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 1st and the 2nd 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 colostral 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
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.
Alkaline phosphatase activity in human milk during the first month of lactation

Our longitudinal biochemical analysis showed that alkaline phosphatase activities decreased from 166.66 ± 11.563 Units/L at day 1 of lactation and 173.478 ± 12.462 U/L at day 2 (colostrums) to 78.125 ± 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 ± 0.049 at the end of day 1 of lactation and 2.177 ± 0.110 mmol/L at day 2 (colostrums) to 2.336 ± 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, lyosyme- lacteins, catalase, xanthine oxidase, sulphydryl 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 myoepithelial 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, diacylglicerol phosphate or complex lipids, phosphatydilethanolamine (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 than 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 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 1st and the 2nd 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

AKTIVNOST ALKALNE FOSFATAZE U HUMANOM MLEKU U TOKU PRVOG MESECA LAKTACIJE

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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 kolostrumnog mleka do kraja prvog meseca (30. dan laktacije). Promene u aktivnosti alkalne fosfataze između kolokstruma uključuju smanjenje aktivnosti ALP i povećanje neorganskog fosfora.

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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