

Cord blood α -fetoprotein as a predictive index for indirect hyperbilirubinemia in term neonates

Yadollah Zahedpasha (MD)¹
Mousa Ahmadpour-Kacho (MD)¹
Jafar Khalafi (MD)^{*1}
Ali Bijani (MD)¹

1- Non-Communicable Pediatric Diseases Research Center, Babol University of Medical Sciences, Babol, Iran.

* **Correspondence:**
Jafar Khalafi, Non-Communicable Pediatric Diseases Research Center, Babol University of Medical Sciences, Babol, Iran

E-mail: jafarkhalafi@yahoo.com
Tel: 0098 111 2207918
Fax: 0098 111 3242151
Postal code: 47317-41151

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Abstract

Background: Prediction of severe neonatal hyperbilirubinemia is very important for early treatment and prophylaxis of neurologic sequels. The aim of this study was to evaluate the predictive role of umbilical cord α -fetoprotein (UCAFP) as a marker of an increased risk for neonatal hyperbilirubinemia in full term babies.

Methods: Umbilical cord blood was collected from 400 term singleton full term well newborn babies who met our inclusion criteria and stored in -20°C . Those who developed jaundice and admitted for phototherapy (34 newborns: 22 males and 12 females), considered as case group and 31 non-jaundiced infants (13 males and 18 females) gestational age-weight-matched considered as the control group. The serum level of UCAFP was checked in these 65 newborns and was compared between these two groups.

Results: Mean UCAFP in case group was 523.429 ± 174.158 and in control group was 664.548 ± 154.894 $\mu\text{g/L}$. In the non-jaundiced group, mean UCAFP values was higher than neonate with hyperbilirubinemia (664.548 vs. 523.429 $\mu\text{g/L}$). The mean UCAFP in males was 519.023 $\mu\text{g/L}$ and in females was 531.508 $\mu\text{g/L}$ ($p=0.066$). Sixty (92.3%) babies delivered by cesarean section (CS) and 5 (7.7%) by normal vaginal delivery ($p=0.566$).

Conclusion: According to our study, there was no significant positive association between UCAFP and subsequent neonatal indirect hyperbilirubinemia or serum bilirubin level.

Key words: Alpha-fetoprotein, Hyperbilirubinemia, Term neonate.

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Neonatal Jaundice (indirect hyperbilirubinemia) is the most common problems that can occur in over half of all full term and most premature newborn infants. Although most jaundiced newborn infants are healthy, we should monitor them for signs of hyperbilirubinemic encephalopathy or kernicterus (1).

There are several methods of predicting severe hyperbilirubinemia: hour-specific serum bilirubin level, that is a percentile based bilirubin level nomogram using specific age in hour (2); or AAP guidelines for the clinical risk factors of developing severe hyperbilirubinemia (3). There are several risk factors in term newborn like exclusive breast-feeding, particularly if nursing is not going well and/or weight loss is excessive ($>8-10\%$), isoimmune or other hemolytic disease (e.g. G6PD deficiency, hereditary spherocytosis), previous sibling with jaundice, cephalhematoma or significant bruising, East Asian race, short hospital stay after birth or early discharge that predict severe hyperbilirubinemia (1-15).

There are some researches about biochemical predictors of hyperbilirubinemia like decreased cord level of Ceruloplasmin or elevated cord level of Alpha-fetoprotein (AFP) (16-21). AFP is a glycoprotein that is normally produced during gestation by the yolk sac, gastrointestinal tract, and fetal liver and is similar to albumin. It is one of the first serum protein markers which serve in the dual capacity of fetal defect marker and tumor marker.

AFP synthesis nearly ceases at parturition and serum levels present an exponential fall to adult levels of ≤ 10 ng/mL during the first year of life. The usefulness of AFP as a marker for the detection and/or differentiation of a great number of diseases are well established, especially for some of malignant tumors and liver diseases (16-31). Although there is not a general consensus, in this study we investigated the effect of cord level of AFP as a predictor of hyperbilirubinemia in term neonates.

Methods

From January, 2011 to March 2011, umbilical cord blood was collected at the time of delivery from 400 term singleton full term newborn babies who met our inclusion criteria and were delivered in Rouhani and Babol Clinic Hospital, Babol, Iran. After centrifugation, all serum samples were stored at -20°C until assayed.

Parental informed consent was taken prior to inclusion and the study was approved by the Ethics Committee of Babol University of Medical Sciences. The exclusion criteria were prematurity, evidence of congenital malformations, multiple pregnancy, low Apgar score (with an Apgar score < 7 at 1st and 5th minutes), diabetic mother, and newborn babies who later developed respiratory distress, sepsis, direct hyperbilirubinemia, and hemolytic disease like Rh or ABO incompatibility.

All of the 400 newborns were followed up by phone call for any yellow discoloration. All jaundiced ones were visited by the researchers at Outpatient clinic in Amirkola Children's Hospital and those who met our inclusion criteria, were admitted for phototherapy according to Amirkola Children's Hospital's protocol.

Those babies admitted were selected as case group. This group consisted of 34 newborn infants (22 males and 12 females). From non-jaundiced infants, 31 (13 males and 18 females) gestational age-weight-matched infants were used as control group. Then their respective umbilical cord blood samples were separated from 400 stored samples and recalled from the laboratory (diagram 1).

Apart from these samples (34 cases & 31 control), the rest of samples were discarded. Serum AFP concentrations were determined by ELISA technique using ELISA reader, statfax, USA. (AFP) assayed using CanAg-ELISA kit for AFP (Sweden). The assays were done according to the manufacturer's instructions. The results of AFP were

expressed as $\mu\text{g/L}$. Statistical analysis was performed using the SPSS 18.0 software for Windows XP. The serum level of AFP has been compared in the two groups using the t-test and Mann Whitney. A P Value less than 0.05 was considered significant. Pearson correlation was used for correlation of AFP and bilirubin in case group. For categorical variables χ^2 and for continuous variables t-test was used.

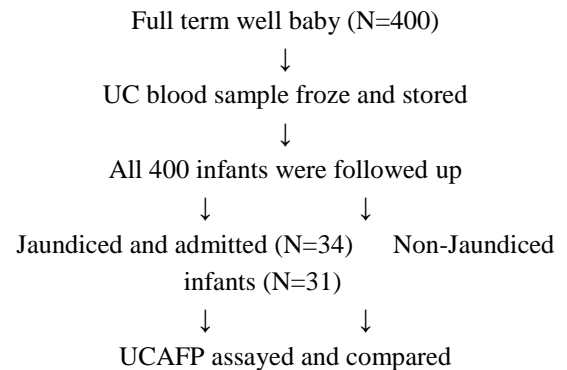


Diagram 1: Flow-diagram of Neon in this study

Results

Out of 400 umbilical cord blood samples taken and stored, 65 samples analyzed for UCAFP were compatible with our inclusion criteria (figure 1). Full term neonates (gestational age between 38-40 weeks) who were recruited into our study were 35 (54%) females and 30 (46%) males. From these 65 newborn babies, 34 ones developed hyperbilirubinemia and were admitted in Amirkola Children's Hospital.

Thirty one non-jaundiced newborns were considered as control group. UCAFPs were compared between the two groups. Statistical analysis showed mean UCAFP in case groups were $523.429 \pm 174.158 \mu\text{g/L}$ and mean UCAFP in control groups are $664.548 \pm 154.894 \mu\text{g/L}$ (figure 2). In the group of non-jaundiced infants, mean UCAFP values were higher than neonate with hyperbilirubinemia. There was no correlation between UCAFP and bilirubin level in case group ($p=0.060$). The mean UCAFP in males was $519.023 \mu\text{g/L}$ and $531.508 \mu\text{g/L}$ in females. There was no significant statistical difference between two genders ($p=0.066$). Sixty (92.3%) babies delivered by cesarean section (CS) and 5 (7.7%) babies delivered by normal vaginal delivery (NVD). There was no significant difference between the two modes of delivery ($p=0.566$) (table 1, 2).

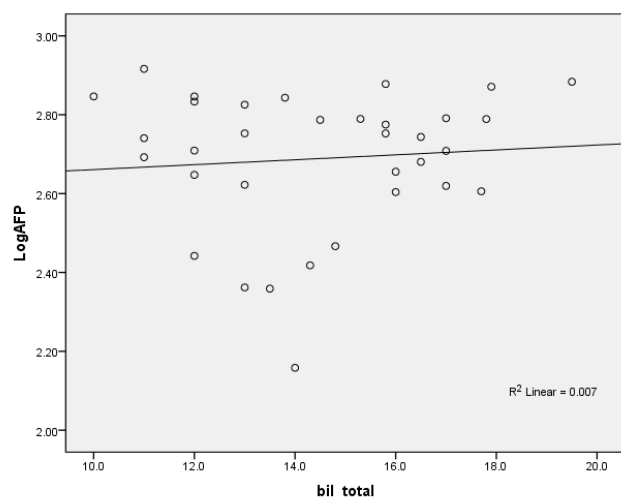


Figure 1. Scattered plot, regression analysis of UCAFP with serum bilirubin level.

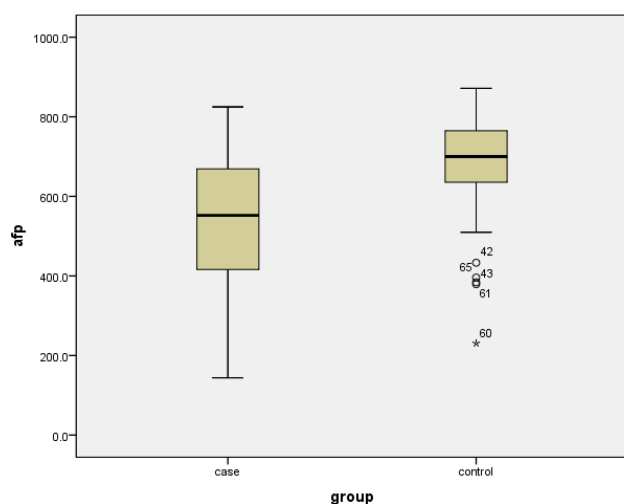


Figure 2. Box plot, comparison of UCAFP in case and control group

Table1. Comparison of variables with mean α FP level

Variable	Number	Mean α FP \pm SD(μ g/L)	pValue	
Groups	jaundiced	34	523.43 \pm 174.16	0.001
	Non-jaundiced	31	664.55 \pm 154.90	
Gender	Male	35	571.80 \pm 194.39	0.066
	Female	30	612.82 \pm 194.55	
Type of delivery	C/S	60	596.93 \pm 177.17	0.566
	NVD	5	516.40 \pm 201.02	

Table 2. Demographic and outcome variables in case and control (Jaundiced and non-jaundiced)

Variable	Jaundiced	Non-jaundiced
Gestational age (days)	271.94 \pm 4.25	272.87 \pm 5.34
Birth weight (gr)	3372.06 \pm 356.16	3377.42 \pm 5.34
Gender		
Male	22 (64.7%)	13 (41.9%)
Female	12 (35.3%)	18 (58.1%)
Mode of delivery		
NVD	2 (5.9%)	3 (9.7%)
C/S	32 (94.1%)	28 (90.3%)
UCAFP level (μ g/L)	523.43 \pm 174.16	664.55 \pm 154.89

Discussion

According to our findings, there is no association between UCAFP and development of jaundice or bilirubin level. This is compatible with findings of Salehzade and Ikonen. Salehzadeh et al in 2010 checked serum AFP and serum bilirubin in 100 term neonates under 28 days at the same time, they concluded there is no statistical significant correlation between serum bilirubin and AFP (22). Ikonen et al in 1980 studied 15 full term babies with hyperbilirubinemia and 15 controls matched for gender and gestational age. They found no correlation between serum bilirubin and AFP concentrations in hyperbilirubinemia neonate (23).

In our study, mean UCAFP in males was 519.023 μ g/L and mean UCAFP in females was 531.508 μ g/L. There was no significant statistical difference between the two genders in this study ($p=0.066$). These results are the same as the study of Salehzadeh et al. and Carlo Valerio Bellieni et al, Goraya et al, Mizejewsk et al. and in large study on 260 neonates by Bader (22, 24-27).

In spite of our study, Obiekwe et al. measured maternal AFP, cord arterial and venous blood from 105 women at 36-42 weeks' gestation. Umbilical cord arterial and venous AFP levels were considerably higher in male babies than in females (28). We only studied term neonates but Obiekwe studied both term and preterm babies. This may explain the reason for our different findings.

We also compared the modes of delivery, CS vs. NVD. There was no significant difference between two modes. From 65 infants, 7.7% delivered by NVD and 92.3% were delivered by CS. ($p=0.566$). In our research in literature, we did not find such study. In fact, we could not emphasize on the analysis of the mode of delivery, because most babies in our study (92.3%) were born by CS and this highlights the

increased rate of CS deliveries nowadays. In our study, AFP was compared between 34 jaundiced and 31 non-jaundiced infants. In the non-jaundice group mean UCAFP values was higher than in neonate with hyperbilirubinemia but Salehzadeh and Ikonen found no correlation between AFP and bilirubin level (22, 23). This difference may be explained by different sampling ages (0-28 days old in Salehzadeh's study), probable genetic and ethnic differences, or probable protective effect of AFP.

In spite of our findings, Manganaro in 2007 studied serum AFP in 98 jaundiced breastfed infants over 20 days age. Mean serum concentration of AFP was significantly higher than 30 control infants (3548 vs. 1095 ng/mL, $p < 0.001$). He concluded serum AFP levels of jaundiced infants were directly associated with serum indirect bilirubin. He studied serum AFP but we studied AFP in umbilical cord (17). Another study in Czech, on a group of 154 full term neonates were done by Hodr et al. The authors recorded significantly higher mean AFP values in children with jaundice than in other neonates. Development of hyperbilirubinemia and need for phototherapy was more likely significantly higher in neonates with UCAFP blood above 100 mg/L and they concluded these infants must be carefully observed. When the concentration was above 130 mg/L, the development of hyperbilirubinemia may be assumed with certainty. Examination of UCAFP is a useful indicator of the functional maturity of the liver, but does not ensure reliable prediction of jaundice in individual neonates. A concentration of alpha-fetoprotein above 100 mg/L exceptional among neonates without indirect hyperbilirubinemia, while lower values do not rule out the development of hyperbilirubinemia (18).

Riskin et al. in 2004 checked UCAFP as a marker of hepatic immaturity in 174 term babies to predict an increased risk for neonatal indirect hyperbilirubinemia. Mean UCAFP was 60.2 ± 45.9 mg/L. Its levels were linearly correlated with subsequent bilirubin levels, and statistically significantly higher bilirubin levels were found in neonates whose UCAFP levels were ≥ 100 mg/L. But in spite of significant correlation between UCAFP and subsequent bilirubin levels this was not recommended for use in clinical practice because of its inability to serve as a screening tool for significant hyperbilirubinemia in the newborns (19).

A study in France by Tourne et al. showed there was significant correlation between the AFP levels during the last days of the pregnancy and UCAFP with subsequent

physiologic jaundice. They concluded fetal hypoxia causes increase in AFP level and also capable of inducing an over stimulation of the fetal erythropoiesis and subsequent jaundice (20). Malan et al. in their article found the relationship between the concentration of UCAFP in 259 neonates and the incidence of severe jaundice (21).

The probable reasons for our different results include: exclusion of preterm and low birth weights (they have much higher AFP than terms), using umbilical cord blood sampling rather than serum level, small sample size, different sampling ages in other studies, and ethnic and genetic differences in normal neonatal AFP level.

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