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ORIGINAL ARTICLE

Advances in Prediction of Neonatal Hyperbilirubinemia severity Using Cord Blood Hydrogen Peroxide

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Abstract

Background Unconjugated hyperbilirubinemia is the most common cause of hospital admissions and readmissions among the newborns. The study aimed to evaluate weather levels of hydrogen peroxide in cord blood can be used to confirm the predictive warning sign of neonatal hyperbilirubinemia and identify which babies require follow-up leaving the hospital. **Methods:** a cross-sectional study involved 30 healthy neonates. They were classified into two groups according to bilirubin level at the 5th day: less severe hyperbilirubinemia with a bilirubin level ≤ 17 mg/dl and severe hyperbilirubinemia with a bilirubin level > 17 mg/dl. Measurement of hydrogen peroxide in cord blood, at birth, and on the third and fifth days of life by colorimetric method was done. **Results:** in our study show that at the cutoff point of cord blood hydrogen peroxide ≥2.685 uMol/L, sensitivity was 90.0%, specificity was 83.3%, predicted values (both positive and negative) were 78.3% and 92.6%, positive and negative likelihood ratios were 5.4 and 0.12, and accuracy was 86.0%. **Conclusions**: The levels of cord hydrogen peroxide and further sampling were considerably greater in neonates with severe hyperbilirubinemia. It is therefore possible to identify a subset of newborns who may be at risk of jaundice and require phototherapy or exchange at delivery by measuring the amounts of hydrogen peroxide in the umbilical cord blood.

Key words: Neonatal jaundice; Severe hyperbilirubinemia; Hydrogen peroxide; Fullterm

INTRODUCTION

The most frequent reason for hospital admissions and readmissions among the neonatal population globally is still significant unconjugated neonatal hyperbilirubinemia [1].

Jaundice continues to be a significant factor in both neonatal morbidity and mortality, particularly in Southeast Asian and African countries [2].

When total bilirubin exceeds the 95th percentile on an hourly basis in neonates ≥35 weeks gestational age, it is referred to as neonatal hyperbilirubinemia on Bhutan´s [3] hour-specific nomogram. The majority of jaundiced babies have no underlying medical conditions, known as "physiological jaundice," which is usually benign. But for some babies, jaundice may worsen and lead to acute bilirubin encephalopathy, or kernicterus, which has a high risk of newborn death [4]. The risk of newborn jaundice is higher at lower gestational ages. According to Campbell and Mena [5], the risk of severe hyperbilirubinemia rises dramatically for every week of gestation that is less than 40 weeks [6].

Natural hydrogen peroxide (H2O2) is found in all living cells. Several human disorders result in an increase in the concentration of hydrogen peroxide in bodily fluids, despite the fact that the hydrogen peroxide level in cells is rigorously confined during the metabolism of the cell. Monitoring the body's hydrogen peroxide levels is helpful for diagnosing diseases early [7].

Increased levels of oxidative free radicals, including nitrous oxide (NO) and hydrogen peroxide (H2O2), can cause oxidative damage to fetal tissues, which are permanently oxygenated during intrauterine life. Soon after delivery, some of these free radicals target the circulating erythrocytes and thrombocytes, potentially

causing thrombolysis and hemolysis [8]. Therefore, creating a quick, easy, and affordable way to identify hydrogen peroxide in body fluids would be beneficial for primary health assessment. To date, hydrogen peroxide detection is mainly performed by using fluorescence analysis [9].

Aim of the work

Cord blood (H2O2) levels can be used to determine which newborns need to be monitored after discharge from the hospital and to validate the predictive warning sign of neonatal hyperbilirubinaemia.

METHODS

All parents gave their informed consent, and the Zagazig University ethical committee authorized the study. After obtaining approval from the Institutional Review Board (IRB) (N1167-5-11-2023), the pediatric departments of Zagazig University Hospitals granted permission to conduct the study. The study was conducted in compliance with the Declaration of Helsinki, the World Medical Association's code of ethics for human subjects' research. A cross sectional study was carried on all term healthy neonates born at Obstetric Hospital, Zagazig University from November 2023 to March 2024.

Thirty healthy babies who met the study's requirements were included in the study. They were born at Obstetric Hospital Zagazig University, attending for follow up at Pediatric Outpatient Clinic, and admitted in Neonatal Unit of Children Hospital Zagazig University for cases which were in need for admission.

They were classified into two groups according to bilirubin level at the 5th day after birth, according to the Bhutani nomogram: less severe hyperbilirubinemia, this group comprises 18 healthy newborns, with bilirubin level ≤ 17 mg/dl. They were 10 males and 8 females and severe hyperbilirubinemia: This group comprises12 healthy newborns, with bilirubin level > 17 mg/dl. They were 7 males and 5 females.

Criteria for inclusions included breastfed and appropriate for gestational age (≥37–42 weeks). The following conditions must be met in order for a baby to be excluded: severe congenital malformations; enclosed hemorrhages; maternal diabetes; eclampsia or preeclampsia; birth asphyxia; sepsis; hemolytic jaundice, as proved by: abnormal peripheral blood smear, blood count, reticulocytic count, Rh incompatibility, blood group iso-immunization , abnormal Coombs test, and a typical activity of glucose-6-phosphate dehydrogenase.; maternal drug consumption during delivery; and a total serum bilirubin level that increased by more than 5 mg/dl per day or was higher than 10 mg/dl in the following 24 hours after birth.

Every baby registered in the study will endure the following: comprehensive history, prenatal, natal, and postnatal, Detailed clinical examination, Measurement of cord blood and capillary blood at birth, 3rd day, and 5th day of age for hydrogen peroxide assay by colorimetric method, the principle: in the presence of peroxidase (HRP), H2O2 reacts with 3, 5-dichloro-2-hydroxybenzensulfonic (DHBS) acid and 4-aminophenazone (AAP) to form a chromophore. Laboratory investigations include: total serum bilirubin and direct bilirubin at the start of phototherapy, follow up of total serum bilirubin every day, total serum bilirubin before discharge from the neonatal intensive care unit. Other investigations include: cord hematocrit, neonatal blood grouping and Rh for mother and newborn, reticulocyte count, Coombs test and Glucose-6-phosphate dehydrogenaze assay.

Statistical Analysis

A computer using the Statistical Package of Social Services version 24 was used to evaluate the data that had been gathered (SPSS). Data were displayed as graphs and tables. P values, correlation tests and receiver operating characteristic (ROC) curves were generated. Continuous quantitative variables, such as age, were expressed as the mean ± SD and median (range), and categorical qualitative variables as absolute frequencies (number) and relative frequencies (percentage).

RESULTS

In our study, the mean cord blood hematocrit was 61.0%, the mean reticulocyte count was 1.3 cells/mm3×10, 100% of the neonates had negative Coombs test results, 57.0% of the neonates were male, the mean gestational age was 38.9 weeks, the mean weight was 3.3 kg, and 54.0% were delivered vaginally **(Table 1)**.

There was a statistically significant difference between the median level of bilirubin in studied neonates at birth, the 3rd day, and the 5th day (1.2, 12.3, and 14.7 mg/dl, respectively) **(Table 2)**.

There is a significant statistical difference between median hydrogen peroxide levels in studied neonates at birth, the 3rd day, and the 5th day (2.4, 4.6, and 7.6 uMol/L, respectively) (**Table 3).**

As regarding the correlation between bilirubin and hydrogen peroxide in the studied neonates at birth and follow-up**,** there is a strong statistically

significant positive association between bilirubin and hydrogen peroxide in the neonates **(Table 4)**.

In terms of the association between severity of hyperbilirubinemia at the 5th day and the characteristics of studied neonates**,** there is a statistically significant association between the severity of hyperbilirubinemia at the 5th day and weight, cord blood hematocrit, and cord blood hydrogen peroxide of the studied neonates **(Table 5)**.

Regarding the cord blood hydrogen peroxide performance in the prediction of severe hyperbilirubinemia**,** at the cutoff point of cord blood hydrogen peroxide ≥2.685 uMol/L, sensitivity was 90.0%, specificity was 83.3%, positive and negative predictive values were 78.3% and 92.6%, positive and negative likelihood ratios were 5.4 and 0.12, and accuracy was 86.0% **(Table 6).**

There was a high statistically significant positive correlation between cord blood hydrogen peroxide and cord blood hematocrit in the studied neonates **(Figure 1)**.

Table (1): Demographic, clinical and laboratory characteristics of studied neonates

Variables	Study participants(n=30)
Sex:	
Males	17 57.0%
Females	13 43.0%
Gestational age (weeks):	
X ± SD	38.9± 0.9
Range	37.0 – 41.0
Weight (kg):	
X ± SD	3.3± 0.3
Range	2.7 – 3.9
Mode of delivery:	
Vaginal	16 54.0%
Cesarean	14 46.0%
Apgar scoreat 1st minute:	
Median	8
Range	7 – 9
Apgare score at 5th minute:	
Median	9
Range	8 – 10
Breast feeding	30 100%
Cord Blood Hematocrit (%):	
X ± SD	61.0 ± 5.2
Range	54.0 – 74.0
Reticulocyte count (cell/mm$^{3\times10}$):	
X ± SD	1.3 ± 0.1
Range	1.0 – 1.6
Combs test:	
Negative	30 100%

Table (2): Bilirubin level in studied neonates at birth and follow up

Bilirubin level (mg/dl)	At birth	At 3rd day	At 5th day	Friedman	P
X ± SD	1.3 ± 0.6	12.4 ± 3.1	15.2 ± 3.4		
Median	1.2	12.3	14.7	396.0	**<0.001**
Range	0.4 – 3.9	0.5 – 18.8	9.1 – 23.1		**HS**

Table (3): Hydrogen Peroxide level in studied neonates at birth and follow up

Hydrogen Peroxide level (uMol/L)	At birth	At 3rd day	At 5th day	Friedman	P
X ± SD *Median* *Range*	2.7 ± 1.6 2.4 0.99 – 9.12	5.3 ± 2.3 4.6 2.22 – 14.0	8.3 ± 3.1 7.6 4.12 – 20.7	335.2	**<0.001 HS**

Table (4): Correlation between Bilirubin and Hydrogen peroxide in studied neonates at birth and follow up

Timing of measurement	r	P
At birth	0.9	**<0.001 (HS)**
At 3rd day	0.5	**<0.001 (HS)**
At 5th day	0.5	**<0.001 (HS)**

Table (5): Association between severity of hyperbilirubinemia at 5th day and characteristics of studied neonates

Variables	Hyperbilirubinemia at 5th day		Test of sig.	P
	Less severe (n=18)	*Severe (n=12)*		
Sex: *Males (n=17)* *Females (n=13)*	10 58.6% 8 61.9%	7 41.4% 5 38.1%	χ^2 0.2	0.6
Gestational age (weeks): *X ± SD* *Range*	39.0 ± 1.0 37.0 – 41.0	38.8 ± 0.8 37.0 – 41.0	**T** 1.3	0.2
Weight (kg): *X ± SD* *Range*	3.3 ± 0.3 2.7 – 3.9	3.2 ± 0.3 2.7 – 3.7	**T** 2.1	**0.04 (S)**
Mode of delivery: *Vaginal (n=16)* *Cesarean (n=14)*	9 59.6% 9 60.4%	7 40.4% 5 39.6%	χ^2 0.01	0.9
Apgar scoreat 1st min.: *Median* *Range*	8 7 – 9	8 7 – 9	**MW** 4463	0.4
Apgar score at 5th min.: *Median* *Range*	9 8 – 10	9 8 – 10	**MW** 4411	0.3
Cord Blood Hematocrit (%): *X ± SD* *Range*	59.8 ± 5.2 54.0 – 74.0	62.8 ± 4.6 54.0 – 73.0	**T** 4.2	**<0.001 (HS)**
Reticulocyte count (cell/mm$^{3\times10}$): *X ± SD* *Range*	1.3 ± 0.1 1.0 – 1.5	1.3 ± 0.2 1.0 – 1.6	**T** 0.2	0.8
Cord blood Hydrogen peroxide (uMol/L) *Median* *Range*	1.3 0.9 – 4.8	3.8 2.0 – 9.1	**MW** 620	**<0.001 (HS)**

Table (6): Performance of Cord blood Hydrogen peroxide in prediction of severe hyperbilirubinemia

Cutoff point	AUC	Sensitivity	Specificity	PPV	NPV	LR+	LR-	Accuracy	P
≥2.685uMol/L	0.94	90.0%	83.3%	78.3%	92.6%	5.4	0.12	86.0%	**<0.001 (HS)**

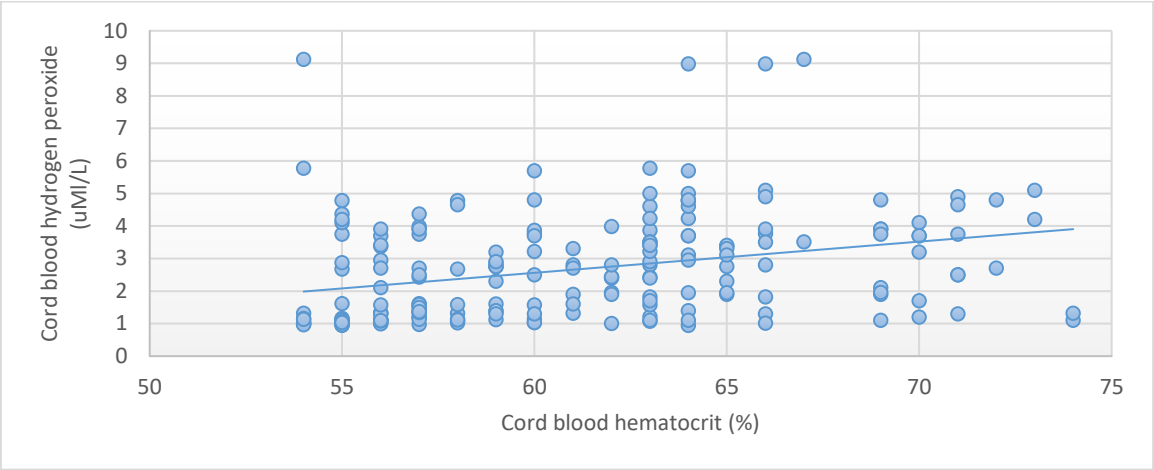

Figure (1): Correlation between cord blood Hydrogen Peroxide and cord blood hematocrit of studied neonates

DISCUSSION

Early jaundice prediction is an idea that would enable safe, economical, targeted intervention and follow-up. It is imperative to identify neonatal hyperbilirubinemia as soon as possible and to provide the best care possible to avoid bilirubin encephalopathy, which can cause brain damage and neuromotor impairment [10].

Newborns discharged from the hospital too soon run the danger of being readmitted, usually for neonatal hyperbilirubinemia. It is unacceptable to extend a patient's stay in the hospital. Predicting which newborns are at high risk of developing hyperbilirubinemia later on is therefore necessary [11].

The aim of the present study was to evaluate the significance of umbilical cord hydrogen peroxide as a predictive indicator of severe neonatal jaundice. The study was conducted on thirty healthy neonates. They were classified into two groups according to serum bilirubin level (≤17 less severe and >17 severe hyperbilirubinemia) and consisted of 17 (57%) males and 13 (43%) females. Forty-eight (48%) were born by cesarean section and 52%) by vaginal delivery. Median Apgar scores were 9 and had a range of 8–10 at the 5th minute; the mean gestational age was 38.9 weeks; and the mean birth weight was 3.3kg. All cases were breastfed.

Regarding sex, Bhutani et al. [12] found that male infants have a higher chance of experiencing severe neonatal jaundice, which validated our findings. The rates of bilirubin generation, which are comparable to those in female babies, do not seem to be associated with this. There was a statistically significant difference between median bilirubin levels in studied neonates at birth, the 3rd day, and the 5th day (1.2, 12.3, and 14.7 mg/dl, respectively). Our findings agreed with Banasia and Jain [13], who described a significant difference between cord blood bilirubin and serum bilirubin at the 3rd and 5th days.

There was a statistically significant difference between the median hydrogen peroxide levels in the studied neonates at birth, the 3rd day, and the 5th day (2.4, 4.6, and 7.6 uMol/L, respectively). Iuchi et al. [14] discovered that during the first few days of life, the quantity of hydrogen peroxide rises quickly in tandem with an increase in bilirubin concentrations. They interpreted these alterations by stating that rising oxygen levels during the shift from the fetal to the extra-fetal environment is what causes an increase in hydrogen peroxide levels. The red blood cell membranes could be harmed by hydrogen peroxide, which would cause bilirubin to be produced.More bilirubin is formed in response to a reactive oxidative product, such as a higher quantity of hydrogen peroxide following birth.

There was a statistically significant association between the severity of hyperbilirubinemia at the 5th day and cord blood hydrogen peroxide in the studied neonates. In agreement with our results, Chou et al. [15]. Hydrogen peroxide (H2O2) levels in cord blood and bilirubin concentrations during the first few days of life were highly correlated. Additionally, their findings imply that the amounts of hydrogen peroxide in cord blood

may be utilized as a predictor of severe hyperbilirubinemia in newborns. It would be obviously beneficial from a clinical standpoint to be able to identify neonates at birth who are likely to have eventual severe hyperbilirubinemia. Moreover; Nageh et al. [16] discovered that during the first few days of life, the quantity of hydrogen peroxide rises quickly in tandem with an increase in bilirubin concentrations.

Pearce et al. [17] and Schneider H. [18] reported that the fastest shift from a low-oxygen environment (the fetus) to a high-oxygen environment (the newborn) occurs after birth. There is a greater than three-fold rise in oxygen concentration during this environmental shift. Part of the delivery process is oxidation. Antioxidant levels are low during birth, but this triggers heme metabolism and a cascade of RBC breakdowns. Reductive and oxidative processes are correlated with bilirubin concentrations.

A high statistically significant positive correlation between bilirubin and hydrogen peroxide was found in neonates at birth and follow-up. Chou et al**.** [15] described the same result: the amounts of bilirubin and hydrogen peroxide in cord blood are highly correlated. Similar growing trends were seen in the early neonatal period for hydrogen peroxide levels and bilirubin concentrations, which both rise simultaneously during the first few days of life.

There was no difference between the studied groups with regard to sex. In this study, the studied groups were distributed with 10 male and 8 female babies in the less severe group and 7 male and 5 females in the severe group of hyperbilirubinemia. Hence, the present study infers that neonatal hyperbilirubinemia (>17 mg/dl) is independent of sex.

There was a statistically significant association between the severity of hyperbilirubinemia at the 5th day and weight, cord blood hematocrit, and cord blood hydrogen peroxide of the studied neonates, which enables the prediction of the severity of hyperbilirubinemia based on both variables. In agreement with our results, Huang et al. [19] indicated that there were undoubtedly more factors contributing to the development of severe hyperbilirubinemia; hence, no one factor has a high sensitivity for predicting severe hyperbilirubinemia at this time. To improve the sensitivity of identifying hyperbilirubinemia, additional risk variables such as cord blood bilirubin concentrations, hematocrit levels, or early body weight loss may need to be taken into account. Between cord blood hematocrit and cord blood hydrogen peroxide in the neonates under study, there was a strong statistically significant positive association.

Our study's findings suggest that measuring the hydrogen peroxide level in cord blood may be a predictive indicator for highly jaundiced newborns, which can surpass 17 mg/dL and require medical attention within the first week of life. With a cutoff level of ≥2.685 uMol/L, cord blood hydrogen peroxide has a 90% sensitivity and 83% specificity, respectively, indicating the need for treatment.

Cord blood hydrogen peroxide level (≥2.685 uMol/L) with high specificity and sensitivity is chosen using ROC curve analysis, with the probability that a neonate develops severe hyperbilirubinemia with a positive predictive value of 78.3%. The negative predictive value, the probability of less severe hyperbilirubinemia given a cord blood hydrogen peroxide lower than 2.685 uMol/L, was 92.6%. If a child becomes severely hyperbilirubinemic,the probability that the cord hydrogen peroxide is ≥2.685 umol/L is 90% (sensitivity). Given a less severe hyperbilirubinemic baby, the probability that the cord hydrogen peroxide was <2.685 umol/L was 83.3% (specificity). The present study showed a significant correlation between cord blood hydrogen peroxide (>2.685 umol/L) and neonatal hyperbilirubinemia (>17 mg/dl) on the 5th day (p <0.001).

Conclusions: The present study discovered that newborns with hyperbilirubinemia had significantly higher levels of cord hydrogen peroxide. Thus, it is possible to identify a group of newborns who are at risk of jaundice and need phototherapy or exchange at delivery by analyzing the levels of hydrogen peroxide in the umbilical cord blood. This enables close monitoring of neonates identified as hyperbilirubinemia-risk, thereby preventing consequences based on early hospital release.

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