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Original Article

Early Serum Creatine Kinase-BB (CK-BB) Levels as Predictors of Hypoxic-Ischemic Encephalopathy Severity and Outcome in Term Neonates: A Prospective Observational Study

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ABSTRACT

Background: Hypoxic-ischemic encephalopathy (HIE) remains a leading cause of neonatal mortality and long-term neurodevelopmental impairment. Early identification of moderate to severe HIE is essential to initiate timely neuroprotective interventions. Creatine kinase-BB (CK-BB), a brain-specific isoenzyme, has been suggested as a biochemical marker of neuronal injury, but its reliability as a diagnostic tool remains uncertain in routine clinical settings. **Objectives**: To assess whether early serum CK-BB levels help to predict the severity of HIE and to determine if CK-BB correlates with clinical severity of HIE.

Methodology: A prospective observational study was conducted in a tertiary NICU over six months, enrolling 48 term neonates diagnosed with HIE and 48 gestational age–matched healthy controls. Serum CK-BB levels were measured at 6–24 hours and 48–72 hours of life. Group-wise comparisons and trend analysis across severity grades were performed.

Results: Mean CK-BB levels at 6–24 hours were 38.07 ± 11.13 U/L in HIE cases and 37.62 ± 11.27 U/L in controls. At 48–72 hours, mean levels were 37.70 ± 12.24 U/L in cases versus 35.24 ± 13.82 U/L in controls with no statistically significant difference ($p > 0.05$), and CK-BB levels did not correlate with severity of HIE. Conclusion: In contrast to earlier reports, this study found no significant elevation of CK-BB in neonates with HIE compared to controls suggesting that CK-BB alone, may not be a reliable early diagnostic or prognostic marker for HIE in term neonates. Its routine use in clinical decision-making warrants re-evaluation, particularly in low-resource settings.

Keywords*: Neonate, Hypoxic-ischemic encephalopathy (HIE), Creatine kinase-BB (CK-BB), Biomarkers, Sarnat and Sarnat staging.*

INTRODUCTION

Hypoxic-ischemic encephalopathy (HIE) remains a significant cause of neonatal mortality and long-term neurodevelopmental disability worldwide. Globally, it accounts for over 1 million neonatal deaths annually and contributes substantially to the burden of childhood neurological impairment, particularly in low- and middle-income countries (LMICs) where access to timely and advanced neonatal care remains limited.[1,2] Survivors of moderate to severe HIE are at high risk for cerebral palsy, epilepsy, cognitive delays, and sensory deficits, many of which could be mitigated by prompt identification and intervention.[3]

Therapeutic hypothermia is currently the only evidence-based neuroprotective strategy for HIE, yet its efficacy is highly time-dependent and must be initiated within the first six hours of life.[4] This imposes a critical demand on clinicians to accurately recognize and stratify affected neonates during the immediate postnatal period. Traditional diagnostic tools—including Apgar scores, cord blood gases, and clinical staging systems such as Sarnat and Sarnat—lack the sensitivity, objectivity, and predictive precision required for early risk stratification. [5,6]

In this context, there is a growing interest in identifying affordable, rapid, and widely applicable biochemical markers that can aid early diagnosis and outcome prediction in HIE. One such marker is creatine kinase-BB (CK-BB), a brain-specific isoenzyme released into the circulation following neuronal injury due to hypoxia-ischemia.[9] The pathophysiological plausibility of CK-BB as a biomarker is compelling: it reflects early neuronal membrane disruption and is measurable in the serum within hours of onset of injury.

Several clinical studies have reported elevated CK-BB levels in cord blood or early neonatal samples of infants with HIE, often correlating with the severity of encephalopathy and long-term neurodevelopmental outcomes. [10–13]. The clinical reliability of CK-BB remains a subject of debate. Contradictory findings have emerged across settings, raising concerns about its diagnostic consistency.

This paradox is especially relevant in LMICs, where low-cost, early biomarkers could fill a critical diagnostic gap if proven reliable. However, data from such real-world settings, particularly from Indian neonatal units, remain limited. Most existing studies have been conducted in controlled or high-resource environments, leaving a translational gap in applying CK-BB findings to broader clinical practice.

This prospective observational study is aimed to determine whether CK-BB levels differed significantly between neonates with and without HIE, and whether they correlated with clinical severity of encephalopathy. This study contributes critical data to an ongoing international debate on the marker's clinical validity and applicability.

MATERIALS AND METHODS

This was a prospective observational study conducted in the Neonatal Intensive Care Unit (NICU) of Rangaraya Medical College, Kakinada, Andhra Pradesh, India, from October 2023 to March 2024. Term neonates (≥37 weeks) admitted within the first 24 hours of life were screened.

Term neonates with clinical diagnosis of HIE based on modified Sarnat staging were enrolled as cases and healthy term neonates without perinatal asphyxia, matched for gestation and sex were taken as controls

Neonates with congenital anomalies, suspected inborn errors of metabolism, clinical evidence of sepsis, and those who received anticonvulsant therapy were excluded. Neonates with a history of birth trauma and those for whom parental consent was not given were also excluded.

Sample Size Estimation
All enrolled neonates underwent detailed clinical and neurological assessment upon admission and daily thereafter. The modified Sarnat and Sarnat criteria was used to classify severity of HIE into Stage I (mild), Stage II (moderate), or Stage III (severe). Seizures, tone abnormalities, level of consciousness, and autonomic function were assessed serially by trained neonatologists.

A total of 96 neonates were enrolled—48 in the HIE group and 48 in the control group. The sample size was based on feasibility and prior biomarker studies in HIE populations, and was expected to provide >80% power to detect a mean CK-BB difference of 10 U/L between groups at $\alpha = 0.05$.

Blood samples were collected at 6–24 hours and 48–72 hours for estimation of CKBB levels. 2 mL venous blood was centrifuged and analyzed using an immunoinhibition-based kinetic assay (e.g., Beckman Coulter AU480). Reference range: 0–45 U/L. Blinded analysis with internal QC was followed.

Additional data collected for each neonate included gestational age and mode of delivery, Apgar scores at one and five minutes, the need for therapeutic hypothermia, mechanical ventilation, or anticonvulsant therapy, and the duration of NICU stay. Information regarding survival status and short-term neurological outcome at discharge was also documented. All enrolled neonates were followed up until either discharge or in-hospital mortality.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 26.0. Continuous variables were presented as mean ± standard deviation (SD) and compared using the independent samples t-test or Mann–Whitney U test, depending on distribution. Categorical variables were analyzed using the Chi-square or Fisher's exact test.

Correlations between CK-BB levels and HIE stage were assessed using Pearson's correlation coefficient. A p-value <0.05 was considered statistically significant.

Ethical Considerations

The study was approved by the Institutional Ethics Committee of Rangaraya Medical College, Kakinada (Approval No. RMCH/HIE/CKBB/23). Written informed consent was obtained from the parents or legal guardians of all enrolled neonates.

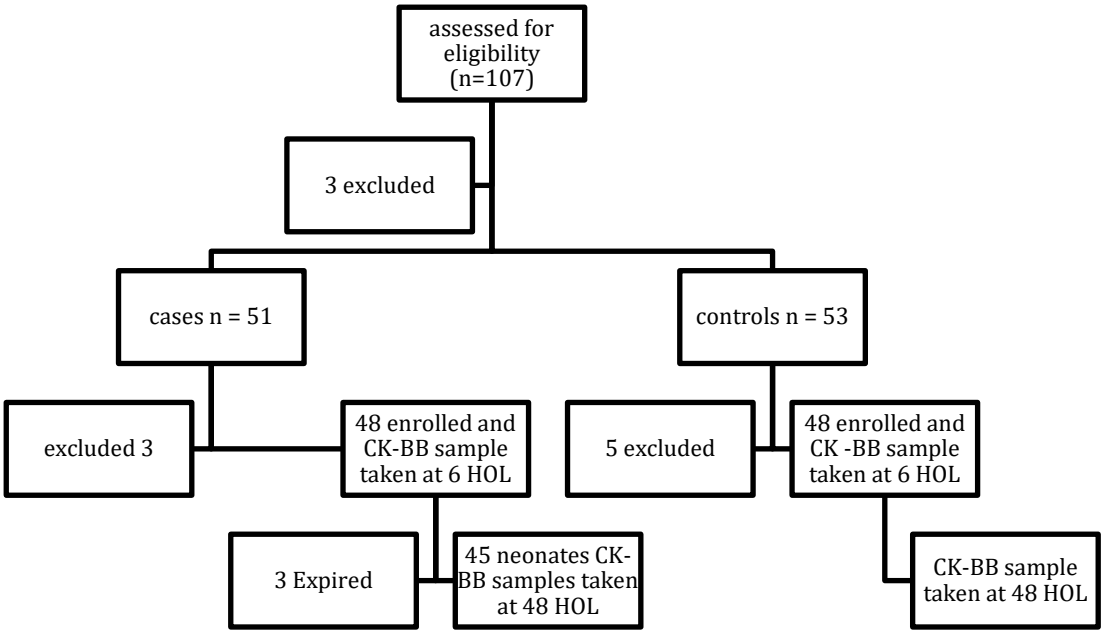

Figure 1. CONSORT Flow Diagram

RESULTS

Study Overview and Baseline Characteristics

A total of 96 term neonates were enrolled in the study, divided equally into 48 cases with hypoxic-ischemic encephalopathy (HIE) and 48 term controls without evidence of perinatal asphyxia. All neonates were inborn and delivered at gestational age ≥37 weeks. Blood samples were obtained at two time points—6 to 24 hours of life and 48 to 72 hours of life—for the measurement of creatine kinase-BB (CK-BB) levels.

Among the 48 HIE cases, 35 (72.9%) were early term and 13 (27.1%) were full term. In the control group, 36 (75%) were early term and 12 (25%) were full term. In the HIE group, 9 neonates (18.8%) had a birth weight between 1.8 and 2.499 kg, 36 (75%) between 2.5 and 3.499 kg, and 3 (6.2%) weighed more than 3.5 kg. In the control group, 15 neonates (31.2%) weighed between 1.8 and 2.499 kg, 29 (60.4%) between 2.5 and 3.499 kg, and 4 (8.3%) had a birth weight above 3.5 kg. Among HIE cases: 38 (79.2%) were appropriate for gestational age (AGA), 9 (18.8%) were small for gestational age (SGA), and 1 (2.1%) was large for gestational age (LGA).

- Among controls: 33 (68.8%) were AGA, 14 (29.2%) were SGA, and 1 (2.1%) was LGA. Among HIE cases, 21 neonates (43.8%) were male and 27 (56.2%) were female. Among controls, 22 (45.8%) were male and 26 (54.2%) were female. In the HIE group, 31 neonates (64.6%) were delivered via vaginal delivery (unassisted or assisted), while 17 (35.4%) were born via lower segment cesarean section (LSCS). In the control group, 29 (60.4%) were delivered vaginally and 19 (39.6%) were delivered by LSCS.

Table 1: Demographic characteristics of the study population

Variable	HIE Cases (n=48)	Controls (n=48)
Gestational Age		
Early term (37–38 +6/7)	35	36
Full term (39–40 +6/7)	13	12

Birth Weight		
1.8–2.499 kg	9	15
2.5–3.499 kg	36	29
>3.5 kg	3	4
Weight-for-GA Classification		
AGA	38	33
SGA	9	14
LGA	1	1
Gender		
Male	21	22
Female	27	26
Mode of Delivery		
Vaginal delivery (Un/assisted)	31	29
LSCS	17	19

At 6 hours of life, the mean CK-BB in cases was 38.06 ± 11.13 IU/L, while in controls it was 37.61 ± 11.26 IU/L (p = 0.842). At 48–72 hours, the mean CK-BB levels were 37.69 ± 12.24 IU/L in cases and 35.23 ± 13.82 IU/L in controls (p = 0.358). These differences were not statistically significant.

Further subgroup analysis based on Sarnat staging revealed that the mean CK-BB level at 6 hours in stage 1,2 and 3 was 39.24, 38.46, 29.59 respectively and mean CKBB level at 48 hours in stage 1,2and 3 was 39.03, 40.16, 24.00 respectively.

Table 2: CK-BB Levels in Cases, Controls, and by Sarnat Stage

Group / Sarnat Stage	No. of Cases	CK-BB @ 6 HOL (Mean ± SD)	CK-BB @ 48–72 HOL (Mean ± SD)	p-value
Controls	48	37.61 ± 11.26	35.23 ± 13.82	
All HIE Cases	48	38.06 ± 11.13	37.69 ± 12.24	
Comparison (Cases vs Controls)				0.842 (6 HOL), 0.358 (48 HOL)
Sarnat Stage 1	33	39.24	39.03	
Sarnat Stage 2	10	38.46	40.16	
Sarnat Stage 3	5	29.59	24 (30)	

CK-BB Levels and Morbidities in HIE Cases

The relationship between CK-BB levels and clinical morbidities was analyzed among neonates diagnosed with HIE using the independent two-sample t-test (Welch's t-test). Mean CK-BB levels at 6–24 hours and 48–72 hours of life were compared between neonates affected and unaffected by each morbidity.

Neonates requiring inotropic support had significantly lower CK-BB levels at 6 hours (mean 32.87 IU/L) compared to those who did not (mean 39.8 IU/L), with a p-value of 0.046. However, this difference was not statistically significant at 48 hours (p = 0.343). For other morbidities—including blood product transfusion, AKI, and meconium aspiration syndrome (MAS)—no statistically significant differences in CK-BB levels were observed.

P-values could not be calculated for mechanical ventilation >24 hours and MAS due to low group size or absence of variance in CK-BB levels.

Table 3: CK-BB Levels in HIE Cases by Morbidity Status

Morbidity	Affected (n)	CK-BB @6 HOL	CK-BB @48 HOL	Unaffected (n)	CK-BB @6 HOL	CK-BB @48 HOL	p-value (6 HOL)	p-value (48 HOL)
MV >24 hours	4	30.05	29.7	44	38.8	38.43	NA (n<2)	NA (n<2)
Shock & Inotropes	12	32.87	34.97	36	39.8	38.61	0.046	0.343
Blood products transfused	7	36.38	40.54	41	38.36	37.21	0.715	0.566
AKI							-	-
MAS	18	36.16	35.17	30	39.21	39.21	NA (no variance)	NA (no variance)

Outcome Correlates in Survivors of HIE Cases

CK-BB levels were analyzed in relation to selected outcome parameters among surviving neonates with HIE. This included time to establish direct breastfeeding or paladai feeding, total hospital stay, and antiepileptic drug (AED) use at discharge. Statistical analysis was performed using Welch's t-test to account for unequal group sizes and variance.

Among survivors, those who attained paladai or direct breastfeeding within 7 days had higher CK-BB levels than those with delayed feeding. This difference was statistically significant at both 6 hours ($p = 0.024$) and 48 hours ($p = 0.001$). The number of neonates discharged on AED was too small to allow for valid statistical comparison. CKBB levels were higher both at 6 hours and 48 hours in those who had hospital stay of >10 days compared to those who stayed for <10 days but this difference is not statistically significant.

Table 4: CK-BB Levels in Survivors Based on Outcome Correlates

Outcome Correlate	No. of Cases Affected	CK-BB @6 HOL (Mean)	CK-BB @48 HOL (Mean)	p-value (6 HOL)	p-value (48 HOL)
Day of attaining paladai/ DBF >7 days	7	36.67	38.39	0.024	0.001
Day of attaining paladai/ DBF ≤7 days	36	39.42	38.70		
Duration of hospital stay >10 days	5	40.47	42.90	0.680	0.419
Duration of hospital stay ≤10 days	38	38.77	38.09		
On AED at discharge	4	36.76	34.70	NA (n<2)	NA (n<2)
Stopped AED before discharge	39	39.19	39.05		

CK-BB Levels in Relation to Mortality

The association between CK-BB levels and mortality was evaluated among neonates with HIE. Out of 48 cases, 43 neonates were discharged, and 5 expired during the NICU stay.

At 6 hours of life, the mean CK-BB level was significantly lower in neonates who expired (30.34 IU/L) compared to those who survived (38.97 IU/L), with a p-value of 0.019. However, no statistically significant difference was observed at 48–72 hours of life (mean 29.53 IU/L in expired vs. 38.65 IU/L in survivors; $p = 0.367$).

Table 5: CK-BB Levels in Survivors vs Expired Cases

Mean CK-BB	Cases Discharged (n=43)	Cases Expired (n=5)	p-value
6 HOL	38.97	30.34	0.019
48–72 HOL	38.65	29.53	0.367

Summary of Key Findings

In this prospective study evaluating CK-BB as a biomarker in neonates with hypoxic-ischemic encephalopathy (HIE), CK-BB levels were measured at two time intervals—6 to 24 hours and 48 to 72 hours of life.

Baseline demographic characteristics were comparable between HIE cases and controls. Mean CK-BB values were higher in HIE cases than in controls at both time points, though the differences were not statistically significant. Within the HIE group, no consistent rise in CK-BB was observed with increasing Sarnat stage; instead, values in Stage 3 were paradoxically lower.

DISCUSSION

This prospective observational study aimed to evaluate utility of serum creatine kinase-BB (CK-BB) in term neonates to predict the severity of hypoxic-ischemic encephalopathy (HIE). While CK-BB is theoretically compelling as a brain-specific isoenzyme released in response to neuronal injury, our findings reveal substantial limitations in its real-world applicability, particularly in low-resource neonatal intensive care units (NICUs).

Contrary to previous studies such as those by Alkholy et al.[9] and Nagdyman et al.[10], which reported significant CK-BB elevation in moderate-to-severe HIE, this study did not demonstrate any statistically significant difference between cases and controls at either 6–24 or 48–72 hours. Furthermore, CK-BB levels did not correlate consistently with Sarnat staging; paradoxically, Stage 3 neonates exhibited lower mean values than those with Stage 1 or 2 encephalopathy. Similar discordant findings have been reported by Fernandez et al.[13] and Sweet et al.[11].

Multiple factors may underlie this discrepancy. First, CK-BB levels are influenced by systemic variables such as perinatal acidosis, mode of delivery, and umbilical manipulation, all of which may contribute to nonspecific elevations.[14,15] Second, the timing of biomarker release is critical; in severe cases, neuronal shutdown or cellular lysis may alter the temporal expression of CK-BB, leading to a biphasic or blunted profile. Third, enzymatic degradation or early clearance may reduce measurable levels by the time routine testing occurs.

Interestingly, CK-BB levels were significantly lower in neonates with adverse clinical profiles—those requiring inotropes, those with delayed establishment of enteral feeds, and those who expired. Although this initially appears counterintuitive, it may reflect either reduced neuronal viability, compromised systemic circulation, or impaired cellular release mechanisms in severely affected neonates. These findings caution against over interpretation of low CK-BB levels as reassuring in isolation.

Results of this study did not identify any meaningful diagnostic thresholds that could guide therapeutic decisions. The extensive overlap between control and HIE subgroups, particularly in mild to moderate encephalopathy, diminishes the utility of CK-BB as a standalone marker. This is consistent with findings from recent systematic reviews questioning the sensitivity and specificity of early serum biomarkers in HIE.[6,7]

From a clinical standpoint, these findings underscore the continued primacy of detailed neurological examination and scoring systems such as the Thompson or modified Sarnat, especially in resource-limited settings. CK-BB, while inexpensive and rapid to measure, should be interpreted cautiously and within the broader clinical context. Our results also highlight the importance of considering paradoxical biomarker behavior in severe disease states, where organ perfusion and cellular integrity may be compromised.

Notably, this study adds to the scarce Indian literature on CK-BB trends in HIE, providing data from a real-world NICU cohort. Most existing evidence stems from high-resource, controlled environments; our findings reflect the variability and challenges inherent in LMIC settings.

Future directions should include exploration of CK-BB in multi-marker models alongside neuron-specific enolase (NSE), S100B, and amplitude-integrated EEG (aEEG). Composite biomarker panels and machine learning–assisted algorithms may offer superior predictive precision.[17] Cost-effectiveness studies assessing the incremental utility of CK-BB within such models would be particularly relevant for LMICs.

The strengths of this study include its prospective design, gestational age–matched control group, and dual-time point CK-BB assessment. However, limitations must be acknowledged: single-center setting, modest sample size, absence of long-term neurodevelopmental follow-up, and lack of correlation with MRI or electrophysiological data. Sampling did not extend beyond 72 hours, and ultra-early (<3 hour) time points were not explored.

CONCLUSION

In this prospective cohort study, CK-BB levels measured at 6 to 24 hours and 48 to 72 hours of life did not significantly differentiate term neonates with HIE from healthy controls. Furthermore, CK-BB levels did not correlate reliably with clinical severity based on Sarnat staging. Paradoxically, lower CK-BB levels were observed among neonates with poor outcomes, including mortality, inotrope requirement, and delayed enteral feeding, suggesting complex kinetics and systemic influences on biomarker expression.

These findings suggest that CK-BB, when used in isolation, may not serve as a reliable standalone biomarker for early stratification of HIE severity. Its use in clinical practice should remain adjunctive and interpreted in conjunction with neurological examination and other modalities. Caution should be exercised in using CK-BB levels as a sole criterion for therapeutic decisions, particularly in resource-limited settings.

FUTURE SCOPE

Future research should focus on integrating CK-BB into multimodal prognostic frameworks that include other biomarkers such as S100B, neuron-specific enolase (NSE), and electrophysiological tools like aEEG. Serial sampling at earlier and more frequent intervals—especially within the first 3 hours of life—may provide clearer insight into CK-BB

kinetics and peak expression. Larger, multicentric studies with neurodevelopmental follow-up and imaging correlations are essential to validate its clinical application.

The potential inclusion of CK-BB into machine learning–based decision-support models and its role in cost-effective risk stratification protocols in LMIC settings warrant further exploration.

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