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Original Research Article

Correlation of cord blood alkaline phosphatase level with serum bilirubin level in term neonates with jaundice: an observational study

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ABSTRACT

Background: Neonatal hyperbilirubinemia remains a significant concern in neonatal care, affecting newborn health and requiring early identification for timely intervention.
Methods: This observational study was conducted in the Department of Pediatrics at Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, from July 2024 to December 2025. A total of 145 term inborn neonates were enrolled. Cord blood samples were collected at birth for estimation of alkaline phosphatase (ALP) levels. Neonates were followed up for the first 5 d of life with daily transcutaneous bilirubin monitoring, and serum bilirubin estimation was performed when indicated. Statistical analysis included Spearman's correlation and receiver operating characteristic (ROC) curve analysis. Sensitivity, specificity, and predictive values were calculated.
Results: Cord blood ALP levels showed a moderate positive correlation with maximum serum bilirubin levels (ρ=0.541, p=0.001). Of the 145 neonates, 33 (22.8%) required phototherapy. Roc analysis demonstrated fair predictive ability of alp for phototherapy requirement (auc=0.695). The optimal cut-off value of 117.5 u/l yielded sensitivity of 51.85%, specificity of 84.85%, positive predictive value of 91.80%, and negative predictive value of 35.0%.
Conclusions: Cord blood alp is a simple, non-invasive, and cost-effective biomarker that correlates significantly with serum bilirubin levels. It may be useful for early identification of neonates at risk of hyperbilirubinemia, enabling timely monitoring and intervention, particularly in resource-limited settings.

Keywords: Neonatal jaundice, Hyperbilirubinemia, Cord blood alkaline phosphatase, Serum bilirubin, Phototherapy

INTRODUCTION

Neonatal jaundice may have first been described in a Chinese textbook 1000 years ago. Neonatal hyperbilirubinemia/neonatal jaundice is yellowish discoloration of the skin, sclera, and mucous membrane which is mainly caused by increase in serum bilirubin levels. The term jaundice derives from the French word "jaune," which means yellow.[1] The prevalence of jaundice is ~60% in term infants and 80% in preterm infants during the 1st week of life.[2] When neonatal jaundice is clinically identified, the underlying etiology of neonatal hyperbilirubinemia must be determined.[3,4]

The two types of neonatal hyperbilirubinemia are unconjugated hyperbilirubinemia (UHB) and conjugated hyperbilirubinemia (CHB). Bilirubin is produced from the catabolism of heme, a breakdown product of hemoglobin, in the reticuloendothelial system (RES). Newborn infants have higher TSB levels than adults due to higher hemoglobin levels at birth, a shorter RBC life span, and limited conjugating ability of the neonatal liver.[5]

Healthy, full-term newborns typically have peak serum bilirubin concentrations of 5 to 6 mg/dL compared to adult levels of <1 mg/dL.

Pathologic jaundice in neonates is related to increased production of bilirubin in the RES, impaired hepatic uptake, deficient conjugation of bilirubin, and enhanced enterohepatic circulation of bilirubin. In severe hyperbilirubinemia, unbound, unconjugated bilirubin crosses the blood-brain barrier and binds to the brainstem, hippocampus, cerebellum, globus pallidus, and subthalamic nuclei. At the cellular level, bilirubin inhibits certain mitochondrial enzymes, interferes with DNA and protein synthesis, induces breaks in DNA strands, and hampers phosphorylation. Bilirubin also impairs tyrosine uptake and alters the normal functioning of N-methyl-D-aspartate–receptor ion channels.[6] These mechanisms are implicated in the pathogenesis of bilirubin toxicity that clinically manifests as bilirubin-induced neurologic dysfunction (BIND) and bilirubin encephalopathy.[7] Hence, early prediction of significant jaundice and timely interventions have been crucial to minimize bilirubin induced neurological damage.

There is concern about an increasing incidence of kernicterus in healthy term neonates. Several potential strategies that are intended to prevent kernicterus have been proposed by experts. Availability of simple, economical, non-invasive, and reliable markers allowing physicians to recognize which of the neonates discharged early are at higher risk for development of significant hyperbilirubinemia become necessary in these situations so as to initiate treatment as early as possible and thus reduce risk of bilirubin-induced brain damage. This ideal marker should also help physicians in early discharge of neonates and selectively follow-up of high-risk ones. Different methods have been proposed to assess risk of hemolysis and hyperbilirubinemia in neonates.[8]

ALP is one such marker being researched as an early detector of NNH. It is an intracellular hydrolase enzyme. It is associated with erythrocyte membrane activity and hemolysis and has been proposed as a potential early indicator of increased bilirubin production. Increased cord blood ALP levels may reflect enhanced hemolysis, thereby predisposing neonates to higher bilirubin levels. Hence, studying the correlation between cord blood ALP and subsequent serum bilirubin levels in term neonates with jaundice can provide a simple, early, and cost-effective predictive tool, facilitating better monitoring, timely initiation of phototherapy, and improved neonatal outcomes. So, this study was conducted to assess the reliability of cord blood ALP as an early predictor of neonatal jaundice in full-term neonates. Aims and objectives were to correlate cord serum ALP level with serum bilirubin level in term neonates with jaundice.

METHODS

Study design

This is an observational study, conducted in Pediatrics Department of Sri Guru Ram Das Institute of medical sciences and Research, Sri Amritsar.

Study participants, ethical approval and informed consent

All term inborn neonates were enrolled after fulfilling the inclusion and exclusion criteria. Written informed consent was taken from parents/guardians of the eligible neonates after providing all the necessary information about the study and after approval from Institutional Ethics Committee, Sri Guru Ram Das Institute of medical sciences and Research, Sri Amritsar before conducting the study.

Duration of the study was from July 2024 to December 2025.

Inclusion criteria included all term (gestation age ≥37 weeks) inborn neonates.

Exclusion criteria excluded neonates born to mothers with diabetes mellitus, eclampsia, bone disorders, liver and kidney diseases. And neonates who require NICU stay after birth for various conditions other than jaundice.

After providing the baby with routine care in delivery room, the umbilical cord was clamped and wiped with antiseptic. Then 1 ml of venous sample from the cord was obtained in a red top additive free vacutainer for the estimation of serum ALP levels. This sample was sent to biochemistry laboratory of Sri Guru Ram Das Institute of medical sciences and Research, Sri Amritsar, where it was assessed using VITROS Chemistry Products ALKP Slides after ultracentrifugation at 3000 rpm for 17 minutes which quantitatively measured ALP activity in serum in VITROS 5600 Integrated Systems. All the enrolled neonates were followed up in post-natal rounds regularly for assessment of clinical jaundice till day 5 of life or till discharge whichever was earlier. Transcutaneous bilirubin levels from forehead or sternum (average of three readings) were done daily for all Neonates from Day 1 consecutively till day 5 of life or till discharge whichever was earlier. Along with this, neonates developing clinical jaundice and transcutaneous bilirubin levels in the phototherapy range as per Bhutani charts, their serum bilirubin levels were also done. 1 ml of Blood sample drawn from a peripheral vein using standard aseptic precautions in a red top additive free vacutainer. These samples were sent to biochemistry laboratory of Sri Guru Ram Das Institute of medical sciences and Research, Sri Amritsar where serum bilirubin level were then assessed using VITROS Chemistry using VITROS 5600 integrated systems. Reports of serum bilirubin were collected and analysed as per Bhutani Charts. Neonates requiring treatment (Phototherapy or Exchange transfusion) were shifted to NICU of Sri Guru Ramdas Institute of Medical Sciences and Research, Sri Amritsar and those neonates not requiring any treatment were again be followed up on post-natal ward till day 5 of life or till discharge whichever was earlier. Admitted neonates were evaluated further to find out the cause of hyperbilirubinemia, by

sending neonatal jaundice work up as per protocols followed in NICU as per standard guidelines. All the relevant data of these neonates was collected and compiled in proforma and correlated with serum ALP levels.

Statistical analysis

Data was compiled and statistically analysed using descriptive and inferential statistics and valid conclusions were drawn. Data were described in terms of range; mean ±standard deviation (±SD), Median (IQR), frequencies (number of cases) and relative frequencies (percentages) as appropriate. To determine whether the data were normally distributed, a Kolmogorov-Smirnov test was used. Comparison of quantitative variables between the study groups was done using Mann Whitney U test for independent samples for non-parametric data. For comparing categorical data, Chi square ($\chi 2$) test was performed and fisher exact test was used when the expected frequency is less than 5. Spearman's correlation coefficient was used to analyze the correlation Covariates.

Receiver operator characteristics (ROC) curve was done, and criterion value was estimated depending on the specificity and sensitivity. Sensitivity, specificity, accuracy, positive predictive value, and negative predictive value were calculated. A probability value (p value) less than 0.05 was considered statistically significant. All statistical calculations were done using (Statistical Package for the Social Science) SPSS 26.0 version (SPSS Inc., Chicago, IL, USA) statistical program for Microsoft Windows.

RESULTS

The Figure 1 represents the flow diagram of study population which shows out of 158 after meeting inclusion and exclusion criteria a total of 145 candidates were included for our study. Table 1 shows demographic profile of 145 neonatal and maternal cases included in the study. Among the neonates, the gender distribution was almost equal, with 72 (49.7%) males and 73 (50.3%) females. Regarding birth weight, the majority of neonates, 83 (57.2%), had a birth weight between 2500-3000 grams, followed by 27 (18.6%) weighing less than 2500 grams, 26 (17.9%) weighing 3000-3500 grams, and only 9 (6.2%) weighing more than 3500 grams. In terms of gestational age, most neonates, 84 (57.9%), were born before 38 weeks of gestation, while 59 (40.7%) were delivered between 38-40 weeks and only 2 (1.4%) after 40 weeks. Maternal characteristics showed that multigravida mothers constituted the majority with 94 (64.8%) cases, whereas primigravida mothers accounted for 51 (35.2%). The mode of delivery was predominantly lower segment cesarean section (LSCS) in 115 (79.3%) cases, while normal vaginal delivery (NVD) was observed in 30 (20.7%) cases. Regarding maternal blood group distribution, O positive was the most common blood group seen in 56 (38.6%) mothers, followed by B positive in 50 (34.5%) and A positive in 22 (15.2%). Less common groups included AB positive in 7 (4.8%), B negative and O negative in 4 (2.8%) each, and A negative in 2 (1.4%), while no cases of AB negative blood group were reported. Overall, the study population was characterized by nearly equal neonatal gender distribution, predominance of birth weight between 2500-3000 grams, higher preterm births, multigravida mothers, cesarean deliveries, and O positive blood group mothers. (Table 1).

The table depicts the distribution of cord serum ALP (Cord SAP), maximum total serum bilirubin (TSB), and requirement of phototherapy among 145 neonates. With respect to Cord SAP levels, the majority of neonates, 99 (68.27%), had values between 100-200 U/L, followed by 37 (25.51%) with levels below 100 U/L. Only a small proportion had higher values, with 6 (4.1%) neonates in the range of 200-300 U/L and 3 (2.07%) having levels above 300 U/L. Regarding maximum TSB levels, most neonates, 64 (44.1%), had bilirubin levels between 11-14.9 mg/dL, followed by 45 (31.0%) with levels between 7-10.9 mg/dL.

Lower bilirubin levels of less than 7 mg/dL were observed in 13 (9.0%) neonates, while 17 (11.7%) had levels between 15-17.9 mg/dL and 6 (4.1%) had severe hyperbilirubinemia with TSB levels greater than 18 mg/dL. In terms of treatment, phototherapy was required in 33 (22.8%) neonates, whereas the majority, 112 (77.2%), did not require phototherapy. Overall, most neonates had cord SAP levels between 100-200 U/L, moderate bilirubin levels ranging from 11-14.9 mg/dL, and only about the one-fifth required phototherapy (Table 2).

Cord serum SAP levels showed a moderate positive correlation with maximum serum bilirubin (ρ=0.541), indicating that higher SAP levels are associated with higher bilirubin levels. This association was statistically significant (p=0.001), suggesting that cord SAP may be a useful predictor of the severity of neonatal hyperbilirubinemia (Table 3).

ROC curve analysis as shown in Figure 3, identified an optimal cut-off value of 117.5 U/L for cord serum ALP in predicting phototherapy requirement. At this threshold, the sensitivity was 51.85%, specificity was 84.85%, positive predictive value was 91.80%, and negative predictive value was 35.0%, with an overall diagnostic accuracy of 59.57% (Table 4).

Figure 2 shows a scatter plot showing relationship between cord serum ALP and maximum serum bilirubin level among all neonates. The scatter plot shows a positive trend between cord SAP levels and maximum serum bilirubin, indicating that higher SAP values are associated with higher bilirubin levels (Figure 2).

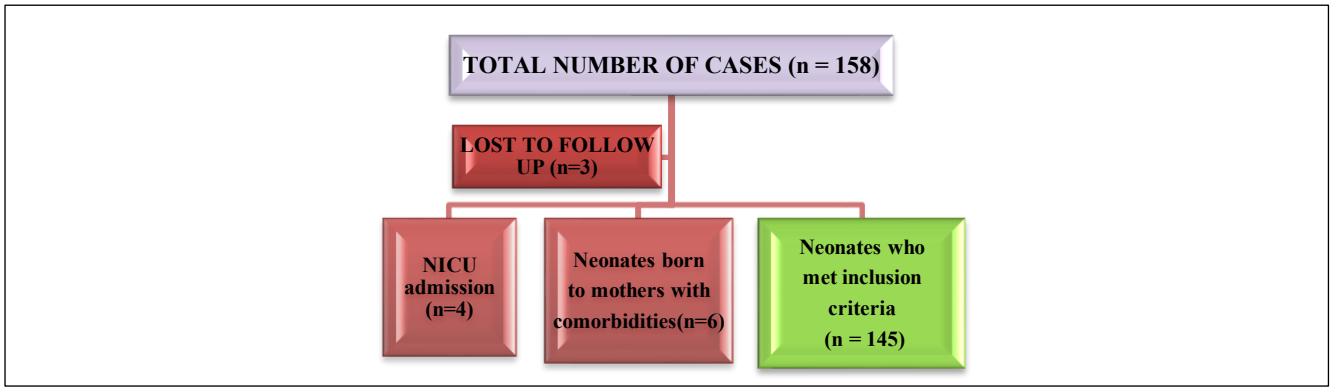

Figure 1: Flow diagram of study population and final study participant inclusion.

Table 1: Demographic details of the study population.

Demographics		N	Percentage
Neonatal			
Gender	Male	72	49.7%
	Female	73	50.3%
Birth weight (gram)	<2500	27	18.6%
	2500-3000	83	57.2%
	3000-3500	26	17.9%
	>3500	9	6.2%
Gestational age (in weeks)	<38	84	57.9%
	38-40	59	40.7%
	>40	2	1.4%
Maternal			
Gravida	Primigravida	51	35.2%
	Multigravida	94	64.8%
Mode of delivery	NVD	30	20.7%
	LSCS	115	79.3%
Maternal blood group	A positive	22	15.2%
	A negative	2	1.4%
	B positive	50	34.5%
	B negative	4	2.8%
	AB positive	7	4.8%
	AB negative	0	0%
	O positive	56	38.6%
	O negative	4	2.8%
	N	145	100%

Table 2: Distribution of cord SAP (U/L) and total serum bilirubin (mg/dL) among the neonates (n=145).

Cord SAP and Max TSB	Value	N	Percentage
CORD SAP (U/L)	<100	37	25.51%
	100-200	99	68.27%
	200-300	6	4.1%
	>300	3	2.07%
Maximum TSB (mg/Dl)	<7	13	9.0%
	7-10.9	45	31.0%
	11-14.9	64	44.1%
	15-17.9	17	11.7%
	>18	6	4.1%
Phototherapy	Yes	33	22.8%
	No	112	77.2%
	N	145	100%

Table 3: Correlation between cord SAP and maximum serum bilirubin levels (Spearman's rho).

Spearman's rho correlation between cord SAP and max TSB	Value	Max S. bilirubin
CORD SAP (U/L)	**Correlation coefficient (ϱ)**	**0.541**
	P value	0.001
	N	145

Table 4: Goodness criteria of cord blood alkaline phosphatase for diagnosis of neonatal jaundice.

Parameters	Value
Optimal cut-off of SAP (U/L)	117.5
Sensitivity	51.85%
Specificity	84.85%
Positive predictive value (PPV)	91.80%
Negative predictive value (NPV)	35.0%
Accuracy	59.57%

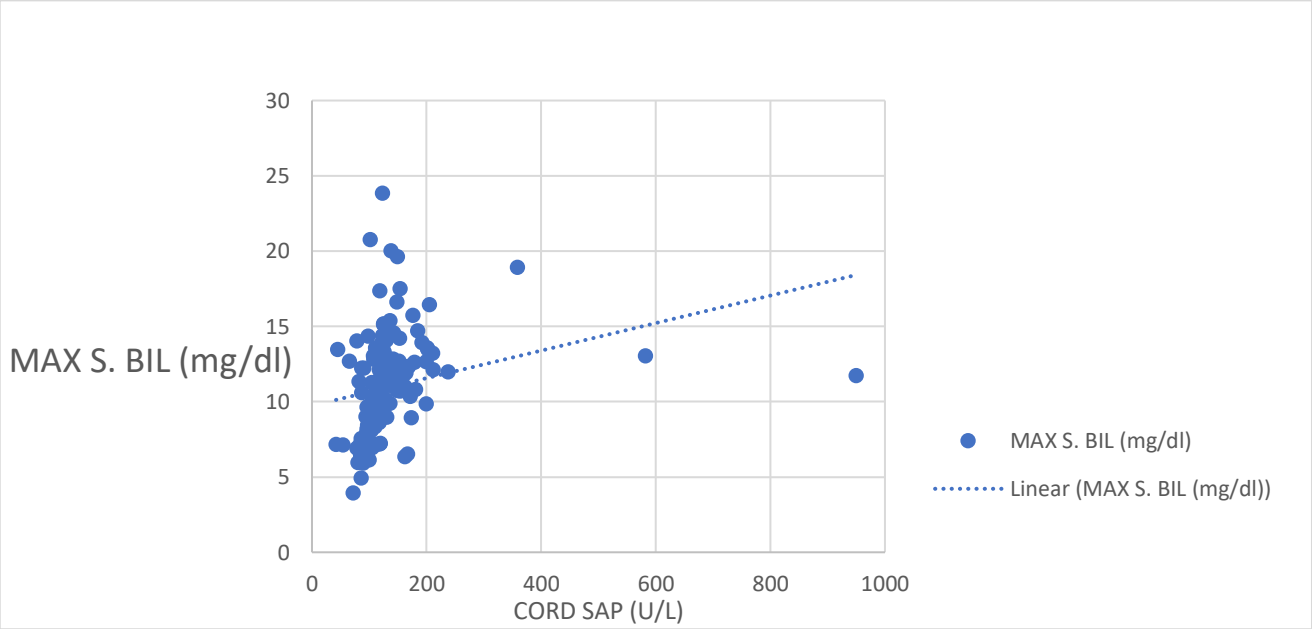

Figure 2: Relationship between cord SAP and maximum serum bilirubin levels.

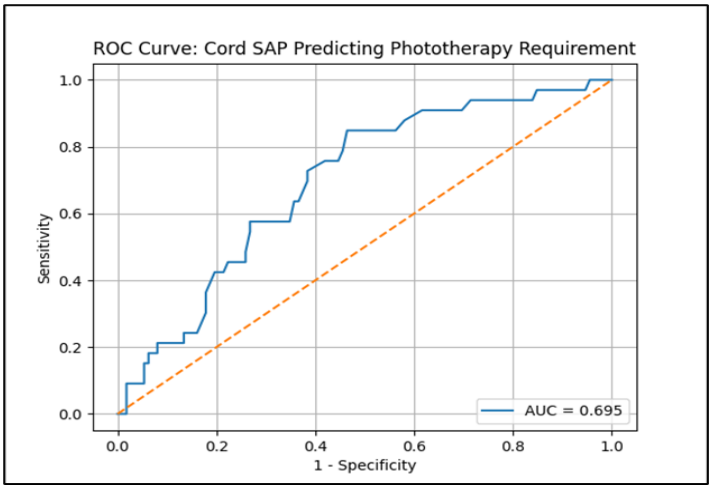

Figure 3 : ROC analysis of cord SAP for predicting phototherapy requirement, (n=33).

DISCUSSION

Different methods have been proposed to determine risk of neonatal hyperbilirubinemia as early as possible to prevent its complications. So, in recent years the research has increasingly focused on non-invasive methods of measuring bilirubin, particularly through transcutaneous bilirubin assessment. Studies have demonstrated a strong correlation between transcutaneous bilirubin and serum bilirubin values across diverse populations, including infants of varying gestational ages. The adoption of this technique has significantly reduced the need for painful venous punctures, making bilirubin monitoring safer and more comfortable for newborns.

However more recently, measurement of cord blood bilirubin level, alpha-fetoprotein and cord blood ALP has also been studied as a marker for detecting this risk at a very early stage. Diagnosing hyperbilirubinemia from cord blood sample helps to detect early, is overall safe and there are less chances of infection.

Therefore, the present study was conducted to evaluate the role of cord serum ALP as an early predictor of neonatal hyperbilirubinemia requiring phototherapy. The findings of this study highlight important associations between cord SAP levels and subsequent bilirubin trends, offering potential clinical utility in early risk stratification.

Our study included 145 neonates with nearly equal gender distribution, minimizing gender-based bias in outcomes. When compared statistically, there was no significant relationship between neonatal gender and hyperbilirubinemia. Similar findings were reported by Awasthi et al and Knudsen et al who also observed no statistically significant relationship between neonatal gender and the development of hyperbilirubinemia.[9] The study done by Doha Mohammed El-Amin et al found also no significant difference between the two groups with respect to sex ($p>0.05$). This observation is consistent with the findings of Rostami, Taks, and Sahu, all of whom reported no significant association between sex and the development of neonatal hyperbilirubinemia. In contrast, El-Gendy identified male sex as a significant independent factor for hyperbilirubinemia, while Satrya also reported a notable difference between genders in neonates who did and did not develop significant hyperbilirubinemia. These discrepancies across studies may be attributed to variations in sample size, population characteristics, or ethnic differences among the groups studied.[10-13]

Most neonates in our study had a birth weight between 2500-3000 g, indicating a predominantly normal birth weight population. A relatively higher proportion of neonates were born at 37-38 weeks of gestation. In our study, there was no difference between the groups with respect to gestational age.

The predominance of LSCS deliveries (79.3%) observed in this study aligns with contemporary obstetric trends in tertiary care settings. While mode of delivery has variable influence on bilirubin levels, increased LSCS rates may indirectly affect early feeding practices and bilirubin kinetics. In terms of mode of delivery, the present study found no significant difference between the two groups ($p>0.05$). This result is consistent with the findings of Rostami et al, Taks et al, Satrya et al and Chary et al who also reported no association between delivery mode and neonatal hyperbilirubinemia. However, Awasthi et al and Rehman et al, previously observed that peak serum bilirubin levels were significantly higher in neonates delivered vaginally.[12,13] Other maternal factors such as multigravidity (64.8%) and predominance of Rh-positive blood groups were noted, but these factors also did not appear to independently influence bilirubin outcomes in this study. These findings are consistent with the known blood group distribution in the general Indian population.

Cord SAP levels demonstrated a wide distribution (mean 134.88±87.47 U/L) with a right-skewed pattern, indicating the presence of higher values in a subset of neonates. Most neonates had cord SAP levels within the 100-200 U/L range (69%), suggesting this as the physiological range in the study population. Nalbantoglu et al however, for the first time used cord ALP level after birth as a measure of bilirubin levels.[1]

Al Assal et al evaluated 200 healthy full-term neonates born to apparently healthy mothers to investigate the predictive role of cord blood ALP levels in neonatal hyperbilirubinemia. Their findings revealed that ALP levels were significantly higher in neonates requiring therapeutic interventions, such as phototherapy or exchange transfusion. The optimal cutoff point for serum ALP was identified as >315 IU/L, with a sensitivity of 84.2%, specificity of 84.48%, and an area under the curve (AUC) of 88.8%. Based on their results, the authors concluded that cord blood ALP levels may serve as a significant predictor of hyperbilirubinemia requiring treatment.[14]

Similarly, as per our study, in the phototherapy subgroup, the correlation between SAP and bilirubin was stronger (ANOVA $F=12.054$, $p=0.001$) compared to the overall population, indicating better predictive value in clinically significant cases. Similarly, ROC analysis in the non-phototherapy group showed comparable performance, reinforcing the consistency of SAP as a biomarker across subgroups.

In our study, 22.8% of neonates required phototherapy, which is consistent with reported incidences in hospital-based studies. Neonates requiring phototherapy had higher mean bilirubin levels compared to those who did not, reinforcing the clinical validity of treatment thresholds. A significant association was observed between cord SAP levels and requirement of phototherapy. More importantly, higher SAP categories

showed progressively increased bilirubin levels, indicating a positive relationship.

Early identification of neonates at risk for hyperbilirubinemia is crucial to prevent severe complications such as bilirubin encephalopathy and kernicterus and cord blood alkaline phosphatase estimation is: non-invasive, easily obtainable at birth, cost-effective and with less chances of sepsis.[15]

Neonates with SAP ≤100 U/L had the lowest mean bilirubin (11.99±2.15 mg/dL). With increasing SAP levels: 101-200 U/L→14.70±2.98 mg/dL, 201-300 U/L→16.04±0.09 mg/dL and 301-400 U/L→18.89 mg/dL.

This shows a clear rising trend, indicating that higher cord SAP is associated with more severe hyperbilirubinemia. Therefore, it may serve as a useful screening tool in resource-limited settings to identify neonates requiring closer monitoring for jaundice.[16]

The present study demonstrates that cord SAP has predictive value for significant NNH. While demographic and clinical factors did not show strong associations, biochemical markers such as SAP may help identify neonates at higher risk for developing jaundice.

The findings of this study support the role of cord SAP as an early, simple, and cost-effective screening marker for predicting neonatal hyperbilirubinemia. Its high sensitivity and negative predictive value make it particularly useful in: identifying neonates who require closer monitoring, guiding early discharge planning, and reducing unnecessary investigations of neonates. However, due to moderate specificity, SAP should be used in conjunction with clinical assessment and other risk factors rather than as a standalone predictor.

CONCLUSION

This observational study evaluated the correlation between cord blood ALP levels and serum bilirubin levels in term neonates, with the aim of identifying ALP as an early predictor of neonatal hyperbilirubinemia. The study found that there was a positive correlation between cord ALP and serum bilirubin levels (Spearman's ρ=0.541, p=0.001). Amongst the study population, 22.8% of neonates required phototherapy, and these neonates had higher ALP levels. ROC analysis showed fair predictive ability (AUC=0.695) for ALP in predicting phototherapy requirement. An optimal ALP cutoff of 117.5 U/L demonstrated: Sensitivity: 84.85%, Specificity: 51.85% and high negative predictive value (91.80%). These findings indicate that cord blood ALP correlates significantly with subsequent bilirubin levels and can help identify neonates at risk early in life. Hence, cord blood alkaline phosphatase is a useful, early, and non-invasive biomarker for predicting neonatal hyperbilirubinemia in term neonates. Therefore, in clinical practice, cord blood ALP estimation can aid in early risk stratification, support timely monitoring, intervention and help reduce delayed diagnosis and deadly complications like kernicterus, particularly in resource-limited settings, where follow-up may be inconsistent, this simple and cost-effective marker can play a valuable role in improving neonatal outcomes.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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