Correlation of Acidosis with Blood Markers (LDH and Nucleated RBCs/100 WBC) and with Mortality and Neurodevelopmental Outcome in Neonates with Perinatal Asphyxia

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ABSTRACT

Introduction: Many infants suffer from perinatal asphyxia even in this century specially in developing countries. This study was undertaken to assess the correlation of serum LDH and nucleated RBCs/100WBCs) with acidosis and to assess their ability in predicting mortality and neurodevelopment in neonates with perinatal asphyxia.

Material and methods: Neonates born at ≥35 weeks of gestation and weighing ≥2000 gm at birth were eligible for enrollment if they had evidence of birth asphyxia(defined as Apgar score < 3 at 1 min in intramural neonates and being born limp with cry > 3 min after birth, in extramural neonates) and encephalopathy within 72 hours of birth. Arterial blood pH, nucleated RBC (nRBC)/100WBCs and serum LDH levels were assessed in all babies after resuscitation in intramural babies and at the time of admission in extramural babies provided they presented within 72 hours after birth. After discharge, the babies were reassessed at 28 days, 3 and 6 months of age for their neurodevelopmental status using the Amiel-Tison Assessment.

Result: A total of 100 neonates were enrolled of whom 29 died before discharge. Neonates who expired had a higher degree of acidosis as compared to those who survived, with mean arterial pH being 6.97+0.16 and 7.063+0.142 respectively (p value <0.01 by unpaired 't' test). nRBC count had a stronger direct correlation with acidosis as compared to LDH (r = 0.71, 95% CI 0.70 to 0.59 versus r =0.54, 95% CI 0.67 to 0.38). Serum LDH ≥810 IU/L and nRBC count >9/100WBCs were able to accurately predict asphyxia and adverse neurodevelopmental outcomes in these babies with a specificity of 95.65%, sensitivity of 54.55% and positive likelihood ratio of 12.54 in the former and specificity of 95.65%, sensitivity of 68.83% and positive likelihood ratio of 15.83 in the latter

Conclusion: Serum LDH and nRBC/100WBC directly correlated with acidosis and both increased as the acidosis increased. They can therefore serve as alternative markers of severity of asphyxia and predict mortality and neurodevelopmental outcome upto 28 days of life.

Keywords: pH, Neurodevelopment, Lactate Dehydrogenase (LDH), nucleated Red Blood Cell (nRBC), Hypoxic Ischemic Encephalopathy (HIE).

INTRODUCTION

World-wide each year four million infants suffer from perinatal asphyxia. Of these, one million die and a significant number develop serious sequelae. Perinatal asphyxia ranks as the second most important cause of neonatal death after infection, accounting for about 30% of neonatal mortality.[1] Of the 1.2 million neonatal deaths in India every year 0.3 million infants die due to perinatal asphyxia.[2]

No single indicator like Apgar score, HIE staging or major organ dysfunctions have good predictive efficiency for perinatal asphyxia. Only a combination of various indices may help in diagnosis. Arterial Blood Gas (ABG) analysis including pH estimation, the most commonly used diagnostic and prognostic parameter, is available in only a few select tertiary care hospitals. It is expensive and requires sophisticated equipment. Moreover, it also requires stringent collection techniques and temperature control after sample collection. There is a need for simpler and cheaper surrogate markers to diagnose asphyxia. nRBC count is a simpler test and can be made available even at Primary Health Care centres. Serum LDH is also a readily available test and does not need stringent storage and transportation conditions. Increase in nRBC count has been reported as a possible marker of perinatal asphyxia as the hypoxia at birth induces erythropoiesis, which results in the release of immature RBCs into the fetal circulation.[3] Hypoxia also leads to leakage of enzymes like LDH, CPK etc. from cells into the blood stream. Amongst these, LDH levels are relatively easy to measure and may serve as a good predictor of HIE in the first few hours of life.[4] The present study was designed primarily to determine the correlation of acidosis (measured by arterial blood pH) with nRBCs/100WBCs and LDH in neonates with perinatal asphyxia and secondarily to assess their ability to predict neonatal mortality and neurodevelopmental outcome at 28 days, 3 months and 6 months of age in these babies.

MATERIAL AND METHODS

A prospective observational study was designed and conducted in the neonatal unit of a tertiary care centre of north India, from June 2012 to September 2013.The sample size was based on inclusion and exclusion criteria and follow up. Neonates of 35 or more completed weeks of gestation, weighing> 2000g at admission and of < 72 hrs post natal age, with perinatal asphyxia were included if bag and mask ventilation was given and clinical features suggestive of encephalopathy (as suggested by Sarnat and Sarnat staging

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1,2,3) along with metabolic or mixed acidemia (pH ≤7.2) were present and Apgar score was less than 3 at 1 minute. Extramural babies with history of being born limp or with cry delayed for more than 3 minutes after birth, having clinical features suggestive of encephalopathy (as suggested by Sarnat and Sarnat staging 1,2,3) with metabolic or mixed acidemia (pH ≤7.2) were also included. Babies with major congenital malformations and Rh incompatibility were excluded. Each subject was enrolled with written informed consent of the parent/guardian. The study was approved by the Institutional Ethical Committee.

Arterial blood pH(as a measure of acidosis), nRBC/100WBC and serum LDH level were assessed at the time of admission. Neurodevelopment was assessed by Amiel-Tison's neurological assessment.[5] ABG sampling was done from the radial artery and analysis was done within 30 minutes of sample collection, the sample being collected and stored under specified norms. Blood film for nRBC analysis was prepared at bed side from fresh venous blood without adding any anticoagulant. The slides were stained with Leishman's stain, fixed with methanol and nRBCs were seen under the microscope by a single pathologist who was blinded to the pH value of the baby. For serum LDH levels, about 2ml of venous blood was collected in a plain vial. Only non-hemolysed serum was analysed, immediately, by spectrophotometry. Statistical analysis was done using STATA 11 software. Variables were described by mean (±SD) and median (IQR – inter-quartile range). Non-parametric Spearman's correlation was used for comparison. Parametric unpaired t-test and non-parametric Kruskal-Walis test were used for comparing means of two groups.'p' value <0.05 was considered significant.

RESULTS

Hundred babies were enrolled for the study. Their mean gestational age was 37.35±0.967 weeks and mean weight at admission was 2706±0.432 g. Other baseline characteristics are shown in table1. Arterial blood pH, nucleated RBC (nRBC)/100WBCs and serum LDH levels were assessed in all babies after resuscitation in intramural babies (mean 1.1 hours/range 1-2 hours) and at the time of admission in extramural babies(mean 18.6 hours/ range 4-72 hours) provided they presented within 72 hours after birth of the 100 patients enrolled, 64 were discharged, 29 expired while 7 left against medical advice.(Fig.1)

Characteristic	Mean/Number*	Standard Deviation / Percent*
Gestational Age (Weeks)	37.35	0.967
Weight(Kg)	2.706	0.432
Sex (Male)	69*	69*
Place of Delivery	56*	56*
(Intramural)		
Mode of delivery (NVD)	57*	57*
Seizures	59/100	
Dyselectrolytemia	52/100	
Coagulopathy	31/100	
Azotaemia	5/100	

Table-1: Clinical profile of study population

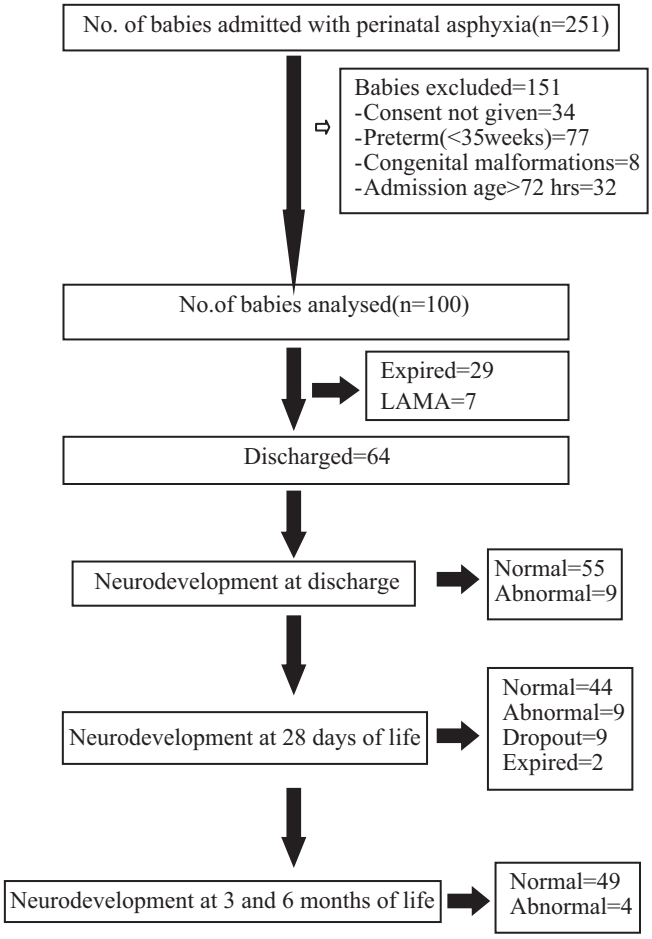

Figure-1: Flow chart of participants enrolled

Correlation of acidosis with mortality and neurological outcome

Babies who subsequently expired had more severe acidosis than in the babies who survived,the mean pH being 6.97 ± 0.163 in the former and 7.063 ± 0.142 in the latter (p value < 0.01 by unpaired t- test). Of the 64 babies who were discharged, 55 (85%) were neurologically normal and 9 (14%) were neurologically abnormal at discharge. Acidosis was also more severe in neurologically abnormal babies as compared to the normal ones (mean pH at admission being 6.96 ± 0.11 in the former and 7.08 ± 0.14 in the latter group (p value = 0.018 by unpaired t test). Of the 55 babies who were normal at discharge, 2 (3.6%) expired while 9 (16.36%) were lost to follow up. At 3 months 49 babies were normal and only 4 were abnormal (5 of the earlier abnormal babies now showed normal development). Acidosis at birth failed to differentiate between babies who were neurologically normal and neurologically abnormal at 3 and 6 months.

Correlation of acidosis with Lactate Dehydrogenase and nucleated RBCs

The mean (SD) of serum LDH was 767.4 (362.2) IU/L and that of nRBCs was 10.45 (8.88)/100WBC. The median (IQR) of LDH was 660 (482.5-1033) IU/L and that of nRBC was 10 (2-16.75)/100WBC. A significant linear correlation existed between acidosis and LDH level (r =0.54) and acidosis and nRBCs in the subjects (r =0.71),(p < 0.01 for both, by non-parametric Spearman's correlation analysis).

"Receiver Operating Characteristic" (ROC) Curve for LDH and nRBCs with pH was made in order to define a cut-off level of serum LDH and nRBCs that can reliably diagnose acidosis(pH ≤7.2) in babies with asphyxia (Table 2). Serum LDH showed excellent discriminating ability in diagnosing acidosis as area under curve was 0.87 (95% C.I. 0.79-0.95) and a value of LDH more than 810 had 95.65% specificity, 54.55% sensitivity and positive likelihood ratio of 12.54 in diagnosing acidosis(pH ≤7.2) (Fig 2). Nucleated RBCs also showed very good discriminating ability in diagnosing acidosis as area under curve was 0.84 (95%C.I. 0.77-0.92) and a value of nRBC more than 9 had 95.65% specificity, 68.93% sensitivity and positive likelihood ratio of 15.83 in diagnosing acidosis (Fig 2).

When nRBCs were analysed in different stages of HIE, it was observed that mean of nRBCs /100WBCs was 5.77 +5.42 in Stage I HIE, 12.20+6.99 in Stage II HIE and 19.63 +8.33 in Stage III HIE whereas the mean LDH level was 851.3+401.4 in Stage I HIE,812.2+389.1 in Stage II HIE and 745.0+297.8 in Stage III HIE. n RBCs/100WBCs correlated better than mean LDH levels with increasing grades of HIE (p value <0.01).

DISCUSSION

Our study demonstrated a statistically significant correlation of acidosis with mortality and neurodevelopment till 28 days of life. It also demonstrated a significant direct correlation of acidosis with both LDH and nRBCs, i.e. with decreasing pH, LDH and nRBCs increased. With worsening of HIE, though mean nRBC count increased significantly, no significant change was observed in LDH levels.

The relation between acidosis at birth and mortality in babies with perinatal asphyxia has been well documented. Low cord arterial pH (acidosis) has been used as a diagnostic biochemical criterion for perinatal asphyxia.[6] However, not many studies have documented the relation of acidosis to neurological outcome, especially in India. Earlier studies[7-10] have reported the relation between adverse neurodevelopmental outcome and stage of HIE and Apgar score, but not with acidosis. We studied the relation of acidosis with neurodevelopment and observed that acidosis relates directly to mortality and abnormal neurodevelopment till 28 days of life, but not beyond that age. This suggests that factors other than acidosis(at birth) gradually start contributing more to neurodevelopment.

Our study also demonstrated that serum LDH increased significantly with increase in acidosis in asphyxiated babies. Reddy et al have reported earlier that LDH can also be used to discriminate asphyxia from other illnesses among neonates who present with signs of encephalopathy. They demonstrated that LDH and other enzyme levels were raised in other sick infants also, but the magnitude of elevation was higher in asphyxiated babies. However, we did not enroll babies with other illnesses. Our study demonstrated that a serum LDH of more than 810IU/L can fairly diagnose acidosis in asphyxiated babies with a sensitivity of 54.55% and specificity of 95.65%. Previous studies have suggested different cut off values for LDH which may be because of different timing of the sample. According to Reddy et al, LDH levels greater than 580 IU/L have a sensitivity of 100% for asphyxia and a specificity of 89% in differentiating asphyxia from other illnesses, if the sample is taken within first 72hrs of life. Karlsson et al have suggested levels >1049 IU/L as having sensitivity of 100% and specificity of 97% while Karunatilaka et al have suggested a much higher cut off of 2948 IU/L of LDH in predicting HIE, along with long term adverse sequelae in asphyxiated babies. The values could be higher in the latter two studies because sampling was done within the first 6 12hrs of birth. We have chosen cut off values with higher specificity so that LDH may be used in diag-

Laboratory Test	Specificity	Sensitivity	Positive likelihood ratio	Negative likelihood ratio
LDH				
≥695IU/L	91.30%	58.44%	6.72	0.45
≥740IU/L	91.30%	57.14%	6.57	0.46
≥770IU/L	91.30%	55.84%	6.42	0.48
≥780IU/L	91.30%	54.55%	6.27	0.49
≥810IU/L	95.65%	54.55%	12.54	0.47
≥823IU/L	95.65%	53.25%	12.24	0.48
≥830IU/L	95.65%	51.95%	11.94	0.50
≥840IU/L	95.65%	50.65%	11.64	0.51
≥845IU/L	95.65%	49.35%	11.35	0.52
nRBCs/100WBCs				
≥6	82.61%	80.52%	4.62	0.23
≥7	86.96%	74.03%	5.67	0.29
≥8	86.96%	71.43%	5.47	0.32
≥9	95.65%	68.83%	15.83	0.32
≥10	95.65%	64.94%	14.93	0.36
≥11	95.65%	55.84%	12.84	0.46
≥12	100%	46.75%	-	0.53
≥13	100%	38.96%	-	0.61

Table-2: Cut off values, specificity and sensitivity of LDH and nRBCs/100WBCs for prediction of acidemia

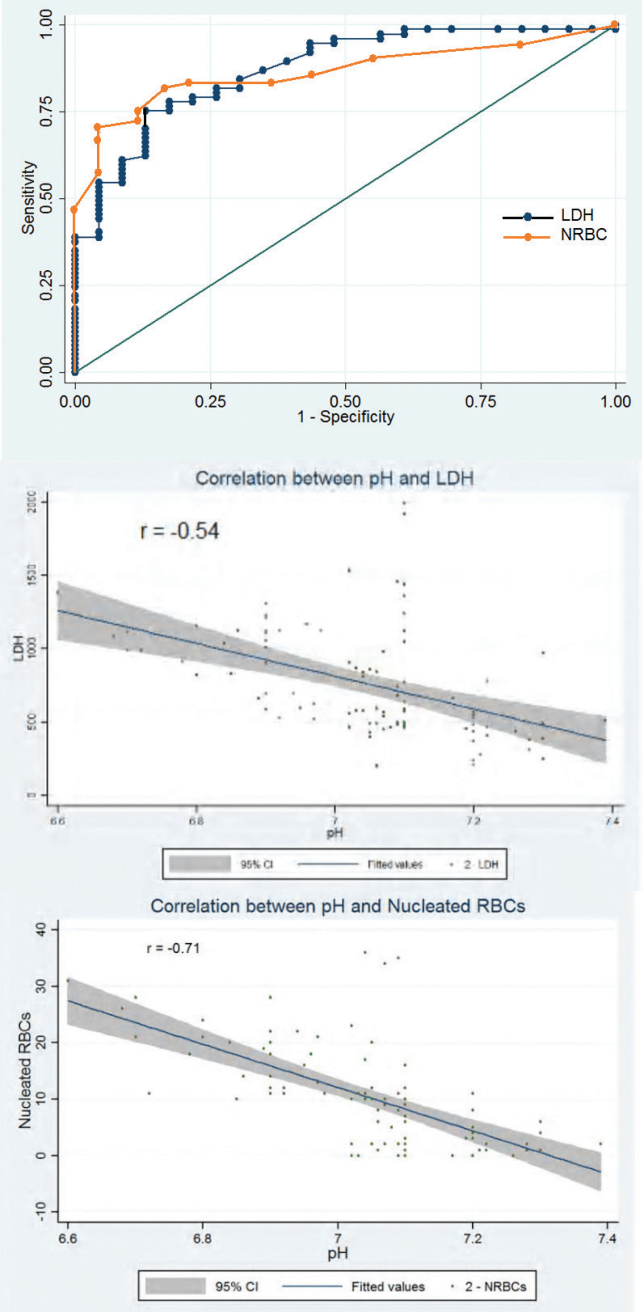

Figure-2: ROC curves for LDH and nRBCs/100WBCs and correlation between pH and LDH and between pH and nRBCs/100WBCs

nosing acidosis. If LDH is to be used for screening, it would not be worthwhile to use values with low specificity, as many babies without acidosis would screen positive and undergo unnecessary neuroimaging and follow up in clinic increasing the clinical burden. Mehta et al in their study have reported significantly higher Salivary LDH levels in the HIE group with a level of 893 IU/L showing excellent discriminating ability in early diagnosis of HIE.[11] Our study found, no relation between LDH levels and worsening stages of HIE. This may be because in our study we have used venous blood, our patient population comprised of extramural babies as well where the mean time of collecting the sample was 18.6 hrs whereas Mehta et al studied only 30 intramural babies and collected their salivary samples within 12 hours of birth.

Our study also demonstrated a strong positive correlation between acidosis and nRBC/100 WBCs in venous samples i.e. as pH decreased, the number of nRBCs increased. Earlier studies[12-17] have shown similar results but were conducted on cord blood samples. We preferred venous samples, as cord blood may not be available if babies were born outside institutional set up and admitted later (extramurally). A cut off value of more than 9 nRBCs/100WBCs was derived by ROC analysis, to have higher specificity and positive likelihood ratio for acidosis. This value is similar to the value of >10 nRBCs/100WBCs, reported by Hassan Boskabadi et al. However, most studies have given only mean value of nRBCs/100WBCs rather than a cut off value, that can help to diagnose acidosis.In our study the mean value of nRBCs in different stages of HIE was lower than in earlier studies.[3,12-17] This could be because of different sites of sample collection and also because venous blood sample was collected at a mean time of 1.10hr in intramural babies but 18.6hr in extramural babies, which could have allowed time for compensation. Our study demonstrated that nRBC/100WBC and LDH levels can serve as predictors of severity of acidemia, where affordability is an issue and at peripheral centers where facilities are limited.

Limitation of our study is that we did not enroll controls due to ethical constraints in sampling otherwise healthy babies. Also,a longer follow-up of at least till 1-2 years of age would have been desirable.

CONCLUSIONS

Serum lactate dehydrogenase and nucleated red blood cells/100WBCs directly correlate with acidosis and can be used to predict the outcome in terms of mortality in asphyxiated babies. Babies with lower mean arterial pH at admission had a worse clinical outcome. Nucleated RBC/100WBC and/or absolute nRBC count may serve as simple surrogate markers for assessment of severity of perinatal asphyxia, as well as for severe HIE whereas LDH levels can serve as a good predictor of severity of acidemia in babies with perinatal asphyxia, though not for HIE. Radial artery pH can also serve as predictor of mortality when cord pH is not feasible, as in extramural deliveries and nRBC/100WBCs and LDH level can serve as predictors of severity of asphyxia where affordability of ABG is an issue.

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