Malondialdehyde as a Prognosticating Marker for Staging of HIE - A Prospective Cohort Study

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Abstract: *Introduction*: Prediction of outcomes of asphyxiated neonates is imperative. Timely intervention and effective resuscitation are beneficial for intact neurodevelopmental outcomes. In this study, we assessed the level of oxidative stress at birth in cord blood and correlated it with the Hypoxic Ischemic Encephalopathy (HIE) staging and outcomes.

Methodology: This Prospective Cohort Study was conducted between January 2020 and June 2022. All neonates requiring resuscitation at birth were enrolled in a study group. The control group included neonates who did not require resuscitation. Cord blood was collected at birth, centrifuged, serum separated, and stored at -20^{0}C. Malondialdehyde (MDA) was read at 535nm on spectrometry.

Results: 102 neonates were enrolled, among which 29 neonates were asphyxiated and 73 non-asphyxiated. The cord blood mean MDA level was significantly high in Severe HIE, with a p-value of <0.001. The serum MDA level in cord blood had a significant difference in asphyxiated neonates (8.59±1.99) and normal neonates (3.18±1.04*)* (P<0.001). **T**here is a significant difference in MDA levels in cord blood; the mean MDA level was significantly higher in HIE III (10.93±2.50), compared to HIE II (9.98±2.34) and HIE I(7.72±1.09) with a p-value of <0.001. Deceased neonates had higher MDA levels than those neonates who survived.

Conclusion: Neonates with high oxidative stress at birth require advanced resuscitation. MDA levels above 7.64 mm/L have 100% sensitivity and 81.4% specificity with respect to mortality in asphyxiated neonates. Hence, cord blood MDA can be a prognostic marker of oxidative stress to predict the outcomes in asphyxiated neonates.

Keywords: Malondialdehyde, asphyxia, cord blood, resuscitation, oxidative stress.

INTRODUCTION

Due to the high energy demand for the growth of the fetus, the mother, during pregnancy, takes a large amount of oxygen for metabolic functions. Oxygen is very necessary but can be toxic if in excess and cause oxidative stress. Many pathophysiological conditions have oxidative stress. In normal conditions, it is balanced by the antioxidant system, but during labor, this balance is disturbed. Malondialdehyde (MDA) is the best indicator of lipid peroxidation and so of oxidative stress. Oxidative stress plays an important role in the pathogenesis of many diseases [1]. Antioxidants are the substances that reduce oxidation of substrates and constitute the body's main protection against free radicals injury. Reducing oxidative stress by supplementation of antioxidants could be an effective option to prevent oxidative stress [2].

Perinatal asphyxia leading to hypoxic–ischemic encephalopathy (HIE) is a devastating neonatal condition leading to significant morbidity and mortality. Although the condition can be mild in some infants, it becomes severe in most affected cases, leading to future disability and impaired quality of life [3,4]. Studies have resulted in an improved understanding of this condition, and promising research has been conducted to determine the pathogenesis of the brain and other damaged organs [3-6]. Oxygen and free oxygen radicals play a crucial role in the development of cerebral tissue damage, and Malondialdehyde (MDA) is one of the key end-products of lipid peroxidation induced by reactive oxygen species [3-7]. Serum MDA (sMDA) concentration changes during HIE and, therefore, could be used as a predictor for determining HIE diagnosis, treatment, and prognosis [5-7].

The aim of this study was to assess MDA concentration in cord blood of all neonates in the study period and evaluate the difference of rise in normal and HIE neonates and the sensitivity of MDA concentration as a predictive marker.

METHODOLOGY

This study was conducted in Level III A NICU of BLDE (DU) Shri B M Patil Medical College, Hospital & Research Centre, Vijayapura, between January 2020 and June 2022 after obtaining Ethical Clearance from BLDE (DU) Institutional Ethical Committee [BLDE (DU)/IEC/09/2021]. The neonates who required resuscitation at birth and with low APGAR scores (APGAR < 8) at 5 mins were categorized into cases, and those who had APGAR >8 at 5 mins were taken as controls. In this study, we had 29 cases and 73

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controls. Baseline Malondialdehyde level was assessed in control healthy neonates, which was not defined in earlier studies. The neonates resuscitated were analyzed with the levels of resuscitation required at birth and categorized into mild, moderate, and severe asphyxia based on Sarnat and Sarnat HIE staging. The cord blood collected was centrifuged, and serum was separated and stored at -20^{0}C; MDA was assessed, and the correlations were done.

Procedure of Estimation of MDA in Cord Blood

MDA is formed by the breakdown of polyunsaturated fatty acids (PUFA), and it serves as a

Table 1: Comparison of Baseline Maternal and Neonatal Characteristics in both Cases and Control Group

Characteristics	Neonates with Birth Asphyxia (n=29)	Neonates without Birth Asphyxia (n=73)	p-value
Mean (SD) maternal age	26.5 (3.7)	26.2 (3.8)	0.693
20 – 24 years	10 (25.0%)	30 (75.0%)	0.826
25 – 29 years	12 (30.8%)	27 (69.2%)	
30 – 36 years	7 (30.4%)	16 (69.6%)	
Gestational Age			
37 – 40 weeks	25 (86.2%)	57 (78.1%)	0.351
>40 weeks	4 (20.0%)	16 (80.0%)	
Parity			
Primi	10 (24.4%)	31 (75.6%)	0.458
Multi	19 (31.2%)	42 (68.8%)	
Birth Weight			
1500 – 2500 gm	8 (80.0%)	2 (20.0%)	< 0.001
2500 – 3500 gm	21 (22.8%)	71 (77.2%)	
Sex			
Male	20 (32.8%)	41 (67.2%)	0.234
Female	9 (21.9%)	32 (78.1%)	
Appropriate for gestational age			
LGA	1 (100%)	0	0.069
SGA	8 (80.0%)	2 (20.0%)	
AGA	20 (22.0%)	71 (78.0%)	
Amniotic fluid			
Meconium stain	11 (57.9%)	8 (42.1%)	0.145
Clear	17 (57.9%)	65 (79.3%)	
Mode of delivery			
LSCS	16 (29.6%)	38 (70.4%)	0.539
Vaginal	13 (28.9%)	35 (72.9%)	
APGAR SCORE			
At 1 min			
<=3	5 (17.2%)	0	<0.001
4-7	24 (82.8%)	0	
≥8	0	73 (100%)	
At 5 mins			
< 8	29 (100%)	0	<0.001
≥ 8	0	73 (76.8%)	

convenient index to determine the extent of lipid peroxidation. It reacts with TBA to give a pink color, which is read at 535nm[56]. Sample: Serum-100µL. 100µL serum was diluted to 500µL with distilled water, and 1ml of TCA-TBA-HCl reagent was added to the diluted sample. The samples were placed in a boiling water bath for 15 minutes. The reaction mixture was then cooled and centrifuged. At 535nm, the supernatant was taken, and the optical density of the pink color formed was read. The plotting was obtained against the standard graph to get a concentration of Malondialdehyde in the sample. The optical density of the pink color formed was directly proportional to the concentration of Malondialdehyde in the given sample in micromol/L.

Statistical Analysis

We categorized the neonates based on the level of neonatal resuscitation required at birth with HIE staging and correlated with cord blood MDA levels. A sample size of 100 neonates was studied, and the anticipated Mean ±SD was 4.38±0.28 of cord blood MDA with a 95% level of confidence and a precision of 10%. The data obtained was entered into a Microsoft Excel sheet, and statistical analysis was performed using SPSS software (Version 20). Receiver operating characteristic (ROC) curves were drawn for MDA levels to predict mortality and severe HIE by plotting sensitivity versus one minus specificity. The area under the ROC curve (AUC) was used to evaluate the discriminatory capacity of the MDA levels to predict mortality.

RESULTS

102 neonates were enrolled, among which 29 neonates were asphyxiated (Cases) and 73 non-asphyxiated (Controls). Baseline Maternal and Neonatal Characteristics documented were not statistically significant except for Birth weight. APGAR scores at 1 minute and 5 minutes were statistically significant among the Cases and Control Group (Table **1**).

Mean values of MDA were higher in neonates with asphyxia (8.59 micromol/L) as compared to neonates without asphyxia (3.18 micromol/L), which was statistically significant (p<0.001) (Table **2**).

Table 2: Comparison of Cord MDA Levels among Neonates with and without Birth Asphyxia

Cord blood	Neonates with Birth Asphyxia (n=29)	Neonates without Birth Asphyxia (n=73)	p-value
MDA micromol/L			
Mean (SD)	8.59 ±1.99	3.18 ±1.04	<0.001
Median (IQR)	8.33 (7.66 – 9.01)	2.96 (2.33 – 3.96)	<0.001

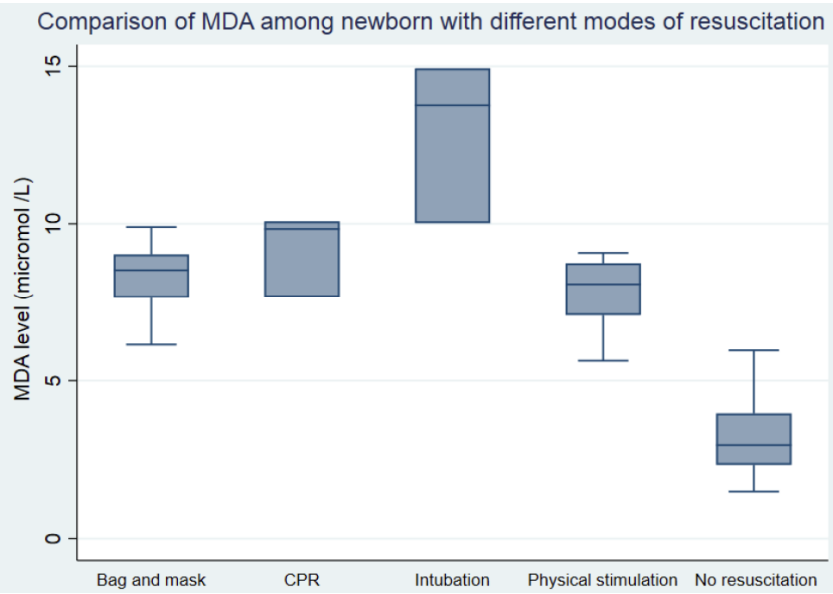

Figure 1: Comparison of cord MDA among Neonates with different HIE Staging.

Cord blood mean MDA levels were high in neonates with HIE stage III (10.93 micromol/L), compared to HIE stage II (9.98 micromol/L) and HIE stage I (7.72 micromol/L) with a significant p-value of <0.001 (Figure **1**).

Based on different levels of resuscitation required at birth, the neonates requiring intubation and CPR at birth showed high levels of cord blood MDA levels of 12.90 micromol/L and 9.18 micromol/L, respectively, which was statistically significant (p<0.001) compared to controls (Table **3**).

Table 3: Comparison of Cord MDA among Neonates Requiring Different Modes of Resuscitation

Type of Resuscitation/MDA level	MDA level Mean (SD)
No resuscitation	3.18 ±1.04
Physical stimulation	7.79 ± 1.13
Bag and mask	8.21 ±1.19
CPR	9.18 ±1.33
Intubation	12.90±2.56
p-value	<0.001

We have also observed the mean MDA levels were higher in deceased neonates (10.87±3.24 micromol/L) than in those neonates who survived (4.40±2.40 micromol/L), which was statistically significant (p<0.001) (Figure **2**).

Receiver operating characteristic curves were drawn for MDA levels to predict mortality and severe HIE by plotting sensitivity versus one minus specificity. The area under the ROC curve (AUC) was used to evaluate the discriminatory capacity of the MDA levels to predict mortality. The ROC curve for predicting mortality among neonates showed a high predictive accuracy of 82.35% with good discriminatory power. The AUC was 0.940 (95% CI: 0.867 – 1.00). The cut-off of 7.64 had a sensitivity of 100% and specificity of 81.4%. The ROC curve for predicting severe HIE among neonates showed a high predictive accuracy of 86.27% with good discriminatory power. The area under the curve was 0.975 (95% CI: 0.943 – 1.00). The cut-off of 7.64 had a sensitivity of 100% and specificity of 85.9% (Table **4**; Figure **3**).

DISCUSSION

The oxidative stress in neonates occurs as a result of the generation of free radicals in excess. Free radicals, like reactive oxygen species, when they attack cellular polyunsaturated membrane lipids, the reactions go on until scavengers of free radicals act on. These scavengers are antioxidants available, and their mechanism is widely studied. Our study considered measuring cord blood MDA levels to see the extent of free radical injury [8,9].

Neonate's antioxidant defense system is ineffective in fighting against oxidative stress; hence, increasing MDA is an indicator of oxidative stress. There is limited literature on the correlation of oxidative stress (MDA levels) and levels of resuscitation required at birth and the staging of HIE, which has been done in this study, which observed significant correlations and high predictive accuracy.

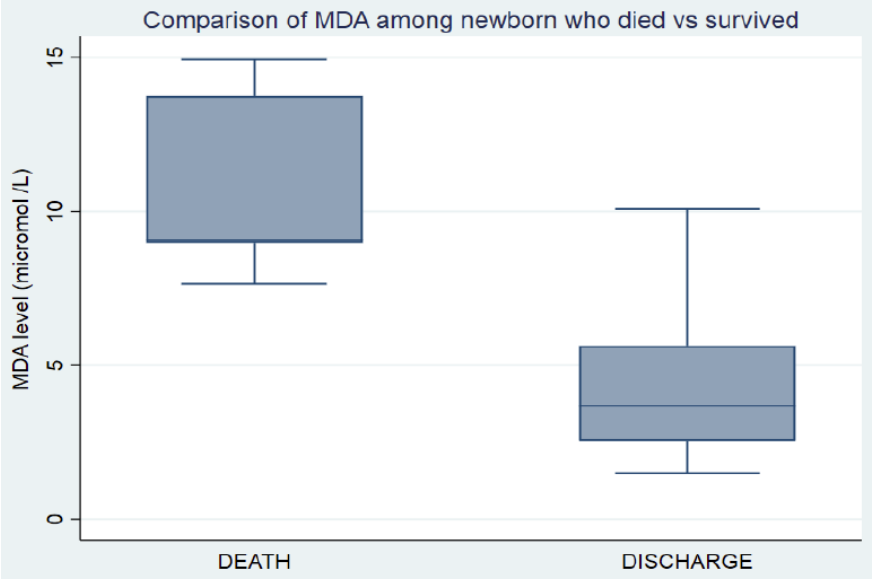

Figure 2: Comparison of cord blood MDA among Survived Neonates and Died Neonates.

Table 4: ROC for Predicting Mortality among Neonates with Birth Asphyxia in Relation to Cord MDA Levels

	Mortality	HIE II & III
ROC – Area under the curve	0.940	0.975
Standard Error	0.037	0.016
95% CI	0.867 – 1.00	0.943 – 1.00
Cut-off	7.64	7.64
Sensitivity	100%	100%
Specificity	81.4%	85.9%
Accuracy	82.35%	86.27%

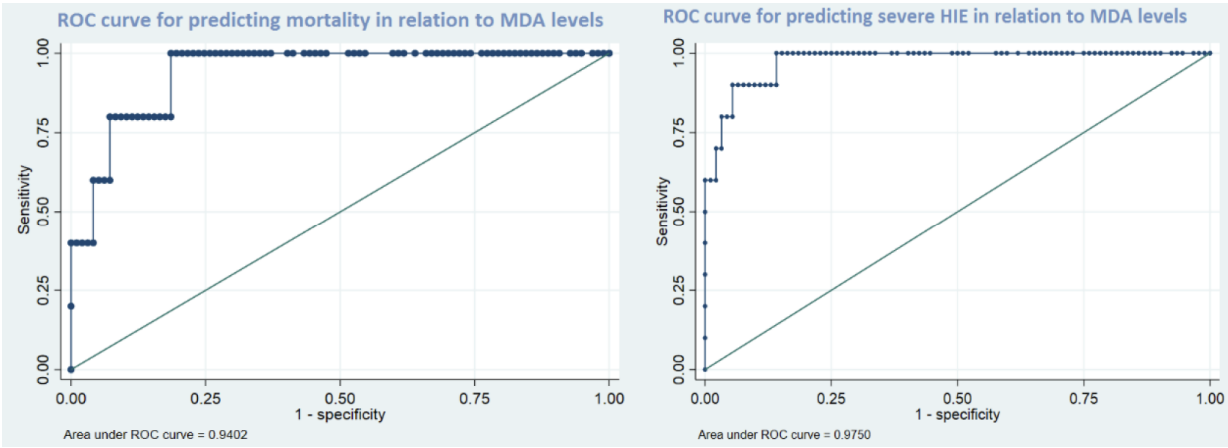

Figure 3: ROC curves for predicting mortality and moderate-severe HIE among neonates with Birth Asphyxia in relation to cord MDA level.

In a study by Nivedita Mondal *et al*., [10] mean MDA (in micromol/L) and protein carbonyl at birth were found to be significantly higher in asphyxiated neonates (5.88 ± 1.401) than in controls (3.11 ± 0.82). In this study, mean values of MDA were higher in neonates with asphyxia (8.59 micromol/L) as compared to neonates without asphyxia (3.18 micromol/L), which was statistically significant (p<0.001).

Based on different levels of resuscitation required at birth, the neonates requiring intubation and CPR at birth showed high levels of cord blood MDA levels, 12.90 micromol/Land 9.18 micromol/L respectively in this statistically significant study (p<0.001) and compared to controls not requiring resuscitation at birth (3.18 micromol/L).

The mean value of MDA at birth was found to increase with worsening stages of HIE, with HIE III having a significantly higher value as compared to HIE I and HIE II. In a study by E Kirimi *et al*., [11] Malondialdehyde levels were 3.46 ± 0.95 micromol/L in Grade I HIE, 6.47 ± 1.21 micromol/L in Grade II HIE, 7.72 ± 2.82 micromol/L in Grade III HIE which correlates with our study findings and the MDA levels were significantly different with stages of HIE. Cord MDA levels for neonates with HIE stage I were significantly lower than for neonates with stages II and III in our study, which was graded according to Sarnat and Sarnat staging. The level of MDA detected in stage III HIE neonates (10.93 micromol/L) was higher than that observed in stage II HIE neonates (9.98 micromol/L) and stage I HIE neonates (7.72 micromol/L). This method may be useful to assess the prognosis of neonates with HIE as the results revealed a substantial association between the cord MDA level and the amount of cerebral injury (as determined by Sarnat's staging).

LIMITATION

This study has a small cohort of cases of moderate and severe asphyxia and has a subjective assessment of APGAR scoring by trained pediatricians. Further studies may lead to the use of antioxidants and modifying maternal risk factors to reduce neonatal asphyxia.

CONCLUSION

Neonates with high oxidative stress at birth required advanced resuscitation. Neonatal outcomes with low APGAR scores at birth were associated with high values of cord blood MDA. Cord blood MDA levels greater than 7.64 micromol/L have 100% sensitivity and 81.4% specificity with respect to mortality in asphyxiated neonates and 100% sensitivity, 85.9% specificity with HIE staging (moderate-severe) with high predictive accuracy. Hence, cord blood MDA levels can be considered as a marker to predict the HIE staging and prognosticate outcomes in asphyxiated neonates.

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