IJN
Iranian Journal of Neonatology
http://ijn.mums.ac.ir

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Original Article

Role of Cord Blood Alkaline Phosphatase as a Predictor for Hyperbilirubinemia in Full-term Neonates

Guslihan Dasatjipta[1], Lili Rohmawati[1*], Bugis Mardina Lubis[1]

1. Department of Pediatrics, Faculty of Medicine, Universitas Sumatera Utara Universitas Sumatera Utara Hospital, Medan, North Sumatera, Indonesia

ABSTRACT

Background: Neonatal hyperbilirubinemia is a common clinical condition among newborns that requires early detection and medical management to avoid bilirubin toxicity. This study aimed to investigate the role of cord blood alkaline phosphatase (ALP) levels as a predictor of hyperbilirubinemia in full-term neonates.

Methods: This cross-sectional study was conducted on full-term newborns within April-August 2019 in several hospitals in Medan, Indonesia. Cord blood ALP levels upon delivery and serum bilirubin levels on 72 h of life were measured.

Results: Out of 147 full-term neonates, the mean cord blood ALP levels and serum bilirubin levels were obtained at 166.3±45.1 IU/l and 9.6±2.3 mg/dl, respectively. There was a significant difference in mean cord blood ALP levels at serum bilirubin levels of >10 mg/dl and ≤10 mg/dl on 72 h of life (196.8±40.7 IU/l and 146.4±37.5 IU/l, respectively; P=0.001). A positive, moderate, and significant correlation was observed between cord blood ALP levels and serum bilirubin levels on 72 h of life (r=0.429; P=0.001). The cut-off value of 163.5 IU/l was associated with 84.7% sensitivity and 77.3% specificity for predicting hyperbilirubinemia in full-term neonates.

Conclusion: There was a positive correlation between cord blood ALP levels and serum bilirubin levels on 72 h of life. Therefore, cord blood ALP may be utilized as a predictor for hyperbilirubinemia in full-term neonates.

Keywords: Alkaline phosphatase, Hyperbilirubinemia, Full-term neonates

Introduction

Neonatal hyperbilirubinemia in the first week of the life of newborns accounts for 60% of full-term neonates (1). Although unconjugated hyperbilirubinemia is normal in the transitional life process of a newborn, in some circumstances, bilirubin toxicity may occur (2). Early detection of neonatal hyperbilirubinemia is important as early intervention may be performed to prevent bilirubin toxicity to the brain organ (kernicterus) (3).

Hyperbilirubinemia also continues to be the most common cause of neonatal readmission to hospitals although meticulous observation is performed before they are discharged from the hospital. In the majority of cases, the underlying etiology of neonatal hyperbilirubinemia is not identified. The high readmission rate after initial discharge warrants further strategies to identify high-risk newborns (4).

Numerous studies have been conducted to predict the occurrence of hyperbilirubinemia among newborns. In practice, the examination of transcutaneous bilirubin after 48 h or prior to newborn discharge is difficult to perform in limited resources countries. Feasible and non-invasive examination to assist clinicians for hyperbilirubinemia risk assessment is an ideal preference. Collection of cord blood, which is determined as a less invasive approach, is beneficial by predicting the hyperbilirubinemia earlier than the collection of newborn's serum, as a more invasive approach that may cause parental refusal. The results of some studies have indicated that cord blood alkaline phosphatase (ALP) levels may serve as a predictor of neonatal jaundice (5-7). This study aimed to investigate the role of cord blood ALP as a predictor for hyperbilirubinemia in

* *Corresponding author:* Lili Rohmawati, Department of Pediatrics, Faculty of Medicine, Universitas Sumatera Utara Universitas Sumatera Utara Hospital, Medan, North Sumatera, Indonesia. Tel: +6281356185367; Email: lily_rahmawati234@yahoo.com

Please cite this paper as:
Dasatjipta G, Rohmawati L, Mardina Lubis B. Role of Cord Blood Alkaline Phosphatase as a Predictor for Hyperbilirubinemia in Full-term Neonates. Iranian Journal of Neonatology. 2022 Jan: 13(1). DOI: 10.22038/IJN.2021.46268.1779

full-term neonates.

Methods

Study design

This cross-sectional study was conducted in Universitas Sumatera Utara and Stella Maris hospitals in Medan, North Sumatera, Indonesia, within April-August 2019. The inclusion criteria for this study were full-term neonates with a gestational age of ≥37 weeks and an Apgar score of >7 at 5 min assessed by expanded new Ballard Score. The patients were enrolled using a consecutive sampling technique. On the other hand, full-term neonates born to mothers with diabetes mellitus, eclampsia, bone disorders, liver and kidney diseases, and newborns with congenital anomalies, asphyxia, treated at neonatal intensive care unit (NICU), and sepsis were excluded from the study.

Informed consent was obtained from the parents of all participating neonates. This study was approved by the Health Research Ethical Committee, Faculty of Medicine, Universitas Sumatera Utara (No.2761/TGL/KEPK FK USU-RSUP HAM/2019).

Cord blood alkaline phosphatase measurements

Cord blood ALP levels were measured with 3 ml cord blood samples prior to delivery. The serum was separated by centrifugation at 3000 rpm for 15 min. Alkaline phosphatase serum was assessed using a colorimetric assay with a standardized method using p-nitrophenyl phosphate as substrate and diaethanolamine as a buffer (International Federation of Clinical Chemistry method) by Architect i2000 (Abbott). Sample collection and analysis were performed according to Prodia clinical laboratory standards, Indonesia. The range of normal values was 150-300 IU/l (8).

Bilirubin measurements

Bilirubin measurements were performed by heel prick-capillary blood sampling upon 72 h of life. During the procedure, the full-term neonates were lying in a secure and safe position, either in the cot or securely held by parents. Heel lancing was performed by a trained nurse using a sterile lancet and limited to the medial and lateral borders of the heel. Blood droplet was touched into the capillary tube. Bilirubin measurements were performed with the Jendrassik Grof method in the respective hospital laboratory. Hyperbilirubinemia was classified as serum bilirubin levels of >10 mg/dl (7).

Statistical analysis

Results were presented as mean ± standard deviation (SD) values and percentages. Bivariate analysis was performed with an independent t-test. Pearson correlation coefficients (r) were used to measure the correlation between variables. The cord blood ALP levels measured having the highest sensitivity for predicting hyperbilirubinemia were determined with the receiver operating characteristic (ROC) curve analysis. Statistical data were analyzed in SPSS version 23.0 (SPSS Inc., Chicago) with a 95% confidence interval. P-values of <0.05 were considered statistically significant.

Results

A total of 154 full-term neonates were enrolled during the study period; however, 7 newborns were excluded from the study since 3 cases refused for heel prick-capillary blood sampling collection and 4 subjects were admitted to NICU due to neonatal asphyxia. Therefore, the total subjects involved in this study were 147 full-term infants consisting of 80 (54.4%) males and 67 (45.6%) females at the maternal age range of 22-41 (mean 30.7±4.1) years old (Table 1). The mean scores of gestational age and birth weight were obtained at 38.3±0.6 weeks and 3,188.6±388.2 g. There were 9 (12.7%) full-term infants with jaundice who needed further in-patient interventions. The ranges of cord blood ALP level and serum bilirubin levels were estimated at 95-307 (mean 166.3±45.1) IU/l and 4.8-17.0 (mean 9.6±2.3) mg/dl, respectively.

Based on the results of this study, 59 (40.1%) full-term infants had serum bilirubin levels of >10

Table 1. Maternal and neonatal characteristics

Characteristics	n (%)	Mean±SD (Range)
Maternal age (years)		30.7±4.1 (22-41)
Gestational age (week)		38.3±0.6 (38-41)
Neonatal gender		
Male	80 (54.4)	
Female	67 (45.6)	
Neonatal birth weight (g)		3,188.6±388.2 (2,080-4,200)
Cord blood ALP (IU/l)		166.3±45.1 (95-307)
Serum bilirubin (mg/dl)		9.6±2.3 (4.8-17.0)

mg/dl on 72 h of life and mean cord blood ALP levels of 195.8±39.8 IU/l. The mean cord blood ALP levels were significantly different between the two groups (196.8±40.7 IU/l and 146.4±36.8 IU/l; P=0.001, respectively) (Table 2).

Table 3 presents a positive, moderate, and significant correlation of cord blood ALP levels and serum bilirubin levels (r=0.429; P=0.001) on 72 h after birth. Figure 1 and Table 4 show the comparison of the ROC curves of the ALP levels with serum bilirubin levels on 72 h of life. It was revealed that cord blood ALP level of ≥163.5 IU/l was the cut-off value for predicting hyperbilirubinemia in full-term neonates. This cut-off value had a sensitivity of 84.9%, a specificity of 84.7%, and an area under the curve of 0.84. Out of 147 neonates whose cord blood ALP levels were measured more than 163.5 IU/l, 59 (40.1%) neonates had hyperbilirubinemia on 72 h of life. The positive and negative predictive values of cord blood ALP levels for hyperbilirubinemia were 71.4% and 88.3%, respectively.

Table 2. Cord blood alkaline phosphatase and serum bilirubin levels on 72 hours of the life of full-term neonates

Variable	Cord blood ALP levels (IU/l)		P*
	n (%)	Mean±SD	
Serum bilirubin levels			
>10 mg/dl	59 (40.1)	195.9±39.8	0.001
≤10 mg/dl	88 (59.9)	146.4±36.8	

ALP: Alkaline phosphatase; *Independent t-test

Table 3. Correlation between cord blood ALP and serum bilirubin levels on 72hours

Variable	r	P*
Cord blood ALP Serum bilirubin	0.429	0.001

ALP: Alkaline phosphatase; *Pearson correlation test

Table 4. Screening utilities of cord blood ALP levels as a predictor for hyperbilirubinemia in full-term neonates

	Cut-off value (IU/l)	AUC	Sensitivity (%)	Specificity (%)	PPV	NPV
Cord blood ALP	≥163.5	0.849	84.7	77.3	71.4	88.3

AUC: Area under the curve; PPV: Positive predictive value; NPV: Negative predictive value

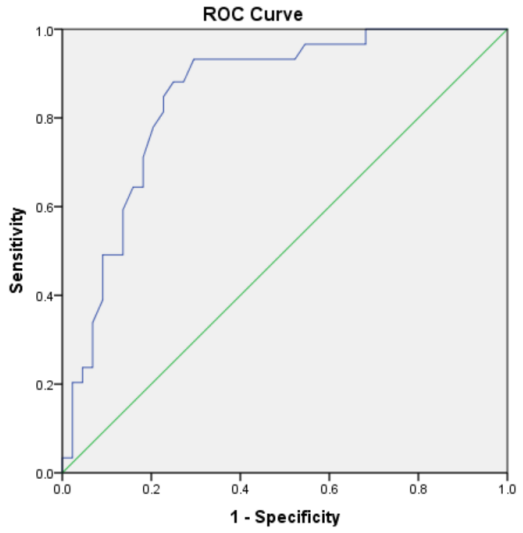

Figure 1. Receiver operating characteristic curve of cord blood alkaline phosphatase levels

Discussion

Clinically stable newborn neonates may be discharged early; as a result, jaundice monitoring and early intervention may become a challenge. The difficulty of collecting blood samples from newborns on the first day of life is a problem related to parents' consent. Screening examination to predict hyperbilirubinemia through cord blood is still a debate. Several methods have been utilized to determine the risk of neonatal hyperbilirubinemia, such as ALP levels, serum bilirubin levels, cord blood alpha-fetoprotein levels, and peroxidase hydrogen (3,6-10). Alkaline phosphatase may be detected in the placenta from 7 weeks of gestational age and will continue to increase along with the pregnancy with various increment patterns (11). Alkaline phosphatase value is reported with the IU/l unit with various references according to laboratory reference, gestational age, and maternal condition during the gestation period. The range of normal value for newborns is determined at 150-300 IU/l (8). This study was conducted to investigate the role of cord blood ALP as a predictor for hyperbilirubinemia on 72 h of life in full-term neonates. All subjects were born via section Caesarea with the mean scores of gestational age and birth weight of 38.3±0.6 weeks and 3,188.6±388.2 g, respectively.

The findings of this study showed that the mean of cord blood ALP levels was obtained at 166.3±45.1 IU/l. Fenton et al. reported the mean

value of laboratory reference interval of cord blood ALP of full-term neonates at 159±49 IU/l, which was similar to that in the current study (12). A different value was reported in a study conducted by Ahmadpour-Kacho et al., in which they reported a higher value (325.24±85.03 IU/l). The higher value of ALP may be due to the incidence of clinical jaundice during follow-up (47%) (7). According to the results of a study performed by Al-Assal et al. in Egypt, the mean values of cord blood ALP in newborns without jaundice, with jaundice, and in need of phototherapy were 205.72±53.49 IU/l, 256.45±62.85 IU/l, and 353.11±48.48 IU/l, respectively. Cord blood ALP levels were significantly higher in neonates requiring therapy, such as phototherapy or exchange transfusion (13).

In the present study, the mean serum bilirubin levels on 72 h after birth was calculated at 9.6±2.3 mg/dl. The value of bilirubin was plotted to the bilirubin risk level curve from the American Academy of Pediatrics. Icteric may be observed on the sclera and skin if the bilirubin levels exceed 5mg/dl. Physiologically, bilirubin levels will increase right after birth, reach peak levels, and decrease after 7 days. An increase in bilirubin levels may be caused by an increase in heme catabolism and immaturity of the liver for conjugation and excretion of bilirubin (5). Approximately, 3-5% of newborns may experience pathological hyperbilirubinemia and develop a higher risk for kernicterus. Several risk factors are associated with hyperbilirubinemia, including hemolytic disorders (ABO isoimmunization and congenital spherocytosis) and other complications (i.e. dehydration, cephalhematoma, sepsis, acidosis, and hypoalbuminemia). The other investigated risk factors are mode of delivery, East Asian race, and maternal age beyond 25 years old (14).

In the present study, there was a significant difference in mean cord blood ALP levels at serum bilirubin levels of >10 mg/dl and ≤10 mg/dl on 72 h of life (146.4±36.8 IU/l and 195.9±39.8 IU/l; P=0.001, respectively). In agreement with the results of this study, Ahmadpour-Kacho et al. (7) and El-Amin et al. (15) reported that the mean cord blood ALP level was lower in the non-jaundiced group than in the clinically-jaundiced group.

The findings of the current study showed a positive and significant correlation between cord blood ALP levels and the increment of serum bilirubin levels 72 h after birth. In line with the results of this study, those of a previous study conducted in Iran showed that cord blood ALP could be a predictor of jaundice with serum bilirubin levels of >10 mg/dl in newborns (7). Accordingly, a significant difference was observed in ALP between non-jaundice and jaundice groups. In the study carried out by Nalbantouglu et al., serum ALP levels on 6 h of life were utilized as a marker to determine hemolysis and hyperbilirubinemia. Based on the findings of the mentioned study, serum ALP levels were significantly higher in newborns requiring therapy (phototherapy or exchange transfusion). Moreover, ALP levels were revealed to raise significantly with an increase in total bilirubin levels (9). However, in our study, cord blood ALP (a less invasive approach) was utilized rather than the serum; nevertheless, the results may be comparable.

In this study, it was found that the cut-off value of cord blood ALP level of 163.5 IU/l was associated with sensitivity, specificity, and negative predictive values of 84.7%, 77.3%, and 88.3%, respectively. Various pieces of research have been carried out to predict hyperbilirubinemia. El-Amin et al. reported that a cord blood ALP level of >145 IU/l was the most suitable cut-off value for predicting jaundice at risk that required interventions, with a sensitivity of 72% and specificity of 85.71% (15). The results of the study conducted by Ahmadpour-Kacho et al. reported a comparison of the ROC curves of the ALP levels between the non-jaundiced and treatment groups. Accordingly, it was revealed that a cord blood ALP level of >314 IU/l was the most suitable cut-off value for predicting severe jaundice that needed treatment. It was concluded that the cord blood ALP levels could be used as a predictor of severe neonatal jaundice (7). In similar studies performed by Al-Assal et al. (13) and Eid et al. (16), it was concluded that cord blood ALP levels might be a significant predictor of developing hyperbilirubinemia requiring treatment.

Conclusion

There was a positive correlation between cord blood ALP levels and serum bilirubin levels on 72 h of life. Therefore, cord blood ALP may be utilized as a predictor for hyperbilirubinemia in full-term neonates.

Acknowledgments

The authors gratefully acknowledge that the research is funded by Universitas Sumatera Utara. The fund is under the research grant TALENTA USU of the Year 2019 Contract Number 4167/UN5.1.R/PPM/2019. The authors would also thank Universitas Sumatera Utara Hospital,

Stella-Marris Hospital, and Prodia Clinical Laboratory, Indonesia, for technical support during the conduction of this study.

Conflicts of interest

The authors declare that there is no conflict of interest in this study.

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