9%	0.228
No	27	62.8%	13	48.1%	

* Chi square test.

Table 5: Comparison of mean serum calcium between HIE-II and HIE-III neonates.

	HIE-II (n=43)	HIE-III (n=27)	p value*
	Mean ± SD	Mean ± SD	
Serum calcium level (mg/dl)	7.58 ± 0.98	6.45 ± 1.78	0.005

*Mann-Whitney U test.

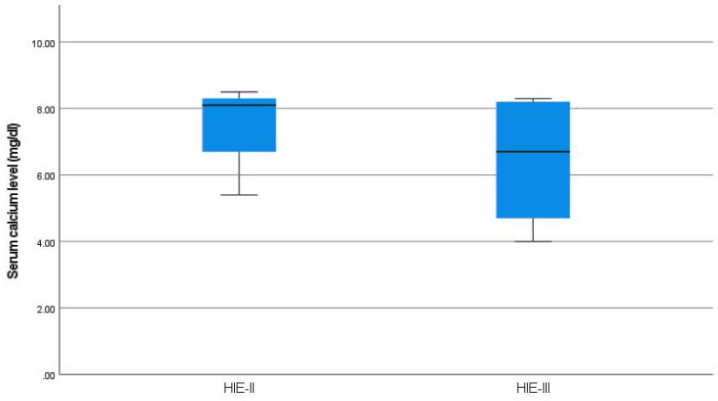

Figure 4: Serum calcium levels among HIE neonates.

DISCUSSION

Hypoxic-ischemic encephalopathy (HIE) is a serious condition in neonates, often leading to long-term neurological damage or death. Calcium plays a crucial role in cellular function, and its dysregulation, particularly hypocalcemia, is a common complication in neonates with HIE. This study focuses on understanding the relationship between serum calcium levels and the severity of HIE in stages II and III. Identifying calcium imbalances early could be vital for improving outcomes in affected neonates. The findings aim to contribute to better clinical management strategies for neonates with HIE.[12]

Our study found a higher proportion of males among both cases (65.7%) and controls (70.0%), which is consistent with the findings by Mahajan et al. (2020), who reported 58% males among cases and 62% among controls.[13] Similarly, Zaman et al. (2021) observed a male predominance, with 63.3% males in cases and 73.3% in controls.[14] The mean gestational age in our cases was 37.4 ± 1.37 weeks, which was significantly lower than the 39.3 ± 1.64 weeks in the controls. This is in contrast to Mahajan et al. (2020), who reported a smaller difference in mean gestational age between cases (2.72 ± 0.44 kgs) and controls (2.80 ± 0.40 kgs). Zaman et al. (2021) reported slightly higher gestational ages in both cases (39.37 ± 1.47 weeks) and controls (39.83 ± 1.64 weeks). Regarding the mode of delivery, our study found a significantly higher rate of C-sections among cases (67.1%) compared to controls (26.7%), which aligns with Zaman et al. (2021), who observed 76.7% of cases delivered via C-section versus 26.7% of controls. [13,14]

The mean APGAR scores at 1 and 5 minutes were significantly lower in our cases (2.6 ± 1.26 and 5.6 ± 1.19, respectively) compared to the controls (8.4 ± 0.96 and 8.7 ± 1.08, respectively). This finding is in line with Zaman et al. (2021), who reported a mean APGAR score of 3.13 ± 1.45 in cases and 8.60 ± 1.03 in controls, indicating that low APGAR scores are a strong predictor of HIE severity. The distribution of HIE stages in our study (61.4% in HIE-II and 38.6% in HIE-III) is comparable to the findings of Khan et al. (2024), who reported 36.6% in HIE-II and 17.5% in HIE-III, although Khan et al. observed a higher proportion of HIE-I cases (39.7%), which were absent in our cohort.[15]

Our study revealed a significantly higher prevalence of hypocalcemia (serum calcium < 8 mg/dl) in the cases, with 42.9% of the cases affected compared to only 6.7% in the control group ($p < 0.001$). Additionally, the mean serum calcium level was significantly lower in the cases (7.14 ± 1.44 mg/dl) compared to the controls (8.43 ± 0.35 mg/dl), with a p-value of < 0.001. This finding is consistent with Khan et al. (2024), who also reported significantly lower mean serum calcium levels in cases (7.54 ± 1.41 mg/dl) compared to controls (8.65 ± 0.62 mg/dl), highlighting a similar trend of hypocalcemia in HIE-affected neonates.[15]Ali et al. (2019) found that 17.5% of their study population had hypocalcemia, a prevalence that is lower than what we observed in our cases but is consistent with the recognition of hypocalcemia as a common electrolyte imbalance in neonates with HIE.[16] Bashir et al. (2016) examined the incidence of electrolyte imbalances in neonates undergoing therapeutic

hypothermia (TH) for HIE, reporting a lower incidence of hypocalcemia (12% in the TH group and 21% in the no TH group) compared to our findings. The difference in hypocalcemia prevalence could be due to the varying effects of therapeutic interventions like hypothermia, which may help mitigate some of the metabolic disturbances associated with HIE.[17]

In our study, we found that the prevalence of hypocalcemia was higher among HIE-III neonates (51.9%) compared to HIE-II neonates (37.2%), although this difference was not statistically significant ($p = 0.228$).Our findings are consistent with those reported by Hassan et al. (2022), who observed a significant correlation between HIE stage and mean serum calcium levels, with progressively lower calcium levels as the severity of HIE increased (7.89 ± 0.54 mg/dl in HIE-I, 7.36 ± 0.28 mg/dl in HIE-II, and 6.88 ± 0.28 mg/dl in HIE-III; $p = 0.001$).[18] Schedewie et al. also reported significantly lower plasma calcium concentrations in asphyxiated infants compared to controls, which is in line with our observation of lower mean serum calcium levels in HIE cases.[19] Similarly, Pyati et al. found that calcium levels were significantly lower in asphyxiated newborns (6.85 ± 0.95 mg/dl) compared to healthy controls (9.50 ± 0.51 mg/dl; $p = 0.001$), supporting the trend of hypocalcemia being associated with birth asphyxia and its severity.[20] Getaneh et al., in their case-control study, also demonstrated significant variations in mean serum calcium levels between asphyxiated and healthy newborns, with asphyxiated neonates having lower calcium levels (7.37 ± 0.10 mg/dl) compared to controls (8.04 ± 0.09 mg/dl).[21] Mollah et al. (2017) reported similar findings, where the mean serum calcium levels were lower in HIE-II (7.14 ± 0.94 mg/dl) and HIE-III (6.58 ± 0.78 mg/dl) neonates, with hypocalcemia prevalence being 35.82% in HIE-II and 57.14% in HIE-III. Their results align closely with our study, particularly in the lower calcium levels observed in HIE-III neonates.[22]

The study provides valuable insights into the relationship between hypocalcemia and the severity of hypoxic-ischemic encephalopathy (HIE) in neonates, with a clear focus on quantitative analysis of serum calcium levels. The study's cross-sectional design and small, single-center sample size limit its generalizability, and the lack of long-term follow-up prevents assessment of the lasting impact of hypocalcemia in these patients.

CONCLUSION

Our study highlights a significant association between hypocalcemia and the severity of hypoxic-ischemic encephalopathy (HIE) in neonates, emphasizing the need for early detection and management of calcium imbalances to improve outcomes in affected infants.

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