Evaluation of cord blood alkaline phosphatase as a predictor of hyperbilirubinemia in Egyptian neonates

Sally R. Eid[a], Amal G. Mohamed[b], Heba T. Okda[b], Hala M. Abdel Salam[c]

[a]Department of Pediatrics, Research Institute of Ophthalmology, Giza, Egypt, [b]Department of Pediatrics, Faculty of Medicine for Girls, Al-Azhar University, Cairo, [c]Department of Clinical Pathology, National Nutrition Institute, Cairo, Egypt

Correspondence to Sally R. Eid, MD, Department of Pediatrics, Research Institute of Ophthalmology, 2 El-Ahram Street, Giza 11516, Cairo, Egypt. Tel: +20 122 372 8215; fax: +20 235735688; e-mail: sallyeid60@gmail.com

Received: 24 December 2019
Revised: 30 November 2
Accepted: 19 April 2020
Published: xx xx 2020

Journal of The Arab Society for Medical Research 2020, 15:11–17

Background/aim
Hyperbilirubinemia is a major cause of neonatal morbidity. It affects nearly 60% of term and 80% of preterm neonates during the first week of life. Early prediction of hyperbilirubinemia will help in early discharge and prevent hospitalization of babies and mothers for longer periods. The aim of this study was to verify whether the cord alkaline phosphatase (ALP) levels can predict the development of neonatal hyperbilirubinemia.

Participants and methods
This prospective study was conducted at Alglaa Teaching Hospital from June 2016 to May 2018. A total of 200 healthy term and late preterm neonates meeting the inclusion criteria were enrolled in this study (gestational age >35 weeks of either sex, from any mode of delivery, and Apgar score ≥7 at first and fifth minutes of life). After birth, cord blood was collected for the estimation of cord blood ALP and serum total bilirubin.

Results
The incidence of clinical jaundice in our study was 50.54% (94 cases). Only 28.72% (27 cases) of them required treatment. There is a significant association between cord blood ALP levels and the development of hyperbilirubinemia requiring treatment. Receiver operating characteristic curve analysis demonstrates that ALP level of the cord blood greater than 342 IU/l was the most appropriate cutoff value for predicting significant jaundice (that needs treatment). It was associated with 91.07% sensitivity and specificity 61.54%. The area under the curve was 0.791, indicating the high significance and the usefulness of this assay in predicting significant jaundice requiring medication.

Conclusion
This study concludes that cord blood ALP levels reliably predict the occurrence of pathological hyperbilirubinemia requiring therapy as defined by the current operational guidelines.

Keywords:
cord blood alkaline phosphatase, hyperbilirubinemia, neonates, prediction, preterm, term

J Arab Soc Med Res 15:11–17

1687-4293

Introduction

Neonatal hyperbilirubinemia (NNHB) is a common challenge in neonates. It is usually a benign condition occurring during the first week of life in ~80% of preterm and 60% of term neonates. Although most cases are physiological, it is imperative to identify those at risk of adverse outcomes of severe NNHB to initiate early therapy, so as to prevent long-term morbidity [1]. Inability to recognize and treat pathological hyperbilirubinemia may lead to kernicterus with its possible neurodevelopmental disability, so an assessment of the risk of development of hyperbilirubinemia and prompt treatment is crucial [2]. However, pathological hyperbilirubinemia that requires treatment may occur in some healthy neonates without any apparent cause, and some of them may develop kernicterus [3].

Early discharge of healthy term neonates particularly after normal labor has become a routine practice, owing to many causes like prevention of nosocomial infections, some social reasons, and also owing to economic constraints. The American Academy of Pediatrics (AAP) recommends risk assessment and measuring of bilirubin concentration before hospital discharge, and also AAP recommends that discharged newborns within 48 h after delivery should be followed up after 2–3 days for any identified jaundice or any other complication [4,5].

The practice of predischarge bilirubin assessment recommended by AAP has restricted practical applicability in resource-restricted countries, such as Egypt. Owing to limited follow-up facilities in the country, there may be a delay in recognition of

pathological hyperbilirubinemia in these newborns. Therefore, it is difficult to predict which infants are at increased risk for significant and relatively late hyperbilirubinemia with the potential high risk of developing bilirubin encephalopathy or even kernicterus [5].

Availability of simple, economical, noninvasive, and reliable markers allowing physicians to recognize which of the neonates discharged early are at a higher risk for development of significant hyperbilirubinemia has become necessary in these situations so as to initiate treatment as early as possible and thus reduce the risk of bilirubin-induced brain damage. This ideal marker also can help physicians in early discharge of the neonates and selectively follow-up of the high-risk ones. Different methods have been used to assess the risk of hemolysis and hyperbilirubinemia in the neonates. Measurement of bilirubin level and alpha-fetoprotein in cord blood has been used as a marker for detecting this risk [6,7]. Moreover, alkaline phosphatase (ALP) level after birth was used for the first time by Nalbantoglu *et al*. [2] for this purpose.

ALP is an intracellular hydrolase enzyme. It is responsible for removing phosphate from many types of molecules. It is found in all body cells including red blood cells and is released to plasma upon the damage of these cells. Therefore, it is postulated that it could be used as an early predictor of hemolysis of red blood cells and assessment of critical hyperbilirubinemia [8]. The aim of this study was to assess the reliability of cord blood ALP as an early predictor of NNHB in full-term and late preterm neonates

Participants and methods

Patients

This prospective study was carried out in the Neonatal Department in Conjunction with the Department of Gynecology and Obstetrics in Al-Galaa Teaching Hospital from June 2016 to May 2018.

Ethical consideration

This study protocol was approved by the Medical Ethical Committee of the Hospital. Written informed consent was obtained from parents of each participant before inclusion in the study. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Inclusion criteria

A total of 200 healthy neonates with gestational age greater than 35 weeks (based on last menstrual period and neonatal assessment by expanded new Ballard Score) with an Apgar score greater than or equal to 7 at first and fifth minutes, irrespective of sex or mode of delivery were enrolled in this study.

Exclusion criteria

Newborn babies with any apparent significant congenital anomaly and those with prolonged rupture of membranes (>24 h), sepsis, prenatal hypoxia, cephalohematoma, or contusions were excluded from the study. Babies who required neonatal intensive care unit admission or showed any complication that could aggravate hyperbilirubinemia (respiratory distress syndrome, asphyxia, intrauterine growth retardation, sepsis, and diabetic mothers) or cholestatic jaundice were also excluded.

Study design

A total of 200 cases were initially enrolled in this study. Of them, nine did not comply with the follow-up schedule, and five others were admitted in the neonatal intensive care unit (one for severe sepsis and four for respiratory distress syndrome) and so were excluded from the study. The remaining 186 participants (39 preterm and 147 terms) were followed up for the first 5 days of life with clinical assessment and laboratory investigations.

The neonates studied were divided into two groups:

(1) Group 1: it included neonates who did not develop hyperbilirubinemia at all (92 neonates).
(2) Group 2: it included neonates who developed hyperbilirubinemia (94 neonates). They were further divided into subgroups: group 2A, which included 67 neonates who did not develop significant hyperbilirubinemia (≥17 mg/dl), and group 2B, which included 27 neonates who developed significant hyperbilirubinemia requiring interventions like phototherapy or exchange transfusion during follow-up (treatment group).

Methods

All the study population was subjected to the following: complete history taking, complete clinical examination, and laboratory investigations. Full clinical examination immediately after birth included the following: vital signs, anthropometric measures, and the presence of cephalohematoma or bruises. Cardiac, abdominal, chest, and neurological examinations were also carried out. Demographic profile and relevant information were collected by interviewing the

mother and from the mother's case sheet. Relevant information from history, physical findings, and laboratory investigations was recorded. Demographic information of all infants including mode of delivery, gestational age, birth weight, and Apgar score was also recorded.

Blood sampling and laboratory methods

After enrollment, umbilical cord blood samples were collected from all newborns who fulfilled the inclusion criteria. Overall, 5 ml of cord venous blood was drained by a sterile syringe, and put in a clean capped tube. Samples were protected from light during processing and storage, and hemolysed samples were excluded. To avoid hemolysis, after the sample was sent to the clinical laboratory, samples were promptly placed in a water bath under the condition of 37°C for 30 min, then it was placed in a centrifugal machine for 5 min whose speed was 3000 turn/min to separate the serum, and then the serum was used to detect alkaline phosphates (ALP), and total and direct bilirubin. Moreover, another portion of the blood was added to an EDTA tube to detect the complete blood picture. The following laboratory tests were carried out:

(1) Serum ALP level by the colorimetric method according to Thomas [9], using diagnostic kit supplied by Biotecnica Instruments S.p.A, Via Licenza (Rome, Italy).
(2) Blood grouping by slide method. Rhesus factor was determined using monoclonal immunoglobulin (Ig) M anti-D antisera [10]
(3) Total serum bilirubin by the colorimetric method, according to Tietz [11], using diagnostic kit supplied by Biotecnica Instruments S.p.A, Via Licenza.

Discharged neonates before the fifth day were motivated to come for follow-up if parents noticed their baby to be significantly icteric for clinical evaluation and serum bilirubin screening. Interventions such as phototherapy and exchange transfusion were based on AAP recommendations for treating significant NNHB. Significant NNHB was identified as the need for phototherapy or exchange transfusion based on the AAP guidelines [5].

Statistical analysis

Data were expressed as means±SD and percentage. χ^2-Test was used for finding association between two or more categorical variables. Analysis of variants was used as appropriate to compare the mean values of the variables. A test with *P* value of less than 0.05 was statistically significant. A receiver operating characteristic (ROC) curve was generated to determine a cutoff value of serum ALP level for predicting significant NNHB.

Results

Of a total of 200 cases that were initially enrolled in this study, only 186 cases completed the study. The study population had been divided into two groups: group 1 included 92 healthy neonates, who did not develop hyperbilirubinemia at all, and group 2 included 94 neonates who had developed any level of hyperbilirubinemia, whether it was mild, requiring no treatment (group 2A), or significant, requiring treatment (group 2B).

In the present study, Apgar scores were normal (9–10) at birth in all cases. There was no significant relationship among the three groups regarding; sex, mode of delivery, blood groups, and RH distribution, as shown in Table 1.The incidence of clinical jaundice during follow-up was 50.54%, whereas it was 28.72% in the treatment group (group 2B). Treatment was

Table 1 Sex, mode of delivery, and blood group distribution in different groups

Variables	Group 1 (92 neonates) [*n* (%)]	Group 2A (67 neonates) [*n* (%)]	P_1	Group 2B (27 neonates) [*n* (%)]	P_2
Sex			0.537		0.537
Male	49 (53.3)	37 (55.22)		16 (59.26)	
Female	43 (46.7)	30 (44.78)		11 (40.74)	
Mode of delivery			0.369		0.432
Vaginal	35 (38.04)	24 (35.82)		8 (29.6)	
CS	57 (61.96)	43 (64.18)		19 (70.4)	
Blood group			0.313		0.218
A	32 (34.78)	26 (38.8)		9 (33.33)	
B	25 (27.17)	19 (28.4)		8 (29.62)	
AB	15 (16.30)	10 (14.9)		4 (14.81)	
O	20 (21.74)	12 (17.9)		6 (22.22)	
RH^+	84 (91.30)	61 (91)		25 (92.6)	

P_1, *P* value of group 1 vs group 2A; P_2: *P* value of group 1 vs group 2B; CS, cesarean section.

Table 2 Comparison of clinical variables (gestational age, birth weight, and Apgar score) between group 1 and group 2A

Variables	Group 1 (92 neonates) [*n* (%)]		Group 2A (67 neonates) [*n* (%)]		*P*
Gestational age	92	39.21±2.24	67	37.8±1.51	0.28
Preterm (<37 weeks)	13 (14.13)	36.1±0.83	17 (25.37)	36.1±2.1	0.39 0.74
Term (≥37 weeks)	79 (85.87)	40.12±072	50 (74.63)	39.91±1.2	0.49
Birth weight	92	3291.195±29.2	67	3479±138.19	0.63
Preterm (<37)	13	3021.543±11.1	17	2915.346±39.1	0.47
Term (≥37)	79	3423.754±19.2	50	32319.239±43.7	0.47
Apgar					
1 min		8.410±71		8.24±0.61	0.379
5 min		9.370±62		9.120±52	

Table 3 Comparison of clinical variables (gestational age, birth weight, and Apgar score) between group 1 and group 2B

Variables	Group 1 (92 neonates) [*n* (%)]		Group 2B (27 neonates) [*n* (%)]		*P*
Gestational age	92	39.21±2.24	27	37.6±1.42	0.59
Preterm (<37 weeks)	13 (14.13)	36.1±0.83	9 (33.3)	35.7±1.1	0.43
Term (≥37 weeks)	79 (85.87)	40.12±072	18 (66.7)	39.41±1.23	0.63
Birth weight	92	3291.195±29.2	27	3453±127.17	0.43
Preterm (<37 weeks)	13	3021.543±11.1	9	2795.437±51.1	0.001
Term (≥37 weeks)	79	3423.754±19.2	18	3215.437±3.1	0.61
Apgar					
1 min		8.410±71		8.13±0.52	0.294
5 min		9.370±62		9.01±043	

Table 4 Comparison of clinical variables (gestational age and birth weight) between nontreatment groups (group 1 and group 2A) and treatment group 2B

Variables	Nontreatment groups (1 and 2A) [*n* (%)]		Treatment group 2B [*n* (%)]		*P*
Gestational age	159	39.21 ±2.14	27	37.6±1.42	0.49
Preterm (<37 weeks)	30 (18.87)	36.3±0.74	9 (33.3)	35.7±1.1	0.53
Term (≥37 weeks)	129 (81.13)	40.23 ±051	18 (66.7)	39.41 ±1.23	0.41
Birth weight	159	3395.174 ±21.9	27	3453 ±127.17	0.52
Preterm (<37 weeks)	30	3032.354 ±12.2	9	2795.437 ±51.1	0.021
Term (≥37 weeks)	129	3478.434 ±18.9	18	3215.437 ±3.1	0.39

essentially by phototherapy, and none of the neonates in this study needed an exchange transfusion.

Comparison of clinical variables was as follows: gestational age and birth weight between group 1 and group 2A showed no statistically significant difference between the two groups in both term and preterm neonates (Table 2).

There was no significant association between gestational age and NNHB in either of the two groups, that is, group 1 and group 2B in both term and preterm neonates. In the term group, there was no significant association between birth weight and significant NNHB. Among preterm neonates, there was a significant association between birth weight and the development of significant NNHB ($P<0.05$), as shown in Table 3.

There was no significant association between gestational age and NNHB in the treatment group (group 2B) and the nontreatment groups (group 1 and group 2A) in both term and preterm neonates. In the term group, there was no significant association between birth weight and significant NNHB. Among preterm neonates, there was a significant association between birth weight and the development of significant NNHB ($P<0.05$), as shown in Table 4.

Comparison of cord blood ALP levels between nonjaundiced (group 1) and jaundiced newborns in whom no treatment was needed (group 2A) revealed no significant difference ($P=0.214$). Comparison of neonates who did not develop jaundice at all (group 1) with neonates needing treatment according to AAP protocol showed a significant difference in cord blood ALP levels ($P=0.001$). In our study, the ALP level was significantly higher in neonates with pathological hyperbilirubinemia necessitating intervention (phototherapy) (Table 5).

ROC curve analysis shows that a cord blood ALP level greater than 342 IU/l was the most suitable cutoff value

Table 5 Mean cord blood alkaline phosphatase and serum total bilirubin levels in different groups

Variables	Group 1 (*N*=92)	Group 2 (*N*=94) (50.54%)	
		Group 2A (*N*=67)	Group 2B (*N*=27)
Alkaline phosphatase	204.89±34.5	217.94±9.51	352.1153±49[a,b]
Total bilirubin	3.75±1.89	10.22±2.41[a]	16.84±2.86[a,b]

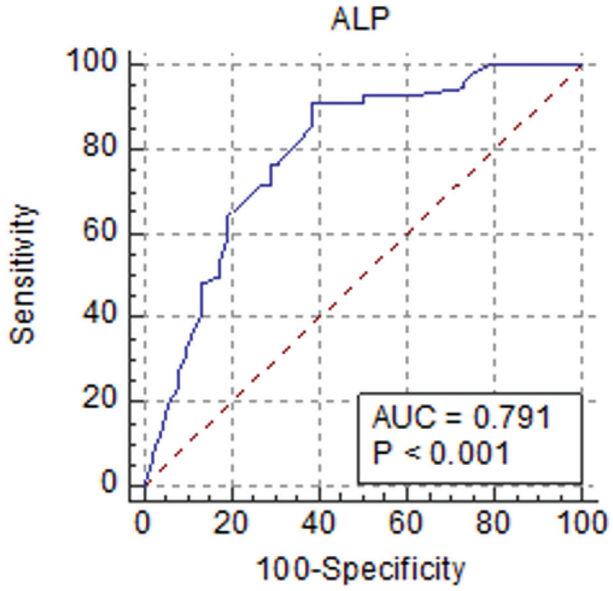
All data are expressed as mean±AD. [a]Significant difference than group 1 at $P<0.05$. [b]Significant difference than group 2A at $P<0.05$.

Table 6 Alkaline phosphatase cut off point, sensitivity, specificity, negative predictive value, and positive predictive value

ALP cut off point	Sensitivity (TP) (%)	FN	Specificity (TN) (%)	FP	PPV	NPV	AUC	*P*
>342	91.07	8.93	61.54	38.46	92	98.05	0.791	0.001

$P<0.001$ indicating the usefulness of the test in predicting hyperbilirubinemia. AUC, area under the curve; FN, false negative; FP, false positive; NPV, negative predictive value; PPV, positive predictive value; TN, true negative; TP, true positive.

Figure 1



Receiver operating characteristics (ROC) curve of cord blood ALP.

for predicting significant jaundice (that needs treatment). It was associated with 91.07% sensitivity, which means that 91.07% of diseased cases can be predicted (true positives), whereas 8.93% of diseased cases cannot be detected (false negatives), and specificity of 61.54%, which means that 61.54% of nondiseased cases were identified as test negative (true negatives), whereas 38.46% of nondiseased cases are incorrectly reported as test positive (false positives). The area under the curve was 0.791, showing the high significance and convenience of the test in anticipating cases of significant jaundice that require intervention (Table 6 and Fig. 1).

Of 154 neonates whose cord blood ALP level was less than 342 IU/l, only three neonates required treatment; thus, the negative predictive value of cord blood ALP for the detection of hyperbilirubinemia needing treatment (phototherapy) was 98.05%.

Discussion

Many efforts have been made to detect newborns who are liable to develop significant neonatal jaundice. Effective strategies can decrease hospital stay for normal babies and detect cases of severe hyperbilirubinemia that may occur later on [12]. The current study was performed to assess the reliability of umbilical cord blood ALP and the development of hyperbilirubinemia in healthy full-term and late preterm neonates, in the first week of life.

In the present study, the incidence of clinical jaundice (groups 2A and 2B) during follow-up was 50.54% (94 cases). The treatment group consisted of 27 (14.52%) neonates, and the nontreatment group consisted of 159 (85.48%) neonates. Treatment was essentially by phototherapy, and none of the neonates in this study needed an exchange transfusion. In this study, the incidence of significant hyperbilirubinemia that required treatment was 28.72%. This is found to be higher than the incidence between 6 and 10.3% reported by other studies [13,14].

The present study concludes that the occurrence of pathological NNHB is independent of the sex of the baby. This is in correlation with the study done by Taksande *et al.* [14], who reported that there is no relation between NNHB and the sex of the newborn. However, studies done by Satrya *et al.* [15] and Maisels and Kring [16] had shown that male babies are at a higher risk of developing icterus and subsequent intervention for icterus.

In this study, no statistically significant association was found between the mode of delivery and the development of significant hyperbilirubinemia in both term and preterm neonates, similar to the observation made by Taksande *et al.* [14] and Satrya *et al.* [15].

There was no significant association between gestational age and NNHB in the treatment group (group 2B) and the nontreatment groups (group 1 and group 2A). This is consistent with the results reported by some other authors, who did not find any significant effect of gestational age on hyperbilirubinemia [17,18]. Moreover, this finding is similar to that concluded by Dhanwadkar *et al*. [19] This is in contrast to the finding of Singhal *et al*. [20] and Narang *et al*. [21], who observed that gestational age has a significant effect on hyperbilirubinemia.

In our study, in the term group, there was no significant association between birth weight and significant NNHB. Among preterm newborns, there was a significant association between birth weight and the development of significant NNHB ($P<0.05$). Satrya *et al*. [15] in a study on 88 newborns reported that there is no association ($P=0.885$) between birth weight and NNHB among term newborns. Romagnoli *et al*. [22] and Onwuanaku *et al*. [23], in their respective studies, concluded that there was a significant association between birth weight and NNHB among preterm newborns. The results of our study are in accordance with the results of these studies.In our study, the levels of cord blood ALP were significantly higher in cases with significant hyperbilirubinemia requiring treatment according to AAP protocol, whereas in cases of mild jaundice that require no treatment, a significant correlation was not detected. The mean cord blood ALP for neonates who required phototherapy (group 2B) was found to be significantly higher than those who did not require such treatment: 352.11±53.49 vs 204.89±34.5 in the group 1 and 217.10±49.51 in the group 2A. This is not consistent with the finding of some authors, such as Fenton *et al*. [24], who found the mean level of cord blood ALP was 159±49 IU/l, and another study by Abbasian *et al*. [25] showed that mean cord blood ALP level was 314.34±122.42 IU/l. These findings confirm the results of Nalbantoglu *et al*. [2], who used blood ALP levels 6 h after birth. They found that ALP levels were significantly higher in neonates with hyperbilirubinemia needing treatment (phototherapy) ($P=0.0001$). However, the advantage of this study is that cord blood sample predicts hyperbilirubinemia earlier than a sample that was taken hours after birth.

ROC curve analysis shows that a cord blood ALP level greater than 342 IU/l was the most suitable cutoff value for predicting significant jaundice (that needs treatment). It was associated with 91.07% sensitivity and 61.54% specificity. The area under the curve was 0.791, showing the high significance and the convenience of the test in anticipating significant jaundice requiring treatment.

The umbilical cord blood ALP level greater than 342 IU/l will predict almost all healthy babies who may develop significant hyperbilirubinemia that requires treatment later during the first week of life. So, close follow-up of newborns with cord blood ALP level greater than 342 IU/l is needed either in a hospital or in an outpatient clinic on days 3–5 if possible, whereas those having cord blood less than 342 IU/l can be discharged early.

Conclusion

Measurement of cord blood ALP level might be used as a critical predictor of the development of hyperbilirubinemia requiring therapeutic intervention in healthy term and preterm newborns. In our study, ROC analysis demonstrates that ALP levels of cord blood greater than 342 IU/l was the most appropriate cutoff value for predicting significant NNHB. We recommend that routine estimation of cord blood ALP should be emphasized in all term and preterm newborns in institutional delivery. It will help to design and implement the follow-up program in high-risk groups effectively and to plan early discharge of babies and mothers. More work and prospective wider studies should be carried out with larger numbers of newborns to further determine the efficacy of ALP enzyme as an early predictor of neonatal jaundice.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Gregory ML, Martin CR, Cloherty JP. Neonatal hyperbilirubinemia. In: Cloherty JP, Eichenwald EC, Hansen AR, Stark AR, editors. Manual of Neonatal Care. 7th ed. Philadelphia, PA: Lippincott William'sand Wilkins; 2012 304–309.

2 Nalbantoglu A, Ovalı F, Nalbantoglu B. Alkaline phosphatase as an early marker of hemolysis in newborns. Pediatr Int 2011; 53:936–938.

3 Ahire N, Sonawane R, Gaikwad R, Patil S, Sonawane T. Study of correlation of cord blood bilirubin with neonatal hyperbilirubinemia. MVP J Med Sci 2016; 3:60–66.

4 American Academy of Pediatrics. Practice parameter: management of hyperbilirubinemia in the healthy term newborn. Pediatrics 1994; 94:558–565.

5 American Academy of Pediatrics. Subcommittee on Hyperbilirubinemia. Management hyperbilirubinemia in the newborn infant 35 or more weeks of gestation.Pediatrics 2004; 114:297–316.

6 Zeitoun AA, Elhagrasy HF, Abdelsatar DM. Predictive value of umbilical cord blood bilirubin in neonatal hyperbilirubinemia. Gaz Egypt Paediatr Assoc 2013; 61:23–30.

7 Tan KL, Loganath A, Roy AC, Goh HH, Karim SM, Ratnam SS. Cord plasma alpha-fetoprotein values and neonatal jaundice. Pediatrics 1984; 74:1065–1068.

8 Tinnion RJ, Embleton ND. How to use alkaline phosphatase in neonatology. Arch Dis Child Educ Pract Ed 2012; 97:157–163.

9 Thomas L. Clinical Laboratory Diagnostics. 1th ed. Frankfurt: TH-Books Verlagsgeselischaft; 1998:136–146.

10 Moghaddam ES, Khosravi S, Dorgalaleh A. Discreoancies in ABO and Rh grouping in southeast Iran, an analysis of 3 years' experience. J blood Disord Transfus 2016; 7:364–366.

11 Tietz NW. Fundamentals of Clinical Chemistry. Philadelphia, PA: W.B. Saunders Co. 1976. 1040.

12 Rostami N, Mehrabi Y. Identifying the newborn at risk for developing significant hyperbilirubinemia by measuring cord bilirubin levels. J Arab Neonatal forum 2005; 2:81–85.

13 Agarwal R, Kaushal M, Aggarwal R, Paul VK, Deorari AK. Early neonatal hyperbilirubinemia using first day serum bilirubin level. Indian Pediatr 2002; 39:729–730.

14 Taksande A, Vilhekar K, Jain M, Zade P, Atkari S, Verkey S. Prediction of the development of neonatal hyperbilirubinemiaby increased umbilical cord blood bilirubin. Curr Pediatr 2005; 9:5–9.

15 Satrya R, Hidayat S, Effendi SH, Gurnida DA. Correlation between cord blood bilirubin level and incidence of hyperbilirubinemia in term newborns. Paediatr Indonesia 2009; 49:349–354.

16 Maisels MJ, Kring E. Length of stay Jaundice and hospital readmission. Pediatr 1998; 101:995–998.

17 Awasthi S, Rehman H. Early prediction of neonatal hyperbilirubinemia. Indian J Pediatr 1998; 65:131–139.

18 Alpay F, Sarici SU, Tosuncuk HD, Serdar MA, Inanc N, Gokcay E. The value of first-day bilirubin measurement in predicting the development of significant hyperbilirubinemia in healthy term newborns. Pediatrics 2000; 106:16–21.

19 Dhanwadkar SS, Rasalam CS, Masoodet Z. Effectiveness of early clinical assessment and bilirubin estimation for prediction of neonatal hyperbilirubinemia. Int J Contemp Pediatr 2016; 3:477–484.

20 Singhal PK, Singh M, Paul VK, Deorari AK, Ghorpade MG. Spectrum of neonatal hyperbilirubinemia: an analysis of 454 cases. Indian Pediatr 1992; 29:319–325.

21 Narang A, Gathwala G, Kumar P. Neonatal jaundice: an analysis of 551 cases. Indian Pediatr 1997; 34:429–432.

22 Romagnoli C, De Turris P, Zuppa AA, Currò V, De Carolis M, Zecca E. Physiologic hyperbilirubinemia in low birth weight newborn infants: relation to gestational age, neonatal weight and intra-uterine growth. Pediatr Med Chir 1982; 5:299–303.

23 Onwuanaku CA, Okolo SN, Ige KO, Okpe SE, Toma BO. The effects of birthweight and gender on neonatal mortality in north central Nigeria. BMC Res Notes 2011; 4:562.

24 Fenton TR, Lyon AW, Rose MS. Cord blood calcium, phosphate, magnesium, and alkaline phosphatase gestational age specific reference intervals for preterm infants. BMC Pediatr 2011; 11:76–84.

25 Abbasian M, Chaman R, DelvarianZadeh M, Amiri M, Raei M, Norouzi P, *et al.* Investigating the prevalence of calcium deficiency and some of its influencing factors in pregnant women and their neonates [in Persian]. Knowledge Health 2012; 7:39–43.