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Research Article

Study of Lactate and Nucleated Red Blood Cells as Early Predictors of Neonatal Hypoxic Ischemic Encephalopathy

Mohamed Shawky Elfarargy[1,2]*, Elsharkawy HM[1], Elgendy MM[1], Nassar MA[1], Attia GF[3], Abu Elmaaty MK[4], Mohamed SA[5], Abd-Ellatif AA[6,7], Alaa A Mohamed[8,9] and Kelleni MT[10,11]

[1]*Pediatric Department, Faculty of Medicine, Tanta University, Egypt*

[2]*Pediatric Department, College of Medicine, Jouf University, KSA*

[3]*Clinical Pathology Department, Faculty of Medicine, Tanta University, Egypt*

[4]*Obestetric and Gynecology Department, Faculty of Medicine, Tanta University, Egypt*

[5]*Pediatric Department, Faculty of Medicine (Damietta) – Al-Azhar University, Egypt*

[6]*Physiology Department, Faculty of Medicine, Beni-Suef University, Egypt*

[7]*Physiology Department, College of Medicine, Jouf University, KSA*

[8]*Biochemistry Division, Pathology Department, College of Medicine, Jouf University, KSA*

[9]*Department of Biochemistry, Faculty of Medicine, Beni-Suef University, Egypt*

[10]*Pharmacology Department, Faculty of Medicine, Minia University, Egypt*

[11]*Pharmacology Department, College of Medicine, Jouf University, KSA*

***Corresponding Author:** Mohamed Shawky Elfarargy, Pediatric Department, Faculty of Medicine, Tanta University, Egypt.

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Abstract

Background: Hypoxic ischemic encephalopathy (HIE) after prenatal asphyxia is an important cause of neonatal morbidity.

Aim: Study nucleated red blood cells per 100 white blood cell (NRBC/100 WBC) counts and lactate levels in cord blood as early markers of neonatal HIE.

Patients and Methods: This is a prospective case control study conducted on 60 HIE neonates, nucleated red blood cells count/100 WBCs and lactate level in the cord blood was measured. These were compared to 60 neonates matched in age, sex and body weight apparently healthy neonates as a control group.

Results: Nucleated red blood cells per 100 white blood cell (NRBC/100 WBC) counts and lactate levels in cord blood were higher in HIE neonates than control group with a significant difference (P value < 0.0001).

Conclusion: NRBC/100 WBC counts and lactate levels in cord blood could be used as an early markers of neonatal HIE

Keywords: Lactate; Nucleated Red Blood Cells; Hypoxic Ischemic Encephalopathy (HIE)

Introduction

Hypoxic ischemic encephalopathy (HIE) after prenatal asphyxia is an important cause of neonatal morbidity, neurological disability and mortality. The early prediction of hypoxic ischemic encephalopathy is particularly important because of the brief therapeutic window and possible side effects of neuroprotective intervention [1].

HIE is clinically defined as a syndrome of disturbed neurological function in neonate after birth, manifested by difficulty with initiating and maintaining respiration, depression of tone and reflexes, subnormal level of consciousness and seizures. Neonatal encephalopathy that results from systemic hypoxemia and decreased cerebral perfusion leading to ischemia is termed HIE [2].

The fate of birth asphyxia may include death or other complication like seizures, epilepsy, cerebral palsy and developmental de-

lay. The incidence is higher in infants of diabetic or toxemic mother, intrauterine growth retardation (IUGR) and breech presentation as the previous events are associated with increased incidence of asphyxia [3].

Based on the previous studies and clinical experience therapeutic window in human neonatal HIE seems to be within 6 hours after birth. Thus, it is important to look for useful predictors early in the course of the disease [4].

Nucleated red blood cells count per 100 white blood cells in umbilical venous blood of newborn has been reported as a simple, quick and cheap marker of prenatal asphyxia, based on the fact that hypoxic events induce fetal compensatory response in the form of exaggerated erythropoiesis and influx of immature red blood cells into fetal circulation [5].

Lactate is produced in the event of hypoxia and poor tissue perfusion. Any reduction of oxygen and substrate delivery to the fetus, aerobic metabolism through krebs cycle cannot be sustained and tissue need anaerobic metabolism to meet energy requirement, this in turn leads to increase in the production and accumulation of blood lactate [6].

Blood lactate concentration in critically ill and injured patients can be used to detect tissue hypoxia at an early stage which is simple, cheap and quick marker to predict and asses illness severity and outcome [7].

Patients and Methods

This is a prospective case control study conducted on 60 HIE neonates delivered either vaginally or by cesarean section in Tanta University Hospital during the period of twelve months from January 2017 to December 2017. These were compared to 60 neonates matched in age, sex and body weight apparently healthy term neonates as a control group.

Inclusion criteria

1. Term neonates
2. Newborns were suggested to be hypoxic if they met these criteria guided by the AAP [8]:
   a. Persistence of an expanded Apgar score of 0-3 for longer than 5 minutes.
   b. Neonatal neurologic sequelae (e.g. seizures, coma, hypotonia).
   c. Multiple organ involvement (e.g. kidney, lungs, liver, heart, intestines).
   d. Base deficit > 10.

Exclusion Criteria

1. Preterm neonates delivered before 36 weeks gestation.
2. Newborn delivered with major congenital anomalies or chromosomal abnormality.
3. Multiple pregnancies.

Both groups of patients and control were subjected to Full maternal history taking, full detailed history of resuscitation, Apgar score at 1 and 5 minutes. System examination with special emphasis on neurological examination with assessment of severity of hypoxic ischemic encephalopathy using Sarnat and Sarnat staging [9].

Investigations

All case were subjected to umbilical cord sampling at time of birth, 5 cc of umbilical cord blood were taken and divided as follow: 2 cc for CBC, and Nucleated RBCs, 2 cc for Lactate and electrolyte and 1 cc for arterial blood gas

1. **Routine laboratory investigations:** Complete blood count, Electrolytes (Na, K, random blood sugar), Umbilical cord arterial blood gas.

2. **Specific investigations**: Nucleated red blood cells count/100 WBCs. Samples of cord blood was collected in tubes after centrifugation, lactate level of umbilical arterial blood was measured. Complete blood count was done by sysmexXS 500 and counted by the way of light scattering technique (scattered light is detected by photo multiplier or photodiode which converts it into electrical impulses which are accumulated and counted) Blood films was prepared from EDTA-anticoagulated blood, and stained by Giemsa and Leishman stain. The lactate is changed into pyruvate and hydrogen peroxide by lactate oxidase. The hydrogen peroxide reacts in the presence of peroxidase with chromogen precursors giving a purple compound. The intensity of the color is proportional to concentration of lactate in the tested sample.

Statistical Analysis

The data were coded, entered and processed on computer using SPSS (latest version). The level P < 0.05 was considered the cut-off value for significance.

Results

This study was conducted on neonates delivered at Tanta University Hospital during the period of 12 months from January 2017 to December 2017. This study was conducted on 2 groups:

1. **Group 1 (HIE group):** 60 full term neonates diagnosed as (HIE) according to the criteria set by AAP.
2. **Group 2 (control group):** 60 age matched appa rently healthy full term neonates as control group with no obstetrical problems.

Table 1 showed that there was no statistical significant difference between both groups as regard the sex (P value > 0.05). There was no statistically significant difference as regarding weight and gestational age (p value > 0.05). There was significant low Apgar score in HIE neonates in relation to the controls (P value < 0.0001).

N		HIE neonates (n = 60)		Control (n = 60)		Test	P-value
		%	N	%			
Sex	Male	34	56.67	32	53.33	X^2: 0.067	0.795
	Female	26	43.33	28	46.67		
Weight (mean ± SD)		3.216 ± 0.320		3.176 ± 0.233		T: 0.548	0.586
Gestational age (mean ± SD)		38.667 ± 1.373		38.900 ± 1.155		T: -0.712	0.479
Apgar score	1minute	2 (1 - 4)		6 (4 - 8)		Z: 6.919	< 0.001*
	5 minutes	6 (4 - 8)		9 (7 - 10)		Z: 6.953	< 0.001*

Table 1: Demographic data and Apgar score of HIE and control newborn infants.

Table 2 showed that patients were divided according to Sarnat and Sarnat scoring system into three stages, 30% were classified as grade I HIE, 30% as grade II HIE and 40% as grade III HIE.

Grades of hypoxia	N	%
Grade I	18	30
Grade II	18	30
Grade III	24	40
Total	60	100

Table 2: Classification of HIE neonates (group 1) according to grades of hypoxia.

Table 3 showed that there was a highly significant difference between both groups in relation to nucleated red blood cells and serum lactate level(higher in HIE than in control) (P value < 0.001).

	Groups		Mann-Whitney Test	
	HIE	Control	Z	P-value
Lactate level (mmol\ dl) [median (IQR)]	7	1.9	6.677	< 0.001*
NRBCs No. [median (IQR)]	18	2	6.705	< 0.001*

Table 3: Comparison between HIE neonates and control group as regarding nucleated red blood cells and serum lactate level.

Table 4 showed that there was highly significant difference in outcome among different grades of hypoxia (P value < 0.001). It also showed significant difference between levels of serum lactate and nucleated red blood cells (P value < 0.001).

		HIE Grade						Test	P-value
		Grade I (n = 18)		Grade II (n = 18)		Grade III (n = 24)			
		N	%	N	%	N	%		
Outcome	Died	0	0	0	0	14	58.33	X^2: 13.696	< 0.001*
	Discharged	18	100	18	100	10	41.67		
Lactate level (mmol\dl) [median (IQR)]		4.5 (4 - 6)		7 (5 - 9)		9 (8 - 10)		KWχ^2: 12.532	< 0.001*
NRBCs No. [median (IQR)]		13 (10 - 15)		20 (15 - 24)		45 (40 - 50)		KWχ^2: 15.641	<0 .001*

Table 4: Comparison between the three grades of hypoxia as regarding nucleated red blood cells and serum lactate level and outcome.

ROC curve between Cases and Control						
	Cut off	Sens.	Spec.	PPV	NPV	Accuracy
Lactate level (mmol\dl)	> 3.6	87.0	100.0	100.0	88.0	93
NRBCs No	> 4.5	77.0	83.0	82.0	78.0	80

Table 5: ROC curve between Cases and Control.

The sensitivity and specificity were computed by establishing receiver operating characteristic (ROC) curves. The clinical values corresponding to the combination of highest sensitivity and specificity determined at the apex of the (ROC) curves were chosen. An ideal curve of a reasonable test should have the larger area under curve of the ROC graph.

The best cutoff of nucleated red blood cells to detect hypoxia was > 4.5/100 WBCs with a sensitivity of 77%, specificity 83%, PPV 82% and NPV 78% with a diagnostic accuracy 80%.

Serum lactate level were reliable to predict hypoxia as P value < 0.0001 and area under the curve (ROC) was 95% and the best cut off of serum lactate to detect hypoxia > 3.6 mmle/L yielded a sensitivity of 87%, specificity 100%, PPV 100% and NPV 88% with a diagnostic accuracy of 93%.

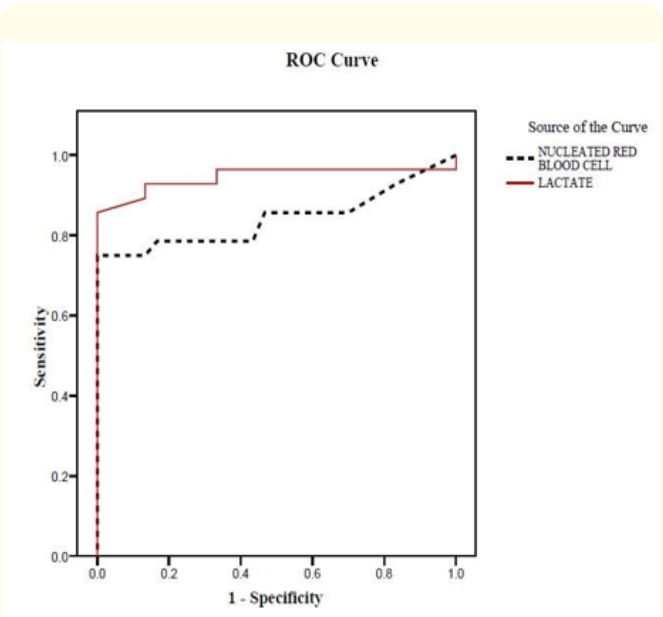

Figure 1: Receiver Operating Characteristic (ROC) curve to define the best cutoff to detect hypoxia.

Discussion and Conclusion

Hypoxic ischemic encephalopathy after prenatal asphyxia is an important cause of neonatal morbidity, neurological disability and mortality. The early prediction of hypoxic ischemic encephalopathy is particularly important because of the brief therapeutic window and possible side effects of neuro protective interventions [1,10].

In spite of major advances with sophisticated monitoring technology and knowledge of fetal and neonatal pathology, perinatal asphyxia or more appropriately, HIE remains a serious condition, that leaves a significant handicaps in the survivors [11].

In our study the median of Apgar score at 1 and 5 minutes was 2 and 6 respectively and it was significantly lower than the control which had normal Apgar score (7 - 9 at 1 and 5 minutes), in agreement with our study some studied showed that low Apgar score was associated with many problems [12].

In our study the median of nucleated red blood cells in the HIE group was 18/100WBCs with a range from 13 - 53 while the median of NRBCs in control group was 2/100WBCs. This in agreement with some studies who found that the median of NRBCs in asphyxiated newborn was higher than the control healthy group [13].

In our study the Levels of nucleated red blood cell per 100 white blood cells were higher in HIE group than in control healthy group this was in agreement with some studies who stated that the Levels of nucleated red blood cell per 100 white blood cells was related to the severity of asphyxia and clinical outcome [14].

In our study the median of serum lactate level in hypoxic group was 7 mmol/L while in the control group the level of lactate was 1.9 mmol/dl which was statistically significant as p value < 0.0001. This in agreement with some studies who found that serum lactate was higher in hypoxic group than healthy group [15,16].

In our study the serum lactate level is higher in neonatal hypoxia than the healthy control group and the more grade of HIE the higher serum lactate. This in agreement with some studies who found that serum lactate was higher in neonatal hypoxia with the more sever hypoxia the more serum lactate [17].

In our study nucleated red blood cells could be used with other markers to detect HIE and best cut off of nucleated red blood cells to diagnose hypoxia was > 4.5/100 WBCs with a sensitivity of 77%, specificity 83%, PPV 82% and NPV 78% with a diagnostic accuracy of 80%. This in concordance with some studies who stated that NRBC counts can predict brain injury and neurological outcomes in asphyxiated neonates [18].

In our study serum lactate level to diagnose HIE was > 3.6 mmol/L which yield a sensitivity of 87%, specificity 100%, PPV 100% and NPV 88% with a diagnostic accuracy of 93%.This is in agreement with some studies which concluded that Umbilical lactate can be used in a middle-low resource setting as a measurement of intrapartum hypoxia, with reasonable sensitivity and specificity [19,20].

In conclusion, we found that both nucleated red blood cells and lactate could be used as early predictors in diagnosis of hypoxic ischemic encephalopathy being very easy, cheap and non-invasive measure.

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