ORIGINAL PAPER

Evaluation of Lead and Cadmium levels in breast milk in Sanandaj, Iran

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ABSTRACT

Although breastfeeding is the main nutrition source of infants, it could also be a source of exposure to toxic metals. The present study aimed to evaluate the levels of lead (Pb) and cadmium (Cd) in breast milk, investigate the effects of some sociodemographic parameters on these concentrations, and assess the correlations between these metals and infant growth. Breast milk samples (20-30 ml) were obtained from 100 women in the second month postpartum. Pb and Cd levels were determined using inductively coupled plasma mass spectroscopy (ICP-MS). Mean concentration of Pb in the samples was 6.8 µg/l, and the concentration of Cd in the collected breast milk was lower than the limit of detection (0.1 µg/l). In 74% of the samples, Pb level was higher than the recommended limit by the World Health Organization (2-5 µg/l). In addition, mean weekly intake of Pb (6.65 µg/kg/week) and Cd (0.098 µg/kg/week) was lower than the tolerable weekly intake for infants. No correlations were observed between the Pb concentration in breast milk and the weight, length, and head circumference of infants at birth and after two months. According to the results, exposure to Pb exceeded the standard limit in the infants in the present study. Therefore, it is recommended that breast milk monitoring programs be conducted on larger sample sizes longitudinally.

Keywords: Lead, Cadmium, Breast Milk, Infants, Sanandaj

Introduction

Population growth and rapid industrialization, urbanization, and economic development have increased exposure to environmental pollutants.[1,2] Breast milk is the optimal nutrition for newborns.[3] The constituents of breast milk (e.g., lactoferrin, lysozyme, and α-lactalbumin) protect neonates against harmful environmental factors, while enhancing their defense mechanisms and stimulating their immune system.[4] The World Health Organization (WHO) recommends the exclusive breastfeeding of infants during the first six months of birth.[5,6] Nevertheless, breast milk could act as a pathway of toxicity excretion from mothers, adversely affecting the health of infants.[5,7,8]

Neonates are more vulnerable to toxic substances and the subsequent poisoning compared to adults.[1,9] Consumption of some heavy metals is considered essential within specific limits, such as iron, zinc, copper, chromium, cobalt, and manganese. On the other hand, substances such as lead (Pb) and cadmium (Cd) are among toxic heavy metals, which exert hemotoxic and neurotoxic effects even at low concentrations. Furthermore, some studies have suggested that these elements may also be teratogenic.[10,11]

Lead may have devastating effects on the nervous system of infants.[12,13] Accumulated lead in bones due to past exposure is released into the blood along with calcium and excreted into breast milk.[14] Exposure to lead is associated with anxiety and depression in adults.[15,16,17] Moreover, it adversely influences the growth of newborns.[18]

Cadmium has been reported to cause neurotoxic and behavioral changes in humans and experimental animal models.[16,19] In addition, it impairs the kidney function and may

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cause neurological disorders, including hyperactivity, gestational hypertension, and increased aggression in humans.[20,21] Cadmium has also been reported to be a significant carcinogen and disrupt bone formation processes.[4]

Unlike lead, a small amount of cadmium could cross the placenta and enter breast milk.[22] In the lactation period, toxic metals (Pb and Cd) are mobilized from the maternal stores and transferred from the maternal blood into the breast milk, which is the key nutritional source for infants.[7,23]

In Iran, few studies have determined the concentrations of Pb and Cd in breast milk and the associated factors.[24,25,26] The present study aimed to evaluate the levels of Pb and Cd in breast milk, investigate the effects of some sociodemographic parameters on these concentrations, and assess the correlations between these metals and infant growth.

Materials and Methods

This study was descriptive-cross sectional which was conducted during July-September 2017 in 22 health centers in Sanandaj, located in the northwestern of Iran, and 10 centers were randomly selected. In each center, 10 medical records of the mothers in the second month postpartum were randomly selected. Breast milk samples were also collected at the second month postpartum. The selected mothers were not occupationally exposed to toxic metals and were residents of urban, non-industrial areas.

In order to provide the details of maternal characteristics, a questionnaire was completed by the participants. Level of maternal hemoglobin on the first day postpartum was recorded based on the medical files available in the health centers. Moreover, neonatal data were obtained from their medical records, including weight, length, and head circumference at birth and two months after birth.

The study protocol was approved by the Ethics Committee of the School of Medicine at Kurdistan University of Medical Sciences, Iran. Written informed consent was obtained from the participants, and the objectives of the research were also clarified in the consent forms.

Sample Collection

Breast milk samples (20-30 ml) were collected manually during seven days. Sample collection was performed in the morning (two hours after the last breastfeeding). Each sample was sealed in a polyethylene tube and frozen at the temperature of -20ºC for further analysis.[22] The polyethylene tubes had been decontaminated using 20% nitric acid baths for 24 hours and rinsed in deionized distilled water.[27]

Analysis of Heavy Metals

Five milliliters of each sample were dried at the temperatures of 65 ºC (12 hours) and 105 ºC (24 hours) and mineralized in a muffle furnace. The samples were mineralized at the temperature of 450 ºC for 12 hours, and the obtained ash was mixed with hydrogen peroxide (H_2O_2) (2 ml), evaporated until drying, and burnt at the temperature of 450 ºC for 12 hours. The ashed samples were diluted with pure HNO_3 (1M).[4] Afterwards, the digested samples were transferred to clean acid-washed polyethylene vials and diluted with deionized water to 50 milliliters.

Concentrations of Pb and Cd were measured using the inductively coupled plasma mass spectrometry technique (ICP-MS). Urinary levels of the toxic metals were reported as microgram per liter (µg/l). With each batch sample, a blank reagent was included and analyzed to determine the appropriate elements. The lowest limits of detection (LOD) for Pb and Cd were considered 0.2 and 0.1, respectively.

Calculation of Heavy Metal Intake

Mean intake of the toxic metals in infants was calculated using Equation 1, as follows:[8]

$$\text{Mean Intake} = \frac{IR \times C}{BW} \quad (1)$$

where *IR* is the ingestion rate (l/week), *C* represents the concentrations of toxic metals in breast milk (µg/l), and *BW* shows the body weight of the neonates (kg). Weekly intake of toxic metals was calculated based on the body weight of the infants, toxic metal concentrations, and amount of consumed breast milk, assuming the consumed breast milk to be 4,500 ml/week in the two-month-old newborns.

Statistical Analysis

Data analysis was performed in SPSS version 20 (SPSS Inc, Chicago, IL, USA). The results were presented as mean values and 25-75th percentiles. The Kolmogorov-Smirnov test was applied to evaluate the normal distribution of data, and nonparametric Mann-Whitney U test was applied to compare the levels of Pb and Cd between the subgroups defined by various maternal and neonatal characteristics. In addition, Spearman's correlation-coefficient was used to assess the correlations between the concentrations of the elements in breast milk and numerical variables. Anthropometric indices of the neonates, including the Z scores of weight-for-age (WAZ), length-for-age (LAZ), head circumference-for-age (HAZ), and weight-for-length (WLZ), were calculated based on the WHO Child Growth Standards (2006).[28] In all the statistical analyses, the significance level was considered 5%.

Results and Discussion

Characteristics of the studied mothers and infants are presented in Table 1. Mean maternal age was 27.5±4 years, and 35% of the mothers were aged more than 30 years. Mean maternal weight was 64.2±6.1 kilograms during pregnancy. In terms of education status, 77% of the mothers had less than 12 years of education (incomplete diploma). Only 9% of the mothers were employed, and none of the women reported prior exposure to specific sources of metals at home or work. Only 6% of the infants had birth weight of <2500 grams. Mean birth weight was 3.3±0.38 kilograms, mean length at birth was 48.9±3.8 centimeters, and mean head circumference at birth was 35.1±2.5 centimeters.

Table 1. Characteristics of the study population

Characteristics	Mean ± SD (rang), (%)
Mothers	
Maternal age (y)	27.5 ± 4 (17-37)
Pregnancy weight (Kg)	64.2 ± 6.1 (48.2-98.7)
Housewife	(91%)
Education level ≤12 years	(77%)
Infants	
Gender, girls/boys	(47%)/ (53%)
Gestational age (wk)	38.2 ±1.4 (31.5-40.0)
Birth weight (g)	3273.37 (±378) (2200-4250)
Birth Length (cm)	48.84 (±3.8) (33-54)
Head circumference at birth (cm)	35.15 (±2.53) (32-52)
Weight at 2 months of age (g)	4582.7 (±582) (3080-5951)
Length at 2 months of age (cm)	68.37 (±3.9) (42.75-69.63)
Head circumference at 2 months of age (cm)	38.3 (±2.62) (34.56-56.16)

Distribution of Pb concentration in the breast milk samples (n=100) collected at the second month postpartum is depicted in Figure 1. Mean concentration of Pb in breast milk was 6.8 µg/l (range: 1.9-6.3 µg/l). In 1989, the WHO recommended standard Pb levels of 2-5 µg/l in human breast milk.[27] In the present study, mean Pb levels were higher than the recommended limit in 74% of the samples. In addition, the Pb levels in our breast milk samples at the second month postpartum were lower compared to the values reported by Rahimi et al. (10.39 µg/l) in Zarrinshahr city (Iran).[24] In another study, Vahidian et al. reported that the Pb levels in 94% of the breast milk samples in Hamadan (Iran) were higher than the recommended values by the WHO, with the mean concentration estimated at 41.9 µg/l.[29] In Sanandaj, the Pb levels in breast milk were observed to be lower compared to the reported values in the studies conducted in various regions in Iran.[25,30]

In general, Pb levels in breast milk largely vary across the world (0.5-126.6 µg/l).[26,30] Differences in this regard could be due to various factors, such as the time of sampling (morning/night), time of lactation, and method of sampling (pump/manual).[17] However, several other factors including analysis method and contamination of the samples during sampling might influence the final results.[17] Moreover, regional differences (e.g., place of residence, factories in regions, air currents, mixing of water sources with metals, use of leaded gasoline and lead pipes in drinking water transport, and traditional methods of food storage) could explain the variable content of Pb in breast milk between the present study and previous research in this regard.[31,32]

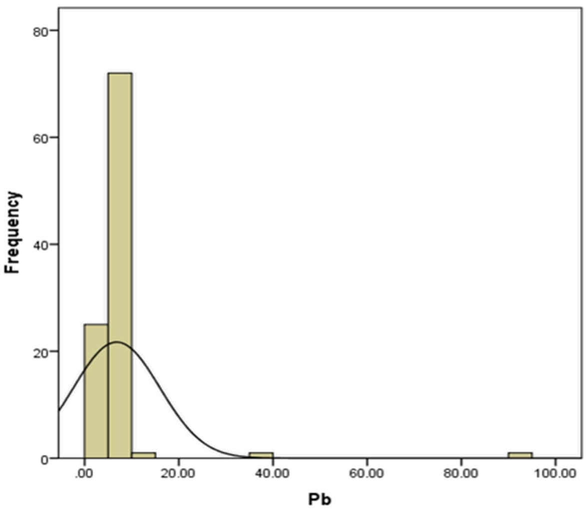

Fig. 1. Distribution of Pb in breast milk at 2nd postpartum, µg/l (n=100)

In the current research, Cd levels were lower than LOD. According to the literature, mean concentration of Cd in 100 samples of breast milk in Isfahan (Iran) was 12.1 µg/l,[33] while Nazarpour et al. reported the Cd levels in human breast milk to be 5.0 µg/l in Varamin city (Iran).[34] According to another research in Ghana, mean level of Cd was below the LOD in 60% of the samples.[35] In general, the Cd levels detected in the breast milk samples in the present study were lower compared to the obtained values in the aforementioned studies.[11,36] As mentioned earlier, this discrepancy may be due to the differences in sampling and analytical techniques.

Previous studies have indicated that smoking has a significant effect on the Cd level in breast milk.[4,24] Considering that none of the mothers in the current research had smoking habits (Table 2), Cd concentration was lower than the LOD in all the samples. Sociodemographic characteristics and Pb levels in breast milk at the second month postpartum are presented in Table 2. Correspondingly, factors such as maternal age, education level, and smoking habits during pregnancy and at the second month after pregnancy had no effects on the Pb levels in breast milk. Nonetheless, Pb levels were observed to be lower in the breast milk of younger mothers (aged ≤25 years) compared to the older ones (aged >25 years); however, the difference was not statistically significant. Conversely, Orun et al. (2011) claimed that the concentration of Pb was comparatively higher in the breast milk of young mothers (P>0.05).[17]

Table 2. Maternal sociodemographic characteristics related to Pb level in breast milk (µg/l)

		N	Mean Pb levels (25-75 p)
Age (y)	≤25	25	6.2 (1.7-6.9)
	>25	75	6.8 (1.9-6.3)
Education (y)	Elementary	24	6.8 (2.1-7.3)
	Secondary	53	5.1 (3.5-7.3)
	University	23	6.2 (1.3-4.2)
Working status	Employee	9	6.9 (0.7-3.1)
	Housewife	91	7.8 (2.2-6.5)*
Smoking during pregnancy and postpartum 2 months	Yes	0	0.0
	No	100	5.95 (1.9-6.3)
	Two or more	28	5.4 (0.23-6.2)
Using hair dye	Yes	40	6.8 (1.7-5.3)
	NO	60	6.3 (0.8-6.9)
Hb value on 1 st day postpartum (g/dl)	≤12	16	6.9 (2.2-10.5)
	>12	84	6.4 (1.82-6.22)
Intake of vitamin supplement in pregnancy	Yes	95	6.2 (1.1-4.2)
	No	5	6.6 (3.2-2.2)
Intake of vitamin supplement in postpartum 2 months	Yes	45	6.4 (0.5-2.1)
	No	55	6.7 (3.1-5.2)

*P<0.05

According to the present study, housewives had significantly higher Pb concentrations in the breast milk compared to employed mothers ($P<0.05$). This could be attributed to the inadequate aeration and possible smoking in housewives ($P<0.05$). According to the results of Spearman's correlation-coefficient, there was no correlation between the use of hair dye in the participants and Pb levels in breast milk ($P>0.05$). Similarly, Koyashiki et al. observed no significant association between the use of hair dye and Pb levels in breast milk.[37]

Some studies have denoted a correlation between iron deficiency and high serum Pb levels in humans.[38,39] However, no significant association was observed between maternal hemoglobin levels on the 1st day postpartum and Pb concentration in breast milk (r_s=-0.131; P=0.22). Consistently, Koyashki et al. reported no correlation between hemoglobin and Pb concentrations in the breast milk and blood samples obtained within the 15-210th day postpartum.[37] In addition, use of vitamin supplements during pregnancy or the second month postpartum had no significant effect on the Pb level in breast milk in the current research ($P>0.05$), which is in line with the studies in Greece.[7]

According to the information in Table 2, none of the mothers were smokers, which might be due to the dominant culture in Iran, where smoking is not common among women. Chao et al. and Orun et al. denoted an association between smoking habits and Cd levels, while Gundacker et al. reported higher Pb levels in the breast milk of the mothers with smoking habits compared to non-smokers.[1,17,23] Other studies have shown no correlations in this regard.[7,40]

Table 3 shows the mean weekly intake of Pb and Cd by the infants per kilogram of their body weight. Accordingly, mean weekly intake of Pb and Cd was 6.65 and 0.098 µg/kg/week, respectively based on the consumption of 4.5 l/week of milk by a two-month-old neonate with the body weight of 4.6 kilograms. According to the Food and Agriculture Organization (FAO) and WHO (2011), the tolerable weekly intake (TWI) for Pb and Cd is 25 and 6.7 µg/kg/week, respectively.[41] In the present study, mean weekly intake of Pb was lower than the TWI (27%), while it was higher compared to the value reported in the study by Leotsinidis.[7] According to the information in Table 3, mean weekly intake of Cd was 0.098 µg/kg/week, which was lower than the TWI.

Table 3. Mean weekly intake of Pb and Cd from breast milk, per kg of infant's body weight

Toxic metals	Mean weekly intake (µg/kg.wk)	TWI (µg/kg.wk)
Pb	6.65	25 (FAO/WHO)
Cd	0.098	6.7 (FAO/WHO)

In the current research, 6% of the neonates had a birth weight of <2,500 grams, while 47% and 43% were born with the length and head circumference <50 and <35 centimeters, respectively (Table 4). No correlations were observed between the Pb levels in breast milk at the second month postpartum and neonatal gestational age, gender, birth weight, length, and head circumference.

Table 4. Influence of selected infant factors on Pb level in breast milk

		N	Mean Pb levels (25-75 p)
Gender	girl	47	6.0 (1.32-5.7)
	boy	53	6.8 (1.2-3.1)
Gestational age (wk)	<37	32	6.2 (0.8-2.2)
	≥37	68	6.4 (2.1-4.1)
Birth weight (g)	< 2500	6	6.05 (1.47-3.8)
	≥ 2500	94	7.15 (1.9-6.4)
Birth length (cm)	<50	47	7.2 (2.2-6.4)
	≥50	53	6.8 (1.6-6.2)
head circumference at birth (cm)	<35	43	7.7 (2.2-6.6)
	≥35	57	6.2 (1.6-6.2)
Weight at 2 months of age (g)	< 4000	43	6.14 (0.9-1.7)
	≥ 4000	57	7.2 (1.2-2.3)
Length at 2 months of age (cm)	< 60	49	6.5 (2.6-4.4)
	≥ 60	51	6.8 (0.9-1.3)
Head circumference at 2 months of age (cm)	< 45	38	7.3 (2.0-5.7)
	≥ 45	62	6.1 (1.7-6.2)

In a study in this regard, Kordas et al. reported a negative correlation between the serum Pb levels and head circumference of the infants that were born in the vicinity of a casting plant in Mexico.[42] The discrepancy could be attributed to the level of intoxication, environmental conditions, and genetic factors. On the other hand, Orun et al. reported no association between neonatal characteristics (birth weight, gestational age, and gender) and levels of Pb and Cd in breast milk.[17] However, Gundacker et al. stated that gestational age had no effect on the Cd level in breast milk.[43]

In the present study, we investigated the correlations between Pb levels in the breast milk and HAZ, WAZ, LAZ, and WLZ at birth and two months after delivery in terms of gender (Table 5). The obtained results showed no correlations between the mentioned anthropometric indices in the neonates upon birth and at the second month postpartum. In this regard, the findings of Orun et al. indicated negative associations between HAZ, WAZ, and Cd concentration in breast milk.[17] Reducing exposure to toxic metals for the general population, especially mothers and infants, is the most effective strategy to prevent the adverse effects. Other preventive strategies in this regard are behavior modification and proper nutrition, which should be directed toward the women who are at a high risk of toxicant exposure.[1]

Table 5. Correlation between breast milk Pb level and z scores of anthropometric measurements at birth and 2 months postpartum

	Pb	
	Girls	Boys
Birth		
HAZ	0.081	0.029
WAZ	0.031	0.054
LAZ	0.163	0.060
WLZ	0.134	0.054
2 months		
HAZ	0.152	0.002
WAZ	0.184	0.065
LAZ	0.120	0.041
WLZ	0.105	0.043

*$p<0.05$

Conclusion

Examination of breast milk provides the information on the level of toxic metal exposure in mothers and their infants. According to the results, mean Pb levels in the breast milk samples were higher than the recommended safety limits. Moreover, our findings revealed substantial lead exposure in the non-occupationally exposed Iranian mothers living in urban areas and their neonates, which might greatly jeopardize their health. Further research is required in order to explore the contributing factors to maternal exposure to lead and cadmium, such as dietary patterns, environment, lifestyle, and cultural tendencies. In addition, preventive measures are strongly recommended so as to reduce environmental toxic metal pollution, and efforts should be made to eliminate or lower the levels of toxic metals in breast milk. In this regard, periodic breast milk monitoring programs are essential to evaluating maternal exposure during the course of lactation.

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Conflicts of Interest

None declared.

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