Original Article

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Paediatrics Section

Clinical Spectrum of Neonatal Encephalopathy and the Role of CK-MB Assay in Transient Myocardial Ischaemia in Neonates with Hypoxic Ischaemic Encephalopathy: A Prospective Cohort Study

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ABSTRACT

Introduction: Significant advancements have been made in the field of perinatal medicine through the use of newer monitoring technologies. However, birth asphyxia continues to cause prolonged hospitalisation due to multiple organ dysfunction and unfortunately, sometimes results in death. An elevation of the serum Creatine Kinase Myocardial Bound (CK-MB) fraction in myocardial ischaemia could indicate exposure to asphyxia and shock in neonates.

Aim: The aim of this study was to examine the clinical spectrum of Neonatal Encephalopathy (NE) and investigate Transient Myocardial Ischaemia (TMI) in neonates with Hypoxic Ischaemic Encephalopathy (HIE) using CK-MB assay at birth, 24 hours, and 72 hours.

Materials and Methods: A prospective clinical study was conducted in the Neonatal Intensive Care Unit (NICU) at a Tertiary Care Hospital in Tamil Nadu, India. The study duration was eight months, from January 2014 to August 2014. A total of 70 term neonates admitted with NE symptoms were included. Among them, 65 babies with evidence of asphyxia, such as an Appearance, Pulse, Grimace,Activity and respiration (APGAR) score less than 3 at five minutes, heart rate less than 60 Beats Per Minute (bpm), meconium-stained amniotic fluid, or the need for positive pressure ventilation for more than one minute, were evaluated for TMI. Myocardial involvement was assessed through clinical evaluation, chest X-ray (CXR), Electrocardiogram (ECG), Echocardiography (ECHO), and CK-MB assay at birth, 24 hours, and 72 hours. Statistical analysis was performed using Epi Info software version 7.0.

Results: The mean Gestational Age (GA) of the study participants was 38.97±1.31 weeks. During the study period, 70 babies were admitted with NE symptoms, and HIE was the cause of encephalopathy in 65 babies. Other causes included hypoglycaemia, intraventricular haemorrhage, and bilirubin encephalopathy. Meconium staining of amniotic fluid was the most common intrapartum event in HIE babies. The survival rate in encephalopathy cases was 28.57%. Shock and respiratory failure were common complications in HIE, representing 46.15% and 44.61% respectively. Cardiomegaly in CXR, ST depression in ECG, Tricuspid Regurgitation (TR) in ECHO, and elevated CK-MB were commonly detected in babies with TMI associated with HIE.

Conclusion: NE caused by HIE has a high mortality and morbidity rate. 83.3% of asphyxiated babies with shock had elevated CK-MB levels at birth, but the CK-MB values at 24 hours and 72 hours were not significantly elevated.

Keywords: Birth asphyxia, Ischaemic markers, Neonatal echocardiography, Serum creatine kinase myocardial bound

INTRODUCTION

Neonatal encephalopathy is a clinical term that describes an abnormal neurobehavioral state consisting of an altered level of consciousness. It does not imply a specific aetiology. HIE describes encephalopathy with objective data to support hypoxic ischaemia as the underlying cause [1]. The National Neonatal-Perinatal database defines perinatal asphyxia as “slow gasping breathing or an APGAR score of 4-6 as moderate asphyxia, whereas the absence of breathing or an APGAR score of 0-3 at one minute as severe asphyxia” [2]. According to the National Neonatology Forum of India (NNF), asphyxia is defined as “gasping respiration or ineffective breathing efforts or absence of breathing at one minute of life” [2].

The frequency of perinatal asphyxia is approximately 1.5% of live births in developed countries with advanced obstetric or neonatal care. Perinatal asphyxia accounts for 20% of perinatal deaths (50% if stillbirths are included). The incidence of perinatal asphyxia is estimated to be 10 to 15 times higher in low to middle-income countries [1]. Prolonged total or partial asphyxia commonly results in decreased ventricular contractility and declining cardiac output. In addition to biochemical and radiological evidence of TMI, there is also noted contractile dysfunction of the heart [3-5]. In cases of TMI, the neonate may exhibit tachycardia, a murmur indicating tricuspid valve insufficiency, congestive cardiac failure, and, in severe cases, cardiogenic shock [6]. Due to the decline in cardiac function, the child may develop hypotension following asphyxia [7]. The neonatal heart obtains glucose through glycogenolysis from its own glycogen storage. Experimental data suggests that, because of this characteristic, the immature heart has the capability to recover from short periods of ischaemia compared to the adult heart [8]. The ECG may show ST depression in the mid precordium and T-wave inversion in the left precordium. Tricuspid insufficiency is functional and secondary to acute cardiac dilation. Echocardiographic findings most commonly include decreased left ventricular contractility, elevated ventricular end-diastolic pressures, Tricuspid Regurgitation (TR) (due to papillary muscle ischaemia), and pulmonary hypertension. In severe asphyxia, dysfunction more commonly affects the right ventricle [1].

Creatine Phosphokinase (CPK) or Creatine Kinase (CK) is an enzyme expressed in many cell types. CK-MB, the muscle-brain isoenzyme, is primarily present in cardiac muscle and in small

quantities in skeletal muscle. There are several reports stating that levels of CK-MB are significantly elevated in babies who had birth asphyxia compared to those who did not [9,10]. Gunes T et al., published data on CK-MB and echocardiography. Babies with severe asphyxia had significantly elevated CK-MB values in the first two to four hours compared to healthy babies and babies with mild asphyxia [10]. The CK-MB values on the third day showed no difference, and the echocardiography performed on the first day of life showed involvement in 80% of severely asphyxiated babies. However, echocardiography taken on day seven and day 15 showed normal results in all babies. Boo Y et al., found that levels of CK-MB in asphyxiated infants peaked significantly at 12 hours of life before returning to a low level at 48 hours of life [11]. However, studies with serial measurements of CK-MB values in the first three days are lacking. Therefore, the present study aimed to investigate the clinical spectrum of NE and TMI in neonates with HIE using CK-MB assay at birth, 24 hours, and 72 hours.

MATERIALS AND METHODS

A prospective clinical study was conducted in the NICU at a Tertiary Care referral Hospital in Tamil Nadu, India. The study duration was eight months, from January 2014 to August 2014. Ethical clearance for the study was obtained from the Institutional Ethical Committee (Ref. No. 491/PAED/2013/41).

Inclusion criteria: All neonates with features of NE were included to describe the clinical spectrum. Babies with evidence of asphyxia, such as a five-minute APGAR score less than 3, heart rate less than 60 bpm, meconium-stained amniotic fluid, or the need for positive pressure ventilation for more than one minute, were included in the study [1].

Exclusion criteria: Neonates with congenital malformations and mothers who had received magnesium sulphate injection within four hours prior to delivery or received opioids were excluded from the study.

Sample size calculation: The calculated sample size was 60, using the formula:

$$n=z^2p(1-p)/d^2,$$

where n is the sample size, 'z' is 1.96 for a 95% confidence level, 'p' is the prevalence, and 'd' is the degree of error, based on a previous study by Agarwal J et al., (2012) [12] where CK-MB was raised in 86.6% of asphyxiated babies (with an allowable error of 10%).

Study Procedure

All included neonates were clinically evaluated and underwent CXR, biochemical analysis for CK-MB, microbiological analysis, and neurosonogram to determine the cause of encephalopathy. The outcome of each neonate was recorded as discharged or expired. Term neonates who had suffered perinatal asphyxia and developed HIE were enrolled for the evaluation of TMI. Myocardial involvement was assessed through clinical evaluation, CXR, ECG, ECHO, and CK-MB assay at birth, 24 hours, and 72 hours. Written consent was obtained from parents, and data regarding detailed maternal history, details of meconium staining of amniotic fluid, birth events, APGAR score, gender, and weight of the baby were recorded. The gestational age of the baby was assessed using the New Ballard scoring system [13]. Treatment was provided according to the existing institutional protocol. Neonatal hypotension or shock was defined as a mean arterial pressure less than 30 mmHg or a low Blood Pressure (BP) value accompanied by clinically detectable evidence of circulatory compromise, such as poor peripheral perfusion or decreased urine output [14]. Perfusion Index (PI) using pulsoximetry and BP were measured to detect the presence of shock. Serum CK-MB assay was performed at birth, 24 hours, and 72 hours. ECG and CXR were taken to assess cardiac dysfunction. Echocardiography was performed in 18 babies based on the newborn's transportability to the Department of Cardiology.

STATISTICAL ANALYSIS

Data was entered into an Excel spreadsheet and analysed using Epi Info software version 7.0. Frequencies, percentages, means, and Standard Deviation (SD) were calculated using this software. Statistical analysis was performed using the chi-square test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The mean gestational age of the newborn babies was 38.97±1.31 weeks. Among the 70 cases of NE, there were 35 males and 35 females. Forty (57.14%) Babies were born in the hospital of study (inborn) and 30 (42.85%) babies were born in other hospital and referred here (outborn). Natural labor was the most common mode of delivery, while breech and vacuum-assisted vaginal delivery were the least common methods [Table/Fig-1].

Characteristics	Frequency n (%)
Gender	
Male	35 (50)
Female	35 (50)
Place of birth	
Inborn	40 (57.14)
Outborn	30 (42.85)
Outcome	
Discharged	20 (28.57)
Expired	50 (71.42)
Asphyxia as cause of encephalopathy	
Present	65 (92.85)
Absent	5 (7.14)
Mode of delivery	
Labour natural	36 (51.43)
Lower segment caesarian section	20 (28.57)
Forceps-assisted	10 (14.28)
Vaccum-assisted	2 (2.86)
Breech	2 (2.86)

[Table/Fig-1]: Demographic data and clinical spectrum of NE (N=70).

Among the 70 babies with NE, 65 (92.8%) had HIE. Hypoglycemia and bilirubin encephalopathy accounted for 2 (2.8%) cases each, followed by intraventricular haemorrhage which accounted for only 1 (1.4%) case [Table/Fig-2]. Meconium Staining of the Amniotic Fluid (MSAF) was the most common intrapartum event, while 12 (18.46%) babies had no adverse intrapartum events [Table/Fig-3]. ECG positive findings were only seen in 20 (30.76%) asphyxiated newborns. Using echocardiography, 12 (66.67%) babies were reported to have a normal heart without any structural or functional abnormalities, as determined by the cardiologist.

Causes	No. of babies n (%)	Expired (n=50)
HIE	65 (92.8)	49
Hypoglycaemia	2 (2.8)	nil
Intraventricular haemorrhage	2 (2.8)	nil
Bilirubin encephalopathy	1 (1.4)	1

[Table/Fig-2]: Causes of NE and disease specific mortality (N=70).
HIE: Hypoxic ischemic encephalopathy

Intrapartum events	Number of babies n (%)
MSAF	40 (61.53)
Cord around the neck	6 (9.23)
Obstructed labour	5 (7.69)
Cord prolapse	2 (3.07)
No adverse events	12 (18.46)

[Table/Fig-3]: Intrapartum events in HIE babies.
MSAF: Meconium staining of amniotic fluid

One baby had both Tricuspid Regurgitation (TR) and decreased left ventricular contractility. Three babies had TR alone, and two other babies had decreased left ventricular contractility alone. Blood culture analysis showed growth in 8 (12.31%) babies, with Staphylococcus aureus being the most common organism grown in 3 (4.61%) cases [Table/Fig-4].

Investigations	Findings	HIE cases, n (%)
Chest X-ray	Normal	25 (38.46)
	Cardiomegaly	27 (41.54)
	Hyperinflated lungs	9 (13.84)
	Meconium aspiration syndrome	4 (6.15)
ECG	Normal	45 (69.23)
	T wave inversion	6 (9.23)
	ST depression	14 (21.53)
Echocardiographic findings*	Normal	12 (66.67)
	Decreased LV contractility	3 (16.66)
	Tricuspid regurtitation	4 (22.22)
Blood culture analysis	No growth	57 (87.69)
	Staphylococcus aureus	3 (4.61)
	Klebsiella	2 (3.08)
	CONS	2 (3.08)
	Acinetobacter	1 (1.54)

[Table/Fig-4]: Investigation findings in HIE babies.
*In ECHO, one baby had both regurgitation and decreased Left Ventricular (LV) contractility
CONS: Coagulase negative staphylococcus (N=65)

In the present study, the mean CK-MB value was lowest at birth, increased to a maximum after 24 hours, and then decreased after 72 hours. The sensitivity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) of CK-MB were highest at birth [Table/Fig-5]. In the present study, 30 (83.33%) babies with shock had significantly elevated CK-MB values at birth, with a p-value <0.001. Only 7 (25%) of the HIE babies with shock had elevated CK-MB at 24 hours (p-value >0.05). Likewise, only 4 (50%) of the HIE babies with shock had elevated CK-MB at 72 hours (p-value >0.05) [Table/Fig-6].

CK-MB	Mean±SD	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
At birth	13.14±12.03	86	80	83	83
At 24 h	40.96±25.92	37	38	25	52
At 72 h	27.97±14.87	36	82	50	72

[Table/Fig-5]: CK-MB values at birth, 24 hours and 72 hours.
PPV: Positive predictive value; NPV: Negative predictive value

Shock	Elevated n (%)	Not elevated n (%)	Chi-square value	p-value
CK-MB at birth				
Present	30 (83.33)	6 (16.67)	25.63	<0.001
Absent	5 (17.24)	24 (82.76)		
CK-MB at 24 hours				
Present	7 (25)	21 (75)	2.12	>0.05
Absent	12 (48)	13 (52)		
CK-MB at 72 hours				
Present	4 (50)	4 (50)	0.51	>0.05
Absent	7 (28)	18 (72)		

[Table/Fig-6]: CK-MB association with shock at birth, 24 hours and 72 hours.
p-value calculated using Chi-square test

Among the 65 babies with HIE, 29 (44.61%) babies had respiratory failure, 28 (43.07%) babies had shock alone, 6 (9.23%) babies had sepsis alone, and 2 (3.07%) babies had both sepsis and shock, along with acute kidney injury. In the present study, the survival rate among babies affected by NE and HIE in the neonatal unit during the study period was 28.57% and 24.61%, respectively. Shock and respiratory failure were the two major complications in HIE cases, present in 30 (46.15%) and 29 (44.61%) babies, respectively. While respiratory failure caused death in 24 (82.75%) babies with severe respiratory distress, cardiac dysfunction caused death in 17 babies (56.66%) with shock. Sepsis complicated with Neonatal Necrotising Enterocolitis (NNEC) and Acute Kidney Injury (AKI) increased the mortality rate [Table/Fig-7].

Diagnosis/complications in HIE	No. of cases* n (%)	Death	Fatality rate (%)
Shock	30 (46.15)	17	56.66
Respiratory failure	29 (44.61)	24	82.75
septicaemia	8 (12.3)	8	100
AKI	1 (1.53)	1	100
NNEC	1 (1.53)	1	100

[Table/Fig-7]: Morbidity and mortality in HIE babies.
*four babies presented with multiple morbidities; NNEC: Neonatal necrotising enterocolitis; AKI: Acute kidney injury

DISCUSSION

The higher percentage of inborn newborns accounting for NE may be due to the presence of maternal risk factors, leading to referrals to a tertiary institute before or during labor. MSAF occurred in 40 (61.53%) neonates with HIE in the present study, while a study by Mundhra R and Agarwal M reported MSAF in only 12-16% of deliveries [15]. Another study by Reddy S et al., reported MSAF in only 8% of deliveries [16]. The difference can be attributed to differences in inclusion criteria.

TR and left ventricular dysfunction were the most common findings in the echocardiography of asphyxiated babies, consistent with studies by Ranjit MS, Omokhodion SI, and Losekoot TG [17,18]. These studies examined CK and CK-MB activities in perinatally asphyxiated newborns and healthy controls during the first 100 hours of life. The babies with asphyxia had significantly raised mean CK and absolute CK-MB values. Fractional CK-MB values did not show such a rise in asphyxiated babies, but healthy controls showed a steady decline in the activities of these enzymes from birth [18].

The mean CK-MB value at birth in the present study was 13.14 U/L (±12.03). In a study by Omokhodion SI and Losekoot TG, the mean value was 16.36 (±3.0) in the immediate postpartum period [18]. Higher values of 176.1±243 and 121±77.4 were obtained in studies by Reddy S and Dutta S, and Rajakumar PS et al., respectively [16,19]. A comparison of the diagnostic performance of CPK-MB in various studies is shown in [Table/Fig-8] [9,16,19,20]. In a study by Heba A et al., in Egypt, the sensitivity and specificity of CK-MB were 99% and 97.5%, respectively [9]. However, Sachin C

S. No.	Authors name and ref no.	Place and year of the study	Diagnostic performance			
			Sensitivity	Specificity	PPV	NPV
1	Reddy S and Dutta S [16]	Chandigarh (2007)	36	100	100	52
2	Rajakumar PS et al., [19]	Puducherry (2007)	56.5	75.7	-	-
3	Sachin C et al., [20] (CK-MB at birth)	Nepal (2019)	18.06	100	100	60.93
4	Heba A et al., [9]	Egypt (2021)	99	97.5	-	-
4	Present study (CK-MB at birth)	Tirunelveli (2014)	86	80	83	83
5	Present study (CK-MB at 24 hours)	Tirunelveli (2014)	37	38	25	52
6	Present study (CK-MB at 72 hours)	Tirunelveli (2014)	36	82	50	72

[Table/Fig-8]: Comparison of diagnostic performance of CK-MB in various studies [9,16,19,20].
PPV: Positive predictive value; NPV: Negative predictive value

et al., from Nepal reported a sensitivity of 18.06% and specificity of 100% for samples collected at birth [20]. In the present study, the corresponding values were 86% and 80%. Sensitivity and specificity for CK-MB at 24 hours analysis in a study by Reddy S and Dutta S were 36% and 100%, respectively. In the present study, the corresponding values were 37% and 38%.

In the present study, shock (46.15%) and respiratory failure (44.16%) were the leading causes of morbidity and mortality in HIE-affected babies. A study by Reddy S and Dutta S reported cardiogenic shock in only 16% of cases [16], while Rajakumar PS et al., reported congestive heart failure in 36.7% of cases and respiratory failure in 66.7% of cases [19]. In the present study, out of the 65 HIE babies admitted, 49 expired (75.4%). In the study by Rajakumar PS et al., only 16% of babies expired [19]. The high mortality in the present study can be attributed to differences in inclusion criteria. Respiratory failure caused death in 82.75% of newborns with severe respiratory distress, while cardiac dysfunction resulted in only 56% of cases. This can be attributed to the transient nature of myocardial ischaemia. Early detection and prompt initiation of treatment can help improve the prognosis of these asphyxiated newborns.

Limitation(s)

Echocardiography is considered the gold standard test for assessing myocardial dysfunction, but it was not performed in all HIE babies for the evaluation of TMI due to the difficulty in mobilising sick/ventilated babies.

CONCLUSION(S)

In the present study, HIE accounted for the majority of cases of NE. The NE caused by HIE is associated with high mortality and morbidity rates. The case fatality rates for HIE due to shock, respiratory failure, and sepsis are 56.66%, 82.75%, and 100%, respectively. Approximately 83.3% of asphyxiated babies with shock had elevated CK-MB levels at birth. However, the CK-MB values at 24 hours and 72 hours were not significantly elevated. Further studies comparing CK-MB values with point-of-care echocardiography by neonatologists or intensivists would be preferred.

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