Early prediction of neonatal hypoxic ischemic encephalopathy by detection of umbilical cord nucleated red blood cells and lactate

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Abstract
Hypoxic-Ischemic Encephalopathy (HIE) remains a serious condition that causes significant mortality and long-term morbidity. This study established a simple and quick method thatcould be used to predict the occurrence of HIE as early as possible by investigating the variations of nucleated red bloodcells (RBCs) per 100 white blood cell (NRBC/100 WBC) counts and lactate levels in cord blood of the newborn. The study included 30 full term babies diagnosed as having HIE who fulfilled AAP criteria, compared with 30 agematched apparently healthy term neonates as a control group with no obstetrical problems, delivered either vaginally or bycesarean section. Nucleated RBCs were able to predict hypoxia and to assess the grade of hypoxia, while lactate had better ability to predict hypoxia but could not assess the grade of hypoxia. Nucleated RBCs and lactate were able to predict HIE early in labor as they had better sensitivity and specificity than other alone.
Key Words: prediction, neonatal hypoxic ischemic, encephalopathy

Introduction
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 (Zhang Haiju and Suyan, 2008).

Recently nucleated red blood cells count per 100 white blood cells in umbilical venous blood of newborns has reported as a marker of prenatal asphyxia (Blackwell et al., 2004)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 (Hendrik et al., 2002).

Based on previous studies and preliminary clinical experience therapeutic window in human neonatal hypoxic ischemic encephalopathy seems to be within 1- 6 hours after birth. Thus it is important to look for useful predictors early in the course of disease (Johnston, 2000).
Lactate is invariably produced in the event of hypoxia and poor tissue perfusion .When a clinical reduction of oxygen and substrate deliveryoccurs, aerobic metabolism through Krebs cycle cannot be sustained andtissues need anaerobic metabolism to meet the energy requirement .this inturn, leads to increase in the production and accumulation of blood lactate (Kobayashi et al., 2005)

Blood lactate concentration in critically ill and injured patients can be used to detect tissue hypoxia at an early stage assesses illness severity and predict outcome (Vannucci et al, 2005).

Aim of the work
The aim was to establish a simple and quick method that could be used to predict occurrence of hypoxic ischemic encephalopathy as early as possible to improve efficiency of short term prognosis of asphyxia in neonates and to correlate levels of nucleated red blood cells and cord lactate with the degree of hypoxia.

Subject and Methods

This is a prospective case control study conducted on 30 full termneonates with asphyxia delivered either vaginally or by cesarean section in Minia Gynae, Obstetrics and Pediatric University Hospital . Those were compared to 30 and sex age matched apparently healthy term neonates as a control group with no obstetrical problems, delivered either vaginaly or by cesarean section from January 2016 to June 2016.

Inclusion criteria:

Term neonates 37-42 weeks of gestation according to [new Ballard score] for assessment of gestational age.

Newborns were diagnosed as hypoxic according the criteriaof American Academy of Pediatrics (2012):

1- Low Apgar score for longer than 5 minutes.

2- Neonatal neurologicsequelae (e.g., seizures, coma, hypotonia).

3- Base deficit>10 + Metabolic acidosis.

Exclusion Criteria:

1- Preterm neonates delivered before 37 weeks gestation

2- Newborn delivered with major congenital anomalies or chromosomal abnormality

3- Suspected cases of hemolytic diseases (Rh and ABO incompitabilty) or Septisemia Investigations:

All cases were subjected to the following

1) Routine laboratory investigations

2) Special Investigations:

A) Nucleated red blood cells count/ 100 WBCs in umblical cord blood.

B) Assessment of lactate level of umbilical cord blood

Sensitivity: is the ability of the test to detect those who are truly diseased (true positive rate).

Specificity: is the ability of the test to detect those who are free of disease (true negative rate).

Positive predictive value (PPV): is the proportion of patients with an outcome or disease if the test is positive, is the percentage of truepositive to all positive by the examined test. Negative predictive value (NPV) is the proportion of free cases in negative results.

A receiver operating characteristic (ROC) curve: used to illustratethe diagnostic properties of a test on a numerical scale.

Results

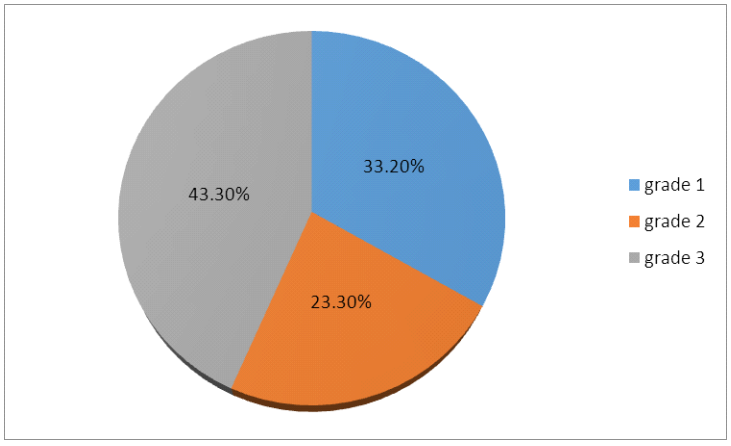

Figure (1) Classification of cases according to grades of hypoxia.

Table (1) Comparison between cases and control group as regarding umblical cord nucleated red blood cells and lactate level.

	Cases N=30		Controls N=30		P value
	Mean±SD	Median	Mean±SD	Median	
Cord lactate(mg/dl)	71±41	68	31±29	20	0.003*
Nucleated RBCs (/100WBCs)	31.3±21.1	14.5	15±19	5	0.000*

P value <0.0001 highly significant

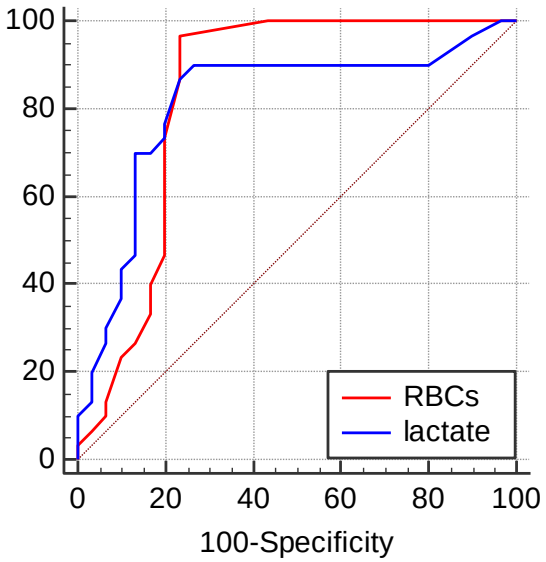

Figure (2): Receiver Operating Characteristic (ROC) curve to define the best cutoff to detect hypoxia.

Table (2): Cut -off of NRBCs and cord lactate level in detecting hypoxia.

	AUC	Std error	P value
Nucleated red blood cells /100WBCs	0.83	0.06	<0.0001
Cord lactate level mg / dl	0.81	0.06	<0.0001

RBCs sestivity 96.7 specificity 76.7 cutoff point >6 PPV 4 NPV 100
Lactate sestivity 86.7 specificity 76.7 cutoff point >28 PPV 3.6 NPV 99.8.

Table (3): Comparison between the grade of hypoxia and cord nucleated red blood cells and lactate level.

Grades of hypoxia	Cord nucleated RBCs (Median)	Cord lactate (Median)
Grade I	13.5	52
Grade II	12	39
Grade III	53	89
P value	0.000	0.000

P value <0.0001 highly significant

Table (3) Outcome of the studied and control group.

Cases N=30		P value
Outcome	**N(%)**	
Died	7(23.3%)	0.005*
Discharged	23(76.7%)	

P value <0.01 highly significant

Discussion

.In our study the median of cord nucleated red blood cells in the asphyxiated group was14.5 /100WBCs while the median of NRBCs in control group was 5 /100WBCs as shown in table. This is in agreement with (Hassan Boskabadi et al., 2016)who carried out a study in cord blood of 75 term asphyxiated newborns to investigate variations in NRBC counts in perinatal asphyxia and they found that the median of NRBCs in asphyxiated newborn was 16.5/100 while in control group was 8.6/ 100 WBCs.

Variations in number of NRBCs depend on the time of occurrence of hypoxia as it was known that the reticulocytic response to hypoxia-induced erythropoietin release is generally not seen until the second or third day after hypoxia, elevated nucleated red blood cell counts could be seen in cord blood only if the hypoxic event occurred or the hypoxic process began at least several days before delivery so the number of nucleated red cells is more in cases with chronic fetal distress than acute fetal distress (Kleinbongard et al., 2006).

Concerning the relation between Apgar score and nucleated red blood cells, cases with low Apgar score showed higher level of nucleated red blood cells. This is in agreement with Perrone et al., (2005).who found that Apgar scores at 1st and 5th minutes were inversely related to the nucleated red blood cell count.

In our study the median of serum lactate level in hypoxic group was 68 mg /dL (7.4 mmol/l) while in the control group it was 20mg / dl(2.2 mmol/l) as shown in table which was statistically significant as p value < 0.0001. This level is lower than that was found in other study done by Shah et al., (2004) who carried out a study on 61 term neonates to estimate lactate level as a predictor for short term outcome after intrapartum asphyxia. They found that the median of lactate level was 11.09 mmol/l in asphyxiated group met all the criteria of hypoxic ischemic encephalopathy , the study also found that plasma lactate levels lower than 5 mmol/l were not associated with severe encephalopathy while plasma lactate levels >15 mmol/Lwere associated with moderate to severe HIE in 100% of cases.

In our study nucleated red blood cells was reliable to detect HIE as area under the curve (AUC) was 83% and best cut off of nucleated red blood cells to diagnose hypoxia was >6/100 WBCs with a sensitivity of 96.7%, specificity 76.7%, PPV 4 and NPV 100% with a diagnostic accuracy of 96%. This is in agreement with Chand R et al., (2016), who reported that sensitivity and specificity were 87% and 81% at a level of 14 NRBCs/100 WBCs to predict fetal hypoxia. A nother study carried out by Blackwell et al., (2004) showed that the best cutoff value for NRBCs counts in predicting HIE was 15 /100 WBCs with sensitivity 100% and specificity of 73%.

In conclusion, we found that both cord 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.

Combined detection of both cord nucleated red blood cells and lactate in diagnosis of HIE gives better sensitivity and specificity than each one alone, also it could be used to detect the prognosis of the newborns with hypoxic ischemic encephalopathy and for detecting the outcome.

Summary

Perinatal hypoxic ischemic cerebral injury remains an important issue partly because it is the most clearly recognized cause of cerebral palsy. Most cases of hypoxic ischemic encephalopathy result from injury in the perinatal period secondary to intrauterine asphyxia, with disturbance of gas exchange across the placenta.

The aim of this study was to assess the patients of hypoxic ischemic encephalopathy and to establish a simple and quick method that could be used to predict occurrence of hypoxic ischemic encephalopathy as early as possible to improve efficiency of short term prognosis of asphyxia in neonates by assessment of level of both nucleated red blood cells and lactate in cord blood in the first few hours after delivery.

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