Mercury exposure via breast-milk in infants from a suburban area of Ankara, Turkey

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The objective of this study was to evaluate mercury (Hg) levels in breast-milk in the postpartum 2nd month and to determine the relationship between Hg levels and sociodemographic parameters, maternal nutritional habits, presence of dental amalgam, maternal depression, maternal anemia, and growth status of infants. One hundred forty-four mothers residing in a suburban area in Ankara were invited at the 2nd month postpartum. A questionnaire concerning sociodemographic characteristics, vitamin intake during pregnancy and in the 2nd month postpartum, consumption of fish and viscera, smoking habits, and presence of dental fillings was applied. Hg could be detected in samples of 18% of the mothers, all levels of which were higher than the Hg limit in breast-milk reported by the World Health Organization. The Hg levels were higher in mothers who consumed viscera than in those who did not. Turkish women can still safely breastfeed their children, but Hg contamination in breast-milk should be monitored during the lactation period.

Key words: ***mercury, breast-milk, fish consumption, dental amalgam, maternal depression.***

Breast-milk is the best source of nutrition for infants; unfortunately, it is not pristine. Contamination of breast-milk is widespread and is the consequence of decades of inadequately controlled pollution of the environment by toxic chemicals. Mercury (Hg) is excreted into breast-milk, thereby exposing breast-fed infants. Exposure to Hg is a public health concern due to its widespread persistence in the environment and its toxic effects[1].

The major effects of inhaled Hg vapor occur in occupationally exposed workers, and dentists have reported mood changes, neurobehavioral deficits, EEG changes, slowed nerve conduction, impaired short-term memory, and disturbances in psychomotor performance in relation to urine Hg levels[2,3]. Subacute occupational exposure gives rise to the triad of tremor, gingivitis and erethism. The latter is a wide spectrum of psychological and personality disturbances. One end of the spectrum involves delirium, hallucinations, excessive shyness, and fits of rage[3]. There is evidence from recent experimental neurodevelopmental studies that methyl mercury (MeHg) can induce depression-like behavior[4,5]. The hematopoietic system, due to intensive cell proliferation, is very sensitive to toxic substances. Hg exerts its toxic effect on the hematopoietic system via eryptosis (the suicidal death of erythrocytes), anemia and hemolytic reactions[6]. Experimental exposure to Channa punctatus, which is a species of fish, to 0.3–1.8 mg/L $HgCl_2$ has been shown to produce a number of pathological alterations such as reductions in blood hemoglobin (Hb) content and erythrocyte count[7]. It was also reported that anemia is a common sign in cases of acute Hg intoxication[8,9]. The safe limit of the Hg level in breast-milk for nursing infants is not known. The World Health Organization (WHO) has reported that 1.4 to 1.7 ngHg/g may exist in the breast-milk at three months postpartum under normal conditions, based on its research conducted in 1989 in six countries (Sweden, Hungary, Zaire, the Philippines, Guatemala, Nigeria)[10]. Turkish data on Hg content in breast-milk are sparse[11,12]. Furthermore, the influence of maternal lifestyle on milk Hg concentrations has not yet been investigated.

In our study, we aimed to detect the Hg levels in breast-milk during lactation in the 2nd postpartum month and to investigate the effects of some sociodemographic parameters, presence of amalgam fillings, fish and viscera (liver, spleen, brain of bovine and sheep) consumption, and smoking habits on the concentrations of Hg in breast-milk. In addition, we evaluated the influence of Hg level on postpartum depression and anemia in the mothers and on the infant's crying/sleep patterns and physical status.

Material and Methods

The mothers residing in a suburban area who delivered their babies at the Zekai Tahir Burak Maternity Hospital in Ankara, Turkey were invited at two months postpartum as a part of a previous study[13]. A total of 144 healthy mothers enrolled voluntarily were evaluated at İhsan Doğramacı Children's Hospital and provided breast-milk samples in the postpartum 2nd month. Levels of Hg in the breast-milk were investigated in this group. These mothers were not occupationally exposed to toxic metals.

All the mothers completed a questionnaire to provide details regarding some of the maternal and infant features, including the mother's occupation, smoking habit, reproductive history, breastfeeding pattern (exclusive or partial), and the intake of iron and vitamin supplementation during pregnancy and within two months postpartum. Postpartum maternal Hb levels analyzed on the postpartum 1st day were noted from the hospital record files. The Brief Symptom Inventory (BSI) was applied to all mothers in the first three days postpartum.

All mothers were evaluated according to the Edinburgh Postpartum Depression Scale (EPDS) to identify postpartum depression risk. The infants were examined, and their weight, length and head circumference were recorded. The mothers were queried regarding the feeding pattern (exclusive or partial breastfeeding), sleep pattern (regular or irregular) and crying characteristics (colic or non-colic) of their babies.

The study was approved by the Ethical Committee of Hacettepe University Faculty of Medicine (HEK 07/97-10). All mothers were informed about the purpose of the study and their written consent was obtained.

Instruments

Brief Symptom Inventory (BSI): The BSI is a 53-item self-report symptom inventory drawn from the Symptom Checklist-90-R (SCL-90-R), which assesses current psychological symptomatology[14]. Each item describes a symptom such as "feeling no interest in things" and is scored from not at all (0) to extremely (4). It results in three global indexes of distress: global severity index (number and severity of symptoms), positive symptom distress index (intensity of symptom) and positive symptom total index (number of symptoms endorsed in a pathological direction without regard to intensity). The BSI was standardized for the Turkish population previously[15].

Edinburgh Postpartum Depression Scale (EPDS): The EPDS is a validated self-rating 10-item questionnaire developed to identify postnatal depression[15]. A validated Turkish version of the EPDS was used in this study[16].

Analysis of the Breast-milk Samples

In this study, the Hg concentration in breast-milk samples was analyzed with Inductively Coupled Plasma Mass Spectrometry (using an Agilent 7500a instrument) and CEM MARS 5 Microwave Digestion[18]. The method used for the analysis of toxic metals in the breast-milk was given in a previous study[19]. The lowest limit of quantitation (LOQ) for Hg was 0.1 μg/L.

Statistics

The data were analyzed using the SPSS Windows ver. 14.0 (SPSS Inc, Chicago, IL, USA). Since the Hg level could not be determined in 82% of the breast-milk samples, the number of cases above the value given by WHO (>1.7 μg/L) and the percentage distribution (n, %) were given and the difference between case distribution within groups was analyzed with chi-square test in the analysis of Hg levels in 2nd-month breast-milk. The Spearman's correlation coefficient was used to determine the relationship between breast-milk Hg concentrations and numerical variables such as anthropometric measurements, EPDS scores, maternal Hb values, and the number of amalgam fillings. The anthropometric measurements evaluated in infants included the z scores of weight-for-age (WAZ), length-for-age (LAZ) head circumference-for-age (HCZ), and body mass index-for-age (BAZ), and were calculated on the basis of the recent WHO Growth References[20]. The level of statistical significance was accepted at $p<0.05$.

Results

The mean age (±SD) of the mothers was 25 (± 5) (range: 17-41) years. Ninety-nine (69%) of the mothers and 72 (50%) of the fathers had less than 9 years of education. Only 11 (7.6%) mothers were working. The mean gravidity (±SD) was 2.0 (± 0.9) and mean parity (±SD) was 1.6 (± 0.8). The birth interval was >2 years in 53 (85.7%) mothers who had parity >1.

Second-Month Breast-milk Hg Levels

Breast-milk Hg levels in 26 (18%) samples were above the limit of detection; the mean (±SD) of these 26 breast-milk samples was 25.8 (±44.6) (range: 1.8-236) μg/L. All of these levels were above the reference level given by WHO for Hg (>1.7 μg/L). In one case, breast-milk Hg level was 236 μg/L and the value was confirmed by repeat measurement. For this case, the breast-milk lead (Pb) level was 36.8 μg/L and cadmium (Cd) level was 4.2 μg/L. He was born at term weighing 3340 g from a 27-year-old mother's first pregnancy. The mother was a housewife, the father was a hairdresser, and the mother's father was a welder. The mother had a history of geophagia since 15 years of age, anemia, a fish consumption frequency of <2 times/week and a viscera consumption of <1 time/month, was not an active smoker but was passively exposed to <5 cigarettes/day, and had worn makeup since 18 years of age. She had no history of amalgam fillings or direct contact with Hg vapor.

Characteristics of Mothers and Infants and Breast-milk Hg Levels

Maternal age (≤20 or >20 years), level of education (≤8 or >8 years), family income (≤250 or >250 US dollars), employment status, gravidity/parity, birth interval (≤2 or >2 years), and family structure (nuclear or extended) were determined to have no effect on breast-milk Hg levels in the 2nd postpartum month (Table I).

Hemoglobin (Hb) value was <11 g/dl in 36.8% of the mothers on the 1st day postpartum. Postpartum anemia in the mothers did not affect the Hg level in breast-milk in the 2nd postpartum month. No correlation between Hb concentration and breast-milk Hg level was determined (Spearman's rho coefficient= -0.052; p=0.536). In addition, no relationship was determined between vitamin supplement intake during pregnancy and the 2nd month postpartum and breast-milk Hg level (Table I).

It was determined that 20.7% and 21.4% of the mothers were active smokers during pregnancy and in the 2nd month postpartum, respectively. Active and passive smokers were combined for the group comparison analysis because most of the active smokers smoked on average <5 cigarettes/day. Exposure to cigarette smoke during pregnancy and the 2nd month postpartum did not affect the breast-milk Hg levels (Table I).

Eighty-eight percent of the mothers stated that they consumed fish <2 times/week during pregnancy. 46.5% of the mothers consumed viscera, with 81% consuming it less than once a month. The percentage of the 2nd month breast-milk Hg levels >1.7 μg/L was higher in mothers who consumed viscera as compared to those who did not (24.2% and 11.7%, respectively; p=0.049). However, it was seen that consuming fish ≥2 times/week during pregnancy did not affect the 2nd month breast-milk Hg levels.

The mean number of amalgam fillings of mothers was 1.2±2.2 (median: 0). Forty-eight (64%) mothers had no amalgam fillings. Hg concentration in breast-milk did not correlate with the number of amalgam fillings ($r_s=0.092$; $p=0.432$). No statistically significant difference was determined in the percentages of Hg levels >1.7 μg/L between the mothers who did or did not have amalgam fillings (Table I).

Eighty-one percent of the infants were fed only breast-milk during the 2nd month. The percentages of 2nd month breast-milk Hg

Table I. The Percentages of Hg Levels in Breast-Milk >1.7 μg/L at 2 Months According to Maternal Sociodemographic Characteristics and Environmental Risk Factors

		Total number of cases	Percentages of levels >1.7 μg/L, n (%)
Age (years)	≤ 20	31	7 (22.6)
	>20	111	19 (17.1)
Education level (years)	≤ 8	99	18 (18.0)
	> 8	45	8 (18.2)
Family monthly income, US $	≤ 250	25	6 (22.2)
	> 250	119	20 (17.1)
Working status	Employed	11	0 (0.0)
	Housewife	133	26 (100)
Parity	Primiparous	78	17 (21.8)
	Multiparous	66	9 (13.6)
Birth interval year	≤ 2	10	2 (22.2)
	> 2	53	7 (13.0)
Intake of vitamin in pregnancy	Yes	83	15 (18.3)
	No	28	4 (14.3)
Intake of vitamin at 2 months postpartum	Yes	31	3 (9.7)
	No	85	17 (20.0)
Smoking during pregnancy	Active+passive	81	17 (20.7)
	No smoking	62	9 (14.8)
Smoking during postpartum 2nd month	Active+passive	95	17 (18.1)
	No smoking	49	8 (16.3)
Number of amalgam fillings	None	48	5 (10.4)
	≥ 1	27	5 (22.7)
Fish consumption during pregnancy	<2/week	127	20 (15.9)
	>2/week	17	5 (29.4)
Consumption of viscera*	Present	67	16 (24.2)
	Absent	77	9 (11.7)*
Hb value on 1st day postpartum g/dl	< 11	54	9 (17.0)
	≥ 11	90	17 (18.7)

*p=0.049

levels >1.7 μg/L in infants who were fed only breast-milk versus those who were partially breastfed were similar (18.5% and 17.2%, respectively).

Relation Between Second-Month Breast-Milk Hg Levels and Early Growth Sleep Pattern and Crying in Infants

Of the infants presenting in the 2nd month postpartum, 67 (45.8%) were male, 10 (6.3%) had birth weight <2500 g, and 18 were born earlier than 37 weeks. The mean birth weight (±SD) was 3226 ± 445 g (range: 2030-4080) and mean head circumference was 35.0 ± 1.4 cm (range: 32.0-38.0). Infant gender, birth weight (<2500 g vs ≥2500 g) and gestational age (<37 weeks vs ≥ 37 weeks) were shown to have no effect on the 2nd month breast-milk Hg level (Table II).

The infants' 2nd month postpartum mean weight (±SD) was 4755 (± 684) g, mean length (±SD) 55.2 (± 2.6) cm, and mean head circumference (±SD) 38.0 (± 1.5) cm. The relationships between head circumference and weight at birth and 2nd month body weight, length and head circumference z scores and breast-milk Hg levels were analyzed separately according to gender. No correlation was determined between gender and the 2nd month head circumference (HCZ), body weight (WAZ), length (LAZ), and body mass index (BAZ) z scores and 2nd month breast-milk Hg levels (for females r_s=0.012, p=0.917; r_s=-0.018, p=0.879; r_s=0.027, p=0.824; r_s=0.002, p=0.985, respectively; for males r_s=-0.012, p=0.921; r_s=-0.122, p=0.326; r_s=-0.199, p=0.165; r_s=-0.218, p=0.077, respectively).

Twenty-nine percent of the infants had colic crying attacks while 38% had irregular sleep

Table II. Infants' Birth Characteristics and Comparison of 2nd Month Colic Crying and Sleep Patterns and Percentages of Hg level >1.7 μg/L

		Total number of cases	Percentages of levels >1.7 μg/L, n (%)
Gender	Male	67	13 (50.0)
	Female	77	13 (50.0)
Birth weight, g	< 2500	10	1 (10.0)
	≥ 2500	134	25 (18.8)
Gestational age, week	< 37	18	3 (16.7)
	≥ 37	124	23 (18.7)
Breast feeding 2nd month	Exclusive	116	20 (18.5)
	Partial	27	5 (17.2)
Sleep pattern 2nd month	Regular	89	18 (20.2)
	Irregular	55	7 (13.0)
Episodes of colic crying 2nd month	Yes	43	9 (21.4)
	No	101	16 (15.8)

pattern. The 2nd month Hg levels did not change according to the presence of sleep disorder and colicky crying (Table II).

Relation Between Breast-milk Hg Levels and BSI Global Indexes Applied During the First Three Days Postpartum and Second-month Edinburgh Postpartum Depression Scale

Brief Symptom Inventory (BSI) global index scores assessed in the first three days after delivery were similar between mothers whose breast-milk Hg level was >1.7 μg/L or ≤1.7 μg/L. The mean (±SD) global severity index score was 0.50 (±0.42) in the group with high breast-milk Hg levels and 0.64 (±0.52) in the group with low levels (p=0.348). The mean (±SD) positive symptom total index score was 15.5 (±9.7) in the group with high breast-milk Hg levels and 19.2 (±11.5) in the low Hg level group (p=0.161). The mean (±SD) positive symptom distress index score was 1.64 (±0.46) in the group with high breast-milk Hg levels and 1.66 (±0.56) in the low Hg level group (p=0.793).

The mothers with an EPDS score ≥13 were considered to be at risk for depression. According to the EPDS applied in the 2nd month, 26 mothers (22%) were at risk for depression. The percentages of mothers having high breast-milk Hg levels according to EPDS score of <13 or ≥13 were similar, at 16% and 17.8%, respectively. Similarly, no significant correlation was detected between the 2nd month EPDS scores and breast-milk Hg levels (r_s=-0.024, p=0.776).

Discussion

In our study, the ratio of detectable Hg levels in the 2nd month breast-milk samples was quite low (18%). A review of the literature revealed that breast-milk Hg level varied in the range 0.2-13.9 μg/L[11,21-31]. Bakir et al.[32] reported poisoning in Iraqi mothers with high concentrations of Hg in breast-milk, ranging from 10 to 200 ngHg/g. This wide variation shows a strong dependence of Hg content on various factors, including the local environment, socioeconomic conditions of the family, local diet and habits.

The lactation stage is an important physiological factor that affects Hg secretion into breast-milk[1]. Inorganic and organic Hg have both been found to be associated with proteins in breast-milk. Differential protein binding may significantly change total Hg transfer from maternal serum to milk. Dorea[33] showed that total protein concentrations decrease during lactation from colostrum to mature milk. As a result, there is a decline in Hg concentrations between colostrum and mature milk. Compared with data from the literature, the rate of Hg detection in breast-milk was low in the present study. This result may be related to taking breast-milk samples in the late lactation stage.

Several maternal sociodemographic factors affect Hg secretion into breast-milk, such as maternal age, parity and birth interval, etc. Drasch et al.[24] reported that the mother's age did not affect milk levels of Hg. In another study, Ursinyova and Masanova[35] did not find a statistically significant influence of parity, birth weight or gender on Hg level taken on the 4th postpartum day. Similarly, Yalçın et al.[11] reported that mother's age, parity, birth interval, infant gender, and birth weight had no impact on breast-milk level at 10-20 days postpartum[11].

The number of studies on breast-milk Hg level and anemia are limited. In contrast to the present study, Yalçın et al.[11] found that there was a statistically negative correlation between maternal Hb and serum iron concentration and Hg levels in transitional breast-milk (r=-0.357, r=-0.331, respectively)[11].

In our study, no relation was found between vitamin intake during the 2nd month and 2nd month breast-milk Hg level. Gundacker et al.[27] in a study on 116 mothers found that breast-milk Hg levels were higher in mothers who took vitamins. They found that the high levels of Hg in breast-milk were related to the mother's body weight being <60 kg, prematurity, frequent consumption of cereals, and living in industrial and urban regions of Austria. In the multivariate analyses, they determined that only prematurity and frequent consumption of cereals affected the breast-milk Hg levels.

The most important known sources of human exposure to Hg are consumption of fish and amalgam fillings. The studies conducted in populations in which fish consumption is high have shown that the frequency of the mother's fish consumption and the type of fish affect breast-milk Hg level[25,34]. However Vimy et al.[30] observed that total breast-milk Hg was not associated with fish consumption. In our study, the mother's consumption of fish during pregnancy did not affect 2nd month breast-milk Hg levels; however, the low consumption of fish in our study group may have partially influenced the statistical comparisons. Only 12% of mothers consumed fish ≥2 times/week during their pregnancy. This situation may be related to not living near the coast, cultural dietary habits, and the avoidance of fish consumption during pregnancy.

While there are studies showing that presence of amalgam fillings and history of procedures to place and remove fillings during the pregnancy and lactation periods affected the breast-milk Hg levels[23,24,30,34,35], others have shown no such relationship[11,12,25]. In our study, no relationship between amalgam fillings and 2nd month breast-milk Hg level was found. In our study, 62.7% of the mothers had no fillings and only five of them had >5 fillings. To determine the effect of amalgam fillings on breast-milk Hg, further studies should be done with larger population samples having more amalgam fillings.

Dorea[1] reported that farming practices in industrialized countries increasingly utilized animal by-products in the food given to animals that are bred for human consumption. Hg can thus also pass to the eggs, milk, meat, and farmed fish fed fish-meal-containing diets. Therefore, fish may not be the endpoint of Hg contamination in the human food chain[11]. In some experiments with animals like sheep and mice/rats that are fed with Hg-supplemented food, it was shown that Hg accumulated in internal organs such as the liver, kidney and brain[36,37]. Thus, consumption of viscera can be an important source of Hg. In our study, almost one-half of the mothers (46%) stated that they consumed viscera. It was found that the percentage of samples with breast-milk Hg level above the WHO reference value was greater in mothers with viscera consumption when compared to those who did not consume viscera. In agreement with our results, Yalçın et al.'s[11] study with 44 mother-infant pairs showed that postpartum 10th to 20th day breast-milk Hg level was correlated with consumption of viscera during pregnancy.

There are contradictory results as to whether exposure to cigarettes affects breast-milk Hg levels. While Gundacker et al.[27] and Ursinyova et al.[35] did not find any correlation between cigarette exposure and breast-milk Hg level, Yalçın et al.[11]revealed that active and/or passive exposure to cigarettes affected the Hg level[11,27,35].

In our study, it was seen that 2nd month breast-milk Hg levels did not affect infant crying attacks or sleep pattern within the first two months postpartum. A review of the related literature revealed no other study that examined the relationship between breast-milk Hg level and colic crying and sleep pattern in infants.

In our study, no correlation was seen between 2nd month breast-milk Hg levels and BSI global index scores or the EPDS scores. Barboni et al.[38] reported a series of workers from a Hg recycling industry in Brazil who were assessed utilizing some neuropsychological tests (Beck Depression Inventory and State-Trait Anxiety Inventory), which demonstrated no significant differences in test scores between Hg-exposed subjects and controls. Nevertheless, they found that the duration of exposure was statistically correlated to depression scores. There is a need for further studies that investigate the relationship between breast-milk Hg level and maternal depression.

In conclusion, while the percentage of detectable Hg in breast-milk among the mothers who took part in our study was low, the Hg levels of whole measurable samples were higher than the recommended WHO level. Even though it is difficult to interpret the Hg level in a single breast-milk sample obtained, the population of this study living in a suburban area was not significantly at risk for Hg exposure. Mother-infant pairs with higher levels in breast-milk should be recruited for follow-up visits to monitor chronic low-level Hg exposure.

We determined that breast-milk Hg levels were higher in mothers who consumed viscera. It should be kept in mind that animal viscera, which are recommended for their rich iron content, can also be a source of toxic metals. Therefore, viscera consumption should be limited in pregnant and/or lactating mothers and infants younger than one year of age. Further systematic research of Hg biomonitoring in breast-milk is needed to increase our knowledge about the bio-transfer of Hg through breast-milk to the infant and the potential adverse effects of Hg exposure in infants.

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