



## Original article

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Ljiljana Bjelakovic<sup>1</sup>, Gordana Kocic<sup>2</sup>,  
Tatjana Cvetkovic<sup>2</sup>, Dusica Stojanovic<sup>3</sup>,  
Stevo Najman<sup>4</sup>, Zoran Pop-Trajkovic<sup>5</sup>,  
Marina Jonovic<sup>5</sup>, Bojko Bjelakovic<sup>1</sup>

<sup>1</sup>Pediatric Clinic, Clinical Center Nis, Serbia,

<sup>2</sup>Institute of Biochemistry,  
Faculty of Medicine in Nis, Serbia,

<sup>3</sup>Department of Hygiene and Epidemiology,  
Public Health Institute Nis, Serbia,

<sup>4</sup>Institute of Biology and Human Genetics,  
Faculty of Medicine in Nis

<sup>5</sup>Clinic of Gynecology and Obstetrics,  
Clinical Center Nis, Serbia

## ALKALINE PHOSPHATASE ACTIVITY IN HUMAN MILK DURING THE FIRST MONTH OF LACTATION

### SUMMARY

Human milk is the main source of nutrients for a child during the first months of life. Alkaline phosphatase (EC 3.1.3.1) is a very important enzyme in clinical chemistry because of its activity in various tissues and biological fluids, being an indicator of physiological or diseased states. Milk contains several phosphatases, the principal ones being alkaline and acid phosphomonoesterases which have no known function or significance in milk, and have been studied extensively so far.

The objective of the present study was to determine the alkaline phosphatase activity and concentration of inorganic phosphorus (P) in colostrum and mature milk obtained from nursing mothers.

Our longitudinal biochemical analysis showed that alkaline phosphatase activities decreased from the 1<sup>st</sup> and the 2<sup>nd</sup> day of lactation (colostrum) to the end of the first month of lactation (at day 30). The amount of inorganic phosphates from colostrum increased to the end of the first month (at day 30) of lactation. The alteration between colostrum and mature milk alkaline phosphatase activity may be a consequence of the ALP transfer from the blood of mother into the colostrum and milk during breastfeeding.

The concentration of nutrients in colostrum and mature milk suffers alterations, including a decrease in alkaline phosphatase activity and an increase in P, probably in order to satisfy the requirements of the nursing infant.

**Key words:** human milk, colostrum, alkaline phosphatase, inorganic phosphor

### INTRODUCTION

Human milk is the main source of nutrients for a child during the first months of life. Milk synthesis starts in the epithelial cells of the mammary gland at the end of pregnancy to support the nutrition and promote the health of newborn infants. Milk contains vital nutrients such as proteins, carbohydrates, lipids, minerals and vitamins, together with bioactive substances including immunoglobulins, hormones and enzymes (1-3). Over 70 indigenous enzymes have been identified in the milk of various

mammalian species (4,5). Some milk enzymes are constitutive components of milk, while others are induced at particular periods of the lactation cycle and some pathological conditions. The enzyme systems normally present in mother's milk may influence the health and nutrition of the newborn infant. The occurrence of a phosphatase in milk was first recognized in 1925 by F. Demuth (6). Subsequently, ALP was characterized as an indigenously in milk (7-9).

Milk contains several phosphatases, the principal ones being alkaline and acid phospho-

monoesterases which have no known function or significance in milk and have been studied extensively so far (10,11).

Alkaline phosphatase (ALP) (EC 3.1.3.1) is a membrane-bound glycoprotein that is widely distributed in human tissues. There are four principal types of human ALP: intestinal, placental, germ-cell, mammary tissue and bone/kidney/liver (tissue non-specific). The intestine and placenta are particularly rich sources and ALP. There are slight differences between the ALPs in the bone/kidney/liver and other tissues, probably including mammary tissue, mainly in the degree of glycosylation. The gene for human bone/kidney/liver (tissue non-specific) ALP is at least five times larger than that for intestinal ALP (12).

Alkaline phosphate (EC 3.1.3.1) is a very important enzyme in clinical chemistry, since its activity varies in different tissues and serves as a specific indicator of diseased states, especially of bones and liver. However, in spite of the intensive research on alkaline phosphates and its widespread distribution, its physiological roles are not yet known (13,14). The aim of the present study was to determine the alkaline phosphatase ALP (EC 3.1.3.1) activity and concentration of inorganic phosphorus (P) in colostrums and mature milk obtained from nursing mothers.

## MATERIAL AND METHODS

This longitudinal study involved 30 healthy women admitted to the Clinic of Obstetrics and Gynecology in Clinical Center Nis, Serbia, for delivery between October and December 2008.

The samples of human milk were obtained from twenty healthy mothers of term infants at days 1,2 (colostrums) and 30 of lactation.

The milk sample was obtained fresh, after the infant had sucked for 5 minutes with a manual breast pump (Ginevri, Milan, Italy). Samples of human milk (n = 90) were stored at -20°C until analyzed.

Alkaline phosphatase (EC 3.1.3.1) activity was measured at pH 10.3 using p-nitrophenyl phosphate as substrate by using commercial Elitech-France kit, ALP-DEA. The concentration of inorganic phosphate (P) was measured with commercial kit Elitech-France on the basis of the formation phosphomolibdate (12, 13). The estimated samples were dissolved ten times before applying to the biochemical analyzer.

## STATISTICS

Descriptive data were presented as a mean values with a standard deviation. The obtained results were statistically analyzed using one way ANOVA trend analysis with SPSS computer statistical program. The value  $p < 0.05$  was considered statistically significant. All statistics were done using the SPSS computer package version 13 (SPSS, Chicago, IL, USA).

## RESULTS AND DISCUSSION

The analysis for alkaline phosphatase (ALP) and inorganic phosphate (P) in human colostrums and milk are presented in Graphs 1 and 2.

Fig.1 Alkaline phosphatase activities in human colostrum and milk

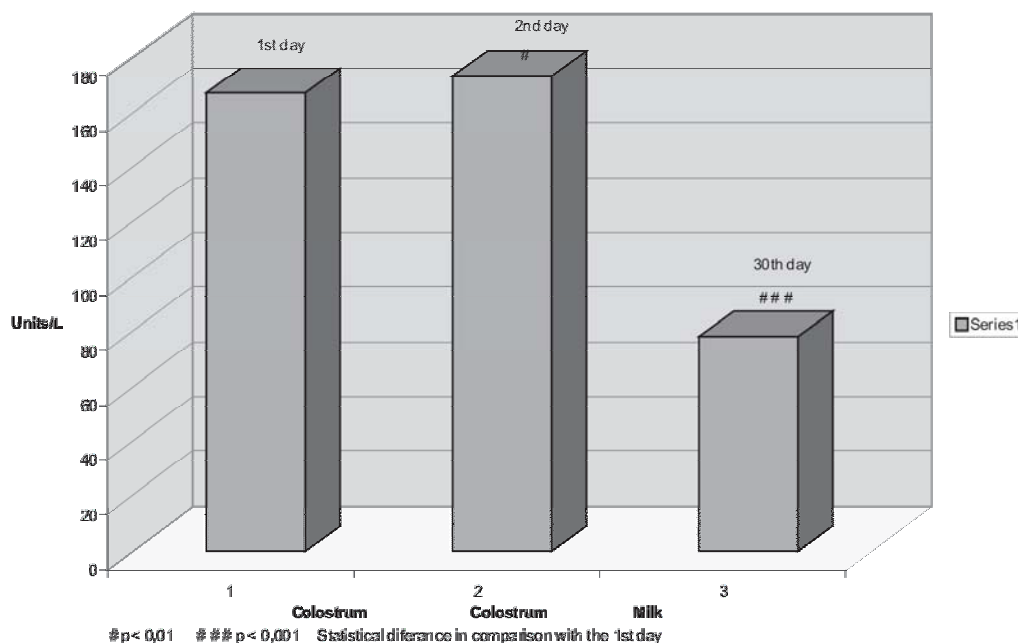
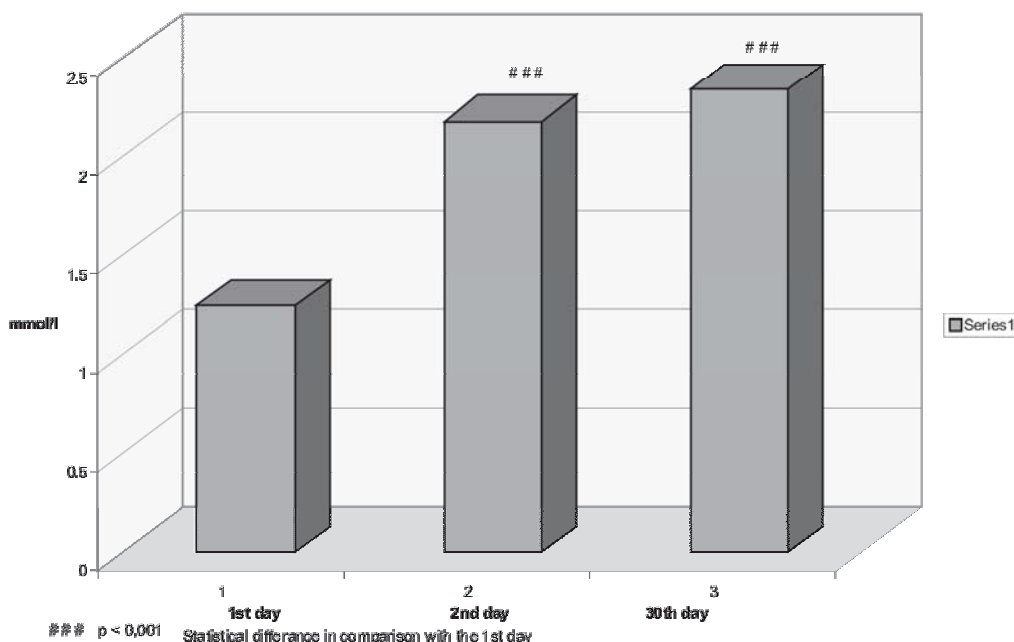


Fig.2 The amounts of inorganic phosphates in human milk



Our longitudinal biochemical analysis showed that alkaline phosphatase activities decreased from  $166.66 \pm 11.563$  Units/L at day 1 of lactation and  $173.478 \pm 12.462$  U/L at day 2 (colostrums) to  $78.125 \pm 4.021$  Units/L at the end of the first month of lactation (at day 30)  $p < 0.05$ .

Inorganic phosphates increased linearly from  $1.242 \pm 0.049$  at the end of day 1 of lactation and  $2.177 \pm 0.110$  mmol/L at day 2 (colostrums) to  $2.336 \pm 0.105$  mmol/L at day 30 of lactation.

## DISCUSSION

About 70 inherited enzymes have been discovered in milk (5,6,8). Those enzymes which are present at the highest levels have been isolated from milk and characterized. Among them are the naturally present enzymes of serum, like transaminases (ALT and AST), phosphatases (alkaline and acid), lactate dehydrogenase (LDH), creatinphosphokinase

(CPK, -amylase, aldolase, ribonucleases, -glutamyl transferase, lysosyme- lachtenins, catalase, xanthine oxidase, sulphhydryl oxidase - SHOX, glutathione peroxidase, *N*-acetylglucosaminidase- NAG, superoxide dismutase-SOD, and others (9). Because of that, enzymology of mammalian milk enzymes is very intensive today.

There are a few recent researches on milk alkaline phosphatase.

The inherited ALP in milk is similar to the enzyme in mammary tissue (14, 15). Most of the ALPs in the mammary gland is in the mycoepithelial cells; the epithelial secretory cells have low ALP

activity, which may suggest a role of these cells in milk secretion (16,17). ALP can exist in blood in the complex with protein, as enzyme- immunoglobulin complex as well as ALP-lipoproteins complex (14,18,19). It is well-known that colostrums are important sources of immunoglobulins and lipids (20).

Colostrum milk is one of the most important foods a newborn can receive from his mother soon after his birth. When the milk produced in the mother's body changes from colostrum milk to normal milk, the levels of total protein, fat, total solids decrease, lactose increases and colostral Ig declines rapidly. The decreasing of ALP in our milk samples may be explained according to decreasing of immunoglobulins and lipids during the lactation (21,22).

ALP act on a large variety of naturally occurring substrates, but the natural substrates on which the enzyme acts in the body are not known. ALP can dephosphorylate casein, phosphoprotein, under suitable conditions (23). As a phosphomonoesterase ALP may act on monophosphate esters of carbohydrates or lipids like glucoso-6-phosphate, galactoso-6-phosphate or 3-phosphoglycerin aldehyde, dioxyacetone phosphate, diacylglycerol phosphate or complex lipids, phosphatidylethanolamine (12). Based on these data we expected that the concentration of inorganic phosphate would follow the ALP activity. However, we obtained the opposite results.

The concentration of micronutrients in colostrum and mature milk suffers alterations, including a decrease in Fe, Zn, K and Na and an increase in Ca and P, probably in order to satisfy the requirements of

the nursing infant (24 - 26). Our results, concerning the decrease of inorganic phosphates levels in milk samples, are in agreement with these data and with the observation that ALP is inhibited by inorganic phosphate (24).

The ALP activity of bovine milk varies considerably between individuals and throughout lactation, minimum at week 1 and maximum at week 28 (26).

Our results considering high alkaline phosphatase activity in colostrum, during the first and second day and lower enzyme activity at day 30 of lactation, are in agreement with the statement that, in general, the enzyme content of human colostrum is higher from that in the corresponding mature milk (21,22). The alteration between colostrum and mature milk alkaline phosphatase activity may be the consequence of the ALP transfer from the blood of mother into the colostrum and milk during breastfeeding.

Namely, it is well-documented that during gravidity, the ALP in mother's blood increases proportionally to the augmentation of the mass of the placenta (12-14).

Some data show that increased dietary fat intake in lactating women increased the levels of lipase, esterase and alkaline phosphatase (17,18) enzymes that play a role in digestion and metabolism of fat; at the same time the protein supplementation of malnourished women increased alkaline phosphatase activity in their milk (27, 28).

Considering the origin of milk enzymes, e.g., from mammary cells or blood, their activities in

milk are not constant, but vary significantly due to different physiological and health states of the lactating mothers: due to the stage of lactation, diet, stress, mastitis or other factors (10). Among the most significant features of milk enzymes are those used as indices of mother's and baby's health.

## CONCLUSION

Alkaline phosphatase (EC 3.1.3.1) is a very important enzyme in clinical chemistry because of its activity in different tissues and biological fluids, serving as an indicator of physiological functions or disease states. Milk contains several phosphatases, the principal ones being alkaline and acid phosphomonoesterases. They do not have precise function or role in milk and until to date have been studied extensively. The objective of the present study was to determine the alkaline phosphatase activity and its concentrations of inorganic phosphorus (P) in colostrums and mature milk obtained from nursing mothers. Our longitudinal biochemical analysis showed that alkaline phosphatase activities decreased from the 1<sup>st</sup> and the 2<sup>nd</sup> day of lactation (colostrum) to the end of the first month of lactation (day 30). The amount of inorganic phosphates increased from colostrums to the end of the first month (day 30) of lactation.

The alkaline phosphate activity which is normally present in mother's milk may influence the health and nutrition of the newborn infant.

## REFERENCES

1. Margaret C, Neville A.- Lactogenesis in Women. A Cascade of Events Revealed by Milk Composition. Handbook of Milk Composition 1995: 87-98.
2. Kocic G. Biohemija tkiva i telesnih tečnosti. Mleko. U knjizi: Koracevic D, Bjelakovic G, Djordjevic V, Nikolic J, Pavlovic D, Kocic G. Biohemija. Savremena Administracija, Beograd. 2006:1018-1026.
3. Kuntz S, Rudloff S, Clemens Kunz C. Oligosaccharides from human milk influence growth-related characteristics of intestinally transformed and non-transformed intestinal cells. Br J Nut 2008;99:462-471.
4. Yamashiro Y, Sato M, Shimizu T, Oguchi S, Maruyama K, Kitamura S. Possible Biological Growth Factors in Breast Milk and Postnatal Development of the Gastrointestinal Tract. Ped International 2007;31:417-423.
5. Fox PF, Kelly AL. Indigenous enzymes in milk: Overview and historical aspects—Part 1. International Dairy Journal 2006;16:500-516.
6. Stewart RA, Platou E, Kelly VJ. The alkaline phosphatase content of human milk. J Biol Chem 1958;777 - 784.
7. Morton RK. Alkaline Phosphatase of Milk. 2. Purification of the enzyme Biochem J 1953; 55:795-800.
8. Fox PF, Kelly AL. Indigenous enzymes in milk: Overview and historical aspects—Part 2. Intern Dairy Journal 2006;16 :517-532.
9. Kelly AL, O'Flaherty F, Fox PF. Indigenous proteolytic enzymes in milk: A brief overview of the present state of knowledge. Intern Dairy J 2006;16: 563-572.
10. Kelly AL, Fox PF. Indigenous enzymes in milk: A synopsis of future research requirements. Intern Dairy Journal 2006;16: 707-715.
11. Silanikove N, Merin U, Leitner G. Physiological role of indigenous milk enzymes: An overview of an evolving picture International Dairy 2006:533-545.
12. Moss DW, Henderson AR, Kachmar JF. Enzymes. In Tietz NW (Ed), The textbook of Clinical Chemistry. W.B.Saunders Company 1986: 619-774.
13. Werner CW, Lott AJ. Alkaline phosphatase. In: Kaplan AL and Pesce AJ (ed). Clinical Chemistry: theory, analysis and correlation. 1984:1094-1098. The C.V. Mosby Company.
14. Koracevic D. Enzimi. U knjizi : Koracevic D, Bjelakovic G, Djordjevic V, Nikolic J, Pavlovic D, Kocic G . Biohemija. Savremena Administracija, Beograd. 2006:1-162.
15. Morton RK. Alkaline phosphatase of milk. 1. Association of the enzyme with a particulate lipoprotein complex. Biochem J 1953; 55(5): 786-795.

16. Chuang NN. Alkaline phosphatase in human milk: a new heat-stable enzyme. *Clin Chim Acta* 1987;169:165-174.
17. Hamilton TA, Górnicki SZ, Sussman HH. Alkaline phosphates from human milk. Comparison with isoenzymes from placenta and liver. *Biochem J* 1979; 177:197-201.
18. Karmarkar MG, Rajalakshni R, Ramakrishnan CV. II. Activities of Certain Milk Enzymes in Relation to Dietary Fat intake. *Acta Paediatrica* 2008;52:554-556.
19. Karmarkar MG, Ramakrishnan CV. Relation between Dietary Fat, Fat Content of Milk and Concentration of Certain Enzymes in Human Milk. *J Nutr* 1959;69: 274-276.
20. Csapo-Kiss Z, Stefler J, Martin TG, Makray S, Csapo J. Composition of Mares' Colostrum and Milk. Protein Content, Amino Acid Composition and Contents of Macro- and Micro-elements. *International Dairy Journal* 1995;5:403-415.
21. Hadom U, Hammon H, Bruckmaier RM, Blum JW. Delaying Colostrum Intake by One Day Has Important Effects on Metabolic Traits and on Gastrointestinal and Metabolic Hormones in Neonatal Calves. *J Nutr* 1997;127:2011-2023.
22. Maden M, Birdane FM, Altunok V, Dere S. Serum and colostrum /milk alkaline phosphatase activities in the determination. *Revue Méd Vét* 2004;11: 565-569.
23. Jasinska BK, Kleczkowski K, Michalak W. Influence of  $\alpha$ -Lactoglobulin on Milk Alkaline Phosphatase Activity Toward the Main Milk Caseins. *J Dairy Sci* 1985;68:2172-2175.
24. Mastroeni SSBS, Okada IA, Rondó PHC, Duran MC, Paiva AA, Neto JM. Concentrations of Fe, K, Na, Ca, P, Zn and Mg in Maternal Colostrum and Mature Milk. *J Trop Pediatrics* 2006 52(4):272-275.
25. Lucas A, Brooke G, Baker BA, Bishop N, Morley R. High alkaline phosphatase activity and growth in preterm neonates. *Archives of Disease in Childhood* 1989; 64: 902-909.
26. Nagra SA. Longitudinal Study in Biochemical Composition of Human Milk During First Year of Lactation. *J Tropical Pediatrics* 1989; 35 :126-129.
27. Hibberd CM, Brooke G, Carter ND, Haug M, Harzer G. Variation in the composition of breast milk during the first 5 weeks of lactation: implications for the feeding of preterm infants. *Arch Dis Child* 1982;57: 658-662.
28. Boss MH, Batt WG. Alkaline phosphatase activity and diet. *J Nutr* 1956: 137-144.

## AKTIVNOST ALKALNE FOSFATAZE U HUMANOM MLEKU U TOKU PRVOG MESECA LAKTACIJE

Ljiljana Bjelaković<sup>1</sup>, Gordana Kocić<sup>2</sup>, Tatjana Cvetković<sup>2</sup>, Dušica Stojanović<sup>3</sup>, Stevo Najman<sup>4</sup>, Zoran Pop-Trajković<sup>5</sup>, Marina Jonović<sup>5</sup>, Bojko Bjelaković<sup>1</sup>

<sup>1</sup>Klinika za dečije interne bolesti, Klinički centar Niš, Medicinski fakultet Niš, Srbija

<sup>2</sup>Biohemijski institut, Medicinski fakultet Niš, Srbija

<sup>3</sup>Institut za higijenu i epidemiologiju, Medicinski fakultet Niš, Srbija

<sup>4</sup>Institut za biologiju i humanu genetiku, Medicinski fakultet Niš, Srbija

<sup>5</sup>Klinika za obstetriciju i ginekologiju, Klinički centar Niš, Srbija  
Medicinski fakultet Niš, Srbija

### SAŽETAK

**Humano mleko je glavni izvor nutritivnih sastojaka za decu u toku prvog meseca života.**

Alkalna fosfataza (EC 3.1.3.1) je veoma značajan enzim u kliničkoj hemiji jer je njena aktivnost u raznim tkivima i biološkim tečnostima indikator fizioloških i patoloških stanja. Mleko sadrži nekoliko fosfataza, od kojih su najznačajnije alkalna i kisela fosfomonoesteraza, čija je funkcija još uvek nejasna i pored intenzivnog izučavanja. Predmet prikazane studije bilo je ispitivanje aktivnosti alkalne fosfataze (ALP), kao i neorganskog fosfora u kolostrumu i mleku koje je dobijeno od majki dojilja. Naša longitudinalna ispitivanja su pokazala da se aktivnost ALP smanjivala počev od prvog i drugog dana laktacije (kolostrum) do kraja prvog meseca laktacije (30. dan). Koncentracija neorganskog fosfora se povećala, počev od kolostralnog mleka (prvi i drugi dan laktacije) do kraja prvog meseca (30. dan laktacije). Promene u aktivnosti alkalne fosfataze između kolostruma i pravog mleka mogu da budu posledica prelaska ALP iz krvi majke u toku dojenja u kolostrum ili mleko.

Koncentracija hranljivih sastojaka u kolostrumu i pravom mleku trpi promene, uključujući smanjenje aktivnosti ALP i povećanje neorganskog fosfora, verovatno prema potrebama bebe koja doji.

**Ključne reči:** humano mleko, kolostrum, alkalna fosfataza, neorganski fosfor