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ORIGINAL ARTICLE

Can Serum Lactate Dehydrogenase Levels be used as a Diagnostic Tool for Hemolytic Hyperbilirubinemia in Neonates?

MUHAMMAD TAUSEEF RAUF[1], BUSHRA FATIMA[2], MAZHAR QADIR KHAN[3], AYESHA KHANUM[4], NISAR AHMAD[5], KHAWAJA AHMAD IRFAN WAHEED[6]

[1]Fellow clinical Neonatology, University of Child Health Sciences, The Children's Hospital, Ferozpur Road Lahore
[2,3]Assistant Professor, University of Child Health Sciences, The Children's Hospital, Ferozpur Road Lahore
[4]Consultant Hematologist
[5]Prof of Pediatric Hematology, University of Child Health Sciences, The Children's Hospital, Ferozpur Road Lahore
[6]Prof of Neonatology, University of Child Health Sciences, The Children's Hospital, Ferozpur Road Lahore
Correspondence to: Muhammad Tauseef Rauf, Email: gims650@hotmail.com, Cell: 03334749165

ABSTRACT

Background & Objective: Neonatal jaundice is common reason for admission in neonatal units requiring further evaluation to prevent adverse neurodevelopmental outcome. Lactate dehydrogenase levels are high in hemolysis. This study was aimed to explore the diagnostic properties of Lactate Dehydrogenase (LDH)in hemolytic causes of neonatal hyperbilirubinemia.

Methods: In this observational cross sectional study, prospective data was collected at Neonatology department of The Children's Hospital and University of Child Health, Lahore fromFebruary 2020 to March 2022. A total of 364 neonates with the diagnosis of indirect hyperbilirubinemia were enrolled through convenient sampling method after excluding the comorbidities and sick neonates.The neonates were categorized into hemolytic and non-hemolytic group and lactate dehydrogenase levels were compared in both the group. Data was analyzed using SPSS version 24

Results: Out of 364 babies, 123 (33.79%) were diagnosed as having hemolytic jaundice while 241 (66.21%) fell in non-hemolytic group. Out of 123 newborns in hemolytic jaundice group, LDH levels of 101 (82.1%) newborns were raised while only 124 (51.5%) newborns in non-hemolytic group (241) had raised LDH (p-value <0.001). Sensitivity and Specificity of LDH levels as marker of hemolysis was 82.11% and 48.55% and positive predictive value and negative predictive value were 44.89% and 84.17% respectively. The practical implication that there are insufficient test s in common clinical usage that cal reliability make the the diagnosis of hemolysis

Conclusion: LDH is an effective diagnostic tool for the diagnosis of hemolytic hyperbilirubinemia in neonates with a higher sensitivity and negative predictive value.

Keywords: neonatal hyperbilirubinemia, hemolytic jaundice,lactate dehydrogenase

INTRODUCTION

Neonatal jaundice is yellowish discoloration of the skin, conjunctiva, and sclera due to elevated serum bilirubin in the newborn period. It develops in 50% term and 80% preterm babies[1]. Serum bilirubin level more than physiological limit for age is called neonatal hyperbilirubinemia[2]. The prevalence of neonatal hyperbilirubinemia is reported in literature as 29.6 to 31.7 %[3]. It accounts for 1309 deaths per 100,000 live births. Its burden was highest in countries with low sociodemographic index especially in Sub-Saharan Africa and South Asia[4]. Neonatal hyperbilirubinemia is an interplay between production and excretion of bilirubin. Increased production occurs in blood group incompatibilities, RBC enzyme deficiencies, structural defects, whereas decreased excretion occurs in impaired hepatic uptake of bilirubin its conjugation and increased enterohepatic circulation. It requires evaluation and management to prevent adverse neurodevelopmental outcome which is mainly due to unconjugated hyperbilirubinemia[5].

Increased reticulocyte counts and positive Coombs test in incompatible blood group setting favors the diagnosis of immune mediated hemolytic disease while negative Coombs test and increased reticulocyte count points towards the non-immune hemolysis including erythrocytes membrane defects and enzymopathies[6].Coombs test has limited usefulness with a positive predictive value of 12–53%, sensitivity of 15–64% and specificity of 65-98%[7].Moreover, reticulocyte counts are not always helpful in diagnosis because of its wide range in neonates[8].Peripheral smear also aids in diagnosis of hemolysis but has a limited value[9].

LDH is an intracellular enzyme released into serum by cell lysis and its physiological levels are present in serum due to routine turnover of cells including RBC's[10]. Its levels are raised in conditions e.g., hemolysis[11], respiratory distress, necrotizing enterocolitis and asphyxia[12]. In the absence of conditions like respiratory distress, asphyxia and necrotizing enterocolitis, the rise of serum LDH points towards hemolysis[11,12.] Therefore, it is recommended for diagnostic workup inhemolysis[12] study was designed to evaluate the effectiveness of serum LDH level in diagnosis of hemolytic jaundice in neonates. LDH isoenzyme level may further help in the accurate diagnosis of hemolytic jaundice.

METHODOLOGY

A cross-sectional study was conducted at The Neonatology department of The Children's Hospital and University of Child Health, from January 2020 to March 2022.Patientswho presented with jaundice in neonatal age group were included and babies with conjugated hyperbilirubinemia, any comorbid conditions like perinatal asphyxia, respiratory distress, necrotizing enterocolitis, malignancy, sepsis, if have received treatment from any other medical facility, bleeding or if already transfused with blood or blood products were excluded. A sample size of 364was calculated using prevalence of neonatal hyperbilirubinemia as 38.5% with 5% margin of error using online WHO calculator for sample size determination for health studies .After approval from the institutional review board and informed consent from the parents, the data of patients was collected on a pre-designed proforma. At presentation blood samples. 0.5 ml each, were collected in standard CBC vial (for CBC, peripheral smear, Coombs and reticulocyte count) and serum vial for total, direct bilirubin levels and LDH) that was kept in transportation box for 15 minutes, after which it was immediately transported to the central laboratory of the hospital for immediate analysis.They were analyzed within 2 hours using the analyzer Beckman Counter AV480 for serum and Sysmex Kx21 for CBC. The results were documented in the proforma.

The neonates were divided into hemolytic and non-hemolytic jaundice groups. Hemolytic jaundice was defined as unconjugated jaundice with positive Coombs or increased reticulocyte count or evidence of hemolysis on peripheral blood film and non-hemolyticjaundice was defined as unconjugated jaundice with negative Coombs, normal reticulocyte counts and no evidence of hemolysis on peripheral smear. LDH levels were compared in both groups and 629 U/L was taken as cut-off value[13]Datawas entered and analyzed using SPSS version 24. Mean and standard deviation was calculated for quantitative data e.g., LDH levels, reticulocyte count, and serum bilirubin. Frequency and the

percentage were calculated for categorical data e.g., gender, blood group and Coombs test. Independent sample t-test was applied to compare LDH levels in relation to different parameters of hemolysis viz. microspherocytosis , presence of bite & blister cells, Coombs test. ABO-Rh incompatibility was compared in relation to LDH level with the help of chi square test. Diagnostic accuracy such as sensitivity, specificity, positive predictive value and negative predictive value was calculated after making 2 x 2 table taking the split bilirubin levels as gold standard. Post stratified diagnostic accuracy was calculated. Patients were investigated and treated as per institutional guidelines.

RESULTS

A total of 446patients with hyperbilirubinemia were initially included. Out of those 82 met the exclusion criteria (Conjugated hyperbilirubinemia = 22, respiratory distress = 15, asphyxia = 20, NEC = 03, sepsis = 22). Therefore, 364 cases of unconjugated hyperbilirubinemia were finally included in the study and used for statistical analysis (Flow diagram).

The study population was divided into two groups, hemolytic and non-hemolytic and LDH levels were compared in both the groups. Out of 364 babies, 123 (33.79%) were diagnosed as having hemolytic jaundice while 241 (66.21%) fell in non-hemolytic group. Both the groups were similar as regards their demographic parameters including gender, age, weight and gestation and the difference was insignificant Serum bilirubin and reticulocyte count showed significant difference for neonates with hemolytic and non-hemolytic jaundice.(Table-1).

Table-1: Demographic parameters in hemolytic & non-hemolytic jaundice groups

Parameters		Hemolytic jaundice (N=123)	Non-Hemolytic jaundice (N=241)	Total (n=364)	p-value
Age (Days)		6.29±4.24	8.61±4.48	7.82±4.53 (1-20)	<0.001
Gender	Male	64 (52%)	149 (61.8%)	213	0.073
	Female	59 (48%)	92 (38.2%)	151	
Weight (Grams)	<1500	0 (0%)	1 (0.4%)	1	0.630
	1500-2499	28 (22.8%)	62 (25.7%)	90	
	2500-400	95 (77.2%)	178 (73.9%)	273	
Gestational Age (Weeks)	<34	2 (1.6%)	9 (3.7%)	11	0.365
	34-36	21 (17.1%)	55 (22.8%)	76	
	37-38	91 (74%)	160 (66.4%)	251	
	39-41	9 (7.3%)	17 (7.1%)	26	
Serum Bilirubin		19.23±4.26	18.24±4.50	18.58±4.44	0.046
Reticulocyte count		7.18±5.43	1.82±1.79	3.63±4.30	<0.001

Out of 123 newborns in hemolytic jaundice group, LDH levels of 101 (82.1%) newborns were raised while only124 (51.5%) newborns in non-hemolytic group (241) had raised LDH which showed that significant number of neonates with hemolytic jaundice had increased level of LDH as compared to neonates with non-hemolytic jaundice (p-value <0.001) (Table-2).

Sensitivity and Specificity of LDH levels as marker of hemolysis was 82.11% and 48.55% and positive predictive value and negative predictive value were 44.89% and 84.17% respectively. The overall diagnostic accuracy of LDH level as a marker of hemolysis was 58.89% (Table-2).

Table-2: Comparison and effectiveness of LDH levels in hemolytic vs non-hemolytic jaundice groups

LDH level	Hemolytic jaundice N = 123	Non-hemolytic jaundice N = 241	Total N = 364	p-value
Increased	101(82.1%)	124(51.5%)	225	<0.001
Normal	22(17.9%)	117(48.5%)	139	

Sensitivity= 82.11% (74.4, 87.88)
Specificity= 48.55% (42.31, 54.83)
Positive predictive value= 44.89% (38.53, 51.42)
Negative predictive value= 84.17% (77.2, 89.31)
Diagnostic Accuracy= 59.89% (54.78, 64.8)

Increased levels of LDH were found to be significantly associated with presence of parameters of hemolysis viz. microspherocytosis, presence of bite & blister cells& Coombs test (Table-3).

Table-3: Association of LDH levels with parameters of hemolysis

Parameter		LDH levels	p-value
Microspherocytosis	Positive	1367.39±551.06	<0.001
	Negative	932.80±592.09	
Bite and Blister cells	Positive	1575.11±422.35	<0.001
	Negative	956.00±594.70	
Coombs Test	Positive	1356.27±621.93	<0.001
	Negative	866.72±538.07	

Similarly, it was observed that the LDH levels were raised in case of blood group incompatibility settings. (Table-4).

Table-4: Association of LDH levels with ABO & Rh blood group incompatibilities

Incompatibility	LDH levels		p-value
	Increased	Normal	
ABO + Rh Incompatibility	91(45.3%)	28(21.9%)	0.000
ABO Incompatibility	40(19.9%)	14(10.9%)	0.032
Rh Incompatibility	51(25.4%)	14(10.9%)	0.001

DISCUSSION

Serum bilirubin levels in neonates are determined by the comparative rates of bilirubin production and elimination. The presence of hemolysis in a jaundiced neonate is the key parameter in the risk stratification as it predicts the chances of hyperbilirubinemia and vulnerability for bilirubin toxicity. American academy of pediatrics recommends identification of hemolysis which may be at times challenging, requiring multiple tests. There are insufficient tests in common clinical usage that can reliably make the diagnosis of hemolysis[14].We used lactate dehydrogenase, as urrogate marker of hemolysis to diagnose hemolytic causes of neonatal hyperbilirubinemia.

In the present study mean age of neonates was 7 days and among them 58.5% were males and 41.5% were females. This can be related to the study in 2015,who reported that mean age of neonates presenting with hemolyticjaundice due to pyruvate kinase deficiency was 7 days and among them 58% were males and 42% were females[15]. Mean weight of neonates in our study was 2771.18±460.96 grams while another study conducted. reported that low-birthweight (<2.5kg) was found in 31% of babies with jaundice.[16]

In our study there were 251 (69%) neonates with gestational age between 37-38 weeks which is comparable to study in 2017.which showed mean gestational age of 35.2±1.4 weeks[17].

Out of 364 neonates,33.79%(123) were diagnosed as hemolytic and 66.21%(241) were non-hemolytic jaundice this can be compared to the study done 2021in which the hemolytic jaundice was found to be 38.5%[18].

In this study we reported that positive blood smear (presence of microspherocytosis, Bite and Blister cells), ABO and Rh incompatibility blood group settings showed significant

association with increased LDH levels as compared to no findings on peripheral smear and without ABO-Rh incompatibility settings. This can be supported by the fact that LD. His raised in hemolytic states and has been recommended for the workup in hemolytic anemia in neonates[19].Waldron etal[20] has reported the raised level of lactate dehydrogenase in ABO hemolytic disease of newborn similar case reports have been published by different studies [21], .[22], [23] .[24]showingncreased level of LDH in different hemolytic states including hemolytic jaundice among neonates.

In our study LDH showed a sensitivity and specificity of 80.95% and 48.21% respectively. Positive predictive value and negative predictive value for LDH level was 42.29% and 84.38% respectively. The overall diagnostic accuracy of LDH level was reported as 58.66%. Our study showed that LDH elevation is sensitive for hemolysis but is not specific, which can be explained on the fact that its ubiquitous nature and can be released from other tissues. Since this is the first study evaluating the role of LDH in jaundiced neonates to our knowledge, we do not have data from other studies to compare. However, in adults a study done by Edward R Burns et al showed a high sensitivity (100%) and a low specificity (59%) of LDH in hemolysis, which is comparable to our results of high sensitivity and low specificity [25].

This can also be related to the diagnostic yield of other tools to diagnose the hemolysis like Coombs, Reticulocyte count and peripheral smear. Coombs have shown to have a positive predictive value of 12%–53%, negative predictive value of 89%-96% and a sensitivity of 15%–64% for the development of hyperbilirubinemia in newborns[7]. However, the value of hematological tests for the identification of hemolysis in the newborn is not great, the reticulocyte count has a wide range, limiting its value and the blood smear in newborns is also not very helpful[8]. The study design lends itself to replicability for a multicenter study so that further study can be carried out with larger sample size to conclusively establish the diagnostic role of LDH for the diagnosis of hemolytic jaundice in neonates.

CONCLUSION

Serum lactate dehydrogenase levels can be used as a diagnostic tool for hemolytic hyperbilirubinemia in neonates and carries a high sensitivity and negative predictive value.

Conflict of Interest: The authors declare no conflict of interest associated with this manuscript.

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