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Determination of zinc, copper, lead and cadmium concentration in breastmilk by anodic stripping voltammetry method and investigating their impact on infants' growth indicators

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Abstract

Background and objective: Breastmilk provides nutritional, immunological, psychological, and developmental benefits for infants. Zinc and copper are essential elements, being involved in many biological processes. Their insufficient intake by infants can detrimentally affect function of body. In comparison, lead and cadmium are harmful trace elements which accumulate in women before pregnancy, transfer to fetus through placenta in pregnancy and then to infant through breastmilk in lactating period. The aims of this study was to investigate effect of zinc, copper, lead, and cadmium in breastmilk on infants' growth and their correlations with the mothers' dietary intake.

Materials and methods: Breastmilk samples and food intake information were collected from 160 lactating women in Tehran. Concentration of copper, zinc, lead and cadmium were determined by anodic stripping voltammetry conducted by microwave digestion.

Results and conclusion: According to the results, concentration of copper, zinc, lead and cadmium were 0.36 ±0.33 mg/l, 2.40 ±2.02 mg/l, 7.15 ±5.96 µg/l, and 1.07 ±1.14 µg/l, respectively. Concentration of zinc and copper in breastmilk were increased by consumption of dairy, vegetables, fruits, bread, and nuts. Infants' height, weight and head circumstance at birthday were directly correlated with zinc concentration while reversed correlation was observed for copper, lead and cadmium in the samples. In conclusion, controlled dietary intake of women before pregnancy and after childbirth has significant impact on healthy status of the infants. Obviously, more attention should be paid on potential sources of lead intake in women of Tehran because of its high concentration in the samples.

Keywords: Cadmium, Copper, Human breastmilk, Lead, Voltammetry, Zinc

1. Introduction

Quality of the foods taken at early ages determines health status of human in adulthood. Therefore, both nutrients and chemical contamination of human breastmilk is of concern for child healthcare practitioners. As reported, amount of maternal zinc retained by fetus during the last trimester of pregnancy is less than the

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amounts secreted into the mothers' milk at the first three months of lactation [1]. Scientific evidences indicate that neurodevelopmental benefits of breastfeeding surpass the potential effects of environmental neurotoxic substances, in terms of immunological, physiological, nutritional, and psychological advantages provided by breastmilk [2]. World Health Organization recommends exclusive breastfeeding of infants until six months of age and continued breast-feeding with complementary foods until two years [3].

Composition of human breastmilk is important for infants' development. Deficiency of essential trace elements results in impaired growth and development during infancy. Zinc deficiency may lead to stunted growth and compromise immune function. Copper is found in all tissues and plays a role in making red blood cells, maintaining nerve cells, and function of immune system. It is recommended to intake 8-11 mg per day zinc and 0.9 mg per day copper in adulthood; even though they are recommended as 11 and 12 mg per day zinc and also 1 and 1.3 mg per day copper in pregnancy and lactation, respectively [4]. In comparison, contamination of breastmilk with toxic chemicals is concerned. Cadmium is one of heavy metals widely dispersed in the environment. Women are exposed to cadmium primarily by smoking or eating the foods contained/contaminated by it [5]. In addition, lead is another potentially toxic heavy metal with hepatotoxic, neurotoxic and nephrotoxic effects. It can also cause hemolytic anemia by disruption of cell membrane. Pregnant women and children living close to waste areas (containing cathode ray tube (CRT) televisions and monitors, desktops, laptops, liquid crystal display (LCD) monitors, cell phones, keyboards, computer mice, printers, and copiers) are at high risk of exposure to heavy metals such as cadmium and lead [6]. World Health Organization set a limit of 5 μg/l for lead and 0.1 μg/l for cadmium in breastmilk [7].

In lactation, heavy metals are transported from the mothers' plasma to mammary glands and then secreted into breastmilk. In this regard, Kippler et al. reported that early-life low exposure to cadmium by breastmilk induces oxidative stress [8]. Despite the study shown no impact of maternal diet on concentration of iron, zinc, and copper in the breastmilk [5], Honda et al. reported that amount of toxic metals in breastmilk obtained from 68 Japanese mothers, aged 19-38 years, at 5-8 days postpartum was significant [9]. Therefore, effect of nutritional status of mothers on quality and quantity of their milk is a controversial issue [10].

Several techniques have been used for analysis of trace metals including flame atomic absorption spectrometry and inductively coupled plasma mass spectrometry (ICP-MS) [11,12]. Among them, differential pulse anodic stripping voltammetry (DPASV) has demonstrated great sensitivity for determination of zinc, copper, lead, and cadmium at low quantities. Therefore, this study aimed to determine concentration of zinc, copper, lead and cadmium by DPASV technique in breastmilk samples collected from 160 women within five months postpartum in Tehran in 2015. In addition, correlation of the heavy metals with infants' growth and dietary intake of the mothers is discussed.

2. Materials and methods

2.1. Sample size and sampling procedure

In the current research, the women who took nutritional supplements were excluded. Breastmilk of 160 healthy lactating women of Fazel, Kan, Yaftabad, and Arash Clinic in Tehran (Iran) were collected. About 10 ml of each sample was stored in acid-washed tube and kept at -20 °C until analysis.

The sample size (n) was calculated by Eq. 1.

$$n = \sigma^2(z_{1-\beta}+z_{1-\alpha})^2/(\mu_0-\mu_1)^2 \quad \text{Eq. 1}$$

$\sigma = 2.02$ $\alpha = 0.05$ $\beta = 0.2$

$\mu_0 = 2.79$ $\mu_1 = 2.40$

2.2. Sample preparation

For mineralization, the samples were transferred to polyethylene tubes and digested by high performance microwave (Berghof Speed wave, Germany) equipped with a rotor designed for pressures up to 50 bar. One ml of breastmilk was poured into the digestion vessel and mixed with 5 ml nitric acid (65%, Suprapur®, Merck). Then, 2 ml of high purity hydrogen peroxide (30%, Suprapur®, Merck) was added and mineralization was done at 175 °C for 50 min. Hydrogen peroxide was further removed by heating and the solution was diluted by 5 ml of 0.1 M nitric acid.

2.3. Instrumental analysis

Concentration of zinc, copper, lead and cadmium were determined by differential pulse anodic stripping voltammetry with a hanging mercury drop electrode (Model 746 VA Trace Analyzer, Metrohm, Switzerland). The analysis was conducted by deposition potential of -1.20 V, deposition time of 30-90 s, scan rate of 6 mv/s, pulse amplitude of 50 mv, and final potential of +0.3 v. Standard solutions of zinc, copper, lead and cadmium at concentration of 1 g/l were prepared. At first, blank polarogram of the supporting electrolyte was recorded by using 10 ml of acetate buffer. Then, 500 µl of the samples were added to the cells and their polarogram was recorded. The analysis was done by standard addition method by adding 100 µl of the standard solutions to the samples [13].

2.4. Statistical analysis

SPSS software ver. 16.0.0 was used for statistical analysis. The data was analyzed by one-way ANOVA method and the means were compared by Tukey test. All of the experiments were repeated three times. Difference of data was significant at $p < 0.05$. The results are presented as mean ±SD.

3. Results and discussion

In the present study, limits of detection of 1.20 µg/l for zinc, 2.80 µg/l for copper, 0.30 µg/l for lead and 0.15 µg/l for cadmium were detected. Results of the heavy metals are shown in Table 1 and descriptive data of the participants is presented in Table 2.

Table 1- Concentration of heavy metals in breastmilk of lactating women in Tehran

Clinic	N	Zn (mg/l)	Cu (mg/l)	Pb (µg/l)	Cd (µg/l)
A	40	1.33 ±1.35	0.64 ±0.77	6.32 ±2.76	0.21 ±0.25
B	40	3.01 ±2.36	1.42 ±1.40	8.36 ±5.12	0.52 ±0.43
C	40	2.62 ±2.00	0.98 ±1.04	5.57 ±5.12	0.29 ±0.27
D	40	2.07 ±1.88	1.07 ±1.10	8.17 ±7.18	0.37 ±0.30
Total	160	2.40 ±2.02	1.07 ±1.14	7.15 ±5.96	0.36 ±0.33

*Zn: zinc; Cu: copper; Pb: lead; Cd: cadmium

Table 2- Descriptive data of the mothers and infants

	Result	Range
Mothers age (years)	26.4 ±4.8	18-41
Infant age (month)	5.6 ±5.2	0-22
Height at birth (cm)	50.5 ±2.96	42-69
Weight at birth (kg)	3.26 ±0.45	2.45-5.25
Head circumference at birth (cm)	34.58 ±2.43	23.20-38

In our study, zinc concentration (2.40 ±2.02 mg/l) was comparable to 4.1 mg/l reported in breastmilk of 12-20 weeks postpartum mothers in Austria [14], 1.17–5.31 mg/l in breastmilk of Canadian mothers [15], and 2.0 mg/l in breastmilk of women in Texas [16]. Copper concentration of our study (1.07 ±1.14 mg/l) was higher than the amounts reported as 0.09–0.27 mg/l in Taiwan [17], 0.36–0.47 mg/l in Korea [18], and 0.86 mg/l in Austria [14]. Our result of lead concentration (7.15 ±5.96 mg/l) was higher than 0.9±0.4 µg/l in Sweden [19] and 0.55 µg/l in Australia, and lower than 20±5 mg/l in Greece [20], and 35.8±15.0 mg/l in Austria [21]. For cadmium, our results (0.36 ±0.33 µg/l) were lower than Saudi Arabia (1.73 µg/l) [22], similar to those found in Japan (0.27±1.82 µg/l) [9] and Slovakia (0.43±0.27 µg/l) [23], and significantly lower than concentrations found in England (17.3±4.9 μg/l) and Germany (24.6±7.3 μg/l). The differences found among various studies might be due to the different assays employed other than variable concentrations of the heavy metals absorbed in body. Entrance of cadmium and lead to breastmilk is universal problem. These toxic metals are abundantly present in the environment and people are exposed to them inevitably [24]. Chou et al. reported that infants of smoking mothers are exposed to cadmium more than infants of nonsmokers [25].

Comparison of our results to other studies is presented at Table 3. We found significant correlation of breastmilk zinc, copper, lead and cadmium with height at birth, weight at birth, head circumference at birth and growth of infants. There was direct correlation between height at birth with zinc concentration but indirect correlation with copper, lead and cadmium in breastmilk. Orun et al. reported that breastmilk cadmium in second month after birth was negatively correlated with z-scores of head circumference and weight of girls at birth while no correlation was observed between lead/ cadmium and Edinburgh Postpartum Depression Scale scores [26].

Table 3- Heavy metals concentration in breastmilk of women from different studies

Country	Zn (mg/l)	Cu (mg/l)	Pb (mg/l)	Cd (mg/l)	References
Iran	2.40 ±2.02	1.07 ±1.14	7.15 ±5.96	0.36 ±0.33	Current study
Iran			3.06–19.47	0.45–5.87	[33]
Iran	2.95	0.36			[34]
Slovakia			4.7	0.43	[35]
Sweden	1.24–5.71	0.32–0.67	0.74–6.4	0.028–0.27	[36]
Spain			0.1–32.3	0.6–11.3	[37]
Portugal	0.39–5.09	0.33–0.97	0.07–4.03		[38]
Croatia	0.62–15.0	0.27–1.35			[39]
Greece	4.90	0.38	0.48	0.19	[3]
Nigeria	0.7	0.83	8.7	9.7	[40]
Kuwait	1.7–3.2	0.41–0.71			[41]
China	40.6	0.67			[42]

De Felip et al. found no significant correlation between consumption of seafood in Italian mothers and the environmental pollutants in their breastmilk. In addition, no health risk in breastfed infants was observed [27].

Tanner et al. reported that selenium correlates with copper positively [28]. In accordance, Kantola et al. showed that selenium addition to fertilizers in Finland increased selenium concentration in human milk and likely had impact on zinc and copper concentration in maternal breastmilk [29]. A comparable study was done on lactating cows exposed to environmental toxicants through which increased lead and cadmium were observed in their blood and milk [30].

In one study conducted in Tehran in 2020, concentration of trace elements in raw and bottled milks was measured by DPASV. Concentration of zinc, copper, lead, and cadmium were estimated as 4.990 ±6.244, 2.424 ±4.017, 0.271 ±0.640 and 0.099 ±0 μg/ml in raw milk, respectively, and 0.999 ±0.873, 0.228 ±0.188, 0.048 ±0.033 and 0.049 ±0 μg/ml in bottled milk, respectively [31]. In 2014, heavy metals were measured by DPASV in four brands of baby food (containing rice and wheat) and powdered milk. Concentration of zinc, copper, lead, and cadmium in baby foods (n = 240) were 11.86 ±1.474 mg/100 g, 508.197 ±83.154 μg/100 g, 0.445 ±0.006 mg/kg, and 0.050 ±0.005 mg/kg, respecttively. Moreover, their concentration was 3.621 ±0.529 mg/100 g, 403.822 ±133.953 μg/100 g, 0.007 ±0.003 mg/kg, and 0.060 ±0.040 mg/kg, respectively, in powdered milk (n = 240) [32].

4. Conclusion

The results showed that breastmilk of Iranian mothers had enough essential elements of zinc and copper. Fortunately, cadmium concentration in the samples was within the safety limits recommended by international agencies. In comparison, lead concentration of breastmilk was high that is a major hazard in public health especially for neonatal and children undergrown at industrial regions. We conclude that there is a need for conducting risk assessment studies to establish national safe limits for heavy metals in breastmilk. Although, more extensive studies with larger sample size should be done to minimize the uncertainties.

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6. Conflict of interest

The authors declare that there is no conflict of interest.

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