ENZYME ACTIVITY OF HUMAN MILK DURING THE FIRST MONTH OF LACTATION

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The dynamic of enzyme activities in human colostrum and milk changes, depending on the lactation stimulation. The aim of the study was to study the activity of alkaline phosphatase, amylase, transaminases (ALT and AST) and lactate dehydrogenase (LDH) in the samples of human colostrum and mature milk obtained by manual squeezing. The study involved 35 women, 18-39 years of age, who had given birth at the Clinic for Gynecology and Obstetrics, Faculty of Medicine in Niš, with normal delivery, without any complications reported. The samples of colostrum and milk were collected by mechanical squeezing of milk during three intervals: the first sample of colostrum was obtained immediately after the appearance of the first drops of milk after childbirth; the second sample was obtained after 24 hours, and the third after one month. The samples were collected in the morning, and kept in sterile test tubes in the freezer until the performance of analysis. The activity of these enzymes was reduced (statistically significant for amylase) in the period after one month, which means that it corresponded to the amount of protein. The activity of transaminases (ALT and AST) and alkaline phosphatase activity showed a tendency to fall after a month, but statistically significant difference was obtained with AST. The dynamic of alkaline phosphatase activity showed statistically significant decrease after one month. Enzyme activity in human milk represents an important field of research, from the aspect of functional importance and benefits of human milk compared to the cow's milk, in which enzymes are generally destroyed by pasteurization. On the other hand, increased activity of certain enzymes may be a useful and valid diagnostic marker of mechanical tissue irritation, epithelial desqamation and increase of the concentration of inflammatory cells during mastitis. Acta Medica Medianae 2010;49(2):20-24.

Key words: *human milk, colostrum, alkaline phosphatase, amylase, transaminase, lactate-dehydrogenase*

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Introduction

Milk represents the basis of nutrition, a medium that embodies the perfection of nature reflected in the survival and upbringing of the offspring of mammals. From the chemical point of view, milk represents a stable fat emulsion in colloidal solution of proteins and genuine solution of lactose and mineral matter. The great nutritional and biochemical-metabolic significance of milk in the nourishment of the newborns and infants stems from its richness in vitamins, enzymes, as well as cellular elements that have an active role in protection from infections (1-4).

Although at first sight milk appears to be a homogenous liquid, its ultrastructural content reveals the following elements: whey (rich in peptides, minerals and carbohydrates), fat globules which represent lipoprotein complexes, protein micelles primarily casein, and membranous vesicular complex known as the exosomes (1-5).

The study of enzyme activity in milk started at the end of the 19th century. The first findings of enzyme activity in colostrum and milk were followed by a number of clinical and experimental studies which aimed at explaining the reasons, i.e. the sources of enzymes in milk. Enzymes may be present during various milk phases depending on their primary localization, physical-chemical properties and the major reason of their existence in milk. The presence of enzymes in milk is a consequence of spontaneous diffusion of low molecular weight enzymes from plasma, active secretion from apical parts of the mammary gland epithelium or a result of enzymes leaving somatic cells which are present in secreted milk, most often leukocytes or other macrophage cells. At the beginning of the 21st century it was already known that human colostrum and milk may contain over 70 different enzymes. Even after more than a century of studying milk enzymes, their exact role and significance in milk cannot be defined with certainty. For some of them, such as xanthine oxidase, lactoperoxidase and lysozyme, the active antimicrobial role in the production of free radicals has been proved. Other enzymes help the digestion of various substances in still undeveloped digestive tract of newborns (pancreatic lipase and amylase). The enzymes from apical parts of epithelial cells increase during inflammatory processes of various etiologies when a greater epithelial desquamation takes place. Another reason for increased activity during inflammatory processes is an extravasation of leukocytes to the area of inflammation. If the inflammation is systemic, the milk enzymes increase due to the transfer from plasma on the basis of concentration gradient. However, the results of some studies showed that the activity of certain enzymes is much greater in milk and cannot be correlated to an increased activity in plasma. One of the reasons for increased enzyme activity in milk is the departure from myoepithelial cells as a consequence of mechanical pressure (milking). The fact that some enzymes are present in milk due to the separation of apical part of epithelial cells is confirmed by the findings that their substrates are often absent from milk, as well as that for a certain number of enzymes the pH balance of milk is far from optimal. Many milk enzymes have some useful effects on the digestive tract of a newborn and this confirms the advantage of breastfeeding over artificial nutrition (4-6).

The dynamics of enzyme activity in human colostrum and milk changes depending on the lactation stimulation, mechanical pressure upon squeezing, as well as in relation to the amount of proteins and cells present in milk. Having this in mind, this study aims at examining the activity of alkaline phosphatase, amylase, transaminases (ALT and AST) and lactate dehydrogenase (LDH) in the samples of human colostrum and milk obtained by manual squeezing.

Examinees and methods

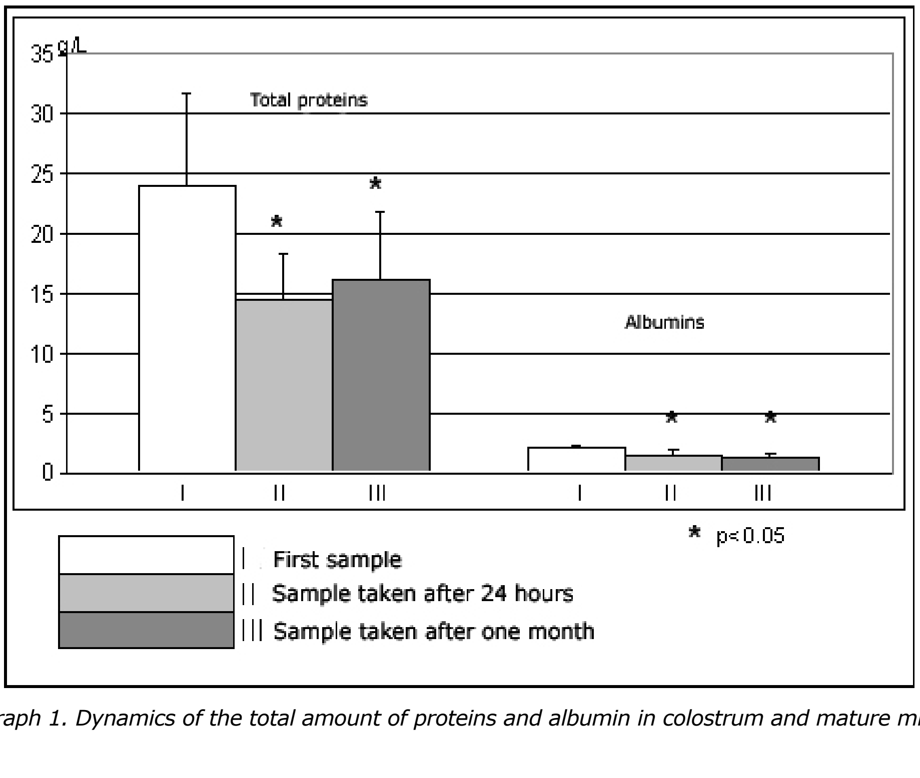
The study included 35 women, aged 18-39 who had given birth at the Clinic of Gynecology and Obstetrics in Niš, with normal delivery without complications such as diabetes, hypertension or eclampsia. The women did not suffer from acute or chronic diseases and had a common, non-vegetarian diet. The colostrum and milk samples were collected by mechanical squeezing in three intervals: the first colostrum sample was obtained immediately after the appearance of the first milk drops upon delivery; the second sample was collected after 24 hours and the third was collected after one month. The samples were collected in the morning before breastfeeding and kept in sterile test tubes in a freezer until the analysis was performed.

The activity of alkaline phosphatase, amylase, transaminases (ALT and AST) and lactate dehydrogenase (LHD) was determined using ready-made tests by Bio Systems on an A25 biochemical analyzer. The total amounts of proteins and albumin were determined in the same way.

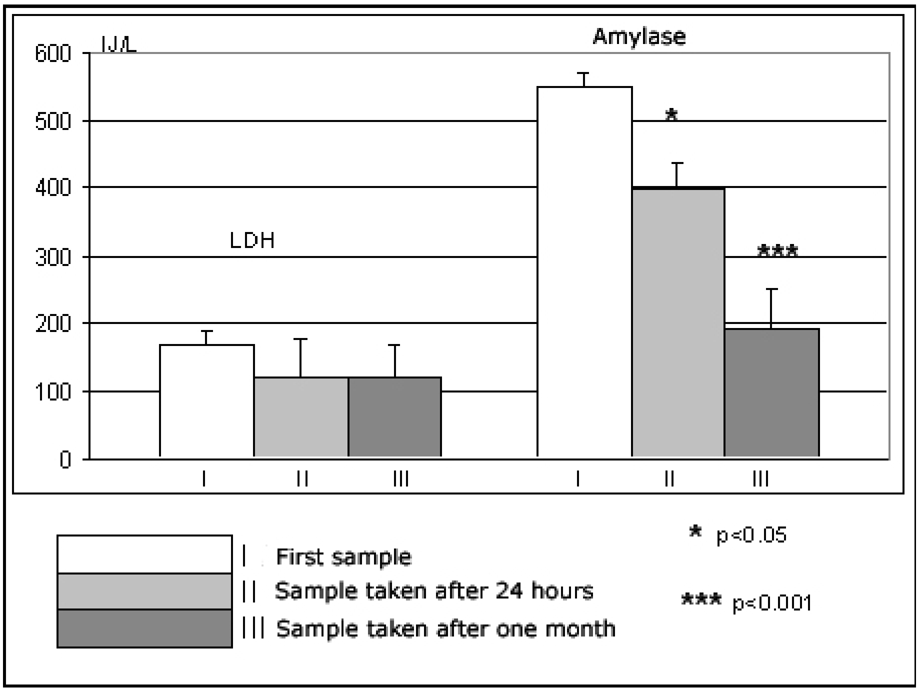
Statistical analysis included determining the mean value and standard deviation and was performed using the Anova test. Statistical significance appeared in the samples with t>0.05.

Results

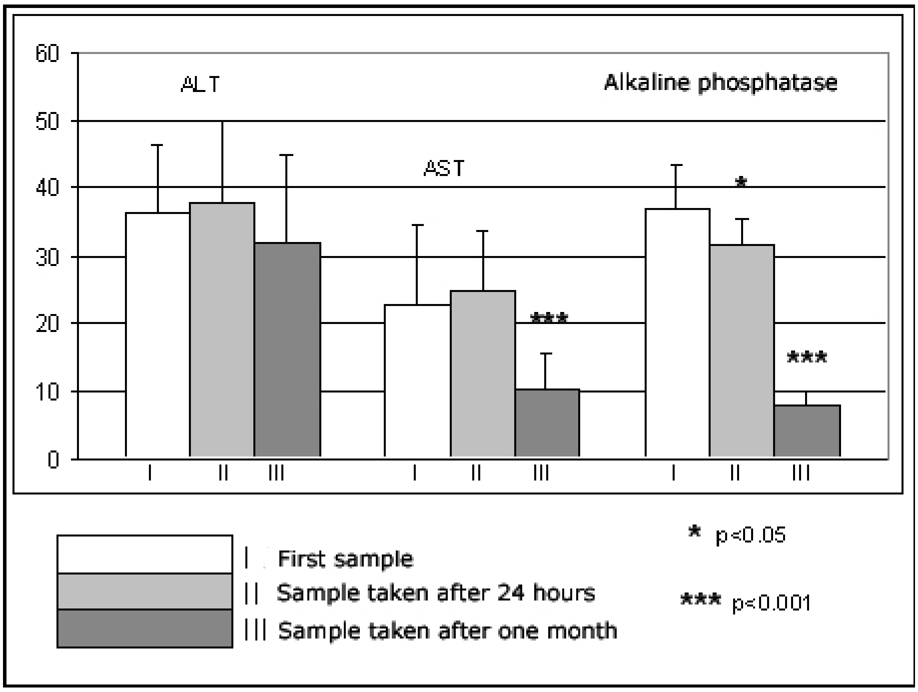
The results of the research are shown in Graphs 1, 2, and 3. Graph 1 displays the total amount of proteins and albumin in milk including the described dynamics. In the first sample, the colostrum is yellowish in color and has a high viscosity. The concentration of proteins in it is usually the highest. Our research had the same results, with the total amount of proteins and albumin significantly different on the second day and after one month, as compared to the first sample.



Graph 1. Dynamics of the total amount of proteins and albumin in colostrum and mature milk



Graph 2. Dynamics of the activity of lactate dehydrogenase (LDH) and amylase in colostrum and mature milk



Graph 3. Dynamics of the activity of transaminases (ALT and AST) and alkaline phosphatase in colostrum and mature milk

Discussion

Colostrum, the first milk, is secreted during the first two to seven days after delivery and it provides the newly born child with all the necessary nutrients. Transitional milk is secreted during the next two weeks and is followed by mature milk which appears in the third week after delivery. The average content of human milk is around 86-87% water and 13-14% dry matter. Dry matter is composed of the following substances: organic matter which is mostly formed of proteins (about 2.5%) in colostrum and up to 1.5% in mature milk. The proteins for the most part include casein, lactalbumin and lactoglobulin. Casein is the major milk protein, present in at least four forms; it represents a complete protein, rich in all essential amino acids. The nutritive value of milk and dairy products is the result of the presence of essential amino acids. Recently, data have shed light on the significance of breastfeeding from the aspect of harmful effects of cow's casein. This particularly refers to β-casein type A. This type of casein is also responsible for immunogenic effects of cow's milk and the development of type 1 diabetes, as well as neuro-psychiatric defects mostly in children who had been fed by cow's milk. Therefore, human milk represents the most natural way of nutrition of newborns and infants and should be valued as much as possible. Albumin is mostly obtained from serum, so it can be taken as a marker of the plasma-milk barrier (5, 6).

The amount of proteins in colostrum is greater because an infant develops quickly but does not possess enough strength to suckle actively for a long time. Therefore, a small amount of milk should satisfy all the needs of a newborn. The results of this study (Graph 1) confirmed the dynamics described in literature.

Lactate-dehydrogenase is an NAD-dependent enzyme included in the metabolism of glucose in aerobic and anaerobic conditions. Therefore, the distribution of isoenzyme forms changes depending on the supply of oxygen to tissues and metabolic activity of tissues. The major source of enzymes in milk is the epithelium of the mammary gland. Some studies have shown that LDH activity is correlated to active milk secretion so that it represents a constitutive component. Increased activity in the first colostrum may be a consequence of an overall increase in the amount of proteins, as well as of proteins leaving myoepithelial and muscle cells caused by mechanical milk squeezing (1,2,4). As for specific enzyme activity in relation to the total amount of proteins, it may be concluded that an enzyme maintains high concentrations even after a period of one month (Graph 2).

Amylase is an enzyme that belongs to the class of hydrolytic enzymes included in the digestion of starch. It was the first enzyme isolated in milk at the end of the 19th century. It was shown later that the main component is α-amylase and it is interesting that amylase activity in human milk is more than 20-40 times higher than in cow's milk. This enzyme is homologous to the salivary amylase and is 15-140 times more active than in plasma. These findings show that the presence of enzymes in milk is the result of neither the passive diffusion from plasma nor their presence in the apical part of outlet ducts but that they are products of active secretion. Considering that milk does not contain starch, one of the assumptions is that amylase is necessary for breaking down the oligosaccharide units which are present in milk (5, 7, 8). But milk oligosaccharides contain fucose and N-acetyl-neuraminic acid, types of monosaccharides which cannot be broken down by α-amylase, the same way it cannot break down lactose, the main disaccharide. One of the hypotheses is that amylase is also produced as a compensatory component in the undeveloped digestive enzymatic system of a newborn child who possesses only 0.2-0.5% of the total amylase activity that takes place in adults. This enzyme is capable of breaking down the polysaccharide bacteria capsules, so its antimicrobial effect is undeniable. The decrease in activity during lactation after a period of one month is in accordance with the decrease in proteins as well as with the development of the digestive system of infants who do not need this enzyme anymore. The richness of mother's milk in amylase explains why children feeding on human milk are able to digest carbohydrate meals earlier and much better than children taking artificial food (9, 10).

Alkaline phosphatase in human milk was discovered at the beginning of the 20th century. It is a membranous enzyme particularly present in lipoprotein structures of the Golgi complex. Therefore, it is not surprising that it is secreted in milk in high concentrations. Considering the structural homology, alkaline phosphatase from milk is very homologous to the enzyme in liver and placenta because, besides structural homology, it also has a similar bond with phosphatidylinositol in the lipoprotein complex of the membrane. These kinds of membranous fat globules are actively secreted in milk. More complex researches of the structure and origin have shown that milk contains two forms of enzymes, one from myoepithelial cells and the other from cytoplasm (11,12). The richness of canaliculi epithelium in this enzyme points to the reason why it can be taken as a marker of mastitis. Our research also had samples of milk with very high alkaline phosphatase activity which is the result of mastitis developed as a consequence of mechanical irritation due to milking and milk retention (Graph 3).

Alanine transferase (ALT-GPT) and aspartate-trasferase (AST-GOT) are transaminases which transfer amino groups from amino acids to α-Ketoglutaric acid, although this reaction is also reversible. The origin of ALT is strictly cytosolar whereas AST is localized in cytoplasm and mitochondria. During mastitis, the activity of these enzymes increases. AST may also be increased as a consequence of irritation of the surrounding muscle tissue and myoepithelial cells. After a period of one month, the activity of milk enzymes decreases but the AST/ALT activity ratio is lower than 1, which means that it does not completely imitate the relation in the cytoplasm (1, 2, 13).

It may be concluded that enzyme activity in human milk represents a significant field of research from the point of view of functional significance and advantages of human milk over cow's milk in which enzymes are mostly destroyed by pasteurization. On the other hand, increased activity of some enzymes may be a useful and valid diagnostic marker of mechanical tissue irritation, epithelial desquamation and increase of the concentration of inflammatory cells during mastitis.

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AKTIVNOSTI ENZIMA U HUMANOM MLEKU U TOKU PRVOG MESECA LAKTACIJE

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Dinamika aktivnosti enzima u humanom kolostrumu i mleku menja se u zavisnosti od stimulacije laktacije. Za cilj studije bilo je postavljeno izučavanje aktivnosti alkalne fosfataze, amilaze, transaminaza (ALT i AST) i laktat dehidrogenaze (LDH) u uzorcima humanog kolostruma i zrelog mleka dobijenog manuelnim istiskivanjem u prvom mesecu laktacije. U studiju je bilo uključeno 45 žena, dobne starosti 18-39 godina, koje su se porodile na Klinici za ginekologiju i akušerstvo Kliničkog centra u Nišu, normalnim porođajem, bez postojanja komplikacija. Uzorci kolostruma i mleka skupljani su mehaničkim istiskivanjem mleka u tri intervala: prvi uzorak kolostruma je dobijen odmah nakon pojave prvih kapi mleka nakon porođaja; drugi uzorak je dobijen nakon 24 časa i treći nakon mesec dana. Uzorci su skupljani u jutarnjim časovima, pre podoja deteta i čuvani u sterilnim epruvetama u zamrzivaču do izvođenja analiza. Aktivnost pomenutih enzima se smanjuje (statistički značajno kod amilaze) u periodu nakon mesec dana, što znači da prati količinu proteina. Aktivnost transaminaza (ALT i AST) i alkalne fosfataze pokazuje tendenciju pada aktivnosti nakon mesec dana, ali je statistički značajna razlika dobijena kod AST. Aktivnost alkalne fosfataze pokazuje dinamiku koja upućuje na visoko statistički značajan pad nakon mesec dana. Aktivnost enzima u humanom mleku predstavlja značajno polje istraživanja, jer se potenciraju značaj i prednosti humanog mleka nad kravljim, u kome su enzimi pasterizacijom uglavnom uništeni. Povećana aktivnost određenih enzima može biti koristan i validan dijagnostički marker mehaničke iritacije tkiva, deskvamacije epitela i porasta koncentracije inflamatornih ćelija u toku mastitisa. *Acta Medica Medianae 2010;49(2):20-24.*

Ključne reči: *humano mleko, kolostrum, alkalna fosfataza, amilaza, transaminaze, laktat-dehidrogenaza*