International Journal of Contemporary Pediatrics
Hemavathi S et al. Int J Contemp Pediatr. 2024 Aug;11(8):1092-1096
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: https://dx.doi.org/10.18203/2349-3291.ijcp20242020

Original Research Article

Alkaline phosphatase isoenzyme analysis in umbilical cord blood of healthy term neonates

S. Hemavathi[1], V. Sathiyapriya[2], C. Rekha[1]*, A. T. Arasar Seeralar[1], Pragna B. Dolia[2], Abinaya P.[3]

[1]Department of Pediatrics, ACS Medical College and Hospital, Velappanchavadi, Chennai, Tamil Nadu, India
[2]Department of Biochemistry, ACS Medical College and Hospital, Velappanchavadi, Chennai, Tamil Nadu, India
[3]Department of Central Research Laboratory, ACS Medical College and Hospital, Velappanchavadi, Chennai, Tamil Nadu, India

Received: 14 June 2024
Revised: 08 July 2024
Accepted: 12 July 2024

***Correspondence:**
Dr. C. Rekha,
E-mail: svrclinic2016@gmail.com

ABSTRACT

Background: Alkaline phosphatases are isoenzymes that hydrolyze the organic phosphate in the extracellular space to free phosphates; mostly they are present on the outer layer of the cell membrane similar to that on hepatocytes. The human body contains four isoenzymes: they are hepatic/bone/renal alkaline phosphatase (L/B/K ALP), intestinal alkaline phosphatase (IALP), placental alkaline phosphatase (PLALP isoenzyme), and germ cell alkaline phosphatase (GCALP). In clinical practice, alkaline phosphatase (ALP) is routinely measured as a marker of bone and hepatic function. In the field of neonatology, it is used in diagnosing and indicating the severity of metabolic bone disease (MBD) in preterm newborns. It is also used as an early predictor of neonatal jaundice. The current study is aimed at detecting the specific isoenzyme form of alkaline phosphatase elevated in the umbilical cord of newborns and its association with hyperbilirubinemia.
Methods: The study included 30-term newborns in a tertiary care hospital in Velappanchavadi, Chennai. After birth, cord blood was collected for the determination of alkaline phosphatase isoenzyme in the serum. The average alkaline phosphatase activity in the cord blood serum was 109±35 IU/l. The average percentage of alkaline phosphatase activity after treatment with heat, phenylalanine, and urea were 6, 11.5, and 87% respectively.
Results: It was evident that the isoenzyme form present in the umbilical cord of term healthy neonates is of intestinal origin.
Conclusions: The elevated intestinal form of alkaline phosphatase indicates an immature liver and thereby predicts the future development of hyperbilirubinemia.

Keywords: Cord blood alkaline phosphatase, Hyperbilirubinemia, Isoenzymes, Neonates, Prediction

INTRODUCTION

Alkaline phosphatases are a group of isoenzymes involved in the hydrolysis of organic phosphates in the extracellular environment. They are ectoenzymes, present on the outer layer of the cell membrane. These enzymes prevail in tissue-specific and tissue-non-specific forms in our body.[1]

Tissue-specific forms are found in the intestinal tract, placenta, and germinal tissue. Under physiological conditions, these enzymes are not present in serum but in certain pathological conditions, their serum levels are elevated.[2] The majority of the circulating form in serum is the tissue-nonspecific form. It is made by the kidneys, liver, and bone from a single gene. Although the amino acid sequences of this form are similar, their lipid and

carbohydrate side chains differ, giving them distinct physicochemical properties following post-translational modifications.[3] These isoenzymes can be differentiated by various physiochemical characteristics like heat stability, inhibition by phenylalanine, and so on.[4,5]

ALP measurements are done routinely in clinical practice to identify various diseases. Elevated ALP levels are observed in children, pregnancy, and diseases like Paget's disease, vitamin D deficiency, hyperparathyroidism, celiac disease, seminomas, and damaged liver cells.[6,7] Research conducted on preterm newborns has demonstrated correlations between elevated ALP levels and low plasma phosphorus and high calcium levels with osteopenia.[8]

Neonatal jaundice is a common feature seen in preterm infants and 60% of full-term infants. Early identification of the disease leads to timely intervention like phototherapy, which can prevent the harmful effects of jaundice in neonates. According to Nalbantoglu et al alkaline phosphatase (ALP) level 6 hours after birth can be used as a marker for determining future development of hyperbilirubinemia.[9] Many similar studies also mention the cord blood ALP levels correlating with the development of neonatal jaundice.[10] However, none of the studies have so far focused on the cause of elevated alkaline phosphatase in neonates susceptible to neonatal hyperbilirubinemia. So, the present study aimed to investigate the source of elevated alkaline phosphatase in neonates by analysing the cord blood alkaline phosphatase isoenzyme pattern.

METHODS

This cohort hospital-based observational study was conducted between September 2023 and December 2023 in ACS Medical College and Hospital, Velappanchavadi, Chennai. This study protocol was approved by our Institute's Human Ethics Committee. Written informed consent was obtained from the mother for collecting cord blood. 30 healthy newborn babies included in the study.

Inclusion criteria

Newborn babies with gestational age between 37 and 42 weeks, birth weight more than 2500g, and APGAR score more than or equal to 7 at 1st and 5th minute of life were included.

Exclusion criteria

Preterm newborns, infants born to mothers diagnosed with eclampsia, diabetes, bone, kidney, and liver diseases, and infants with congenital/acquired diseases were excluded from the study. Newborns with complications like ABO/Rh incompatibility, neonatal sepsis, Birth asphyxia, and neonatal jaundice within 24 hours were also excluded from this study.

Procedure

About 3 ml of cord blood was collected from the placental end of the umbilical cord of the neonates and allowed to clot. Serum was collected from the clotted blood by centrifugation. Serum alkaline phosphatase level was analyzed using a Beckman Colter autoanalyzer. 30 samples with elevated alkaline phosphatase were frozen at -20 °C until further analysis for isoenzymes. For isoenzyme analysis, four different vials were taken with 50 µl of serum in each vial. The first vial was kept as such without any treatment, the second vial was heated at 56°C for 15 minutes, the third vial was treated with L-Phenyl alanine (10 mm) and the fourth vial was treated with 3M urea. All the vials are tested for alkaline phosphatase activity.

From the remaining sample about 20µl from each vial were loaded in 10% polyacrylamide gel. The movement of the proteins was tracked using bromo phenol blue dye. A constant voltage of 50 Volt was applied to the gel and the whole experiment was conducted in the cold temperature to avoid denaturation of enzymes. The activity of alkaline phosphatase in the gel was detected using: 10 mg of β-naphthyl phosphate, 24 mg of MgCl, 30.915 mg of Boric acid (0.05M), and 37.27 mg of KCl (0.05M) in 10 ml of distilled water with pH 9.2 and fast blue B salt -10 mg in 10 ml of distilled water. Alkaline phosphatase gives a purple color. After color development, the gel was allowed to be set by keeping in a fixative solution of isopropyl alcohol, acetic acid, and distilled water in a ratio of 10:10:80.

The enzyme activity of the samples is expressed as Mean±SD. The percentage of enzyme activity retained was expressed as a percentage. The data were analyzed using SPSS 25 software. A p value less than 0.05 is considered significant.

RESULTS

Demographic details of the neonates have been tabulated in Table 1.

The effect of heat, phenylalanine, and urea treatment on alkaline phosphatase activity is shown in Table 2. In the present study, the average alkaline phosphatase activity of the neonate cord blood was 109±35 IU/l. The alkaline phosphatase present in the cord blood was sensitive to heat as Heat treatment for 15 minutes at 56 °C caused a decline in its activity to 7±2 U/l. The enzyme was also sensitive to phenylalanine as indicated by diminished activity to 12±3 U/l. The enzyme activity after urea treatment was 95±39 U/l. The percentage of alkaline phosphatase activity after treatment with various inhibitors and heat is shown in Table 2. The average percentage of alkaline phosphatase activity in the heat, phenylalanine, and urea-treated groups were 6, 11.5 and 87% respectively.

Table 1: Demographic parameters of neonates (n=30).

Parameters	Categories	Frequency	Percentage
Gestational age (weeks)	37-38^{+6}	22	73.3
	39-40^{+6}	8	26.7
Birth weight (kgs)	2.5-3	18	60
	3-3.5	9	30
	3.5-4	3	10
Gender	Male	15	50
	Female	15	50
Mode of delivery	NVD	10	33.3
	LSCS	20	66.7
Parity	Primi	13	43.3
	Multi	17	56.7
APGAR at 1st min	7	2	6.7
	8	28	93.3
APGAR at 5th min	8	1	3.3
	9	29	96.7

Table 2: Alkaline phosphatase activity of the cord blood samples and percentage of alkaline phosphatase activity retained after treatment with heat, phenylalanine, and urea.

Treatment	Alkaline phosphatase activity (IU/l)	Percentage of activity retained after treatment (%)
Untreated serum	109±35	100
Heat treated serum	7±2	6
Phenylalanine treated serum	12±3	11.5
Urea treated serum	95±39	87

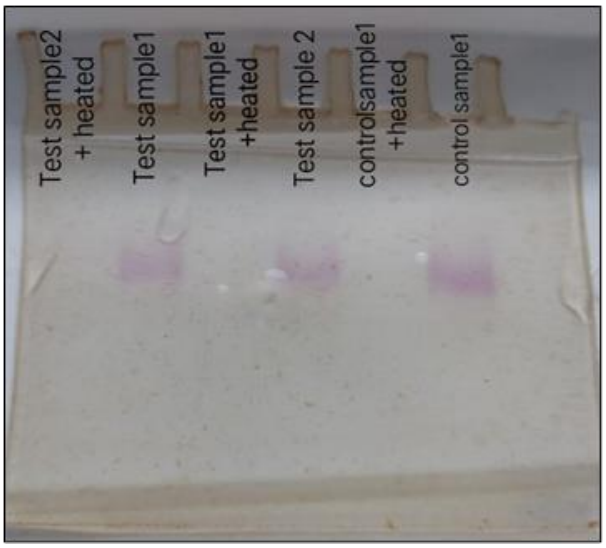

Figure 1: Effect of heat treatment on alkaline phosphatase activity.

The alkaline phosphatase activity was also demonstrated using acrylamide gel electrophoresis. The effect of heat treatment on the activity of alkaline phosphatase is demonstrated in Figure 1. From right to left lane 1: loaded with tracking dye; lane 2: control serum; lane 3: heat treated control serum; lane 4: cord blood sample1; lane 5: heat treated cord blood sample; lane 6: cord blood sample 2; lane 7: heat treated cord blood sample 2.

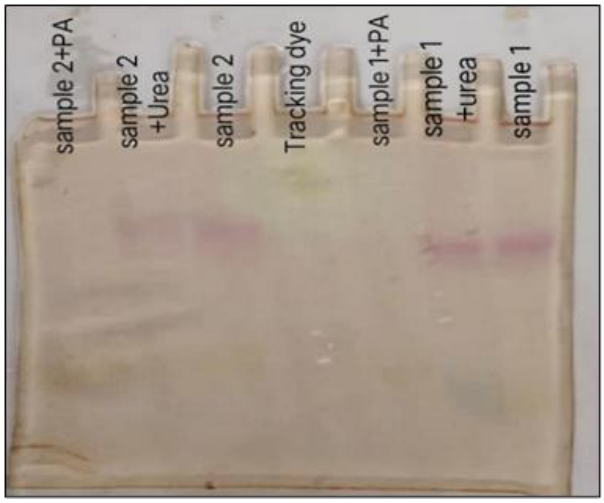

Figure 2: Effect of phenylalanine and urea treatment on the activity of alkaline phosphatase.

The effect of phenylalanine and urea on the activity of alkaline phosphatase is shown in Figure 2: from right to left lane 1: sample 1; lane 2: urea treated sample 1; lane 3: phenylalanine treated sample 1; lane 4: tracking dye; lane 5: sample 2; lane 6: urea treated sample 2; lane 7: phenylalanine treated sample 2.

DISCUSSION

In neonatology, the most commonly encountered problem in newborns is neonatal jaundice. It occurs in the first 2 weeks of life so it contributes to hospital readmission and extended stay in hospital.[11] Approximately 60% of full-term infants experience clinical jaundice.[12] Early prediction of jaundice can improve treatment, prevent hospital readmission, and prevent pathological changes associated with it.[13,14] According to a study done by Nalbantoglu et al 6 hours after birth, blood alkaline phosphatase levels predicted the development of treatment requiring hyperbilirubinemia.[9,15]

Cord blood alkaline phosphatase can be used as an early predictor of the development of neonatal jaundice requiring treatment.[13,15] Though most of the studies mentioned elevated alkaline phosphatase can predict the development of neonatal jaundice none of the studies tried to explore the cause of elevated alkaline phosphatase and its relationship with hyperbilirubinemia.

The present study on the analysis of the isoenzyme pattern of alkaline phosphatase has revealed that the cord

blood alkaline phosphatase was sensitive to heat. Only 6% of activity was retained after heating for 15 minutes at 56°C. Studies revealed that placental and placental-like isoenzymes are thermostable and cannot be inactivated by heating at 56°C.[16] This shows that the cord blood alkaline phosphatase was not of placental origin.

In the present study, treatment with 3.4 M urea did not significantly alter the cord blood alkaline phosphatase activity as 87% of the activity was retained even after urea treatment. Inhibition studies on the effect of urea on the alkaline phosphatase isoenzymes have shown that liver isoenzymes are sensitive to urea.[17] This demonstrates that the cord blood alkaline phosphatase was not of liver origin.

In the present study treatment with phenylalanine retained only 11.5% of the alkaline phosphatase activity. Inhibition studies with low molecular weight substances have shown that placental and intestinal enzymes are 30 times more sensitive to L-phenylalanine than liver, bone, and kidney isoenzymes.[16] This concludes that the cord blood alkaline phosphatase was of intestinal origin.

Studies have demonstrated the occurrence of fetal intestinal alkaline phosphatase in normal amniotic fluid and meconium as free dimers and as anchor-bearing and membrane-bound alkaline phosphatase.[18] Clearance of Fetal intestinal alkaline phosphatase is decreased in premature infants due to immature liver.[19] Normally intestinal alkaline phosphatase is rapidly cleared off from the plasma by the liver and elevated levels of intestinal isoenzyme are seen in chronic liver diseases.[20]

So, it is clear that an immature liver contributes to elevated alkaline phosphatase and thereby it can predict the future development of neonatal jaundice (hyperbilirubinemia) requiring treatment.

This study has few limitations. In the present study, we have analyzed only 30 samples. Though this sample size is statistically sufficient for concluding, including a larger sample size with immunological markers would have given a broader perspective.

CONCLUSION

To conclude, isoenzyme pattern analysis showed a predominant presence of intestinal alkaline phosphatase in cord blood samples. This isoenzyme being an asialoglycoprotein usually gets cleared off rapidly by the liver. So, the elevation of this isoenzyme can predict future development of hyperbilirubinemia as it is an indicator of immature liver.

ACKNOWLEDGEMENTS

Authors would like to thank all the participants and laboratory personnel for their kind and valuable assistance and contribution to this study.

Funding: This study funded by ACS Advanced Medical Research Institute
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Green MR, Sambrook J. Alkaline Phosphatase. Cold Spring Harb Protoc. 2020;2020(8):100768.
2. Vimalraj S. Alkaline phosphatase: Structure, expression and its function in bone mineralization. Gene. 2020;754:144855.
3. Dixon IMC. Tissue non-specific alkaline phosphatase (TNAP): A player in post-MI cardiac fibrosis. EBioMedicine. 2021;68:103430.
4. Komoda T, Sakagishi Y. The function of the carbohydrate moiety and alteration of carbohydrate composition in human alkaline phosphatase isoenzymes. Biochim Biophys Acta. 1978;523(2):395-406.
5. Tsai LC, Hung MW, Chen YH, Su WC, Chang GG, Chang TC. Expression and regulation of alkaline phosphatases in human breast cancer MCF-7 cells. Eur J Biochem. 2000;267(5):1330-9.
6. Millan JL, Manes R. Seminoma-derived nagao isozyme is encoded by a germ cell alkaline phosphatase gene. Proc Natl Acad Sci. 1988;85(9):3024-8.
7. Hofmann MC, Jeltsch W, Brecher J, Walt H. Alkaline phosphatase isozymes in human testicular germ cell tumors, precancerous stage, and three related cell lines. Cancer Res. 1989;49(17):4696-700.
8. Tinnion RJ, Embleton ND. How to use alkaline phosphatase in neonatology. Arch Dis Child Educ Pract Ed. 2012;97(4):157-63.
9. Nalbantoglu A, Ovali F, Nalbantoglu B. Alkaline phosphatase as an early marker of hemolysis in newborns. Pediatr Int. 2011;53(6):936-8.
10. Rostami N, Mehrabi Y. Identifying the newborn at risk for developing significant hyperbilirubinemia by measuring cord bilirubin levels. J Arab Neonatal Forum 2005;2:81-5.
11. Gale R, Seidman DS, Stevenson DK. Hyperbilirubinemia and early discharge. J Perinatol. 2001;21(1):40-3.
12. Mitra S, Rennie J. Neonatal jaundice: etiology, diagnosis, and treatment. Br J Hosp Med (Lond). 2017;78(12):699-704.
13. Ahmadpour-Kacho M, 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):1-5.
14. El-Amin DM, Ahmed YA, Hashim AM, Asmail AH. Evaluation of cord blood alkaline phosphatase levels as an indicator of neonatal jaundice. Al-Azhar Assiut Medi J. 2020;18(2):112-7.
15. Elmonem H, El-DinA, Kholeef E, Eldin S. Alkaline phosphatase and haptoglobin as predictor of

neonatal jaundice. Egypt J Hosp Medi. 2019;74(5):978-87.
16. Harris H. The human alkaline phosphatases: what we know and what we don't know. Clin Chim Acta. 1990;186(2):133-50.
17. Bergström K, Lefvert AK. Practical aspects on the determination of serum alkaline phosphatase isoenzymes using l-phenylalanine and urea. Clinica Chimica Acta. 1975;64(1):95-100.
18. Verpooten GF, Hoylaerts MF, Nouwen EJ, et al. Human fetal intestinal alkaline phosphatase: molecular heterogeneity and immunological detection in amniotic fluids. Clin Chim Acra 1990;186(2):225-37.
19. Fishman WH, Inglis NI, Krant MJ. Serum alkaline phosphatase ofintestinal origin in patients with cancer and with cirrhosis of the liver. Chin Chim Acta 1965;12(3):298-303.
20. Moss DW. Perspectives in alkaline phosphatase research. Clin Chem. 1992;38(12):2486-92.

Cite this article as: Hemavathi S, Sathiyapriya V, Rekha C, Seeralar ATA, Dolia PB, Abinaya P. Alkaline phosphatase isoenzyme analysis in umbilical cord blood of healthy term neonates. Int J Contemp Pediatr 2024;11:1092-6.