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ORIGINAL ARTICLE

Breast Milk Lead and Cadmium Levels in Suburban Areas of Nanjing, China

Kang-sheng Liu¹, Jia-hu Hao², Yu-qing Xu¹, Xiao-qi Gu³, Juan Shi⁴,
Chun-fang Dai⁵, Fei Xu⁶, and Rong Shen^{3*}

¹State Key Laboratory of Reproductive Medicine, Department of Clinical Laboratory, Nanjing
Maternity and Child Health Care Hospital Affiliated to Nanjing Medical University,
Nanjing 210029, China

²Department of Maternity and Child Health Care, School of Public Health,
Anhui Medical University, Hefei 230032, China

³Director's Office, ⁴Child Health Care Department, ⁵Obstetrical Department, Nanjing Maternity
and Child Health Care Hospital Affiliated to Nanjing Medical University, Nanjing 210004, China

⁶Department of Clinical Laboratory, Nanjing Children's Hospital, Nanjing 210008, China

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Objective To evaluate levels of lead (Pb) and cadmium (Cd) in the breast milk in the second postpartum month, to investigate the relationship between Pb/Cd levels in breast milk and some sociodemographic parameters, and to explore whether these levels affect the infants' physical status or the mothers' psychological status (postpartum depression).

Methods A cross-sectional study was conducted between November 2009 and December 2010. Altogether 170 healthy mothers were enrolled from Nanjing Maternity and Child Health Care Hospital. The inclusion criteria were: voluntary to participate in this study, healthy, with no chronic disease, breastfeeding in the second postpartum month, living in a suburban but not non-industrial area of Nanjing, and not occupationally exposed to toxic metals. All the mothers completed a questionnaire and were evaluated based on the Edinburgh Postpartum Depression Scale (EPDS) to identify the risk of postpartum depression. Pb and Cd levels in breast milk were determined by inductively coupled plasma mass spectroscopy. The infants of these mothers were examined for their z scores of weight for age, length for age, head circumference for age, and body mass index for age.

Results The median breast milk levels of Pb and Cd were 40.6 µg/L and 0.67 µg/L, respectively. In 164 (96.5%) of the 170 samples, Pb levels were higher than the limit reported by the World Health Organization (> 5 µg/L). Breast milk Cd level was > 1 µg/L in 54 (31.8%) mothers. The mothers with a his-

tory of anemia had a higher breast milk Pb level than those without a history of anemia (41.1 $\mu\text{g/L}$ vs. 37.9 $\mu\text{g/L}$, $P = 0.050$). The median breast milk Cd level in those who were active and passive smokers during pregnancy was significantly higher than that in non-smokers (0.88 $\mu\text{g/L}$ vs. 0.00 $\mu\text{g/L}$, $P = 0.025$). The breast milk Cd level in the mothers not taking iron and vitamin supplements for 2 months postpartum was higher than in those taking the supplements (iron supplement: 0.74 $\mu\text{g/L}$ vs. 0.00 $\mu\text{g/L}$, $P = 0.025$; vitamin supplement: 0.78 $\mu\text{g/L}$ vs. 0.00 $\mu\text{g/L}$, $P = 0.005$). Breast milk Cd level at the second postpartum month was negatively correlated with the z scores of head circumference ($r = -0.248$, $P = 0.042$) and weight for age at birth ($r = -0.241$, $P = 0.024$) in girls. No correlation was found between the breast milk Pb/Cd levels and the EPDS scores.

Conclusion Considering the high levels of Pb and Cd in breast milk in this study, breast milk monitoring programs are necessary.

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THE rapid industrialization and urbanization have increased the exposure to environmental pollutants.¹ Lead (Pb) and cadmium (Cd) have been reported as among the top 10 toxic metals in the Priority List of Hazardous Substances announced by the Agency for Toxic Substances and Disease Registry.² Pb poses a public health problem due to its adverse effects, mainly those affecting the central nervous system in the most vulnerable populations, such as pregnant and lactating women and children. In adults, Pb exposure has been found associated with anxiety and depression.³ Because of the absence of a safe exposure limit in children and the ability of Pb to accumulate in the body for a long time, a great interest in evaluating its adverse effects in low concentrations has emerged.⁴ Cd has also been found to cause neurotoxicologic and behavioral changes in both human and experimental animal studies.⁵ Cd exposure may be implicated in some neurological disorders including hyperactivity and increased aggressiveness in humans.⁶ Nonetheless, the observational clinical data on the relationship between anxiety or depression and Cd exposure are inadequate.

Pb can negatively influence the growth of newborns. Previous studies have reported an inverse relationship between anthropometric measurements of newborns and the Pb levels in placenta/umbilical cord.⁷ As Pb is readily transferred to the fetus through the placenta,⁸ its interference can be observed on early embryonic development and during the last months of pregnancy.⁹ The primary sources of Pb in breast milk are diet and bone lead. When up to 5% of bone mass is mobilized as a source of calcium during pregnancy and lactation, the lead accumulated in bone from previous exposure is concomitantly released into the blood and excreted into breast milk.¹⁰ Detectable Pb level in breast milk has been documented in population studies of women with no current environmental or oc-

cupational Pb exposures.¹¹

The levels of toxic metals in breast milk can indicate the exposure on the part of the infants and the possible prenatal exposure on the part of the mothers.¹² A limited number of studies have been conducted to determine the toxic metal concentrations in breast milk.⁴ In the present study, we detected the Pb and Cd levels in breast milk in the second postpartum month, investigated the relationship between some sociodemographic parameters and the concentrations of Pb and Cd, and analyzed the influence of these toxic metals on the infants' growth and on the mothers' postpartum depression.

MATERIALS AND METHODS

Study population

This is a cross-sectional study conducted from November 2009 to December 2010. A total of 170 voluntarily enrolled healthy mothers were evaluated at Nanjing Maternity and Child Health Care Hospital, and all of them provided breast milk samples at the second postpartum month. These mothers were all living in a suburban but not non-industrial area of Nanjing, and not occupationally exposed to toxic metals.

All the mothers completed a questionnaire regarding their occupation, smoking habits, childbearing history, history of anemia at any time, and intake of iron and vitamin supplementation during pregnancy and within 2 months after delivery. The level of maternal hemoglobin on the first postpartum day was taken from the hospital records. All the studied mothers were evaluated using the Edinburgh Postpartum Depression Scale (EPDS) to identify the risk of postpartum depression, those with a score ≥ 13 were considered at the risk of depression.¹³ The infants of these mothers were examined, and their z scores of weight for age (WAZ), length for age (LAZ), head circumference

for age (HCZ), and body mass index for age (BAZ) were calculated based on recent World Health Organization Growth References.¹⁴ The study was approved by the Ethical Committee of Nanjing Maternity and Child Health Care Hospital. The mothers were informed about the purpose of the study and written informed consents were obtained from all the participants.

Analysis of the breast milk

The breast milk samples were collected 2 hours after the latest feeding session in the morning. Every mother expressed 10 mL milk directly into clean polyethylene tubes. All the samples were frozen immediately after collection and kept in -20°C until analyzed.

In order to minimize the polyatomic interferences, reagent water equivalent to ASTM Type 1 (ASTM D 1193, $>18\text{ m}\Omega\text{ cm}$ resistivity), 0.5% (v/v) nitric acid, a tuning solution (for sensitivity tuning: $10\text{ }\mu\text{g/L}$ for each of the metals Li, Y, and Tl in 0.1% HNO_3), and a single-element standard stock solution for Pb and Cd were used. Breast milk sample (1 g) was accurately weighed, put into a dry XP1500 vessel, into which 5 mL of 0.1% HNO_3 and 5 mL of distilled water were added. For digestion, this mixture was placed in a microwave oven at 105°C for 10 minutes. Following digestion, the samples were diluted with 25 mL distilled water. The standard stock solution and the milk samples in the polyethylene tubes were placed in the automatic sequencer of the Inductively Coupled Plasma Mass Spectrometer (Yokogawa Analytical Systems, Tokyo, Japan). The lowest limits of quantitation (LOQ) for Pb and Cd were both $0.2\text{ }\mu\text{g/L}$.

Statistical analysis

The data were analyzed using SPSS 14.0. The results were presented as means $\pm$ SD (range), and medians (25th-75th percentiles). The Mann-Whitney U test was applied to compare the Pb and Cd levels between subgroups divided according to the characteristics listed in the questionnaire. The Pearson correlation coefficient was used for the relationship between breast milk Pb and Cd concentrations and numerical variables (anthropometric measurements, EPDS scores, etc.). $P<0.05$ was considered statistically significant.

RESULTS

General information

The mean maternal age was 26 ± 4 years (18-42 years), 7 (4.1%) were over 34 years. One hundred and ten (64.7%) of the mothers had been educated for no more than 8

years. Only 37 (21.8%) mothers were working. The monthly family income was less than 2000 yuan in 20 (11.8%), 2000 yuan in 40 (23.5%), and over 2000 yuan in 110 (64.7%). Mean gravidity was 2.1 ± 0.9 , and mean parity 1.7 ± 0.8 (Table 1). The birth interval was less than 2 years in 10 (5.3%) mothers who had parity >1 . The mean maternal hemoglobin level was $11.4\pm 1.4\text{ g/dL}$ (6.1-14.8 g/dL).

Seventy-five (44.1%) of the infants were female and 95 (55.9%) were male. Nine (5.3%) of the infants were $<2500\text{ g}$ at birth and 21 were born <37 weeks gestation. The mean birth weight was $3216\pm 345\text{ g}$ (2131-4110 g) and the mean head circumference at birth was $34.1\pm 1.7\text{ cm}$ (31.0-39.0 cm). In the second month after birth, the mean weight for age was $4788\pm 634\text{ g}$ (2670-7100 g), the mean length for age was $54.17\pm 2.71\text{ cm}$ (41.5-67.0 cm), and the mean head circumference for age was $37.9\pm 1.4\text{ cm}$ (33.0-42.0 cm) (Table 1).

Pb and Cd levels in breast milk

The Pb level was above the LOQ in 168 (98.8%) breast milk samples, and $>5\text{ }\mu\text{g/L}$ in 164 (96.5%) samples. The median (25th-75th percentile) Pb level of the 170 samples was $40.6\text{ }\mu\text{g/L}$ (22.1-59.2 $\mu\text{g/L}$), which was confirmed by a duplicate analysis.

The Cd level in breast milk was above LOQ in 87 (51.2%) samples, and $>1\text{ }\mu\text{g/L}$ in 54 (31.8%) samples. The median Cd level was $0.67\text{ }\mu\text{g/L}$ ($<\text{LOQ}$ - $1.26\text{ }\mu\text{g/L}$) (Table 2). The highest Cd level in the samples was $43.0\text{ }\mu\text{g/L}$. The mother was 19 years old, and this was her first pregnancy. The baby was delivered by vaginal birth, weighing 3400 g. There was no history of chronic disease.

Table 1. Characteristics of the studied mothers and infants

Characteristics	Values
Mothers ($n=170$)	
Age [year, $\bar{x}\pm s$ (range)]	26 ± 4 (18-42)
Being housewife [n (%)]	133 (78.2)
Education ≤ 8 years [n (%)]	110 (64.7)
Gravidity [times, $\bar{x}\pm s$ (range)]	2.1 ± 0.9 (1-11)
Parity [times, $\bar{x}\pm s$ (range)]	1.7 ± 0.8 (1-9)
Infants ($n=170$)	
Gender (male/female)	95/75
Gestational age [week, $\bar{x}\pm s$ (range)]	38.4 ± 1.4 (32.0-41.0)
Birth weight [g, $\bar{x}\pm s$ (range)]	3216 ± 345 (2131-4110)
Head circumference at birth [cm, $\bar{x}\pm s$ (range)]	34.1 ± 1.7 (31.0-39.0)
Weight for age at 2 months [g, $\bar{x}\pm s$ (range)]	4788 ± 634 (2670-7100)
Length for age at 2 months [cm, $\bar{x}\pm s$ (range)]	54.17 ± 2.71 (41.5-67.0)
Head circumference for age at 2 months [cm, $\bar{x}\pm s$ (range)]	37.9 ± 1.4 (33.0-42.0)

She was diagnosed with anemia during the pregnancy but did not take iron supplements. The maternal hemoglobin level on the first postpartum day was 9.1 g/dL. She had lived in a suburban area since childhood. The heating in her former childhood house was provided by a coal stove. Her family currently lived in a 20-year-old house, heated by a coal stove as well, and close to the main road (< 200 m). She and her husband were both non-smokers and the mother was not exposed to cigarette smoke during her childhood in her family environment.

Relationship between maternal sociodemographic characteristics and breast milk Pb/Cd levels

The maternal age and education level, monthly family income, parity, and birth interval were not related with the breast milk Pb and Cd levels. The employment status of the mothers did not affect the Pb level in breast milk, while the median breast milk Cd level was higher in the unemployed mothers than that in the working mothers (0.71 µg/L vs. 0.01 µg/L, $P = 0.025$).

Active and passive smoking during pregnancy and in 2 postpartum months was analyzed. The mean daily number of cigarettes in active smokers was < 5 and thus active and passive smokers were combined for analysis (active and passive smokers versus non-smokers). Smoking during pregnancy or in 2 postpartum months had no effect on the breast milk Pb level, while the median breast milk Cd level in the active and passive smokers during pregnancy was significantly higher than that in non-smokers (0.88 µg/L vs. 0.00 µg/L, $P = 0.025$, Table 3). Smoking in 2 postpartum months had no significant effect on the breast milk Cd level.

The mothers with a history of anemia at any time had higher breast milk Pb level than those without anemia (41.1 µg/L vs. 37.9 µg/L, $P = 0.050$, Table 3). According to the Spearman correlation analysis, no significant correlation was observed between the maternal Hb level and the breast milk levels of Pb and Cd (Pb: $r_s = -0.031$, $P = 0.644$; Cd: $r_s = -0.117$, $P = 0.160$). The use of iron and vitamin supplements during pregnancy or in 2 postpartum months did not affect the breast milk Pb level. The mothers who did not take iron and vitamin supplements for 2 months postpartum had higher breast milk Cd level than

those who did (iron supplement: 0.74 µg/L vs. 0.00 µg/L, $P = 0.025$; vitamin supplement: 0.78 µg/L vs. 0.00 µg/L, $P = 0.005$; Table 3). Nonetheless, the use of iron and vitamin supplements during pregnancy did not affect the breast milk Cd level.

Twenty-six mothers (15.3%) were at risk of postpartum depression at the second postpartum month. The breast milk Pb and Cd levels in the mothers at risk of depression were not significantly different from those in the mothers not at risk (Pb: $r = -0.055$, $P > 0.05$; Cd: $r = -0.051$, $P > 0.05$).

Relationship between infant factors and breast milk Pb/Cd levels

Breast milk Pb or Cd levels did not change with birth weight, gestational age, or gender of infants (Table 4). In the infants, 61 had crying attacks of colic type and 75 had irregular sleep pattern. Infants with irregular sleep pattern and colic crying had similar breast milk Cd and Pb levels compared with infants without these problems. A total of 117 infants (68.8%) were exclusively breastfed at the second postpartum month. In the comparison between exclusively breastfed infants and mixed fed infants, the Pb and Cd levels in their mothers' breast milk at the second postpartum month showed no significant difference.

Breast milk Cd level at the second postpartum month was negatively correlated with HCZ and WAZ at birth in girls (HCZ: $r = -0.248$, $P = 0.042$; WAZ: $r = -0.241$, $P = 0.024$; Table 5). Breast milk Pb and Cd levels were not correlated with other anthropometric measurements.

DISCUSSION

The WHO has reported that 2-5 µg/L of Pb may exist in the breast milk in 3 postpartum months under normal conditions based on its research conducted in 1989 in 6 countries (Guatemala, Hungary, Nigeria, Philippines, Sweden, and Zaire).¹⁵ Pb level in breast milk varies with regions (0.5-126.6 µg/L).¹⁶⁻¹⁸ The difference in the breast milk Pb level may depend on various factors such as the time of sampling (morning or night), the time of lactation (colostrum/transient/mature milk or foremilk/hindmilk), the method of sampling (pump or manual), maternal factors

Table 2. Levels of lead (Pb) and cadmium (Cd) in breast milk at the second postpartum month (µg/L, $n = 170$)

Toxic metals	Minimum	Percentiles			Maximum	> LOQ [n (%)]	> WHO recommended limit level [n (%)]
		25	50	75			
Pb	< LOQ	22.1	40.6	59.2	1212.0	168 (98.8)	164 (96.5)
Cd	< LOQ	< LOQ	0.67	1.26	43.0	87 (51.2)	54 (31.8)

LOQ: limits of quantitation; LOQ for Pb and Cd are both 0.2 µg/L; WHO recommended limit for Pb is < 5 µg/L, for Cd is < 1 µg/L.

Table 3. Maternal sociodemographic characteristics and Pb/Cd levels in breast milk

Sociodemographic characteristics	Number	Pb level [$\mu\text{g/L}$, median (25th-75th percentile)]	Cd level [$\mu\text{g/L}$, median (25th-75th percentile)]
Age (year)			
≤21	41	40.9 (17.3-59.1)	0.51 (0.00-1.64)
>21	129	40.2 (12.1-59.1)	0.61 (0.00-1.24)
Monthly family income (yuan)			
≤2000	60	41.9 (14.3-59.1)	0.00 (0.00-1.47)
>2000	110	40.42 (14.0-59.1)	0.61 (0.00-1.23)
Education (year)			
≤8	110	40.0 (12.8-56.2)	0.61 (0.00-1.22)
>8	60	41.3 (14.3-55.7)	0.61 (0.00-1.41)
Parity			
1	81	40.7 (11.4-59.7)	0.62 (0.00-1.37)
>1	89	40.1 (10.8-24.3)	0.68 (0.00-1.24)
Working status			
Employed	37	41.1 (9.25-56.7)	0.01 (0.00-0.69)*
Housewife	133	40.2 (11.7-59.4)	0.71 (0.00-1.28)
Birth interval (year)			
≤2	14	43.6 (20.1-61.2)	0.52 (0.00-3.31)
>2	72	40.0 (11.3-49.0)	0.64 (0.00-1.22)
Smoking during pregnancy			
Active and passive	84	40.6 (12.4-51.0)	0.88 (0.00-1.30)*
No smoking	86	40.5 (10.5-58.1)	0.00 (0.00-1.12)
Smoking during 2 postpartum months			
Active and passive	114	40.9 (10.3-61.1)	0.70 (0.00-1.36)
No smoking	56	40.0 (10.7-55.1)	0.24 (0.00-1.22)
Hb value on the first postpartum day (g/dL)			
≤11	41	40.4 (15.3-58.3)	0.84 (0.00-1.53)
>11	129	40.6 (10.1-54.7)	0.67 (0.00-1.20)
Intake of vitamin supplement in pregnancy			
Yes	140	40.6 (12.2-61.4)	0.00 (0.00-1.24)
No	30	41.6 (13.7-58.5)	0.69 (0.00-1.29)
Intake of iron supplement in pregnancy			
Yes	40	46.4 (3.41-54.9)	0.53 (0.00-1.33)
No	130	40.0 (12.8-60.1)	0.67 (0.00-1.14)
Intake of vitamin supplement in 2 postpartum months			
Yes	40	41.2 (15.1-57.4)	0.00 (0.00-0.78) [#]
No	130	40.0 (9.47-59.0)	0.78 (0.00-1.26)
Intake of iron supplement in 2 postpartum months			
Yes	37	42.6 (15.1-57.1)	0.00 (0.00-0.98)*
No	113	39.2 (9.10-59.8)	0.74 (0.00-1.16)
History of anemia at any time			
Yes	67	41.1 (17.8-61.0) [†]	0.51 (0.00-1.34)
No	34	37.9 (6.75-54.3)	0.21 (0.00-1.09)

* $P=0.025$ compared with the housewives/women not smoking during pregnancy/women not taking iron supplement during 2 postpartum months; [#] $P=0.005$ compared with the women not taking vitamin supplement during 2 postpartum months; [†] $P=0.050$ compared with the women without a history of anemia at any time.

Table 4. Relationship between infant factors and Pb/Cd levels in breast milk at the second postpartum month

Infant factor	Number	Pb level [$\mu\text{g/L}$, median (25th-75th percentile)]	Cd level [$\mu\text{g/L}$, median (25th-75th percentile)]
Gender			
Girl	75	40.1 (9.14-41.4)	0.51 (0.00-1.17)
Boy	95	40.9 (14.5-49.4)	0.61 (0.00-1.26)
Gestational age (week)			
<37	34	40.1 (15.2-48.9)	0.01 (0.00-1.39)
≥ 37	136	40.6 (12.2-50.2)	0.70 (0.00-1.26)
Birth weight (g)			
<2500	21	40.1 (8.1-47.2)	0.18 (0.00-1.01)
≥ 2500	149	40.7 (10.8-49.8)	0.68 (0.00-1.27)
Sleep pattern			
Regular	95	40.6 (11.4-48.1)	0.69 (0.00-1.16)
Irregular	75	40.6 (12.0-60.2)	0.51 (0.00-1.21)
Colic crying			
Yes	61	40.6 (11.0-49.0)	0.70 (0.00-1.34)
No	109	40.6 (12.1-49.4)	0.71 (0.00-1.21)

Table 5. Correlations between breast milk Pb/Cd levels and z scores of anthropometric measurements at birth and the second postpartum month

Z scores of anthropometric measurements	Correlation coefficient in girls		Correlation coefficient in boys	
	Pb	Cd	Pb	Cd
At birth				
HCZ	0.074	-0.248*	0.017	0.031
WAZ	0.091	-0.241 [#]	0.038	0.062
LAZ	0.100	-0.014	0.002	0.017
BAZ	0.087	0.026	0.059	0.062
Two months after birth				
HCZ	0.145	0.064	0.061	0.058
WAZ	0.147	0.001	0.057	0.078
LAZ	0.122	-0.037	0.003	0.031
BAZ	0.111	0.030	0.080	0.091

HCZ: head circumference for age; WAZ: weight for age; LAZ: length for age; BAZ: body mass index for age; * $P=0.042$; [#] $P=0.024$

(parity and maternal Pb burden), as well as environmental factors (place of residence and exposure level/duration). However, several other factors (methods of analysis, contamination of the samples, etc.) might interfere in the final results as well.¹⁹⁻²¹ Considering the previously reported range, the present study detected high levels of Pb in breast milk (40.6 $\mu\text{g/L}$), consistent with the results of some previous studies, in which there were risk factors such as occupational exposure, urban living, living close to ore processing plants.^{22,23} The participants in the present study, in contrast, lived in a suburban area, had no occupational exposure, and were not close to ore processing plants.

The median breast milk Cd level at the second postpartum month was 0.67 (< LOQ-1.26) $\mu\text{g/L}$ in this study. The level reported in the literature is 0.06-24.6 $\mu\text{g/L}$.^{24,25} Only 5 breast milk samples in the present study showed a Cd level higher than the minimal risk level (5 $\mu\text{g/L}$) stated

by Abadin et al.¹¹ The breast milk Cd level at the second postpartum month in the present study was lower than that reported by Turan et al.²⁶

The present study did not find maternal age and education level, monthly family income, parity, and birth interval correlated with the breast milk Pb and Cd levels. However, Younes et al.²⁷ detected lower breast milk Pb levels in younger mothers (≤ 20 years) than in older mothers (≥ 36 years) (5.1 $\pm$ 1.4 $\mu\text{g/L}$ vs. 13.4 $\pm$ 3.5 $\mu\text{g/L}$). Several studies have shown that maternal age and parity are not correlated with breast milk Cd concentrations.^{4,28} In the present study, mothers who were housewives had significantly higher breast milk Cd concentrations than the working mothers. A possible explanation is that Cd may bind to the dust particles in indoor air. It has been reported that the amount of Cd in household dust particles was higher than that in outdoor air.²⁹ The inadequate ventilation and the presence of cigarette smoke in the house may

cause the high breast milk Cd level in housewife mothers.

Some studies reported a relation between iron deficiency and high blood Pb level in humans.³⁰ In the present study, mothers with a history of anemia at any time had higher breast milk Pb level than those without anemia history ($P=0.050$). Nonetheless, this study revealed no significant relation between breast milk Pb level and maternal Hb level on the first postpartum day postpartum, or between breast milk Pb level and iron supplementation during pregnancy or in 2 postpartum months. Similarly, Koyashiki et al³¹ observed no correlation between Hb and lead level in milk/blood samples taken between the 15 th and 210 th day after delivery.

Vahter et al³² reported an increase in Cd absorption and its toxicity in the presence of decreased iron stores and iron deficiency. Satarug et al³³ reported that non-smoking mothers with insufficient iron stores had three times more body Cd load than the mothers with sufficient iron stores. However, the present study showed that the breast milk Cd level at the second postpartum month did not change with maternal anemia status, which might be explained by the severity and duration of anemia.

In this study, the breast milk of the mothers not taking vitamin and iron supplements at the second postpartum month contained higher levels of Cd than those of the mothers taking supplements ($P=0.005$ and 0.025). Similarly, Gundacker et al³⁴ identified lower breast milk Cd concentrations at 2-14 postpartum days in the non-smoking mothers who took trace element/vitamin supplements. In contrast, Leotsinidis et al³⁵ reported that taking trace element/vitamin supplements did not affect the Cd levels in breast milk.

One of the most well-known routes of exposure to Pb and Cd is through cigarette smoking.³⁶ In this study, mothers with active and/or passive smoking exposure during pregnancy had significantly higher breast milk Cd level than non-smoking mothers; however, maternal smoking habit in the postpartum period did not affect breast milk Pb level. Although some studies reported a relation between smoking and breast milk Pb levels,³⁴ other studies have shown no such relationship.³⁷ This might be explained by the number of cigarettes consumed or other associated environmental contaminants. Ursinyova et al³⁷ found higher breast milk Cd level in mothers actively smoking before and during pregnancy.

Some clinical studies have been conducted on anxiety and depression status with exposure to Pb.³⁸ Dang et al³⁸ assessed the mental health status of the residents in a mining area in Hubei using the Symptom Checklist 90 (SCL-90) and State-Trait Anxiety Inventory (STAI). They

found that the mental health status of the residents was affected, having higher scores on the S-AI and T-AI scales than those living in a non-mining area. This study found no correlation between the breast milk Pb/Cd levels and EPDS scores. To our knowledge, this is the first study investigating the association between breast milk Pb/Cd levels and maternal mental health in the postpartum period. Further studies are necessary to confirm the finding of the present study.

The present study found no relationship between the breast milk Pb/Cd levels at the second postpartum month and infant characteristics such as birth weight, gestational age, and gender.

Irregular sleep pattern or colic crying in infants were not associated with different breast milk Pb and Cd levels in their mothers at the second postpartum month in the present study. On the other hand, there has been no published study exploring the effect of toxic metals on infantile colic.

The clinical presentation with toxic metals might differ with gender.³⁹ In this study, breast milk Cd level was negatively correlated with HCZ and WAZ at birth in girls. The breast milk Pb level in the second postpartum month was not correlated with the anthropometric measurements of the infants at birth or 2 months after birth in either sex in the present study. Kordas et al⁴⁰ reported a negative correlation between the blood Pb levels and the head circumference measurements of 602 infants aged 8 years and living near a casting plant in Mexico. The possible explanation for these differences might be the level of intoxication, associated environmental conditions, genetic factors, and follow-up duration.⁴⁰

In conclusion, Pb level in breast milk samples in this study were much higher than the currently recommended safety limits. Human milk could be used for research on lead exposure, with the goal to evaluate the risk to children's health using a non-invasive biological procedure. Investigations are urgently required to explore factors such as environment, diet, lifestyle, and/or cultural habits contributing to maternal and fetal exposures to Pb and Cd. Preventive measures must be taken accordingly to reduce environmental exposure and manage anemia in women in all age groups. Furthermore, periodic breast milk monitoring programs could help in evaluating maternal exposure due to ongoing exposure during the course of lactation.

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REFERENCES

1. Wong SL, Lye EJ. Lead, mercury and cadmium levels in Canadians. *Health Rep* 2008;19:31-6.
2. Agency for Toxic Substances & Disease Registry. Priority List of Hazardous Substances. Atlanta: Agency for Toxic Substances and Disease Registry. 2007 [cited 2012 Jul 1]. Available from: <http://www.atsdr.cdc.gov/SPL/index.html>
3. Schnaas L, Rothenberg SJ, Flores MF, et al. Reduced intellectual development in children with prenatal lead exposure. *Environ Health Perspect* 2006;114:791-9.
4. Dursun A. The levels of Pb, Hg, Cd in cord blood, breast milk, and newborn hair. Ankara: Thesis, Hacettepe University; 2008.
5. Sinha M, Manna P, Sil PC. Cadmium-induced neurological disorders: prophylactic role of taurine. *J Appl Toxicol* 2008; 28:974-86.
6. Matés JM, Segura JA, Alonso FJ, et al. Role of dioxins and heavy metals in cancer and neurological diseases by ROS-mediated mechanisms. *Free Radic Biol Med* 2010; 49:1328-41.
7. Llanos MN, Ronco AM. Fetal growth restriction is related to placental levels of lead and arsenic but not with antioxidant activities. *Reprod Toxicol* 2009; 27:88-92.
8. Balleg C, Khan LK, Kaufmann R, et al. Blood lead concentration and children's anthropometric dimensions in the Third National Health and Nutrition Examination Survey (NHANES III), 1988-1994. *J Pediatr* 1999;134: 623-30.
9. Rossipal E, Krachler M, Li F, et al. Investigation of the transport of trace elements across barriers in humans: studies of placental and mammary transfer. *Acta Paediatr* 2000;89:1190-5.
10. Ettinger AS, Téllez-Rojo MM, Amarasiriwardena C, et al. Influence of maternal bone lead burden and calcium intake on levels of lead in breast milk over the course of lactation. *Am J Epidemiol* 2006;163:48-56.
11. Abadin HG, Hibbs BF, Pohl HR. Breast-feeding exposure of infants to cadmium, lead and mercury: a public health viewpoint. *Toxicol Ind Health* 1997;13:495-517.
12. Järup L. Hazards of heavy metal contamination. *Br Med Bull* 2003;68:167-82.
13. Engindeniz N. Validation of Turkish form of Edinburgh Postpartum Depression Scale. Thesis, Ege University, İzmir: 1996.
14. WHO Multicentre Growth Reference Study Group. WHO Child growth standards based on length/height, weight and age. *Acta Paediatr Suppl* 2006;450:76-85.
15. Parr RM, DeMaeyer EM, Iyengar VG, et al. Minor and trace elements in human milk from Guatemala, Hungary, Nigeria, Philippines, Sweden, and Zaire. Results from a WHO/IAEA joint project. *Biol Trace Elem Res* 1991; 29:51-75.
16. Guidi B, Ronchi S, Ori E, et al. Lead concentrations in breast milk of women living in urban areas compared with women living in rural areas. *Pediatr Med Chir* 1992; 14:611-6.
17. Gulson BL, Mizon KJ, Palmer JM, et al. Longitudinal study of daily intake and excretion of lead in newly born infants. *Environ Res* 2001;85: 232-45.
18. Gundacker C, Pietschnig B, Wittmann KJ, et al. Smoking, cereal consumption, and supplementation affect cadmium content in breast milk. *J Expo Sci Environ Epidemiol* 2007;17:39-46.
19. Casey CE, Smith A, Zhang P. Microminerals in human and animal milk. In: Jensen RC, editor. *Handbook of milk composition*. San Diego: Academic Press; 1995.
20. Ettinger AS, Téllez-Rojo MM, Amarasiriwardena C, et al. Levels of lead in breast milk and their relation to maternal blood and bone lead levels at one month postpartum. *Environ Health Perspect* 2004;112:926-31.
21. Iyengar V, Woittiez J. Trace elements in human clinical specimens: evaluation of literature data to identify reference values. *Clin Chem* 1988; 34:474-81.
22. Al-Saleh I, Shinwari N, Mashhour A. Heavy metal concentrations in the breast milk of Saudi women. *Biol Trace Elem Res* 2003; 96:21-37.
23. Souad C, Farida Z, Narda L, et al. Trace element level in infant hair and diet, and in the local environment of the Moroccan city of Marrakech. *Sci Total Environ* 2006; 370:337-42.
24. Hallén IP, Jorhem L, Lagerkvist BJ, et al. Lead and cadmium levels in human milk and blood. *Sci Total Environ* 1995;166:149-55.
25. Sternowsky HJ, Wessolowski R. Lead and cadmium in breast milk. Higher levels in urban vs rural mothers during the first 3 months of lactation. *Arch Toxicol* 1985;57: 41-5.
26. Turan S, Saygi S, Kiliç Z, et al. Determination of heavy metal contents in human colostrum samples by electrothermal atomic absorption spectrophotometry. *J Trop Pediatr* 2001;47:81-5.
27. Younes B, Al-Meshari AA, Al-Hakeem A, et al. Lead concentration in breast milk of nursing mothers living in Riyadh. *Ann Saudi Med* 1995;15:249-51.
28. Honda R, Tawara K, Nishijo M, et al. Cadmium exposure and trace elements in human breast milk. *Toxicology* 2003;186:255-9.

29. Rasmussen PE, Subramanian KS, Jessiman BJ. A multi-element profile of house dust in relation to exterior dust and soils in the city of Ottawa, Canada. *Sci Total Environ* 2001;267:125-40.
30. Wright RO, Tsaih SW, Schwartz J, et al. Association between iron deficiency and blood lead level in a longitudinal analysis of children followed in an urban primary care clinic. *J Pediatr* 2003;142:9-14.
31. Koyashiki GA, Paoliello MM, Matsuo T, et al. Lead levels in milk and blood from donors to the Breast Milk Bank in Southern Brazil. *Environ Res* 2010; 110:265-71.
32. Vahter M, Berglund M, Akesson A, et al. Metals and women's health. *Environ Res* 2002;88:145-55.
33. Satarug S, Ujjin P, Vanavanitkun Y, et al. Influence of body iron store status and cigarette smoking on cadmium body burden of healthy Thai women and men. *Toxicol Lett* 2004;148:177-85.
34. Gundacker C, Pietschnig B, Wittmann KJ, et al. Lead and mercury in breast milk. *Pediatrics* 2002;110:873-8.
35. Leotsinidis M, Alexopoulos A, Kostopoulou-Farri E. Toxic and essential trace elements in human milk from Greek lactating women: association with dietary habits and other factors. *Chemosphere* 2005;61:238-47.
36. Gundacker C, Pietschnig B, Wittmann KJ, et al. Smoking, cereal consumption, and supplementation affect cadmium content in breast milk. *J Expo Sci Environ Epidemiol* 2007;17:39-46.
37. Ursinyova M, Masanova V. Cadmium, lead and mercury in human milk from Slovakia. *Food Addit Contam* 2005; 22:579-89.
38. Dang WM, Ma WJ, Wang S, et al. Investigation on mental health of residents living in a mineral area in Hubei province. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 2008;26:457-60.
39. Vahter M, Akesson A, Lidén C, et al. Gender differences in the disposition and toxicity of metals. *Environ Res* 2007;104:85-95.
40. Kordas K, Lopez P, Rosado JL, et al. Blood lead, anemia, and short stature are independently associated with cognitive performance in Mexican school children. *J Nutr* 2004;134:363-71.