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RESEARCH ARTICLE

THE CONTENT OF HEAVY METALS IN WOMEN'S BREAST MILK AND ITS PROTECTIVE FACTORS

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ABSTRACT

The content of Pb, Cr, Cd, Sb and As was analyzed in breast milk and its protective factors (phagocytosis of breast milk macrophages, lysozyme concentrations, SIgA, IgA, IgM, IgG) of 72 women. There were 52 women with somatic and gynecological diseases. 24 women of them had an increased level of heavy metals in breast milk, and it did not exceed the norm in 28 women. 20 healthy women had a normal composition of the chemical elements of breast milk. The average concentration of IgG, IgM and the phagocytic activity of breast milk macrophages decreased by 1.4-1.8 times among women with a high content of heavy metals in comparison with healthy women. There was a decreased IgM content and the dysfunction of phagocytes in women with various somatic and / or gynecological diseases, but with a normal composition of heavy metals in breast milk. The phagocytes were 2-3.6 times less and significantly less pronounced than in women with a modified composition of breast milk. The frequency of IgG low concentrations was 1.5 times higher in breast milk with a high content of heavy metals. Thus, the protective factors of milk were significantly reduced in women whose heavy metals (Pb and Cr) content was increased and / or they were present in milk in combination with Cd, Sb, As.

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INTRODUCTION

Nowadays one of the most important problems in medicine is the negative impact of toxic substances of the environment on the human organism (Avcin *et al*., 1991; Boersma *et al*., 1991; Williamson, 2016). The toxic intoxication by salts of heavy metal takes the important place among the chronic poisoning. Among them the most often met and toxic are the following: chromium (Cr), cadmium (Cd), plumb (Pb), antimony (Sb), arsenic (As). The chemical compounds are widespread in the environment (Avcin *et al*., 1991; Boersma *et al*., 1991; Haider, 2015). Usually these compounds penetrate into the women's organism with the composition of nutrition, inhaled air, through the skin and mucous membranes. Prolonged continuous contact with even small doses of these harmful compounds can lead to chronic poisoning of the women's body and affects not only her health, but it also affects the health of a newborn baby (Avcin *et al*., 1991, Haider and Bhutta, 2015; Kushnareva *et al*., 2015; Yakushkin, 1992).

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Chemical elements in the form of organic and inorganic compounds capable to damage the organs of the digestive system with the development of the inflammatory process. The kidney damage can lead to acute or chronic kidney failure. It is known the toxic effect of heavy metals on the central nervous system, the organs of hematopoiesis and immune defense (Avcin *et al*., 1991, Boersma *et al*., 1991; Kushnareva *et al*., 2015; Williamson, 2006). Up to the present moment, the study of the effect of heavy metals and their compounds on the health of pregnant women and breast-feeding mother was given insufficient attention, although it is possible to expect serious negative consequences of the toxic effects of these substances on the woman's body and her baby. An insignificant amount of work is devoted to these questions (Avcin *et al*., 1991; Haider, 2015; Yakushkin, 1992). It is known that breast milk contains a large number of factors that protect the baby from infection. Among them the most important are immunoglobulins of classes IgA, sIgA, IgM, IgG, lysozyme, macrophages (Lönnerdal, 2003; Paxson, 1979; Yakushkin, 1992). Heavy metals being entered the woman's body may have a negative effect on her immune system, and as a consequence, on the body of her baby (Avcin *et al*., 1991; Haider, 2015). However, we have not met the studies in the scientific literature on the possible impact of the heavy metals of the breast milk on his protective factors. Conducting research in this direction will allow to determine the degree of

accumulation of dangerous toxic substances in the woman's body, their relationship with the protective factors of women's milk and develop methods for the prevention and neutralization of toxic effects on both the mother and the baby.

Aim: Investigate the relation between the high content of heavy metals in the breast milk and its protective factors in women.

Patients and methods: There were examined 72 women, aged 19 to 42 years old. All women lived in Moscow (Russia). Professional harmfulness was not determined among any of the examined women. All women were divided into 3 groups. The first group included 24 women aged 19 to 38 years old, the heavy metals exceeded the norm or were not normal in the breast milk of these women. The second group included 28 women aged 21 to 42 years, who had normal content of heavy metal or it was not determined at all. The control group was made up of 20 mothers aged 19 to 32 years who are healthy and gave birth to healthy full-term babies. The study of five chemical elements' concentration (heavy metals Cr, Cd, Pb, Sb) by emission plasma spectrometry using atomic-emission spectrometer with inductively coupled plasma (Baird company, the USA). The concentration of the element was evaluated by weight in mg in one kg of breast milk (mg / kg). The analysis of the content of heavy metals in breast milk was conducted in the first and second groups and it was compared with the norm of the control group that we had published earlier (Kushnareva *et al*., 2015). The following protective factors of milk were tested in all women. The concentration of lysozyme, IgA, sIgA, IgM, IgG was determined by the method of enzyme immunoassay and expressed in mcg/ml and mg/ml. The phagocytic activity of breast milk macrophages was determined by their ability to phagocytize the control test - strain of Staphylococcus aureus P209 by a modified method of Ch.Paxson et Cress C.C. (Paxson, 1979). During the original method, the cells were previously washed three times with phosphate buffer. We analyzed the phagocytosis of macrophages in native low-fat breast milk. The indices were calculated when counting 100 macrophages: 1. The phagocytic number (PhN) is the percentage of phagocytic macrophage cells. 2. The index of phagocytosis (PhI) is the average number of microbial cells Taken hold by a single phagocyte, which is involved in phagocytosis. 3. Index of completion of phagocytosis (ICPh). It is calculated as follows: (PhN30 / PhN90 + PhI30 / PhI90) : 2. If the ICPh is more than 1 then the phagocytosis is complete; if it is less than 1 then it is incomplete. It is seen in the text that the percentage of small numbers is given for comparing indicators in groups.

RESULTS

Somatic and / or obstetric-gynecological diseases were diagnosed in women of groups I and II: the habitual miscarriage (7 and 3), the threatened miscarriage (24 and 28), toxicosis in the early stages of pregnancy (16 and 12), gestosis (13 and 14), exacerbation of chronic infectious diseases during pregnancy (bronchitis, pyelonephritis, sinusitis, cystitis - in 23 and 10), poor uterine contraction strength (8 and 4), prenatal discharge of amniotic fluid (6 and 2), placental pathology (8 and 1), gynecological diseases (adnexitis, dysfunction of the ovaries, cervical erosion, the ovarian cyst) (13 and 8). The results of the study of the heavy metals' concentration in breast milk are presented in the table 1. As seen from Table 1, there were found in the breast milk of women in group 1: As, Sb and Co, which were absent in the breast milk of healthy women and women from group 2. Pb was detected in the breast milk of all women of group I. Its concentration on the average exceeded the upper limit of the norm by 121.7% ± 25.33% (20-450%), which was higher by 3 and 4.6 times in comparison with the group II and control group, respectively ($p<0,05$).

An increased concentration of the only one Pb was detected in 13 women (56%). It was simultaneously found the increased concentrations of other elements in 11 women: Pb + Cr in 4 women, Pb + Cd in 1 woman, Pb + Cd + As in 1 woman, Pb + Cr + Cd + Sb in 1 woman. The high concentrations of all five studied elements were found in breast milk of 4 women. The concentrations of Pb in group II were not different from the norm. Pb was detected in 16 women among 28 women (57%) of this group, while in the control group it was met 4 times less as follows only among 3 out of 20 healthy women (14%) at the concentration of ≤0.243 mg / kg. Cr was found in all the examined women. The average concentration of Cr in breast milk among women of Group I had only a tendency to increase in comparison with group II and control group, however, this element exceeded the upper limit of the norm by 11-38% (16.5 ± 4.120%) in 6 women (21%). The level of Cr in breast milk among women of group II was not different from that one in the control group.

The study results of the phagocytic activity of macrophages in breast milk are presented in Table 2. As seen from Table 2, The average PhN30 in Group I was 1.8 times lower than it is among healthy women in Group III, and there was a tendency to decrease this index in comparison with group II. The index of PhN30 in group II was 1.5 times lower than in control group III. PhI30 in group I was 1.4 times lower than in control, and there was a tendency to decrease in comparison with group II. Also there was only a tendency of PhI30 decrease in group II compared to group III. There were no differences for all other average values of macrophage phagocytosis index in the studied groups. PhN30 ≥ 40% and PhI30 ≥ 2,1 was in group III in healthy women. Phagocytosis was completed among all patients of this group. It was revealed that during the individual analysis of phagocytosis indices incomplete phagocytosis was present in only one woman in group I and two women in group II (the difference was not significant, $p> 0.05$). However, all indicators of macrophage phagocytosis were normal only in 3 women in the group I (12.5%). Simultaneously, two indicators such as PhN30 and PhI30 were reduced in six women (25%), including one woman with unfinished phagocytosis. Only PhN30 index' decrease was in 15 women of this group (63%), which indicated on insufficient number of activated macrophages from the total pool of these cells when a pathogen was injected. The normal phagocytic activity of milk macrophages was in 7 women in group II (25%), which was twice more often than in group I. There were only two women (7%) who had a decrease in cell activity in terms of PhN30 and PhI30 indices, therefore, 3.6 times less often than in group I ($p <0.05$). The only decrease of PhN30 index was in 16 women (57%), which was not significantly different from this index in group I. The study results of the concentration of immunoglobulins and lysozyme in breast milk are presented in Table 3. As can be seen from Table 3, there was a significant decrease in the average values of IgM concentration in groups I and II in comparison with the index in group III, as well as the average IgG in group I in

Table 1. The concentration of heavy metals in breast milk among groups with women in the study

Women's Group	Concentration of heavy metals mg / kg (M±m)				
	Pb	Cr	As	Cd	Sb
I Group	0,612±0,0659*▼ (1,267 - 1,54)	1,164±0,0266* (1,06-1,24)	1,5648±0,697 (0,323-4,520)	0,3983±0,119 (0,010-2,467)	0,2304±0,0221 (0,140-0,273)
II Group**	0,201±0,0340	0,662±0,051	<0,100	<0,010	<0,200
Control group (7)**	0,132 ±0,0901 (0,02-0,243)	0,726±0,055 (0,593-0,968)	<0,100	<0,010	<0,200

Notes: 1. * - the differences of the indicators between group I and group II are reliable (p≤,0,05). 2. ▼ - the differences between the control group and the groups I, II are reliable (p≤,0,05). 3. **, the elements As, Cd, Sb in breast milk were not detected within the sensitivity of the method in the control group and group with women II.

Table 2. Phagocytic activity of macrophages of breast milk in the studied groups with women

N	Women's Group	Indicators of phagocytosis: M±m (min-max).				
		PhN30 (%)	PhI30	PhN90 (%)	PHI90	ICPh
1	I Group	27,25±2,52*▼ (12-52)	2,74±0,15 (1,3-3,6)	19,05±1,9 (10-38)	2,1±0,233 (1-3)	1,44±0,084 (0,78-2,46)
2	II Group	32,8±2,10▼ (14-60)	3,23±0,243 (1,7-6,8)	22,9±1,65 (8-40)	2,53±0,778 (1-4,1)	1,45±0,088 (0,84-2,49)
3	Control group	49,4 ±1,91	3,88±0,28	24,4±2,25	2,59±0,14	1,76±0,24

Notes. 1. * - the differences of the indicators between group I and group II are significant (p≤,0,05). 2. ▼ - the differences of the indicators between the control group and I, II groups are significant (p≤,0,05)

Table 3. The concentration of lysozyme and immunoglobulins in breast milk in the study groups

N	Group of women	Indicators of protective factors (M±m, min-max)			
		Lysozyme mcg/ml	sIgA mg/ml	IgM mcg/ml	IgG mcg/ml
1	I group	115.9±4.89 (70-142)	1.15±0.048 (0.7-1.25)	25.3±1.05* (18-37)	38.2±1.36 *▼ (24-78)
2	II group	114.5±6.07 (70-40)	1.04±0.078 (0.75-1.45)	25.5±0.72* (24-27)	50.9±5.39 (25-79)
3	Control group III	112.2±9.61 (90-150)	1.051±0.16 (0.5-2.0)	61.4±2.81 (50-70)	49.0±2.11 (30-50)

Notes. 1. * - The differences between Group III and Groups I or II, reliable (p≤0,05).
2. ▼ - The differences between the indicators between group I and group II (p<0,05).

comparison with groups II and III. The mean indices of lysozyme and sIgA did not differ in the studied groups. However, the detailed individual analysis showed that the reduced level of IgG was in 9 women in group I (38%) and 1.5 times less often in group II - in 7 women (25%). The concentration of IgM was reduced in breast milk in all women in groups I and II. However, the concentrations of sIgA and lysozyme were within the norm of all women in group I and II. It is important to note that violations of the protective factors of milk in Group I were significantly more pronounced than in Group II. Thus, the decrease in indices was 25-75% of the norm (56 ± 6.12%) in group I, while they decreased by 10-40% (26.0 ± 2.34%, p <0.05) in group II.

DISCUSSION

Violation of the protective factors of breast milk in women can reflect both the immune system of the whole organism as well as the direct effect of heavy metals on the function of immune cells in a biological fluid, particularly in breast milk (Avcin *et al*., 1991; Lönnerdal *et al*., 2003; Paxson, 2013; Yakushkin *et al*., 1992). This negative toxic effect of heavy metals may be links with the suppression of the synthesis of humoral protection factors of the body (immunoglobulins) or the enhancement of their catabolism (through the formation of inert complexes and their excretion from the body). There are also possible dysfunctions of the cells of the immune system (macrophages) as a result of the cell membranes' damage and other organelles by heavy metals (Golinelli *et al*., 2014; Kushnareva *et al*., 2015; Lönnerdal, 2013; Yamawaki *et al*., 2015). According to our studies, the increased content of heavy metals in breast milk of women was accompanied in all cases of severe violations from its protective factors (macrophage phagocytosis and / or IgG concentration). Such violations significantly less often and in a smaller degree occurred in women with a normal composition of the studied chemical elements of breast milk, but with various pathological conditions. The comparison of these results demonstrates that, apparently, in the first variant, the reduction of protective factors was associated with both the mother's health as well as the toxic effect of heavy metals, and only with the state of mother's health in the second variant. The decrease in IgM concentration in breast milk was almost the same in women with a normal level of metals in breast milk, as well as in women with a high content of these xenobiotics. Apparently, this violation from the immune system was linked with the presence of an infectious inflammatory pathology in mothers. Further studies of the mechanisms of the heavy metals' damaging effect on the women' immune system will help to develop methods of eliminating or preventing their toxic effect on the macroorganism. To eliminate the toxic load and reduce the concentration of heavy metals in breast milk, it is possible to correct the diet of a pregnant woman and nursing mother with the inclusion of food and / or biologically active additives in the diet that contain chelates. These are dairy products rich in protein, which inactivate heavy metals, as well as products containing vitamin P. Natural chelates (milk proteins and vitamin P) form strong complexes with heavy metals and remove them from the body (Golinelli *et al*., 2014; Paxson *et al*., 1979; Yakushkin, 1992). To protect body cells from the damaging effect of heavy metals, it is useful to enrich the diet with products containing bioantioxidants (vitamins A, C, E, P,

PP) (Agadzhanyan and Skal'nyj, 2001; Avcin *et al*., 1991; Boersma *et al*., 1991; Giribaldi *et al*., 2012). However, for the extensive recommendations on the correction of the breast milk' composition containing heavy metals, additional research is needed.

Conclusion

Thus, the protective factors of milk were significantly reduced in women whose heavy metals (Pb and Cr) content was increased and / or they were present in milk in combination with Cd, Sb, As. There was a decrease in the average concentration of IgG and phagocytosis of breast milk macrophages in these women in comparison with healthy women and women with somatic diseases and/or gynecological pathology, but who had a normal level of heavy metals in breast milk. The IgM content was reduced in women with pathologies and apart from the amount of heavy metals in breast milk. The phagocytic activity of milk macrophages could be reduced both by the number of activated macrophages and by the number of captured cells of the test strain Staphylococcus aureus P209, as well as on intensity of intracellular destruction of the pathogen. There was also a decrease in phagocytosis by the macrophage in women with pathological conditions, but with the normal composition of heavy metals in breast milk. However, these disorders were much less often (2-3.6 times) and less pronounced than in women with a changed composition of breast milk.

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