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Original Article

Correlation of cord Blood pH with Nucleated RBC and Serum LDH in Neonates with Perinatal Asphyxia

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Abstract

Introduction: Perinatal asphyxia is one of the leading causes of neonatal mortality in developing countries. No single indicator has good diagnostic efficacy for perinatal asphyxia and only a combination of various indices may help in diagnosis. This study aims at determining the utility of serum lactate dehydrogenase (LDH) and cord blood nucleated red blood cell (nRBC) count as surrogate markers to pH in perinatal asphyxia.

Methods: It is an observational study done over a period of 18 months. All newborn babies fitting into inclusion criteria were enrolled into the study. After initial resuscitation, cord blood was sent for pH and nRBC analysis and serum LDH levels was estimated from first collected blood sample (within six hours). Data was collected on excel sheet, Pearson's correlation coefficients and ROC curves were used to tabulate results.

Results: Our study included 74 neonates with perinatal asphyxia. Mean pH was 6.99 (SD - 0.138). Mean nRBC count was 38 / 100 WBC (SD-17.9) and mean LDH level was 1478 IU / L (SD - 429). Cord blood pH showed significant ($p < 0.001$) correlation with raising nRBC count and LDH levels ROC plot showed nRBC count (AUC: 0.7866) and Serum LDH (AUC: 0.8143) as very good predictors of severe acidosis in perinatal asphyxia.

Conclusions: Cord blood nRBC count and serum LDH levels are significantly increased in perinatal asphyxia and correlate well with severity of acidosis. Nucleated RBC count and serum LDH can be used as surrogate markers to diagnose perinatal asphyxia.

Introduction

Birth asphyxia is the most common and important cause of preventable cerebral injury during neonatal period. According to WHO, around 3% of 120 million infants born every year develop birth asphyxia while 900,000 of these newborns die each year.[1] Around one million children who survive birth asphyxia live with chronic neuro developmental morbidities.[2] In India, 8.4% of inborn babies have a one minute Apgar score less than 7 and 1.4% suffer from hypoxic ischemic encephalopathy (HIE).[3] Asphyxia is the simultaneous combination of both hypoxia and hypo perfusion, which impairs tissue gas exchange leading to tissue acidosis.[4] In the absence of perinatal records, it is difficult to retrospectively diagnose perinatal asphyxia.[3]

Parameters that have been used to predict perinatal asphyxia include: intrapartum electronic fetal monitoring, fetal or umbilical cord pH

measurement, meconium stained amniotic fluid, Apgar score, HIE and major organ disorder. The number of nRBC/100 WBC is variable but is rarely greater than 10 in normal neonates. Earlier studies have shown a cut off value of more than 10 nRBCs / 100WBCs have higher specificity and positive likelihood ratio for acidosis. However, most studies have given only mean value of nRBCs / 100 WBCs rather than a cut off value that can help to diagnose acidosis. Hence nRBC count / 100 WBC obtained from cord blood peripheral smear can serve as effective and economical early predictor of perinatal asphyxia in a resource limited countries like India. Injured cells leak intracellular enzymes like lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and increased levels are seen after perinatal asphyxia as early as in the cord blood directly after birth. Plasma LDH in the first hours of life seems so far to be the best chemical predictor of HIE and its postnatal outcome in hypothermia-treated infants. lDH levels are relatively easy to measure and may serve as a good predictor of HIE in the first few hours of life.

Various biochemical markers have been used to find out organ dysfunction. No single marker of perinatal asphyxia has shown good predictive efficiency and only a combination of various indices can help in the early diagnosis of perinatal asphyxia. In this study, we are trying to find the levels of pH, nucleated RBC's in the cord blood and serum LDH in neonates experiencing perinatal asphyxia. We look forward to establish the values of cord blood nRBC and serum LDH levels which can be used as surrogate marker to pH for predicting the severity of acidosis and hence perinatal asphyxia.

Methods

This is an observational study done over a period of 18 months at a tertiary care neonatal unit of a teaching medical college in south India. Term babies delivered in a medical college hospital who sustained perinatal asphyxia were included in the study. The neonates were identified to have sustained perinatal asphyxia when at least two of the following were present: Apgar score of < 7 at one minute of life, cord blood pH < 7, clinical features suggestive of encephalopathy. Excluded from the study were those with congenital malformations, maternal drug addiction, neonates born to mothers who have received magnesium sulphate within four hours prior to delivery or opioids (Pharmacological depression), congenital or acquired infections, Hemolytic disease of the newborn etc. The study was approved by institutional ethical committee (No. FMMC/FMIEC/4040/2016 dated 04/11/2016. AAP defines perinatal asphyxia when neonate demonstrates all of the following: (a) profound metabolic or mixed acidemia (pH of 7.00) on an umbilical arterial blood sample, if obtained, (b) an Apgar score of 0 - 3 for longer than five minutes, (c) neurologic manifestation, EEG, seizure, coma, or hypotonia, and (d) evidence of multiorgan dysfunction. The criteria that defines asphyxia and include HIE as a cause of neonatal encephalopathy include (a) prolonged (> 1 hour) antenatal acidosis, (b) fetal heart rate less than 60 beats / min, (c) Apgar score 3 at 10 minutes, (d) need for positive pressure ventilation for > 1 minute or first cry delayed for > 5 minutes, (e) seizures within 12 - 24 hours of birth, and (f) burst suppression or suppressed background pattern on EEG or amplitude integrated EEG.[6] All the babies with perinatal asphyxia as defined, meeting the inclusion criteria were included in the study. Informed consent was obtained from the parents for involving baby in the study. Detailed history was obtained from the parents including family history, past history, antenatal history and natal history. Immediately after the birth of the baby, after initial resuscitation at the labour room, 1 ml of blood was collected in heparinised syringe from doubly clamped segment of umbilical cord and transported in ice container to central laboratory to determine pH. Simultaneously 1.5 ml of cord blood was sent in EDTA vacutainer to central laboratory for smear examination to determine nRBC /100 WBCs which was reported by a pathologist. Blood collected in first six hours of life was sent for serum LDH analysis. Babies were managed as per standard NICU protocol after resuscitation. Levels of cord blood pH, nucleated RBC's and serum LDH were correlated with each other. Collected data was entered in the Microsoft excel sheet. Analysis was done using SPSS statistical software version 24. Mean, frequency, percentage, standard deviation was calculated. Pearson's correlation coefficient and student T test was used to correlate the parameters. ROC curves were used to depict the results.

Results

Out of 7256 deliveries, 74 neonates with perinatal asphyxia fulfilling the inclusion criteria were enrolled in the study. Majority of our study neonates were males (59.5%). Mean maternal age in our study population was 27 ± 4.48 years while gestational age of newborns ranged from 37 weeks to 42 weeks (38 ± 1.6). The mean birth weight was 2886.5 ± 355.8 grams with lowest being 2490 gms and highest being 4030 gms. Majority were delivered by lower segment caesarean section (55.4%) while four were delivered by assisted vaginal deliveries either forceps or ventouse or both, remaining being delivered by normal vaginal delivery (39.2%). Meconium stained amniotic fluid was present in majority (70.2%) of the deliveries. The median APGAR score at 1 minute was 5 (Range - 4) with least being 1 and highest being 6 while median APGAR score at 5 minutes was 7 (Range - 6), least being 3 and highest score being 9. Out of 74 neonates included in our study, 32% had stage 1 HIE, 27% had stage 2 HIE and 23% had stage 3 HIE while 18% had no features of HIE. Umbilical cord blood gas pH analysis showed mean pH of

6.99 with minimum being 6.6. 50% of the study population had cord blood pH of less than 7.01. Cord blood smear examination was done and number of nucleated red blood cells (nRBC) per 100 white blood cells was counted. Mean nRBC count was 37.8 ± 17.9 with highest being 106 and least being 12 nRBC / 100 WBC. Serum LDH levels was estimated from the first collected blood sample after admission. Maximum serum LDH level was 2460 IU / L while minimum being 760 IU / L, with mean 1478.5 ± 429 IU / L. Distribution of cord blood pH, nucleated RBC and serum LDH in our study population is shown in table no 1.

Table No 1: pH, nRBC and Serum LDH distribution

Parameters	No.(n)	Mean	Minimum	Maximum	SD
Ph	74	6.997	6.6	7.2	0.138
nRBC / 100 WBC	74	38	14	106	18
Serum LDH (IU/L)	74	1478	760	2460	429

Correlation of umbilical cord blood pH with nRBC and serum LDH

Cord blood pH showed significant negative correlation (-0.7866) with nRBC counts. This indicated that acidosis in perinatal asphyxia is associated with significant ($p < 0.0001$) raise in cord blood nRBC counts. as depicted in figure no 1.

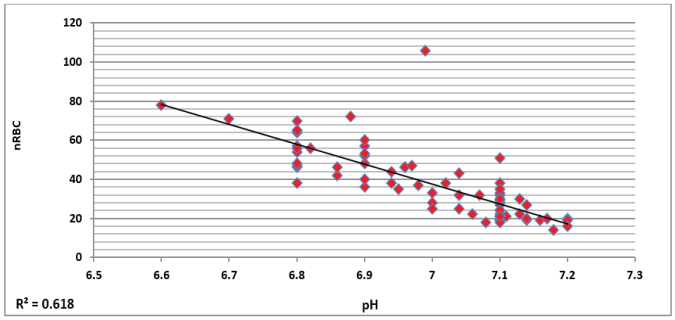

Figure 1: Correlation between pH and nRBC

Cord blood pH showed significant negative correlation (-0.8143) with serum LDH levels. This indicated that acidosis (indicated by low pH) in perinatal asphyxia is associated with significant ($p < 0.0001$) raise in serum LDH levels as shown in table 1 and figure no. 2.

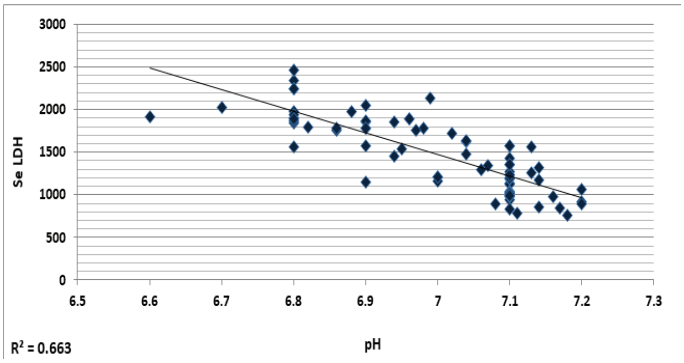

Figure 2: Correlation between pH and Serum LDH

Predictability of severe acidosis by Cord blood nRBC count and serum LDH

Base on the ph values, study population was divided into severe acidosis group (pH, 7.01; n = 37) and moderate acidosis (pH > 7.0) group. Mean values of nRBC count was 50 ± 27 nRBC / 100 WBC and serum LDH was 1761 ± 379 IU / L in severe acidosis group while mean value of nRBC was 26 ± 8.6 nRBC / 100 WBC and serum LDH 1204 ± 281 IU/L was in moderate acidosis group. Paired sample T test showed significant difference between means ($P < 0.001$) of nRBC and serum LDH in severe and moderate acidosis group. The values are depicted in table no 2.

Table no 2: Comparison between means of nRBC and serum LDH in severe (ph < 7.01) and moderate (ph7.01-7.20) acidosis

Parameter	Group	Mean	SD	SE of mean	Paired test
nRBC / 100 WBC	pH < 7.01	50	27	2.86	p < 0.001
	pH > 7.01	26	8.6	1.43	
Serum LDH (IU/L)	pH < 7.01	1761	379	63.2	P < 0.001
	pH > 7.01	1204	281	46.82	

Receiver operator curve (ROC) was plotted and Area under curve (AUC) was determined to evaluate for the predictability of severe acidosis by cord blood nucleated RBC count and serum LDH values. Cord blood nRBC count (AUC - 908) and serum LDH (AUC - 872) showed a very good predictability of severe acidosis in neonates experiencing perinatal asphyxia. Cord blood nRBC count of 32 nRBC / 100 WBC was 90% sensitive and 82% specific in predicting sever acidosis while serum LDH of 1480 IU / L was 82% sensitive and 80% specific in predicting acidosis as presented in ROC curve in figure no 3 and table no 3.

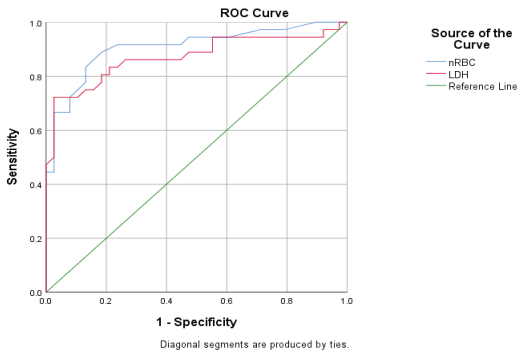

Figure 3: ROC curve showing nRBC and Serum LDH excellent indicators of severe acidosis

Table no 3: ROC curve showing nRBC and Serum LDH as excellent indicators of sever acidosis

Parameters	Area under curve (AUC)	Significance (CI 95%)	Cut off value	Sensitivity/ Specificity
nRBC / 100 WBC	0.908	p < 0.001	32	90%
				82%
Serum LDH (IU/L)	0.872	P < 0.001	1480	82%
				80%

Discussion

A disturbance in acid-base balance is one of the earliest and most sensitive signs of fetal distress. The degree of acidosis is measured by scalp or cord pH, with acidosis being used to determine the need for intervention. A pH of < 7.00 gives a 50% chance of abnormal outcome; however the positive predictive value for significant encephalopathy is low.[7] The mean pH in this study were 6.99 with severe acidosis of pH less than 7.01 were found in 50% of the study population. This is similar to other studies done by Winkler et al[8] and Prasanna et al.[9]

Nucleated RBC in perinatal asphyxia

The number of nRBC / 100 WBC is variable but is rarely greater than 10 in normal neonates. 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.[10] Mean number of nRBC in umbilical cord blood smear in our study was 38 per 100 WBC. Hermansen et al and Phelan et al in their studies had found higher number of cord blood nRBC in severe asphyxia cases.[11-12]

pH values showed significant correlation (p < 0.0001, r of r = 0.786) with cord blood nRBC / 100 WBC. This indicates that low pH in perinatal asphyxia is associated with significant raise in nRBC in cord blood. Mamta Malik et al similarly demonstrated a significant linear correlation between acidosis and nRBCs in the subjects (r = 0.71), (p < 0.01). They 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.[13]

Paired sample T test showed significant difference between means (P < 0.001) of nRBC in severe and moderate acidosis group. Cord blood nRBC count (AUC - 908) showed a very good predictability of severe acidosis with nRBC count of 32 nRBC / 100 WBC being 90% sensitive and 82% specific in predicting severe acidosis in neonates experiencing perinatal asphyxia. M Malik et al demonstrated a cut off value of more than 9 nRBCs / 100 WBCs, derived by ROC analysis, showed very good discriminating ability in diagnosing acidosis with area under curve of 0.84 (95% C.I. 0.77 - 0.92) and 95.65% specificity, 68.93% sensitivity in diagnosing acidosis.[13] Patil et al also inferred that cord blood nRBC count was found to be a good predictor of perinatal asphyxia with sensitivity of 96%, specificity of 98%.[14] This value is similar to the value of >10 nRBCs / 100 WBCs, reported by H Boskabadi et al.[10] Hence we infer that cord blood nRBC count / 100 WBC can serve as an economical as well as sensitive marker in perinatal asphyxia. A cut of value of more than 32 nRBC / 100 WBC would indicate severe acidosis in term neonates experiencing perinatal asphyxia.

Serum LDH in perinatal asphyxia

LDH increases early in newborns in several critical conditions and the LDH activity correlates well with the severity of diseases such as asphyxia and respiratory distress.[3] Further, LDH at admission has been shown to be a good predictor of the need for intensive care procedures. LDH levels are relatively easy to measure and may serve as a good predictor of HIE in the first few hours of life.[3] Serum LDH levels were estimated from first collected blood samples (within six hours of birth) of neonates with perinatal asphyxia. Mean serum LDH values was found to be 1478 IU / L. Around 49% of the neonates with perinatal asphyxia had serum LDH values more than 1500 IU / L with maximum observed being 2460 IU / L. pH values showed significant negative correlation (p < 0.0001, r of - 0.814) with serum LDH levels of neonates with perinatal asphyxia. This indicates that as pH in perinatal asphyxia decreases there is raise in serum LDH levels of neonates. Paired sample T test showed significant difference between means (P < 0.001) of serum LDH in severe and moderate acidosis group. Serum LDH (AUC - 872) done within six hours of birth showed a very good predictability of severe acidosis and serum LDH levels of 1480 IU / L was 82% sensitive and 80% specific in predicting acidosis in neonates experiencing perinatal asphyxia.

M Malik et al demonstrated that a serum LDH of more than

810 IU / L can fairly diagnose acidosis in asphyxiated babies with a sensitivity of 54.55% and specificity of 95.65%.[13] They also demonstrated a significant linear correlation between acidosis and LDH level (r = 0.54), (p < 0.01). 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 810IU / L had 95.65% specificity, 54.55% sensitivity in diagnosing acidosis (pH ≤ 7.2).

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 72 hrs of life. 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.[17] Karlsson et al have suggested levels > 1049 IU/L as having sensitivity of 100% and specificity of 97%. They concluded that in asphyxiated infants area under ROC value of 0.954 indicated it as excellent test to differentiate asphyxiated and non-asphyxiated term neonates.[18] These results are similar to those found in our study. Karunatilaka et al have suggested a much higher cut off of 2948 IU / L of LDH in predicting HIE. They concluded that both the CK and LDH values are raised in birth asphyxia. Cut off values for serum LDH taken within six hours of birth in asphyxiated neonates was 2948 IU / L where as in non-asphyxiated neonates was 1671 IU / L (p < 0.001).[19] Hence serum LDH done within six hours of birth can serve as alternative markers of severity of asphyxia in neonates. From our study we infer that serum LDH could serve as a sensitive and economical marker in term neonates with asphyxia. A cut of value of > 1480 IU / L would indicate severe acidosis in term neonates experiencing perinatal asphyxia and could serve as a prognostication marker in the early neonatal period. Limitation of our study is that controls were not considered in our study. Preterm babies were not included in our study where incidence of perinatal asphyxia and related complications are considerably higher.

Conclusions

Umbilical cord blood pH less than 7.01 is a good indicator of perinatal asphyxia. Cord blood nucleated RBC count per 100 WBC and serum LDH levels are significantly increased in perinatal asphyxia and correlate well with lowered cord blood pH. Nucleated RBC count and serum LDH can be used as simple and cheaper surrogate markers to diagnose perinatal asphyxia.

References

1. World Health Organization Perinatal Mortality A Listing of Available Information. WHO, Geneva. - References - Scientific Research Publishing [Internet]. Scirp.org. 2018 Available from: http:// www.scirp.org/ reference/ References Papers. asphyxia. Referenced 2061292. Acessed on 15 November 2021.

2. Lawn JE, Cousens S, Zupan J; Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: when? Where? Why? Lancet. 2005 Mar 5-11; 365(9462):891-900. DOI: 10.1016/S0140-6736(05)71048-5. PMID: 15752534.

3. National Neonatal Perinatal Database. Report for the year 2002–03. Available at www.newbornwhocc.org/pdf/nnpd_report_2002-03.Accessed July 21, 2022.

4. Low JA. The role of blood gas and acid-base assessment in the diagnosis of intrapartum fetal asphyxia. Am J Obstet Gyne. 1988 Nov;159(5):1235-40. DOI: 10.1016/0002-9378(88)90456-5. PMID: 3056010.

5. Beharier O, Kahn J, Shusterman E, Sheiner E. S100B - a potential biomarker for early detection of neonatal brain damage following asphyxia. J Matern Fetal Neonatal Med. 2012 Sep;25(9):1523-8. DOI: 10.3109/14767058.2012.664200. PMID: 22348227.

6. American College of Obstetrics and Gynecology, Task Force on Neonatal Encephalopathy and Cerebral Palsy, American Academy of Pediatrics. Neonatal Encephalopathy and Cerebral Palsy: Defining the Pathogenesis and Pathophysiology. Edited by Washington, DC, American College of Obstetricians and Gynecologists, 2013

7. Ahearne CE, Boylan GB, Murray DM. Short and long term prognosis in perinatal asphyxia: An update. World J Clin Pediatr. 2016 Feb 8;5(1):67-74. DOI:10.5409/wjcp.v5.i1.67. PMID: 26862504; PMC4737695.

8. Winkler CL, Hauth JC, Tucker JM, Owen J, Brumfield CG. Neonatal complications at term as related to the degree of umbilical artery acidemia. Am J Obstet Gynecol. 1991 Feb;164(2):637-41. DOI: 10.1016/s0002-9378(11)80038-4. PMID: 1992716.

9. Prasanna R, Karthikeyan P, Mani M, Paramanantham P, Sekar P. The strength of correlation between umbilical cord pH and early neonatal outcome. Int J of Contemp Ped. 2016; 134-37. DOI: 10.18203/2349-3291.ijcp20160145

10. Boskabadi H, Maamouri G, Sadeghian MH, Ghayour-Mobarhan M, Heidarzade M, Shakeri MT, et al. Early diagnosis of perinatal asphyxia by nucleated red blood cell count: a case-control study. Arch Iran Med. 2010 Jul;13(4):275-81. PMID: 20597559.

11. Hermansen MC. Nucleated red blood cells in the fetus

and newborn. Arch Dis Child Fetal Neonatal Ed. 2001 May;84(3):F211-5.
DOI: 10.1136/fn.84.3.f211.
PMID: 11320052; PMCID: PMC1721260.

12. Phelan JP, Korst LM, Ahn MO, Martin GI. Neonatal nucleated red blood cell and lymphocyte counts in fetal brain injury. Obstet Gynecol. 1998 Apr;91(4):485-9.
DOI: 10.1016/s0029-7844(98)00039-8.
PMID: 9540927.

13. Malik M, Mital R, Jaiswal V, Malik NP, Agarwal V, Upadhyay A. Correlation of acidosis with blood markers (LDH and nucleated RBCs/100 WBC) and with mortality and neurodevelopmental outcome in neonates with perinatal asphyxia. Int J of Contemp Med Res. 2016; 3(4):938-42.
ISSN (Online): 2393-915X; (Print): 2454-7379

14. Patil SS, Sukanya, Rath S, George CE. Study on umbilical cord arterial blood gas analysis and cord blood lactate levels as predictors for adverse neonatal outcome: an observational study. Int J Reprod Contracept Obstet Gynecol 2018;7:1494-500.
DOI: 10.18203/2320-1770.ijrcog20181342

15. Prabhavathi R, Sandhya V, Govindaraj M, Puttaswamy. M. Study of nucleated RBC count as a marker of severity of perinatal asphyxia in newborns - a case control study. Pediatric Rev: Int J Pediatrics Res. 2018 Feb.28;5(2):55-9.
DOI: 10.17511/IJPR.2018.I02.0

16. Sharma D, Choudhary M, Lamba M, Shastri S. Correlation of Apgar Score with Asphyxial Hepatic Injury and Mortality in Newborns: A Prospective Observational Study From India. Clin Med Insights Pediatr. 2016 May 10;10:27-34. DOI: 10.4137/CMPed.S38503.
PMID: 27199578; PMC4863868.

17. Reddy S, Dutta S, Narang A. Evaluation of lactate dehydrogenase, creatine kinase and hepatic enzymes for the retrospective diagnosis of perinatal asphyxia among sick neonates. Indian Pediatr. 2008 Feb;45(2):144-7.
PMID:18310795.

18. Karlsson M, Wiberg-Itzel E, Chakkarapani E, Blennow M, Winbladh B, Thoresen M. Lactate dehydrogenase predicts hypoxic ischaemic encephalopathy in newborn infants: a preliminary study. Acta Paediatr. 2010;99(8):1139-1144.
DOI: 10.1111/j.1651-2227.2010.01802.x.
PMID: 20236255;

19. Karunatilaka D, Amaratunga G, Perera K, Caldera V. Serum creatine kinase and lactic dehydrogenase levels as useful markers of perinatal asphyxia. Sri Lanka J. Child Health. 2009;29(2):49.
DOI: 10.4038/sljch.v29i2.604.