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Alkaline phosphatase and haptoglobin as predictor of neonatal jaundice

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Abstract

Background: The term kernicterus refers to the clinical features of bilirubin encephalopathy. Its risk is increased in term babies with very high bilirubin levels. Kernicterus is also known to occur at lower levels of bilirubin in term babies who have risk factors and in preterm babies.

Objective: The aim of the present study was to investigate whether serum alkaline phosphatse & haptoglobin (Hp) level could be used for the early diagnosis and prediction of hyperbilirubinemia in newborns.

Methodology: The study is a prospective clinical study design, which was carried out in Pediatric Department, Faculty of Medicine, Aswan University Hospital. The study included 100 newborns with gestational age more than 35wk delivered in obstetric department.

Results: Our study showed no statistically significant difference between jaundiced and non- jaundiced cases regarding the mode of delivery, maternal gravidity and parity. As regards previous sibiling affected, we found a significant relation between clinically appearing jaundice and previous sibling affected with neonatal hyperbillirubinemia, which may reflect genetic susceptibility for hyperbillirubinemia. No significant relationship between gestational age and clinically appearing jaundice. When assessing the relationship between clinically appearing jaundice and birth weight, our results revealed no significant relationship.

Conclusion: The cord blood alkaline phosphatase level is not only a useful predictor for severe neonatal jaundice but also can predict the early onset of neonatal hyperbilirubinemia and expected methods of treatment in healthy newborns more than 35 weeks gestational age.

Keywords: Alkaline phosphatase, Haptoglobin, Neonatal jaundice

Introduction

Elevation of the serum bilirubin level is a common, if not universal, finding during the first week of life. This can be a transient phenomenon that will resolve spontaneously or can signify a serious or even potentially life-threatening condition. Independent of the cause, elevated serum bilirubin levels can be potentially toxic to the newborn infant [(1)].

Bilirubin is one of the biologically active end products of heme catabolism. Its clinical significance in the neonate relates to its propensity for deposition in the skin and mucous membranes, producing easily identifiable jaundice [(2)]. As many as 60% of otherwise healthy, term newborns develop some degree of elevated TB levels. Imbalance between bilirubin production and its elimination may result in increasing jaundice or hyperbilirubinemia [(3)].

At present, for essentially economic reasons, maternal discharges have to be scheduled early within 48 hr of childbirth, so most cases of neonatal jaundice cannot be detected. Therefore, early diagnosis of jaundice and timely actions are necessary. Moreover, In Aswan a major portion of population have a black nubian skin colour which mask jaundice and delay its diagnosis, raising the idea of prediction in accessible applicable ways .

Umbilical cord blood is an important but underutilized resource that can be used in the care of premature neonates. Use of umbilical cord blood results in many improved outcomes and its use for laboratory testing of neonates is an increasingly common practice that has demonstrated benefits [(1)].

Alkaline phosphatase is found in almost all body cells, including red blood cells, liver, bile ducts and bone. It is a hydrolase enzyme responsible for removing phosphate from many types of molecules [(2)]. It is found in many forms depending on its origin within the body. It plays an integral role in metabolism within the liver and development within the skeleton. Due to its widespread prevalence in these areas, its concentration in the bloodstream is used as a biomarker helping in diagnosis such as in cases of hepatitis or osteomalacia [(4)]. Abnormal levels of alkaline phosphatase in the blood could indicate issues relating to the liver, gall bladder or bones. Kidney tumors, infections as well as malnutrition

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have also shown abnormal level of alkaline phosphatase in blood [5].

Haptoglobin (Hp) is an α2-sialoglycoprotein with hemoglobin (Hb)-binding capacity. The best-known biological function of Hp is to capture Hb to prevent both iron loss and kidney damage during hemolysis [5].

Aim of the Work

The aim of the present study was to investigate whether serum alkaline phosphatse & Hp level could be used for the early diagnosis and prediction of hyperbilirubinemia in newborns.

Patients and Methods

1) Patients selection:

The study is a prospective clinical study design, which was carried out in Pediatric Department, Faculty of Medicine, Aswan University Hospital. It included 100 newborns with gestational age more than 35wk delivered in obstetric department. **The study was approved by the Ethics Board of Aswan University.**

2) Methods:

1. Full history taking:

With particular emphasis on:

Maternal mode of delivery, parity, gravidity, blood group, previous abortion, history of previous sibling affected. Baby's gender, birth weight, blood group, history of NICU admission and phototherapy and its duration.

2. Full clinical examination:

With particular emphasis on: Birth weight and full neonatal examination from head to toe immediately after birth and in follow up visits.

3. Laboratory investigations:

Specific investigations:

Procedure for Umbilical Cord Blood Sampling

Before initiation of the umbilical cord blood sampling, all of the supplies needed should be gathered. Next, the following schedule of events was recommended:

1. Remind the delivering provider to clamp the distal end of the umbilical cord. Inspect the fetal side of the placenta for signs of umbilical vessel rupture.
2. Swab the base of the cord insertion on the placenta and up the umbilical cord 8 to 10 cm 3 times with povidone-iodine.
3. Allow the betadine to dry. While waiting, use the alcohol pad to wipe the top of the blood culture bottle. Once the povidone-iodine has dried, grasp the umbilical cord and insert the 18-gauge needle, bevel down, into the umbilical vein 6 to 8 cm above the placental insertion site.
4. 5 ml of umbilical cord blood had been drawn from each case after fetal delivery and the sample had been collected in two plain tubes.
5. The tubes had been placed in water bath to be clotted.
6. Then the tubes has been centrifuged at 3000 rpm for 10 min. for serum collection
7. Collected serum was divided into 2 eppindorf tubes (1 ml) for estimation of:
    a. Alkaline Phosphatase
    b. Haptoglobin (samples stored for maximum of 3 days at 2-8^{o}C)
    c. Sample with haemolysis or with lipaemia were excluded

1. Cord blood alkaline phosphatase:

Alkaline Phosphatase and Bilirubin level has been estimated using the automated chemistry analyzer (BT-3500).

ALP is a zinc-containing metalloenzyme; it is activated by Mg2+ and other divalent ions. ALP was measured in a reaction in which it catalyzed the cleavage of phosphate from 4-nitrophenyl phosphate (4-NPP, 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. AMP is usually included in the reaction mixture as a phosphate acceptor; the reaction proceeds in the absence of a specific acceptor (phosphate being transferred to water) but the rate is increased by such an acceptor. Alkaline phosphatase activity is measured in serum or heparinised plasma. Plasma from blood anticoagulated with citrate, oxalate or EDTA should not be used as these substances bind activating cations. No special precautions are required. Activity is stable in serum for 4 h at room temperature; a slight increase in activity (~2%/24 h) may occur at 4 °C.

2. Cord blood haptoglobin:

Quantitative determination of Haptoglobin has been done using immunoturbidimetric method (Ben-Biochemical Enterprise S.r.l- Italy).

Sample concentration of haptoglobin had been estimated after reading the absorbance of the tested sample against calibrator. Determination of Haptoglobin may be done by an immunoturbidimetric method, by automatic analyzers or in manual. Mixing a sample with a precise antigen to a solution having the corresponding anti-serum (Antibody), in a well-defined ratio. It is possible to have turbidity. The use of undiluted sample may require bichromatism (multipoint calibrator traceable to the CRM 470 International Standard was used). It is possible to prepare a Calibration Curve to refer, generally not rectilinear and not crossing the origin. Plotting on the Calibration Curve absorbance values and concentration for each single sample, may be determined by the concentration of each sample.

All newborns were observed daily for appearance of jaundice in first 2 weeks of life.

When jaundice appeared clinically, the patient was subjected to:

1- 5 ml of fetal venous blood had been collected and divided into 2 tubes:
   1- EDTA tube used for estimation of complete blood picture with reticulocytic count and direct Coombs' test
   2- Plain tube for estimation of:
      a. Bilirubin level (Total and Direct using the automated chemistry analyzer (BT-3500))
      b. Blood group and Rh typing
      c. Direct Coombs' test
3. Maternal blood group also had been determined

Statistical analysis of the data:

- Data were fed to the computer 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 two independent population were done using independent t-test while more than two population were analyzed F-test (ANOVA) to be used. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

The prognostic value of the tests determined by:

1) Sensitivity of the test: the percent of the positives by the test and the true positives.
2) Specificity of the test: the percent of the negatives by the test and the true negatives.
3) Accuracy: the percent of agreement between the two tests.

Results

Table (1): Socio-demographic and obstetric characteristics of the studied cases

Variable	Category	N = 100
Sex	Male	39 (39%)
	Female	61 (61%)
Mode of Delivery (n=98)	Vaginal	18 (18.4%)
	CS	80 (81.6%)
Birth Weight in Kg	Mean ± SD	3.1 ± 0.3
Gravidity	Median & Range	3 (2.5 – 3.5)
	Mean ± SD	2.2 ± 1.2
Parity	Median & Range	2 (1 – 5)
	Mean ± SD	2.02 ± 1.04
	Median & Range	2 (1 – 4)
Gestational age Pre term 39 (39%) **Full term** 61 (61%)		
Previous Siblings Affected with Jaundice	Yes	14 (14%)
	No	86 (86%)

Table (1) showed that males were 39 (39%) and females 61(61%). NVD delivery was 18 (18%) and CS delivery was 80 (81%). Gravidity ranged from 1-5 with mean value 2.2 ± 1.28. Parity ranged from 1-4 with mean value of 2.02 ± 1.03. Birth weight ranged from 2.500-3.500 with mean value of 3.055 ± 0.299 kg. Cases with previous sibling were 14 (14%), and as regards gestational age; preterm was 39 (39.0%), and full term was 61(61.0%).

Table (2): Socio-demographic & Obstetric data differences between non-jaundiced (normal) and jaundiced cases

	Not jaundiced (No.=43)	**Jaundiced (No.=57)**	**P-value**
Sex:			
• **Male**	17 (43.6%)	22 (56.4%)	= 0.544*
• **Female**	26 (42.6%)	35 (57.4%)	
Mode of Delivery:			
• **Vaginal**	8 (44.4%)	10 (55.6%)	= 0.503*
• **CS**	33 (41.3%)	47 (58.8%)	
Birth Weight (Mean ± SD)	3.02 ± 0.29	3.08 ± 0.31	= 0.259**
Gravidity (Mean ± SD)	2.1 ± 1.3	2.3 ± 1.2	= 0.382**
Parity (Mean ± SD)	1.9 ± 1.1	2.1 ± 1.0	= 0.454**
Previous Siblings Affected with Jaundice:			
• **No**	42 (49.8%)	44 (51.2%)	**= 0.003**
• **Yes**	1 (7.1%)	13 (92.9%)	

*Chi-square test was used to compare the percentages between groups
**T-test was used to compare the mean difference between groups
***Mann-Whitney U test was used to compare the median difference between groups

Table (2) showed that:

- There was no statistical significant relation between jaundice appeared clinically and sex of newborns, mode of delivery, birth weight, gravidity, and parity (P value: 0.5, 0.5, 0.3, 0.2, 0.3, 0.4 respectivelly).
- The most risk factor which might affect the appearance of jaundice was previous sibling affected with jaundice (P value: 0.003) and that was highly significant.

Table (3): Comparison between jaundiced and non-jaundiced cases as regarding cord Alkaline phosphatase and Haptoglobin

	Not jaundiced cases "n=43"	**Jaundice cases "n=57"**	**t-test**	**p**
Cord Alkaline phosphatase Mean S.D.	266.12 68.48	392.36 14.76	14.65	0.001*
Cord haptoglobin Mean S.D.	14.23 4.71	5.96 1.56	12.13	0.0001*

* Significant at level 0.05

Table (3) showed that cord blood alkaline phosphatase in non-jaundiced cases ranged from 150.3-420 with a mean value of 266.12 ± 68.48, and in jaundiced cases ranged from 239.5-616.8 with mean a value of 392.36 ± 114.76. Mean value of cord blood alkaline phosphatase in jaundiced cases was statistically significantly higher than in non-jaundiced cases (P value: 0.001). On the other hand, cord blood haptoglobin in non-jaundiced cases ranged from 2.26-9.53 with a mean value of 5.11 ± 1.82 and in jaundiced cases ranged from 4.37-48.4 with a mean value of 12.8 ± 9.47. Mean value of cord blood Hp in jaundiced cases was statistically significantly lower than in non-jaundiced cases (P value: 0.001).

Table (4): Comparison between cord Alkaline phosphatase among jaundiced and non-jaundiced cases as regards onset of the disease

	Not jaundiced		**Jaundiced at D1**		**Jaundiced at D2**		**Jaundiced at D3**	**Jaundiced beyond D3**	**F**	**p**
Cord ALP Mean S.D.	150.3 8.5		397.31* 17.32		361.42* 19.51		417.92* 16.07	368.88* 20.06	18.11	0.035*
p	**P1** **P2** **P3** **P4**	0.023* 0.015* 0.014* 0.456	**P5** **P6** **P7**	0.175 0.438 0.553	**P8** **P9**	0.544 0.275	**P10**	0.613		

* Significant at level 0.05

P1 *comparison between not jaundiced and jaundiced at D1*
P2 *comparison between not jaundiced and jaundiced at D2*
P3 *comparison between not jaundiced and jaundiced at D3*
P4 *comparison between not jaundiced and jaundiced beyond D3*
P5 *comparison between jaundiced at D1 and jaundiced at D2*
P6 *comparison between jaundiced at D1and jaundiced at D3*
P7 *comparison between jaundiced at D1 and jaundiced beyond D3*
P8 *comparison between jaundiced at D2 and jaundiced at D3*
P9 *comparison between jaundiced at D2 and jaundiced beyond D3*
P10 *comparison between jaundiced at D3 and jaundiced beyond D3*

Table (4) showed that cord blood Alkaline phosphatase in non-jaundiced cases ranged from (150.3 - 420.1) with a mean value of 266.12 ± 68.5, in jaundiced cases at D1 ranged from 240.50 to 598.30) with a mean value of 397.31±117.32. Jaundiced cases at D2 ranged from 239.50 to 582.80 with a mean valueof 361.42 ± 109.51. Jaundiced cases at D3 ranged from 244.60 to 616.80. Lastlly, jaundiced cases beyond D3 ranged from 251.10 to 578.00) with a mean value of 368.88 ± 120.06. Level of cord blood ALP was significantly higher in jaundiced cases at D1, D2, D3, and beyond D3 than non-jaundiced cases with P values: P1=0.0023, P2=0.015, P3=0.014, P4=0.045 respectively. Mean value of cord blood ALP in jaundiced cases at D1 was higher than that of D2, and beyond D3 but these relations was found statistically insignificant (P5=0.175, P7=0.553, P6=0.438, respectively). Comparing level of cord blood ALP in jaundiced cases at D2, D3, and beyond D3, mean value at D3 was higher than D2 and beyond D3 but these relations were found statistically insignificant. (P8=0.544**,** P9=0.275, P10=0.613 respectively).

Table (5): Comparison between cord haptoglobin among jaundiced and not jaundiced cases as regards onset of the disease

	Not jaundiced		**Jaundiced at D1**		**Jaundiced at D2**		**Jaundiced at D3**		**Jaundiced beyond D3**	**F**	**p**
Cord Haptoglobin Mean S.D.	14.23 10.71		5.45 1.62		6.17 1.42		6.23 1.57		5.96 1.63	8.069	0.002*
P	**P1** **P2** **P3** **P4**	0.001* 0.002* 0.003* 0.001*	**P5** **P6** **P7**	0.365 0.425 0.331	**P8** **P9**	0.652 0.413	**P10**	0.378			

* Significant at level 0.05

P1 *comparison between not jaundiced and jaundiced at D1*
P2 *comparison between not jaundiced and jaundiced at D2*
P3 *comparison between not jaundiced and jaundiced at D3*
P4 *comparison between not jaundiced and jaundiced beyond D3*
P5 *comparison between jaundiced at D1 and jaundiced at D2*

P6 *comparison between jaundiced at D1and jaundiced at D3*
P7 *comparison between jaundiced at D1 and jaundiced beyond D3*
P8 *comparison between jaundiced at D2 and jaundiced at D3*
P9 *comparison between jaundiced at D2 and jaundiced beyond D3*
P10 *comparison between jaundiced at D3 and jaundiced beyond D3*

Table (5) showed that cord blood haptoglobin in non-jaundiced cases ranged from 2.26 to 48.4 with a mean value of 14.23 ± 10.71. In jaundiced cases at D1 ranged from (3.87-9.04) with mean value (5.45 ± 1.62), jaundiced cases at D2 ranged from (3.93-9.24) with mean value (6.17 ± 1.42), jaundiced cases at D3 ranged from (3.83-9.53) with mean value (6.23 ± 1.57), and lastlly jaundiced cases beyond D3 ranged from (4.32-8.20) with mean value (5.96 ± 1.63). Level of cord blood Hp was significantly lower in jaundiced cases at D1, D2, D3, and beyond D3 than non-jaundiced cases (P1=0.001, P2=0.002, P3=0.003, P4 = 0.001 respectively). Mean value of cord blood Hp in jaundiced cases at D1 was lower than that of D2 and beyond D3, but these relations were found statistically insignificant (P5=0.365, P6=0.425, P7= 0.331 respectively). Comparing level of cord blood Hp in jaundiced cases at D2, D3, and beyond D3; mean value at D3 was lower than D2 and beyond D3 but these relations were found statistically insignificant (P8=0.652, P9=0.413, P10=0.378 respectively).

Table (6): Comparison between cord ALP and cord haptoglobin among jaundiced and non- jaundiced cases as regards gestational age

	Pre term		**Full term**	
	Non jaundice	**Jaundiced**	**Non jaundiced**	**Jaundiced**
Cord ALP				
Mean	282.9	394.5	255.2	391.0
S.D.	72.4	22.5	64.9	111.4
P1	0.021*			
P2	0.583			
P3	0.054*			
P4	0.157			
P5	0.001*			
Cord Haptoglobin				
Mean				
S.D.	16.76	6.12	12.57	5.86
	2.12	1.48	2.57	1.622
P1	0.001*			
P2	0.531			
P3	0.001*			
P4	0.221			
P5	0.001*			

*** Significant at level 0.05**
P1 *comparison between Pre term not jaundiced and Jaundiced cases*
P2 *comparison between Pre term not jaundiced and Full term not jaundiced cases*
P3 *comparison between Pre term not jaundiced and Full term jaundiced cases*
P4 *comparison between Pre term jaundiced and Full term jaundiced cases*
P5 *comparison between Full term not jaundiced and Full term jaundiced cases*

Table (6) showed that regarding level of cord blood ALP, its level in non-jaundiced preterms ranged from 165.1 to 420.1 with a mean value of 282.9 ± 72.4. In jaundiced preterms ranged from 239.5 to 616.8 with a mean value of 394.5 ± 122.5 while its level in non-jaundiced full terms ranged from 150.3 to 417 with a mean value of 255.2 ± 64.9 and in jaundiced full terms ranged from 240.5 to 592.9 with a mean value of 391.0 ± 111.4. Mean value of cord blood ALP in jaundiced pre-terms & full terms was significantly higher than mean value of cord blood ALP in non-jaundiced pre-terms & full terms (P1= 0.001, P5=0.001 respectively). Mean value of cord blood ALP in jaundiced full terms was significantly higher than non-jaundiced preterm (P3=0.054). No difference between non- jaundiced pret-erms & full terms or jaundiced pre-terms & full terms as regards ALP. (P2=0.583, P4=0.157 respectively)**.**

Regarding level of cord blood Hp**,** its level in non-jaundiced pre-terms ranged from 3.02 to 48.4 with a mean value of 16.76 ± 12.12. In jaundiced preterms ranged from 3.93 to 9.04 with a mean value of 6.12 ± 1.48) while its level in non-jaundiced fullterms ranged from 12.26 to 41.4 with a mean value of 12.57 ± 9.57 and in

jaundiced full terms ranged from 3.83 to 9.53 with a mean value of 5.86 ± 1.622. Mean value of cord blood Hp in jaundiced pre-terms & full terms was significantly lower than mean value of cord blood Hp in non-jaundiced pre-terms & full terms (P1= 0.001, P5=0.001 respectively). Mean value of cord blood Hp in jaundiced full terms was significantly lower than non-jaundiced preterms (P3=0.003). No difference between non-jaundiced pre-terms & full terms or jaundiced pre-terms & full terms as regards Hp (P2=0.531, P4=0.221 respectively).

Table (7): Correlation between cord ALP, cord haptoglobin among jaundiced cases as regards bilirubin level at onset and max bilirubin level.

		Cord ALP	**Cord Haptoglobin**
Max. Bilirubin level	Pearson Correlation	0.311**	-0.362**
	Sig. (2-tailed)	0.021	0.001
Bilirubin level at onset (jaundiced cases)	Pearson Correlation	0.411	-0.377*
	Sig. (2-tailed)	0.001*	0.027

Table (7) showed that cord blood ALP correlates positively with max. bilirubin level and bilirubin level at onset, and such relation was statistically significant. On the other hand, cord blood Hp correlates negatively with max. bilirubin level and bilirubin level at onset, and such relation was statistically significant.

Table (8): Cut off value, sensitivity, specificity, PPV, NPV and accuracy of ALP and hepatoglobin in prediction the jaundice.

Area Under the Curve

Test Result Variable(s)	**Area**	**Std. Error**	**Sig.**	**Asymptotic 95% Confidence Interval**	
				Lower Bound	**Upper Bound**
Cord ALP	.842	.040	.0001*	.763	.921
Cord Haptoglobin	.887	.027	.0001*	.809	.968

Coordinates of the Curve

Test Result Variable(s)	**Cut off value**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
Cord ALP	240.00	98.2	72.8	95.0	92.0	93.0
Cord Haptoglobin	<5.5	80.6	86.0	82.0	81.0	85.0

*Sensitivity (true positives/all diseased); specificity (true negatives/all non-diseased); PPV (true positives/all test positives); NPV (true negatives/all test negatives).

Table (8): showed Goodness criteria of Cord blood ALP & Haptoglobin for diagnosis of neonatal jaundice in the studied sample:

Cut off value of Cord blood ALP & Hp was 240.00, 5.5repectively.

Sensitivity of Cord blood ALP & Hp was 98.2%, 80.6% respectively

Specificity of Cord blood ALP & Hp was 72.8%, 86.0% respectively

PPV of Cord blood ALP & Hp was 95.0 %, 82.0% respectively

NPV of Cord blood ALP & Hp was 92.0 %, 81.0respectively

Accuracy of Cord blood ALP & Hp was 93.0%, 85.0% respectively in prediction the jaundice.

Discussion

The term kernicterus refers to the clinical features of bilirubin encephalopathy. Its risk is increased in term babies with very high bilirubin levels. Kernicterus is also known to occur at lower levels of bilirubin in term babies who have risk factors and in preterm babies **(6).**

While jaundice per se is not preventable, nonetheless early detection of threatening bilirubin levels permits initiation of phototherapy and prevents higher risks and high cost exchange transfusion therapy or kernicterus. The concept of prediction of jaundice offers an attractive option to pick up babies at risk for neonatal hyperbilirubinemia. Therefore, early diagnosis of jaundice and timely actions are necessary. Several methods have been used to determine risk of neonatal hyperbilirubinemia **(5).**

In our study, we used alkaline phosphatase (ALP) and haptoglobin (Hp) levels at birth as a biochemical marker for determination of hyperbilirubinemia. This study was carried out in Neonatal Intensive Care Unit of Aswan University Hospital on 100 neonates. Our study is a prospective study to assess umbilical cord blood ALP and HP levels to significantly predict hyperbilirubinemia in newborns more than 35 weeks on the basis of serum bilirubin measurements performed within7 days of life.

In terms of demographic data, males represented 39 % of the jaundiced cases in the study and females represented 61 %. The difference was statistically insignificant. This is inagreement with a study carried out by ***Ahire et al.*** **(7)** who studied correlation of cord blood bilirubin with neonatal hyperbilirubinemia and found no statistical significance in the gender of his study newborns. Also, in agreement with ***Huang et al.*** **(8)** study in which 72 neonates with hyperbilirubinemia were monitored for their bilirubin values. He stated that no gender difference was demonstrated for any of the nine risk factors except for G6PD deficiency.

Our study showed no statistically significant difference between the jaundiced and the non-jaundiced cases regarding the mode of delivery, maternal gravidity and parity. This is in agreement with ***Alkan et al.*** **(9)** in which 168 infants delivered by C⁄ S and 155 by normal spontaneous vaginal delivery where he stated that the route of delivery had no effect on TBL. Also, in agreement with ***Betul et al.*** **(10)** study**,** who reported that the mode of delivery was not a risk factor for rate of hyperbilirubinemia.

As regards previous affected sibiling, we found a significant relation between clinically appearance of jaundice and previous sibling affected with neonatal hyperbillirubinemia, which may reflect genetic susceptibility for hyperbillirubinemia. This is in agreement with ***Radha et al.*** **(11)** who studied 216 neonates, 57 % showed significant jaundice and 36% of the jaundiced infants whose TSB greater than 15 mg/dL had a previously affected sibiling.

On the other hand, our results revealed no significant relation between gestational age and clinically appearing jaundice. This comes in agreement with ***Betul et al.*****(10)** who stated that in infants born after 34 weeks of gestational age, the 24 and 48 hours' bilirubin levels were found similar in late preterm and term infants. He elaborated that late preterm infants were more frequently delivered by c/s and had insignificantly delayed meconium passage but received higher amounts of formula supplementation. The similarity of the first 48 hours bilirubin levels to term infants may be a consequence of close follow up and early nutritional support of this special group of infants. While was incompatible with ***Wang et al.*****(12)** who concluded that near-term infants had significantly more medical problems and increased hospital costs compared with fullterm infants and that discrepancy with our study data is explained by limited study samples.

In our study, we found that cord blood alkaline phosphatase in jaundiced cases was statistically significantly higher than non-jaundiced cases. This is in agreement with ***Ahmadpour-Kacho et al.*** **(13)** who studied a total of 102 healthy term infants and stated that there was a significant difference in the levels of cord blood alkaline phosphatase between the non-jaundiced and clinically jaundiced newborns.

Furthermore, our results showed that level of cord blood ALP was significantly higher in jaundiced cases at D1, D2, D3, and beyond D3 than non-jaundiced cases. This is in agreement with ***Nalbantoglu et al.*** **(1)** who found that Serum ALP levels rose in proportion to total bilirubin levels on the first, second and third day of life. In addition, our results showed that there was no difference between non-jaundiced preterms & fullterms or jaundiced preterms & fullterms as regard ALP.

This is in agreement with ***Hilderbrand et al.*** [14] who studied the level of ceruplasmin and alkaline phosphatase in cord serum blood of term, preterm, and physiologically jaundiced neonates, and stated that no significant difference was seen in alkaline phosphatase level in cord serum blood from term and preterm infants.

Our study revealed that there was significant positive correlation between cord blood ALP and maximum bilirubin. This is in agreement with ***Ahmadpour-Kacho et al.***[13] who found that ALP level rose parallel to total bilirubin levels reaching its higher values along with maximum values of bilirubin. Also, in agreement with ***Nalbantoglu et al.*** [1] who found that Serum ALP levels rose in proportion to total bilirubin levels on the first, second and third day of life.

In our study, we found that cord blood haptoglobin in jaundiced cases was statistically significantly lower than in non-jaundiced cases. Also in our results, level of cord blood Hp was significantly lower in jaundiced cases at D1, D2, D3, and beyond D3 than in non-jaundiced cases. This was in agreement with ***El-Gendy et al.*** [15] study in which 61 healthy newborns of at least 35 weeks gestation were followed in the first 5 days of life for the development of significant hyperbilirubinemia. They concluded that haptoglobin and TSB values taken from the blood of the umbilical cord can be used as a guiding indicator to demonstrate the future occurrence of significant hyperbilirubinemia in newborns.

In our study, we found that cord blood Hp in jaundiced cases with positive comb test was lower than those with negative comb test but this was statistically insignificant. This was compatible with ***Cakmak et al.*** [3] who stated that it is difficult to obtain laboratory documentation of mild degrees of hemolysis among newborns. Such infants are unlikely to have decreasing hemoglobin concentrations, elevated reticulocyte counts, or abnormalities on peripheral smears. In any case, these laboratory tests are generally nonspecific and insensitive for newborns.

In our study, we found that cord blood Hp in jaundiced cases treated by phototherapy and exchange transfusion were lower than those treated by observation & follow up but this difference was statistically insignificant. This was in disagreement with ***El-Gendy et al.*** [15] who stated that as a result of the study, we can determine in advance that the healthy term neonatal jaundice will increase by checking haptoglobin in the UC blood. Thus, we can prevent problems based on early discharge by closer monitoring of the babies, and prompt treatment accordingly this may be attributable to our small sample size.

In our study, the mean level of alkaline phosphatase in the studied newborns was 392.36 ± 114.76 IU/L, which was more than that in the existing reports. ***Fenton et al.*** [16] found the mean level of cord blood alkaline phosphatase 159 ± 49 IU/L. Compared to our results, there is a big difference. Another local study by ***Abbasian et al.*** [17] in Shahrood, Iran showed that mean cord blood alkaline phosphatase level was 314.34 ± 122.42 IU/L, which is compatible with our findings. The average level of cord blood alkaline phosphatase in Egyptian and Iranian newborns seems to be higher than in other populations.

A cut-off point level of ALP in cord blood of 240 mg/dl in our study was determined to have the highest sensitivity 98.2%, specificity 72.8%, and PPV 95%. Thus, prediction of neonatal hyperbilirubinemia can be performed in newborns with cord blood ALP levels more than 240 mg/dl, which is slightly lower than ***Ahmadpour-Kacho et al.*** [13] in which cord blood alkaline phosphatase level was with sensitivity and specificity of 80% and 63% respectively in cutoff level > 314 IU/L predicts a need for treatment.

A cut-off point level of Hp in cord blood of 5.5 mg/dl in our study was determined to have the highest sensitivity 80.6%, specificity 86.0%, and PPV 82.0%. Thus, prediction of neonatal hyperbilirubinemia can be performed in newborns with cord blood Hp levels less than 5.5 mg/dl which is slightly lower than ***El-Gendy et al.*** [15] in which cut-off level of Hp in cord blood was 7.5 mg/dl as obtained by the ROC curve and it was determined to have the highest sensitivity (100%), specificity (81%), and positive predictive value (PPV) (86%) in the prediction of occurrence of significant hyperbilirubinemia on the fifth day.

Conclusion & Recommendations

From our work, we conclude that:

1. The cord blood alkaline phosphatase level is not only a useful predictor for severe neonatal jaundice but also can predict the early onset of neonatal hyperbilirubinemia and expected methods of treatment in healthy newborns more than 35 weeks gestational age.
2. The cord blood haptoglobin is a useful predictor for neonatal hyperbilirubinemia.

3. We recommend using UC blood for measurement of ALP and Hp as a predictor of neonatal hyperbirubinemia as it is a painless and easy method.
4. Prevention of problems based on early discharge by closer monitoring of the babies, who we determined as risky in terms of hyperbilirubinemia. Besides, with this method, it is possible to determine the newborns with low hyperbilirubinemia risk and to prevent unnecessary monitoring and care of numerous cases.

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