

## ORIGINAL ARTICLE

### Cord Blood Alkaline Phosphatase as a Predictor of Neonatal Jaundice in Full Term Neonates.

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#### ABSTRACT

**Background:** The use of umbilical cord blood has proven to be a suitable tool for laboratory testing of neonates. In addition, it has been widely used in many regions due to its significant importance. Alkaline phosphatase is a common enzyme found in almost all body cells, including red blood cells, liver, bile ducts and bone. The objective of this research was to investigate whether cord alkaline phosphatase (ALP), an intracellular enzyme abundant in red blood cells, could be used to predict the early hyperbilirubinemia in newborns. This study aimed to investigate whether cord alkaline phosphatase (ALP), which is an intracellular enzyme found abundantly in red blood cells, could be used for the early prediction of hyperbilirubinemia in newborns.

**Methods:** The study is a prospective clinical study model that was performed from March 2019 to September 2019 in the Pediatric Department, Faculty of Medicine, Zagazig University Hospitals. The research included 56 newborns from 37 to 42 weeks of gestational age. The studied newborns were categorised into jaundiced (N=24) and non-jaundiced (N=32) groups. Samples of cord blood were obtained directly after birth to test alkaline phosphatase levels. ALP and Bilirubin levels has been estimated using the automated chemistry analyzer (BT-3500). All newborns were checked daily to find out the occurrence of jaundice in first 2 weeks of life. Neonates were followed-up for the emergence of jaundice. ALP was re-estimated after treatments of jaundiced group according to American Academy of Pediatrics (AAP) protocols.

**Results:** The obtained results of gestational age, APGAR, mode of delivery, sex, combs test score, weight, WBCs and platelets showed that there was no significant difference between jaundiced and non-jaundiced groups. On the other hand, the levels of ALP and hemoglobin showed significant differences between the studied groups. A point to note that, the levels of ALP was significantly decreased following the treatment of jaundice. ALP levels were significantly correlated positively and negatively with Bilirubin and hemoglobin levels, respectively.

**Conclusions:** It could be concluded that, cord blood alkaline phosphatase level is a useful indicator in predicting subsequent jaundice in healthy term newborns.

**Keywords:** Neonatal jaundice; Alkaline phosphatase (ALP); Bilirubin; Hemoglobin and cord blood samples.



#### INTRODUCTION

In newborns, hyperbilirubinemia or jaundice is a life-threatening phenomena. In spite of this condition can be treated with recovery rates of up to 20%, it can still be harmful and medical intervention is required. The treatment of neonatal hyperbilirubinemia can be occurred through

phototherapy, exchange transfusion and reduction of serum bilirubin concentration by drugs [1]. Jaundice is the most common problem experienced in newborn babies, and kernicterus and permanent neuro-developmental impairment can result from failure to mis diagnose and treatment of severe hyperbilirubinemia. In addition, evaluating the risk of hyperbilirubinemia development and the

optimum time of treatment are of great concern. Different methods and biochemical analyses have been proposed to evaluate risk of hyperbilirubinemia. The measurement of bilirubin levels in cord blood is therefore, important for the discovery of hyperbilirubinemia on the first day of life [2]. Alkaline phosphatase (ALP) is a hydrolase enzyme that is responsible for phosphate removal from many molecular types. Alkaline phosphatase is existed in nearly all body cells, including red blood cells, bones, intestines and kidneys. ALP is formed in the placenta in pregnant females [3]. The level of alkaline phosphatase in cord blood is not only a useful predictor for severe neonatal jaundice but treatment approaches in stable newborns over 35 weeks of gestational age may also be anticipated [4]. This study was conducted to determine whether cord alkaline phosphatase (ALP) levels, an intracellular enzyme found abundantly in red blood cells, could be used for early prediction of hyperbilirubinemia in newborns. Examine the validity of cord blood alkaline phosphatase to predict neonatal hyperbilirubinemia.

#### METHODS

This study is a prospective clinical study design that was carried out from March 2019 to September 2019 in the Pediatric Department, Faculty of Medicine, Zagazig University Hospitals- Egypt. This study was approved by the Zagazig University Hospital Medical Committee, and prior to enrollment in the study, written and verbal consent was obtained from each parent's case. This research was carried out in accordance with the Code of Ethics of the World Scientific Association (Declaration of Helsinki) for studies involving humans.

##### ***Inclusion and exclusion criteria:***

The study included 56 healthy full term newborns with gestational age between 37 and 42 weeks with an average weight over 2,500g, and was born from healthy mothers. While, neonates who were born to mothers with diseases such as: eclampsia, diabetes, diseases of different organs were excluded from this study.

##### ***Sample size and design:***

The participants were chosen by systematic random sampling Serial numbers from 1-56 and were randomly randomized divided into two groups: (G1) jaundiced group (N=24) and (G2) non-jaundiced group (N=32) using a web-based randomization tool ([www.randomizer.org](http://www.randomizer.org)) from patients admitted to operation room in Zagazig University Hospitals.

##### ***Full history and clinical examination:***

The assessment of the complete maternal history was performed by defining (mode of delivery, parity, gravidity, blood group, previous abortion

and history of previous sibling affected). Also, birth weight and full neonatal examination from head to toe were recorded immediately after birth and in follow up visits were performed.

***Laboratory investigations:*** Procedure for Umbilical Cord Blood Sampling the distal end of the umbilical cord provide to clamp with inspection the fetal side of the placenta for signs of umbilical vessel rupture. Then swab the base of the povidone-iodine insertion cord and allow it to dry. Thereafter, the umbilical cord was grasping, and the 18- gauge needle was inserted bevel down, into the umbilical vein 6 to 8 cm above the placental insertion site. A 5 ml of umbilical cord blood had been drawn from each case after fetal delivery and the sample was collected in two plain tubes in water bath to be clotted then centrifuged at 3000 rpm for 10 min. for serum collection. The collected serum samples were used for measuring Alkaline Phosphatase (ALP).

***Cord blood alkaline phosphatase:*** Alkaline Phosphatase (ALP) and Bilirubin levels were measured by using the automated chemistry analyzer (BT-3500). ALP was measured in a reaction in which it catalyzed the cleavage of phosphate from 4-nitrophenyl phosphate (colourless) to form 4- nitrophenoxide (benzenoid form), also colourless, which undergoes spontaneous rearrangement at alkaline pH to the quinonoid form (yellow). The reaction was followed by measuring absorbance at 405 nm. All newborns were observed daily for appearance of jaundice in first 2 weeks of life. When jaundice appeared clinically, the patient was subjected to: a 1- 5 ml of fetal venous blood had been collected and subjected in plain tube for measuring bilirubin level. Clinical jaundice neonates were recalled and the amount of serum bilirubin was assessed and then was treated according to American Academy of Pediatrics (AAP) protocols.

***Other laboratory tests,*** i.e.: complete blood count, reticulocyte count, mother and neonate blood group estimate, peripheral blood smear, G6PD level evaluation and Coombs test were performed.

##### ***Statistical Analysis:***

The obtained results were statistically analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using mean and standard deviation for normally distributed data. For normally distributed data, comparison between populations was performed using independent t-test while more than two populations, F test (ANOVA) was applied. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level. Roc curve test and r

spearman correlation which evaluates the linear association between 2 quantitative variables were performed.

**RESULTS**

The obtained results of gestational age, APGAR, mode of delivery, sex, combs test score or weight showed no significant differences between Table 1. The levels of alkaline phosphatase was significantly increased in jaundiced group compared to non-jaundiced group with average values of (324.9±82.8 IU/L versus 210±81.8 IU/L), respectively Table 2.

A significant reduction of hemoglobin level was occurred by 12. 14% in jaundiced group compared to the non-jaundiced one. While, there was no significant difference between jaundiced groups and non -jaundiced cases for WBCs or platelets values Table 3.

There is a reduction of ALP level after the treatment with an average value of 200.3±58.1 IU/L compared to its level prior to the treatment Table 4.

Significant positive correlation was observed between the levels of ALP and bilirubin (Figure 1). There is a significant negative correlation between ALP and hemoglobin level (Figure 2).

A comparison of the ROC curves of ALP levels between the non-jaundiced and non -jaundiced and the treated group revealed that a cord blood alkaline phosphatase level > 311 IU/L was the most suitable cutoff value for predicting severe jaundice (that needs treatment). This cut-off value was associated with 86.3% sensitivity and 69.7% specificity (Figure 3)

**Table (1):** Comparison between jaundiced and non jaundiced cases a regard baseline data:

Variable	Non-Jaundiced n=32		Jaundiced n=24		T test	P value
<b>Gestational age (Weeks):</b>						
Mean ± SD	38.9±0.51		38.7±0.50		1.97	0.057
<b>Weight (g):</b>						
Mean ± SD	3663.2±424.1		3623.3±428.3		0.482	0.674
<b>Apgar score at 1<sup>st</sup> minute:</b>						
Median	8		8		Mw	0.891
Range	7-9		7-9		162.2	
<b>Apgar score at 5<sup>th</sup> minute:</b>						
Median	9		9		Mw	0.941
Range	8-10		8-10		167.3	
	No.	%	No.	%		
<b>Mode of delivery:</b>						
Vaginal Cesarean	17	53.1	13	54.2	0.932	0.911
	15	46.9	11	45.8		
<b>Sex:</b>						
Male	16	50.0	14	58.3	1.01	0.713
Female	16	50.0	10	41.7		
<b>Combs test:</b>						
Negative	32	100	18		0.0	1.0
Positive	0		6			

**Table (2):** Comparison between jaundiced and non jaundiced cases a regard Alkaline phosphatase:

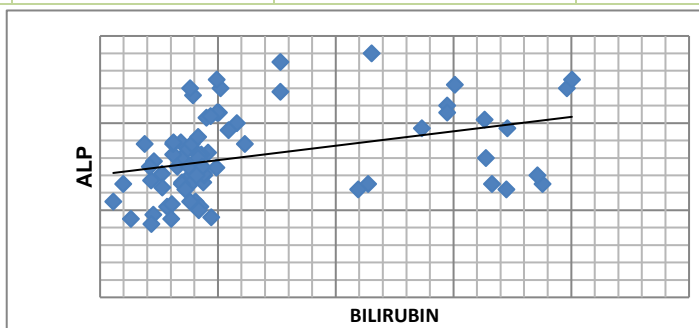
Variable	Non jaundiced n=32		Jaundiced n=24		T test	P value
<b>Alkaline phosphatase (IU/L)</b>						
Mean ± SD	210±81.8		349.1±71.2		2.53	<b>0.013 (S)</b>
	No.	%	No.	%		
<b>ALP groups:</b>						
<100	12	37.5	0	0.0	56.0	<b>&lt;0.001 (HS)</b>
200	8	25	0	0.0		
200-300	12	37.5	0	0.0		
>300	0	0	24	100		

**Table (3) :**Comparison between jaundiced group and non jaundiced cases a regard laboratory analysis:

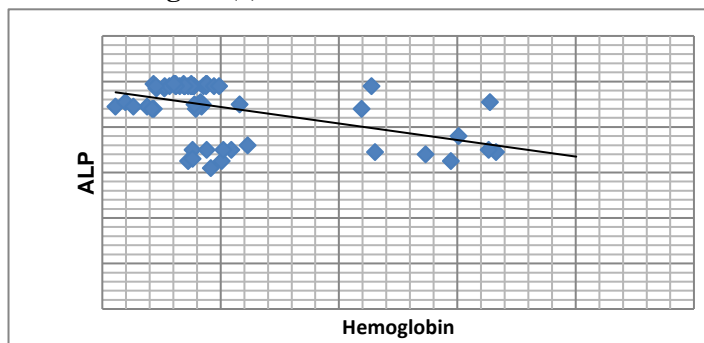
Variable	Non jaundiced n=32	Jaundiced n=24	T test	P value
<b>Haemoglobin g/dl:</b>				
Mean ± SD	16.47±3.13	14.47±3.14	3.18	<b>0.003 (S)</b>
<b>WBCs*10<sup>2</sup>:</b>				
Mean ± SD	5.36±0.11	5.38±0.1	0.913	0.821
<b>Plateletes*10<sup>3</sup>:</b>				
Mean ± SD	4.97±2.22	4.91±2.15	0.137	0.897

**Table (4) :**Comparison between jaundiced group before and after treatment a regard ALP:

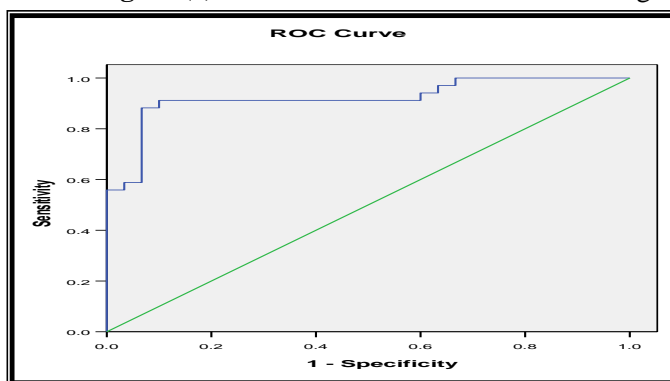
Variable	before	After	Paired T test	P value
<b>Alkaline phosphatase (IU/L)</b>				
Mean ± SD	352.2±69.2	200.3±58.11	57.6	<b>&lt;0.001 (HS)</b>



**Figure (1):**Correlation between ALP and Bilirubin



**Figure (2):**Correlation between ALP and Hemoglobin



**Figure (3):**Roc curve analysis of ALP in predicting post natal jaundice

## DISCUSSION

Hyperbilirubinemia in neonates is normal and considered benign problem. Jaundice is present in around 60 per cent of term infants and 80 per cent of preterm infants during the first week after birth..While bilirubin can play a physiological role as an antioxidant, indirect, unconjugated increases of bilirubin are potentially neurotoxic. Although the conjugate form is not neurotoxic, direct hyperbilirubinemia indicates a potentially serious liver disorder or systemic disease. Researchers have reported conflicting findings on many measures of extreme neonatal jaundice, including alpha-fetoprotein, umbilical cord bilirubin level and alkaline phosphatase. An alkaline phosphate is nearly detected in all body cells, including red blood cells, liver, bile ducts, and bone, are found with alkaline phosphatase. It is a hydrolase enzyme that is responsible for phosphate removal from several kinds of molecules. Depending on its origin within the body, it is used in several ways. It plays an important role in liver metabolism and skeleton development [6].

In this study,56 healthy neonates were involved in the study with mean gestational age of cases (38.7±0.61) weeks, mean birth weight of cases of (3641.1±423.1)g and mean alkaline phosphatase (324.9±82.8) IU / L, median Apgar score at 1st minute was 8 with a range of 7-9,The median Apgar score at 5 minutes was 9 with a range of 8-10, 53.6 per cent were given vaginally and the Combs test was negative in 87.3 per cent and positive in 10.7 per cent of cases, 51.8 per cent for males and 48.2 per cent for females.

Our results are consistent with Ahmadpour-Kacho et al. [5] as they reported a mean gestational age of 38.7 weeks and a mean birth weight of 3649.59 grams. The mean alkaline phosphatase level in the newborns studied was  $325.24 \pm 85.03$  IU / L, which was higher than those of existing reports. Fenton et al. [7] found a mean level of  $159 \pm 49$  IU / L of alkaline phosphatase in cord blood. There's a big gap compared to these outcomes. Another local study in Shahrood, Iran by Abbasian et al. [8] showed that the mean alkaline phosphatase level in cord blood was  $314.34 \pm 122.42$  IU / L, which is compatible with our findings. In Iranian newborns, the average amount of cord blood alkaline phosphatase appears to be higher than in other populations.

There was significant difference of alkaline phosphates levels between jaundiced and non - jaundiced groups. AbdElmonem et al.[4] went almost to similar findings, they have cord blood alkaline phosphatase in jaundiced cases was statistically significantly higher than non-jaundiced cases.Nalbantoglu et al.[9] found that, in patients with hyperbilirubinemia needing

treatment, ALP levels were significantly higher with either phototherapy or exchange transfusion (P value 0.0001).

Ahmadpour-Kacho et al.[5] reported that there was a substantial difference between non-jaundiced and clinically jaundiced newborns in levels of cord blood alkaline phosphatase and slightly higher in patients seeking care with hyperbilirubinemia. In addition, ALP levels were significantly higher in newborns whose serum bilirubin level was 10 mg / dL or higher. The results of Nalbantoglu et al.[9] matching well with our results.

There was significant difference of hemoglobin levels between jaundiced and non-jaundiced groups ;while there was no significant difference of WBCs platelets between jaundiced and non-jaundiced groups. Significant reduction in ALP levels was observed following the treatment. Our findings were in accordance with Al Assal et al.[3] as they stated that there was a substantial difference in cord blood alkaline phosphate levels (P value = 0.040) when compared to the non-jaundiced community with neonates needing care according to the AAP protocol (the treatment group).

Our results revealed a positive correlation between levels of ALP and Bilirubin. However, There is significant negative correlation between ALP and hemoglobin level. ROC curve analysis showed that ALP sensitivity for diagnosis of neonatal jaundice was 86.3%, specificity was 69.7% with area under the curve 0.9, and at cut off point of 311 IU/L.This results was in agreement with Ahmadpour-Kacho et al.[5] who concluded that of 60 neonates whose cord blood alkaline phosphatase measured less than 314 IU/L, only two neonates needed treatment. Thus, the negative predictive value of cord blood alkaline phosphatase for occurrence of hyperbilirubinemia was 96.6%.

## CONCLUSIONS

Jaundice neonates had higher levels of alkaline phosphatase in cord blood compared to non-jaundice neonates cases. Cord blood alkaline phosphatase level is a useful indicator for predicting subsequent jaundice in healthy neonates.

## REFERENCES

- 1- Rylance S, Yan J,Molyneux E. Can transcutaneous bilirubinometry safely guide phototherapy treatment of neonatal jaundice in Malawi? *PaediatrInt Child Health*.2014; 34:101–7.
- 2- El-Sayyad H, Al-Haggag MM, El-Ghawet HA, Bakr IH. Effect of maternal diabetes and hypercholesterolemia on fetal liver of albino Wistar rats, *Nutrition*2014; 30(3),326-36.

3- Al Assal MH, Hablas HR, Afia A. Utility of Cord Blood Alkaline Phosphatase Enzyme as a Predictor of Significant Neonatal Jaundice in Well Term Infants, *N Y Sci J*. 2017;10(4):70-4.

4- AbdElmonemHM, Fakhr El-Din AR, Kholeef EF, Nasr Eldin S. Alkaline phosphatase and haptoglobin as predictor of neonatal jaundice. *The Egypt. J. Hosp. Med.* 2019; 74 (5), 978-87.

5- Ahmadpour-KachoM, Pasha YZ, Haghshenas M, Rad ZA, Firouzjahi A, Bijani A, et al. Cord blood alkaline phosphatase as an indicator of neonatal jaundice. *Iran J Pediatr*. 2015; 25(5).

6- Lubin BH, Shearer WT. Cord blood banking for potential future transplantation. *Pedia.*, 2007; 119 (1): 165-70.

7- 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(1):76.

8- Abbasian M, Chaman R, Delvarian Zadeh M, Amiri M, Raei M, Norouzi P. Investigating the prevalence of calcium deficiency and some of its influencing factors in pregnant women and their neonates [in Persian]. *Knowledge Health.* 2012; 7(1):39-43.

9- Nalbantoglu A, Ovali F, Nalbantoglu B. Alkaline phosphatase as an early marker of hemolysis in newborns. *Pediatr Int.* 2011; 53 (6): 936-8

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