Research Note

Determination of cadmium and lead in human milk

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ABSTRACT: The objectives of this study were to determine cadmium and lead concentrations in the breast milk of healthy lactating women who were living in Zarrinshahr, an industrial area of Iran and to investigate the effect of mother's age, parity and smoking habits in families living in the vicinity of areas contaminated with heavy metals. Cadmium and lead concentrations were determined by graphite furnace atomic absorption spectrometry in 44 milk samples from healthy lactating women collected on first to sixth postpartum week. Accuracy of the analysis was checked by various methods including the use of reference material. The mean ± standard deviation of cadmium and lead concentrations in human milk were 2.44 ± 1.47 µg/L (range 0.62 - 6.32 µg/L) and 10.39 ± 4.72 µg/L (range 3.18-24.67 µg/L), respectively. There was a positive correlation between the concentrations of lead in milk samples and mother's age and parity ($P < 0.05$). Also the concentration of cadmium in breast milk significantly increased ($P < 0.05$) in mothers who were actively and/or passively exposed to smoking. The results of this study showed that lead and cadmium concentrations in milk samples from lactating women in Zarrinshahr were high. This can be a major public health hazard for the inhabitants living in this industrial region of Iran.

Keywords: *Breast milk; Heavy metals; Industrial area; Pollution; Toxic trace elements*

INTRODUCTION

Human milk is usually the only source of food for infants during the first four to five months of their lives. Many chemicals can be transferred from the body stores and from blood into the breast milk of a lactating mother. Despite the attention focused on environmentally persistent organochlorine compounds in human milk, levels of toxic metals in milk are also of significance (Wappelhorst *et al.,* 2002; Koizumi *et al.,* 2008). Each metal is distributed in a characteristic way between the milk fractions. Cadmium and lead are of considerable interest due to their toxicity and widespread use (Abdel-Ghani *et al.,* 2007; Gueu *et al.,* 2007; Samarghandi *et al.,* 2007; Karbassi *et al.,* 2008). In rat, lead is almost exclusively found in the casein fraction (96 %) of milk whereas cadmium is distributed mostly between fat (49 %) and casein (43 %) fractions (Oskarsson *et al.*, 1998).

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The kidneys are the major targets of cadmium toxicity following oral exposure. A specific indicator of cadmium–induced renal effects is tubular proteinuria. An increase in urine cadmium correlates well with low molecular weight proteins in urine. Other effects include disturbances in calcium metabolism, hypercalciuria, formation of renal stones (Hayano *et al.*, 1996) and hypertension during pregnancy (Kosanovic *et al.*, 2002).

The nervous system is the principal target organ for lead, although lead can adversely affect most organs in the body. The nervous system of the fetus and young children is particularly vulnerable to lead because of its rapid growth during this time. Many studies have demonstrated neurobehavioral impairment in children even at low lead exposure levels (Rice, 1996; Tripathi *et al.*, 1999). A decrease of IQ in children was associated with an increase in blood lead

(Schwartz, 1994) and tooth lead (McMichael *et al.*, 1994).

The objectives of this study were to determine cadmium and lead concentrations in the breast milk of healthy lactating women who were living in Zarrinshahr, an industrial area of Iran and to investigate the effect of mother's age, parity and smoking habits in the family on the concentration of these heavy metals.

MATERIALS AND METHODS
Human milk samples

From August 2006 to March 2007 a total of 44 milk samples were collected from 44 healthy women who were living in Zarrinshahr, Iran, at least two years before pregnancy. Zarrinshahr, located in the center of Iran, is an industrial city near a large iron foundry, a steel plant, an ordnance plant and a cement plant. The milk samples were taken during first to sixth postpartum week using a conventional breast pump at the Hygiene and Treatment Center of Zarrinshahr. All the milk samples were stored at -18 ºC in clean polyethylene containers which were previously soaked in 1:1 nitric acid for one day and well rinsed with doubly distilled water (ddH$_2$O). The samples were grouped based on age ($\leq$ 30 y vs. > 30 y) and parity (primiparous vs. multiparous). Eleven milk samples were taken from lactating women who were smoking or were passively exposed to smoke from other family members during pregnancy.

Determination of cadmium and lead

A total of 25 mL of the milk samples were acid digested with 7 mL of HNO$_3$ (Merck, Germany) and 7 mL of 30 % H$_2$O$_2$ (Merck, Germany) in a semi–closed glass digestion apparatus. After cooling, volume was adjusted to 50 mL with ddH$_2$O. The concentrations of cadmium and lead were determined by the graphite furnace atomic absorption spectrometry method using UNICAM 939 (Unicam, Cambridge, UK) apparatus with electrothermic atomizer GF-90 according to the method described previously (Ursinyova and

Masanova, 2005). The detection limits calculated as three times the standard deviation were 0.038 µg/L and 0.043 µg/L of milk for cadmium and lead, respectively. The accuracy of the analysis was checked by various methods including the use of reference material (BCR No 150). The certified and observed metal concentrations are presented in Table 1.

Statistical analysis

Using SPSS/16 statistical software; SPSS Inc., USA, a t-test analysis was performed and differences were considered significant at values of $P < 0.05$. For the statistical analysis, values below the detection limit were set to half that level.

RESULTS AND DISCUSSION

The cadmium and lead concentrations in 44 human milk samples, taken on the first to sixth postpartum week in Zarrinshahr are presented in Table 2. The mean ± standard deviation (SD) of cadmium and lead concentrations in human milk were 2.44 ± 1.47 µg/L (range 0.62 - 6.32 µg/L) and 10.39 ± 4.72 µg/L (range 3.18-24.67 µg/L), respectively.

In human milk, average background concentrations of cadmium and lead are between 0.05-2 µg/L and 5-20 µg/L, respectively, while in the heavily polluted areas they may be up to 20 times higher (Frkovic *et al.*, 1997). In table 3, the concentrations of cadmium and lead in human milk determined in the present study were compared with those reported from other countries.

The results of cadmium concentration in milk in this study are similar to those found in Croatia (Frkovic *et al.*, 1997), Poland (Sikorski *et al.*, 1989) and Turkey (Turan, 2001). The results of lead concentration in milk are between the low levels found in Sweden (Palminger Hallen *et al.*, 1995), Australia (Gulson *et al.*, 2001), China (Li *et al.*, 2000) and Austria (Gundacker *et al.*, 2000) and high levels found in Italy (Guidi *et al.*, 1992), Mexico (Namihiria *et al.*, 1993) and Singapore (Ong *et al.*, 1985). The differences found between different studies conducted in different countries might also be due to different assays employed.

Table 1: Cd and Pb concentration in standard reference material (BCR 150)

Element	Units	Concentration	
		Expected result	Observed result
Cd	mg/g	0.218	0.209 (0.198-0.221)
Pb	mg/g	1.0	1.16 (0.98-1.23)

Table 2: Pb and Cd concentrations (µg/L) in milk from healthy lactating women from Zarrinshahr, Iran

	No.	Cd		Pb	
		Mean ± SD* (range)	*P* value	Mean ± SD (range)	*P* value
Age					
< 30 y	22	2.02 ± 1.09 (0.86-4.62)		8.92 ± 3.28 (3.18-14.27)	
≥ 30 y	22	2.85 ± 1.69 (0.62-6.32)	0.059	11.86 ± 5.51 (4.70-24.67)	0.38
Parity					
Primiparous	22	2.06 ± 1.26 (0.62-4.62)		8.71 ± 4.11 (3.18-19.88)	
Multiparous	22	2.81 ± 1.59 (0.76-6.32)	0.089	12.07 ± 4.78 (4.67-19.88)	0.016
Smoking habits					
Smokers	11	3.39 ± 1.70 (0.76-5.94)		12.22 ± 5.70 (5.78-24.67)	
Nonsmokers	33	2.12 ± 1.25 (0.62-6.32)	0.011	9.78 ± 4.28 (3.18-21.08)	0.140
Total	44	2.44 ± 1.47 (0.62-6.32)		10.39 ± 4.72 (3.18-24.67)	

*SD: Standard deviation

Table 3: Cd and Pb concentrations in human milk reported internationally

Country	No.	Cd (µg/L)	Pb (µg/L)	Reference
Australia	15	-	0.55[a]	Gulson *et al.*, 2001
Austria	51	-	35.8 ± 15.0	Plockinger *et al.*, 1993
	23	-	1.5 ± 2.6	Gundacker *et al.*, 2000
China	165	-	4.74[d]	Li *et al.*, 2000
	21	-	52.7[e]	Li *et al.*, 2000
Croatia	29	2.54 ± 2.52[a]	7.3 ± 8.3	Frkovic *et al.*, 1997
Egypt	120	-	30.6[f]	Saleh *et al.*, 1996
England	39	-	30[f]	Richmond *et al.*, 1993
Germany	10	17.3 ± 4.9[a, g]	9.1 ± 2.5[b, g]	Sternowsky and Wessolowski, 1985
	10	24.6 ± 7.3[a, h]	13.3 ± 5.5[h]	Sternowsky and Wessolowski, 1985
Greece	47	-	20 ± 5[a]	Nashashibi *et al.*, 1999
Italy	34	-	45.62[b, g]	Guidi *et al.*, 1992
	20	-	126.55[b, h]	Guidi *et al.*, 1992
Japan	22	-	27[b]	Ding *et al.*, 1993
	68	0.277±1.82[a]	-	Honda *et al.*, 2003
Malaysia	91	-	21.1[b, g]	Huat *et al.*, 1983
Mexico	35	-	61.8[b, d]	Namihira *et al.*, 1993
	33	0.62 ± 0.28[a]	-	Rydzewska and Krol, 1996
Poland	110	2[b]	-	Sikorski *et al.*, 1989
Saudi Arabia	5	-	6.5 ± 2.2[a]	Younes *et al.*, 1995
	31	-	8.9 ± 5.3[a]	Younes *et al.*, 1995
	11	-	6.3 ± 3.8[a]	Younes *et al.*, 1995
	168	1.73[b]	31.67[b]	Al-Saleh *et al.*, 2003
Singapore	114	-	47.7[b]	Ong *et al.*, 1985
Slovakia	158	0.43 ± 0.27[a]	4.7 ± 4.1[a]	Ursinyova and Masanoua, 2005
Sweden	39	0.05 ± 0.04[a, d]	0.9 ± 0.4[a, d]	Palminger Hallen *et al.*, 1995
	35	0.07± 0.04[a,c]	0.5 ± 0.3[a, c]	Palminger Hallen *et al.*, 1995
Turkey	30	2.8[b]	14.6[b]	Turan *et al.*, 2001
Iran	44	2.44 ± 1.47[a]	10.39 ± 4.72[a]	This study

a: Mean ± SD; b: mean; c: control area; d: smelter area; e: occupationally exposed to lead; g: rural; h: urban

The results of t-test analysis for group comparison regarding mother's age, parity and smoking habits in family and relation to cadmium and lead concentrations in milk are presented in Table 2. In the present study, mean concentration of cadmium and lead in women age ≤ 30 y was lower than women aged > 30 y. This difference is statically significant for lead ($P < 0.05$) but not for cadmium ($P > 0.05$). Younes *et al.*, (1995) reported that the lead concentration was significantly lower in milk samples obtained from mothers aged ≤ 20 years (5.1 ± 1.4 µg/L) compared to mothers aged ≥ 36 years ($13.43.5$ µg/L). On the contrary, Frkovic, *et al*. (1997) and Ursinyova and Masanova (2005) observed higher cadmium and lead concentrations in the milk obtained from younger mothers. However, the differences were not statistically significant. Drasch *et al*., (1998) and Sikorski *et al*., (1989) did not find mother's age effective on concentration of cadmium in milk.

In this study, 22 women were primiparous and had somewhat lower concentration of cadmium and lead in breast milk compared to women with two or more deliveries. The difference was significant for lead ($P \leq 0.05$) but not for cadmium. Other studies also showed no influence of parity on cadmium concentration in milk and a slight increase of lead concentration in milk from lactating multiparous women (Sikorski *et al*., 1989; Frkovic *et al*., 1997; Ursinyova and Masanova, 2005).

Smoking habits in the family (smoking during pregnancy and/or being exposed to smoke from other family members) increased cadmium concentration in milk ($P < 0.05$). A tendency toward higher cadmium concentration in smokers was observed also in the Swedish and Creation lactating women (Plaminger Hallen *et al*., 1995; Frkovic *et al*., 1997) Eynon *et al*., (1985) estimated that the infants of smoking mothers were exposed 20 - 40 % more to cadmium than the infants from non–smoking mothers and the concentration of cadmium in milk were increased by 8 %, 17 % and 28 % due to the mother's smoking habits before pregnancy; father's smoking habits and smoking at home, respectively. Leotsinidis *et al*., (2005) and Ursinyova and Masanova (2005) observed higher cadmium concentration in the milk in women from families with smoking habits, but differences were not statistically significant.

The results of this study show that smoking habits do not have a significant influence on the lead concentration in breast milk ($P > 0.05$) which is in accordance with the results obtained in Sweden, Croatia, Greece and Slovakia (Palminger Hallen *et al*., 1995; Frokvic *et al*., 1997; Ursinyova and Masanova, 2005). However, Gundacker *et al*. (2000) found that smoking increased lead concentration in milk significantly.

CONCLUSION

The results of this study show that the cadmium and lead concentration in milk samples obtained from healthy women in Zarrinshahr are high which a major public health hazard is for inhabitants of industrial locations especially neonatal and children. Cadmium and lead in milk are better absorbed into the body than other dietary components, therefore high cadmium and lead concentration in breast milk is the first source of poisoning with these heavy metals in neonates (Frkovic *et al.,* 1997). In view of all potential consequences of advanced lead and cadmium intoxication, it is necessary to point out the importance of an early diagnosis before morphological changes have been developed. Moreover, the results of the present study indicate a need for establishing safe intake values of heavy metals in human milk.

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