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ANTIOXIDANT STATUS AND METAL CONTENTS IN HUMAN BREAST MILK IN RELATION TO AGE AND COURSE OF LACTATION

STATUS ANTYOKSYDACYJNY ORAZ ZAWARTOŚĆ METALI W MLEKU LUDZKIM U KOBIET W RÓŻNYM WIEKU I OKRESIE LAKTACJI

Abstract: The aim of the study was to investigate the concentration of chosen trace elements, activity of catalase (CAT) and glutathione content (GSH) in human milk. The study subjects were recruited from among the lactating women from Malopolska province in Poland. Milk was taken from 23 mothers classified into three groups of age (20–25, 26–31 and 32–37 years) and two groups of lactation period (colostrums; 1–3 days; and transitional milk, over 4th day). CAT and GSH values were determined by the spectrophotometric method. Cu, Zn, Mg, Fe and Cd concentrations were determined by atomic absorption spectrophotometry (AAS). The results indicate no significant differences between activity of catalase taking into account the age of mothers and the day of lactation. Similarly glutathione level showed insignificant differences between studied groups. Our results indicate that human milk of women from Malopolska province, Poland, may contain significant amount of cadmium. We found statistically significant correlation between some metals in milk from women in different age and period of lactation. It concerned: Fe vs Cd and Cd vs Cu correlation**.** The result of our study indicate that the activity of antioxidant enzymes did not change during the course of lactation and in relation to mother's age.

Keywords: catalase, glutathione, breast milk, trace elements, heavy metals

Human milk is considered as optimal food for newborn infants during the first months of life in relation with its nutritional and protective characteristics. Properties of breast milk change during the course of lactation. Breast milk has three different stages: colostrum, transitional milk, and mature milk. Colostrum is the first stage of breast milk which lasts for several days after the birth. It is rich in proteins, fat-soluble vitamins, minerals, and immunoglobulin. The protein concentration is much higher in colostrum

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than in mature milk but much of it has the form of secretory immunoglobulin A (IgA). The immunoglobulin is probably not absorbed by the gut and therefore it is not nutritionally available. Although, it is thought to have a protective function against infections [1]. Transitional milk occurs after colostrum, lasts for approximately two weeks and has high level of fat, lactose, water-soluble vitamins, and contains more calories than colostrum. Mature milk is the final milk that is produced. It contains about 90 % of water, which is necessary to maintain hydration. The other 10 % is comprised of carbohydrates, proteins, and fats which are necessary for both growth and energy.

Breast milk is also a source of antioxidants. They have specific properties to neutralize free radicals which can damage cellular structures like DNA, proteins, carbohydrate and cause lipid peroxidation. Vitamins, proteins and enzymes are involved in the antioxidant protection [2]. One of the most active antioxidant enzyme is catalase (CAT, E.C.1.11.1.6). Catalase is a hemoprotein composed of four identical subunits which contains haem-bound iron in its activate site. The heme group is responsible for enzymatic activity of the enzyme. The presence of CAT is documented in human and cow milk [3, 4]. It plays very important role in the decomposition of hydrogen peroxide into molecular oxygen and water to prevent accumulation of toxic peroxides in the cell. Milk from mothers of preterm and full term infants has equal resistance to oxidative stress. Catalase activity in human milk increases with time [5].

Protective mechanisms against oxidation involves also tripeptyde glutathione. Glutathione (γ-glutamylocysteinyloglicyn) is a non-enzymatic antioxidant which exhibits reductive properties conditioned by the thiol group. Breast milk becomes an important source of dietary (GSH) since infant's GSH synthetic capacity may not be well developed [6]. Glutathione and its precursors are present in the colostrum. Additionally, GSH takes part in detoxification processes of metals like Cu, Ag, Cr in organism and form stable complexes with them [2].

Breast milk includes nutritional and trace elements, on the other hand there is documented that xenobiotics like cadmium and lead are also present [7]. A plentiful supply of breast milk from a mother eating an adequate diet should provide all the neonate's requirements of minerals and trace elements because they have a high level of bioavailability so even low concentration can be well utilized [1]. We investigated concentration of some trace elements (Fe, Cu), nutritional elements (Mg, Zn) and pollutant Cd. Zinc and copper are present in several enzymes and proteins and interact in various tissues. Metallothionein is considered to regulate the transportation of zinc and copper [7]. Zinc and copper have been found to be bound partially to the same proteins, eg lactalbumin in colostrum and transitional milk [8]. Zinc is present mainly in association with the low-molecular-weight components and proteins may contribute to its high bioavailability [9]. The bioavailability of some elements can have an influence on other elements level, for example high zinc/copper ratio can cause a decrease in the absorption of copper from diet and vice versa [10]. Moreover, the stage of lactation is a very significant factor which can cause changes in trace elements' level what is well documented for copper, iron and zinc. Iron concentration in milk is not dependent on maternal iron status, similarly zinc level [10–13]. Milk can also contain heavy metals like cadmium.

During lactation, Cd is transported from maternal plasma to mammary gland and secreted into breast milk [7].

The aim of the study was to investigate the concentration of nutritional and trace elements, and also activity of catalase (CAT) and glutathione content (GSH) in human milk in relation with mother's age and period of lactation.

Materials and methods

The study subjects were recruited from among the lactating women from Malopolska province in Poland who had given birth to a mature baby at the Czerwiakowski Hospital in Krakow. Women had been informed about the aim of the study and gave their permission for collecting milk. All women were in good health condition. We classified mothers taking into account two factors: age and stage of lactation. According to the various age of the women they were segregated into three groups: 20–25, 26–31 and 32–37 years. In addition women were segregated into two groups of different stage of lactation. First group consisted of women in early stage of lactation (1 to 3 days – colostrum). The women from the second group had lactation for more than 4 days (transitional milk).

Milk samples were collected into 5 cm^3 sterile polypropylene containers by manual expression, always in the morning hours between 9 am and 11 am.

Enzymatic analyses were performed immediately after the samples collection. Catalase activity was measured using method described by Bartosz (1995). Fresh milk was centrifuged at 12.000 g (10 min) and then was skimmed by vacuum suction. Briefly, 0.1 cm^3 of the supernatant was mixed with 0.9 cm^3 potassium – phosphate buffer (pH = 7.0) and 0.5 cm^3 solution of 54 mmol H_2O_2 in this buffer. Then we recorded a decrease in the absorbance at λ = 240 nm during 1 minute. We assumed that one unit of catalase decomposed 1 μmol H_2O_2 during one minute. We also measured total protein level using Lowry method.

Glutathione was measured by Ellman's method (1959). The volume of 500 mm^3 of each sample was mixed with 500 mm^3 TCA and 500 mm^3 EDTA to get rid of proteins. Then the samples were incubated in a fridge temperature for 10 minutes. Next samples were centrifuged at 6300 g for 5 minutes. The volume of 200 mm^3 of supernatant was mixed with 2.3 cm^3 H_2O, 100 mm^3 EDTA, 300 mm^3 TRIS and 100 mm^3 DTND. Blank assay was prepared by mixing 2.3 cm^3 H_2O, 200 mm^3 EDTA, 300 mm^3 TRIS, 100 mm^3 DTND and 100 mm^3 TCA. The absorbance was measured spectrophotometrically towards blank assay at λ = 412 nm. Glutathione content was expressed in milimol per liter of human milk.

For metals determination aliquots of milk (2 cm^3) were placed in a separate mineralization tubes and mixed with 2 cm^3 of concentrated HNO_3 and heated at 120 ^oC for 240 minutes in a thermostat-controlled digestion block. After cooling the samples were filled to the volume of 5 cm^3 with demineralized water. Trace elements: iron, copper; nutritional elements: magnesium, zinc and xenobiotics: cadmium were measured by atomic absorption spectrofotometry (AAS). The concentration of the elements were expressed in miligrams per 1 dm^3 of milk.

Statistical analysis of catalase activity and glutathione content was performed using one-way ANOVA. Correlation coefficients, between six elements (Cd, Fe, Zn, Cu, Mg), catalase and glutathione were calculated by Spearman's correlation test.

Results and discussion

Milk samples were classified into three groups of age (20–25, 26–31, 32–37) and two groups of stage of lactation (1–3 days colostrum, over 4 days transitional milk). Average concentration, mean and standard error of nutritional and trace elements in relation with age are listed in Table 1. The same characteristics in relation with stage of lactation are listed in Table 2.

Table 1

Elements concentration in breast milk in relation with maternal age. Values expressed in mg/dm^3, Cd in $\mu g/dm^3$

	20–25 age			26–31 age			32–37 age		
	mean	min.–max	S.E.	mean	min.–max	S.E.	mean	min.–max	S.E.
Mg	26.00	16.31–37.92	3.67	41.28	33.79–53.32	2.78	44.10	30.58–56.02	6.71
Zn	4.60	3.04–7.77	0.74	3.83	1.43–7.35	0.99	5.62	1.40–8.30	1.56
Cu	0.31	0.23–0.49	0.04	0.41	0.17–0.60	0.07	0.44	0.17–0.97	0.14
Fe	3.15	1.01–8.93	1.33	1.16	0.66–2.93	0.44	12.37	1.57–26.94	5.58
Cd	0.06	0.04–0.07	0.01	0.06	0.05–0.07	0	0.06	0.05–0.06	0

Table 2

Comparison of elements concentration between colostrum and transitional breast milk. Values expressed in mg/dm^3, Cd in $\mu g/dm^3$

	Colostrum			Transitional milk		
	mean	min.–max	S.E.	mean	min.–max	S.E.
Mg	33.68	21.08–55.23	5.33	45.96	29.02–66.67	6.06
Zn	5.59	3.04–8.30	0.80	3.99	1.43–7.77	1.19
Cu	0.33	0.17–0.49	0.04	0.39	0.17–0.60	0.07
Fe	1.42	0.37–2.93	0.43	0.94	0.66–1.57	0.17
Cd	0.06	0.05–0.07	0.00	0.06	0.04–0.07	0.00

The concentrations of all tested elements were not statistically significant in relation with mothers age, however our results show differences in mean concentrations of the metals. We have found higher mean concentrations of Zn and Cu in older women (32–37) than in younger ones (20–25). This data is in accordance with those presented in earlier studies [14, 15]. In other studies Fe level of 12.37 mg/dm^3 was higher in older women (age of 32–37) than in younger ones. The obtained results for iron, copper and zinc in colostrum are similar to those published by Costa et al [16]. Well known decrease in milk zinc level during lactation [10, 12, 17, 18], is confirmed by our study. The decline of that element can be explained by the fact that trace elements are bound by proteins in milk and play role as cofactors of enzymes. Thus the observed changes may result from the changes in women metabolism, nutrition and decreasing zinc requirements of the growing infants. Regarding the changes of elemental concentrations during breast feeding, also Fe and Cu showed decreasing tendency. Copper level found in our study is somewhat higher than copper level reported by other authors, but the compared values are in similar range. Almeida et al reported [17] that there is dependence between Zn and Cu concentration in serum and in colostrum. While copper level is high in the mother's blood and low in colostrum, contrary Zn level is the highest in colostrum because of its essential function for development of the infant [19]. The presence of iron and copper is important for the bacteriostatic properties of human milk. Newborn infants require large amounts of these minerals, but they intake low volume of milk, so colostrum contains high concentration of copper and iron [11, 12]. Mean magnesium level tend to increase with age (26.00–44.10 mg/dm^3). Such a tendency may be confirmed by Lipsman et al [20]. This fact can be caused by differences in bone mineralization between young and adolescent pregnant women [21]. Magnesium concentration in milk increased during the course of lactation, the same tendency is shown by Karra et al [22]. However, Dorea [23] indicates that factors like stage of lactation, maternal metabolic condition, dietary habits and magnesium intake do not have influence on Mg concentration in milk. We have also found that toxic element Cd was present in tested milk, the presence of cadmium in breast milk is well documented by other research. The cadmium concentrations estimated in our studies were lower than concentrations of range 0.07–1.23 μg/dm^3, indicated by Honda et al [7, 10]. Low cadmium concentrations suggest that there might be some maternal homeostatic processes preventing the transfer of cadmium into milk [17]. All the more that we have not found increase in the concentration of cadmium in relation with mother's age. Moreover we have not observed any changes in cadmium content in milk during the course of lactation. Similar observation were also made by Rossipal et al [10].

No significant correlations were found between the studied elements except for Cd vs Cu and Cd vs Fe (Table 3).

Table 3

Spearman correlation coefficients between nutritional elements and cadmium

	Trace elements	
	Cu	Fe
Cd	0.880*	–0.509*

* $p < 0.05$

The interactions between Cd and Cu in kidneys and liver are well known [23]. On the other hand, Honda et al [7] did not show such relations in milk. Our research point to the strong positive correlation Cd vs Cu (r = 0.880). Such a trend is probably a result of protective function of Cu.

Human breast milk is an essential source of Fe for newborn infants. It is important constituent of hemoglobin and negative correlation between Cd vs Fe (r = –0.509), suggest that cadmium has toxic influence on iron uptake and transportation. Antagonism between cadmium and iron is generally attributed to the divalent metal transporter (DMT1) in digestive tract. It is established that the presence of cadmium decreases iron uptake by DMT-1 transporter [25]. We did not find any significant differences in activity of catalase between women in different age and stage of lactation (p = 0.05). Similarly glutathione content did not show significant fluctuations. Moreover there was no correlation between enzymes (p = 0.05).

The highest mean activity of CAT occurred in milk from mothers in the age of 26–31. We have also observed higher activity of CAT in transitional milk in comparison with colostrum. This results do not confirm clearly generally accepted increase of catalase activity in relation to time progress [5].

Table 4

Catalase activity and glutathione concentration in different groups of age, CAT [U/mg of protein], GSH [$mmol/dm^3$]

	CAT			GSH		
	mean	min.–max	S.E.	mean	min.–max	S.E.
I group	0.12	0.07–0.14	0.01	0.433	0.31–0.65	0.063
II group	0.23	0.05–0.70	0.06	0.297	0.11–0.51	0.054
III group	0.14	0.05–0.37	0.06	0.209	0.06–0.51	0.062

Table 5

Catalase activity and glutathione concentration in colostrums and transitional milk, CAT [U/mg of protein], GSH [$mmol/dm^3$]

	GSH			CAT		
	mean	min.–max	S.E.	mean	min.–max	S.E.
Colostrum	0.30	0.06–0.65	0.05	0.14	0.05–0.37	0.03
Transitional milk	0.29	0.11–0.47	0.06	0.20	0.05–0.70	0.06

We observed the highest mean content of GSH in the youngest women (20–25) and the lowest mean content was detected in the oldest women (31–37). Our values are in the range of data showed by other authors. Some data suggest that the content of GSH decreases over 1 month of lactation. Our study indicate only a slightly decreasing trend between colostrum and transitional milk [6]. GSH plays an important role in detoxification of hydrogen peroxide, other peroxides and free radicals take part in the detoxification of a variety of xenobiotics [26]. Thus it seems that the presence of GHS in human milk reflects the antioxidative status of the mothers' organisms and plays role in the protection of infants against reactive oxygen species.

Conclusion

Breast milk composition is dependant on various factors including the age of lactating woman and the stage of lactation. Catalase and glutathione in milk play very important role in antioxidant defence of the infants in early stage of their life. We can observe some tendencies in changing levels of elements, decreasing tendency with course of lactation for Zn, Fe and increasing for Mg and Cu. Additionally, there are significant positive correlations between Cd vs Cu and negative Cd vs Fe. This suggest that Cd has strong influence on other elements. Cadmium concentration did not change during lactation, what suggest that there are some homeostatic mechanisms in mother's organism which prevent newborn against toxicity of Cd.

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STATUS ANTYOKSYDACYJNY ORAZ ZAWARTOŚĆ METALI W MLEKU LUDZKIM U KOBIET W RÓŻNYM WIEKU I OKRESIE LAKTACJI

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Abstrakt: Celem badań było określenie koncentracji metali ciężkich, a także zawartości enzymów antyoksydacyjnych: katalazy (CAT) i zredukowanego glutationu (GSH) w mleku ludzkim. Kobiety biorące udział w eksperymencie były rekrutowane spośród matek w różnych okresach laktacji, pochodzących z terenu województwa małopolskiego. Próbki mleka zostały pobrane od 23 zdrowych kobiet z klas wiekowych: 20–25, 26–31, 32–37 lat. Dodatkowo został dokonany podział na grupy ze względu na okres laktacji, pierwsza: 1–3 dzień laktacji – siara, druga: powyżej 4 dnia – mleko przejściowe. Aktywność katalazy była mierzona spektrofotometrycznie według metody Bartosza (1995), natomiast poziom glutationu oznaczano spektrofotometrycznie przy użyciu metody Ellmana (1965). Koncentrację pierwiastków, takich jak: Zn, Mg, Cu, Fe oraz Cd oznaczano za pomocą spektrofotometrii absorpcyjnej (AAS). Aktywność katalazy, a także poziom glutationu zredukowanego nie wykazały statystycznie istotnych różnic pomiędzy grupami wiekowymi oraz okresem laktacji. Wyniki naszych badań pokazują, że mleko kobiet z regionu Małopolski zawiera pewne ilości kadmu. Dodatkowo zostały wykazane statystycznie istotne korelacje pomiędzy zawartością metali w próbkach mleka od kobiet w różnym wieku i okresie karmienia, dotyczyło to związku pomiędzy: Fe i Cd oraz Cd i Cu. Pomiędzy pozostałymi badanymi pierwiastki nie zaobserwowano statystycznie istotnych korelacji.

Słowa kluczowe: katalaza, glutation; mleko ludzkie, mikroelementy, metale ciężkie