# Mercury in Hair of Mothers and Infants: Influencing Factors Assessment in the Southern shores of the Caspian Sea (Iran)

*Seyed Mahmoud Ghasempouri[1*], Narjes Okati[2], Abbas Esmaili-Sari[1]*

## ABSTRACT

**Background:** Mercury (Hg) is a hazardous metal responsible for environmental contamination and human intoxication. Methyl mercury bio-accumulation through food chain can be responsible for chronic mercury exposure of South Caspian Sea communities with a diet rich in fish. Uncertainties about exposure levels that could have damaging consequences for nervous system development of infants makes bio-monitoring of Hg a necessity in Southern Caspian Sea populations.

**Methods:** Mercury concentration in the hair of 70 pairs of mothers and their breastfed infants were assessed and its relationship with influencing factors was evaluated.

**Results:** Calculated levels of mercury exposure of both infants and mothers indicated concentrations less that the recommended levels by WHO and EPA reference. Total mean mercury concentrations in infants hair was 0.48±0.32 µg / g and for mothers was 0.19±0.09 µg / g. Correlation analysis showed that mercury concentration in the hair of infants was significantly (P=0.002, R=0.371) associated with mercury levels in the hair of their mothers. The influence of other variables such as living location, age of mothers, infants' sex, weight, the amount of fish and sea food consumption of mother, and the number of dental amalgam fillings, were examined as well as.

**Conclusion:** The amount of fish and sea food consumption by mothers and the living location were the variables that significantly affected hair mercury concentrations of mothers and infants. Also the age of infants (p=0.02) and the number of dental amalgam fillings of mothers (p=0.016) significantly affected the hair mercury levels in infants. Hair

**Key Words:** Mercury, hair, mothers, hnfant, Caspian Sea, Iran

## INTRODUCTION

Mercury is a heavy metal that is widespread in the environment and has many toxic effects (1). The contamination sources of mercury consist of industrial waste water, use of fossil fuels and fungicides, and burning wastes (2). The amount of mercury that is transformed into methylmercury and transferred up in the food chain through bio-accumulation depends on many site-specific factors (such as water chemistry, the complexity of food web, fish fauna, age, size and trophic position) (3,4,5)

The three basic forms of mercury include metallic or elemental mercury, inorganic, and organic compounds. Methyl mercury (MeHg), an organic form of mercury, has been of particular concern because it occurs naturally and is especially toxic to the developing nervous system (6).

Previous studies have shown that a diet rich in fish is the primary pathway of human exposure to MeHg and that statistical differences in MeHg intake exist between high and low fish consumption groups (7,8). Fetuses and neonates are known as high-risk group for MeHg exposure (9,10,6). The effects of MeHg exposure on pregnant and breast-feeding woman remains an important issue for elucidation, especially in populations consuming large amounts of fish (11,12,13,6).

*1- Department of Environment, Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University, Nour, Iran*

*2- Department of Environment, Faculty of Natural Resources, Zabol University,Zabol , Iran*

*[*]Corresponding auther : Mahmoud Ghasempouri,Tarbiat Modares University,Noor*

*Email:ghasempm@modares.ac.ir*

Mercury can be stored and accumulated over time in body fat and then be mobilized in to milk during lactation (14) and be transferred to infants through milk (15). Based on the findings that proved prenatal exposure to MeHg leads to significant behavioral effects during infantile development, World Health Organization (WHO) reduced the MeHg provisional tolerable daily intake from 0.47 to 0.23 µg/kg body weight/day (16). WHO (2003) recommends exclusive breast-feeding of infants during the first six months of life to achieve optimal growth, development and health(17).

Hair is the main biological indicator of MeHg exposure, because it contains the thiol (-SH) group for which Hg cations have high affinity (18) and mercury levels in hair have been shown to reflect mercury level in internal organs (19,20) as well as dietary intake (19).

The objective of this study was to characterize the risk of Hg exposure in mothers and their infants in people who live in the south of the Caspian Sea and have a diet high in fish and its products. We examined the relationship between Hg levels in the hair of mothers and their infants to their ages, place of dwelling e.g. village or town, fish and sea food consumption, sex and number of infants and number of dental amalgam fillings in the mothers.

## MATERIAL AND METHODS

### *Population*

Three populations in the southern shores of the Caspian Sea (Mazandaran Province) were selected to carry out a comparative study between September 2006 and January 2007. Nowshahr, Nur and Chamestan cities and villages of Nur and Nowshahr are located along the south of the Caspian Sea in Iran. People living in the Nur and Nowshahr cities have very similar life styles especially relative to dietary habits. Chamestan is a tiny town with a rustic environment which is more like a village. In all these regions, fishing and local agriculture represent the major occupations and the inhabitants' diet includes large amounts of fish. The subjects for this study were 70 mothers (17-36 years old) and their breastfed infants ranging from 0.1 to 6 months of age.

### *Questionnaires*

Recruited mothers answered the questionnaires, consisting of details such as the age of mother and infant, sex and weight of infant, dietary habits of mother (including the number of servings of fish consumed per week), place of residence, and number of dental amalgam fillings of mother.

### *Sampling*

To analyze mercury exposure, hair samples (about 1 gr) were obtained from mothers and infants. Hair was sampled from occipital area, cut close to the scalp with stainless still scissors, and placed in an envelope properly labeled.

All samples were evaluated in the environmental analysis laboratory of Natural Resources and Marine Sciences Faculty of Mazandaran.

### *Analysis procedure*

At first, the hair samples were washed with distilled water (3 times) and finally with acetone (21). The hair samples were dried at 80° C for 12 h in oven (22) and finally they were grinded into powder.

The mercury content was measured by LECO AMA 254 Advanced Mercury Analyzer (USA) according to ASTM, standard NO.D-6722. Each sample was analyzed 3 times. The LECO AMA 254 is an Atomic Absorption Spectrometer (AAS) that is specially designed to determine total mercury content in various solids and certain liquids without sample pre-treatment or sample pre-concentration. Designed with a front-end combustion tube that is ideal for the decomposition of matrices, the instrument's operation may be separated into three phases during any given analysis: using a program of 60 s (drying), 200 s (decomposition) and 45 s (waiting). In order to assess the analytical capability of the proposed methodology, accuracy of total Hg analysis was checked by running three samples of Standard Reference Materials (SRM) from National Institute of Standards and Technology (NIST), SRM 1633, SRM 2709, SRM 2711 in seven replicates (23,24,25).

### *Statistical analysis*

The statistical analysis was performed by SPSS software (version 14). The data of total

mercury quantities were tested for normality using a Kolmogrov-Smirnov test. Analysis of Variance (ANOVA), followed by Tukey test, and Post Hoc when appropriate were used to compare means between groups, with level of significance set at $P \leq 0.05$. Correlation analysis and liner regression was executed to examine the association between Hg concentration in the hair of mothers and infants and independent factors.

## RESULTS

The concentration of mercury in hair is summarized in Table 1 for mothers and infants. The mean concentrations of mercury for mothers and infants were 0.19 μg / g and 0.48 μg / g respectively. The relationship between mercury concentrations in the hair of mothers and their infants are shown in Table 2 and fig.1 which reveals a statistically significant correlation (R=0.371, P=0.002). The most influential independent factors for Hg contamination are shown in Tables 3 and 4. The amount of fish consumed and residence place were the factors that significantly affected mercury concentration in mothers and infants. Furthermore, the age of infants (p=0.02) and number of dental amalgam fillings of mothers (p=0.016) significantly affected hair mercury content of infants.(Table 5) The effect of the other factors on hair mercury levels in infants was little.

**Tbable 1.** Mercury concentration (μg / g) in the hair of mothers and infants

| | | |
|---|---|---|
| N | 70 | 70 |
| Mean | 0.19 | 0.48 |
| Median | 0.16 | 0.36 |
| SD | 0.09 | 0.32 |
| Range | 0.37 | 1.42 |

**Table 2.** The correlation between mercury in the hair of mothers and infants

| | | Hg concentration in the hair of infants |
|---|---|---|
| Hg concentration in the hair of mothers | Pearson Correlation | .371(**) |
| | Sig. (2-tailed) | .002 |

**Table 3.** Tueky Test for Associations between Hg concentration in the hair of mothers and independent factors (statistically significant factors are in boldface type)

| Characteristic | Variable | N | Mean± S.D | Range | P value |
|---|---|---|---|---|---|
| Age of mother (years) | 17-21 | 15 | 0.21± 0.11 | 0.09-0.40 | 0.66 |
| | 22-26 | 31 | 0. 20± 0.09 | 0.06-0.42 | |
| | 27-31 | 20 | 0.17± 0.08 | 0.08-0.43 | |
| | 32-36 | 3 | 0.20± 0.04 | 0.15-0.23 | |
| Fish consumption | <1 per month | 2 | 0.12± 0.02 | 0.11-0.14 | **0.00** |
| | 1-2 per month | 15 | 0.13± 0.05 | 0.08-0.24 | |
| | 1-2 per week | 34 | 0.18± 0.08 | 0.06-0.43 | |
| | >2 per week | 19 | 0.27± 0.09 | 0.13-0.42 | |
| Number of dental amalgam fillings | 0 | 49 | 0.19± 0.10 | 0.06-0.43 | 0.1 |
| | 1-4 | 17 | 0.18± 0.05 | 0.09-0.27 | |
| | >4 | 3 | 0.31± 0.09 | 0.21- 0.4 | |
| Place of dwelling | Nur | 10 | 0.29± 0.08 | 0.18-0.40 | **0.001** |
| | Nowshahr | 8 | 0.24± 0.12 | 0.12-0.43 | |
| | Chamestan | 17 | 0.14± 0.09 | 0.08-0.42 | |
| | Village of Nur | 13 | 0.16± 0.04 | 0.08-0.24 | |
| | Village of Nowshahr | 22 | 0.11± 0.03 | 0.06-0.38 | |

**Table 4.** Tueky Test for the associations between Hg concentration in the hair of infant and independent factors (statistically significant factors are in boldface type)

| Characteristic | Variable | N | Mean± S.D | Range | P value |
|---|---|---|---|---|---|
| Sex of infant | Mail | 35 | 0.55± 0.34 | 0.09-1.51 | 0.058 |
| | Female | 35 | 0.41± 0.28 | 0.10-1.41 | |
| Age of infant (month) | 0-2 | 14 | 0.32± 0.13 | 0.10-0.55 | **0.02** |
| | 3-5 | 31 | 0.45± 0.26 | 0.09-1.05 | |
| | >5 | 25 | 0.60± 0.41 | 0.18-1.51 | |
| Weight of infant (Kg) | 3-5 | 19 | 0.49± 0.31 | 0.1-1.07 | 0.96 |
| | 6-8 | 36 | 0.47± 0.33 | 0.16-1.51 | |
| | >8 | 15 | 0.47±0.32 | 0.09-1.25 | |
| Place of dwelling | Nur | 10 | 0.72± .0.34 | 0.29-1.25 | **0.00** |
| | Nowshahr | 8 | 0.85± 0.52 | 0.09-1.51 | |
| | Chamestan | 17 | 0.43± 0.26 | 0.10-1.02 | |
| | Village of Nur | 13 | 0.35± 0.19 | 0.13-0.91 | |
| | Village of Nowshahr | 22 | 0.35± 0.13 | 0.18-0.71 | |

**Table 5.** Tueky Test for Associations between Hg concentration in the hair of infant and independent factors in mothers

| Characteristic | Variable | N | Mean± S.D | Range | P value |
|---|---|---|---|---|---|
| Fish consumption | <1 per month | 2 | 0.14± 0.05 | 0.10-0.18 | **0.015** |
| | 1-2 per month | 15 | 0.32± 0.15 | 0.16-0.77 | |
| | 1-2 per week | 34 | 0.48± 0.28 | 0.09-1.25 | |
| | >2 per week | 19 | 0.43± 0.83 | 0.13-1.51 | |
| Number of dental amalgam fillings | 0 | 49 | 0.45± 0.29 | 0.10-1.25 | **0.016** |
| | 1-4 | 17 | 0.48± 0.33 | 0.09-1.51 | |
| | >4 | 3 | 0.99± 0.42 | 0.56-1.41 | |

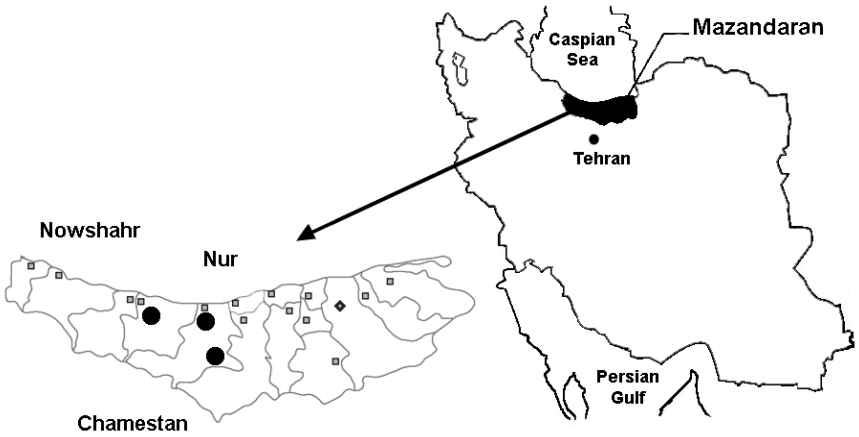


***fig.1.*** *The map of Iran showing sampling sites in the southern shores of Caspian sea.*

## DISCUSSION

Estimated Hg concentration in the hair of mothers (0.19 µg / g ) and infants ( 0.48 µg / g) showed that exposure in south Caspian Sea communities is below recommended WHO (2 µg / g), Health Canada Guideline (1999) (26) (6 µg / g) and EPA Reference dose (5 µg / g) limits.

In the heavily polluted Mina Mata Bay (1953-1971), where the villagers consumed mercury contaminated fish (11.4-39.0 µg $g^{-1}$), the level of mercury in their hair was 191-705 µg / g (27,28); however, in the less contaminated areas of Japan (29), Poland (30), North Sea (31), and Coastal Chile (32), the reported median levels of mercury for women were bellow 4 µg / g of hair.

Figs 2, 3, 4 and 5 show the relationship between Hg in the hair of mothers and independent factors. Compared to the number of dental amalgam fillings of mothers and their age, fish consumption ($R^2$=0.28) was the most influential factor on Hg levels.

Figs 6, 7, 8 and 9 demonstrate the relationship between Hg in hair of infants and independent factors. Again the amount of consumed fish ($R^2$=0.10) was the most important factor.

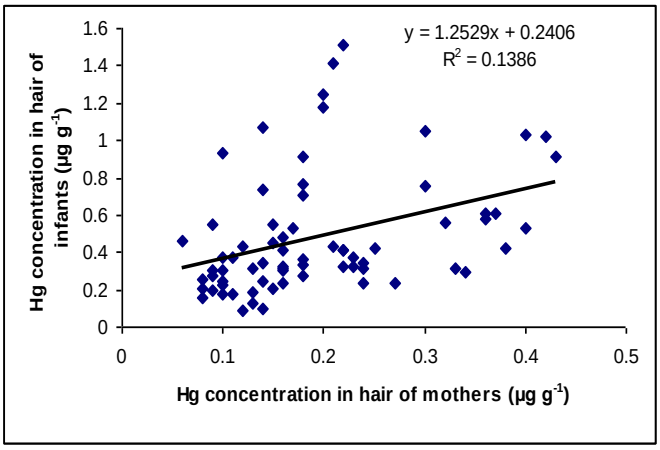


**fig. 2.** The relationship between Hg in the hair of mothers and infants

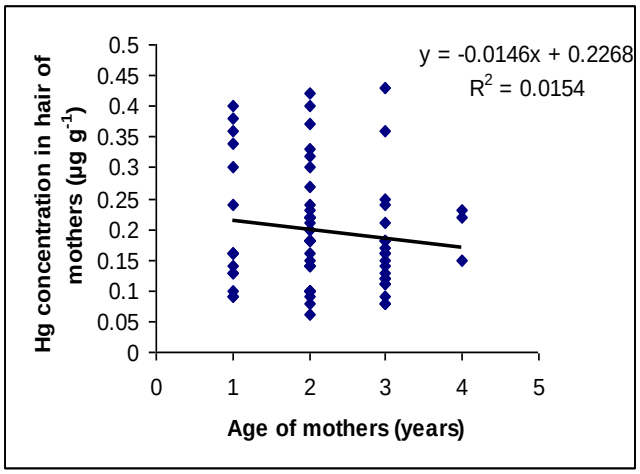


**fig.3 .** The relationship between Hg in the hair of mothers and their age: 1=17-21 years old, 2=22-26 years old ,3=26-31 years old , and 4=32-36 years old

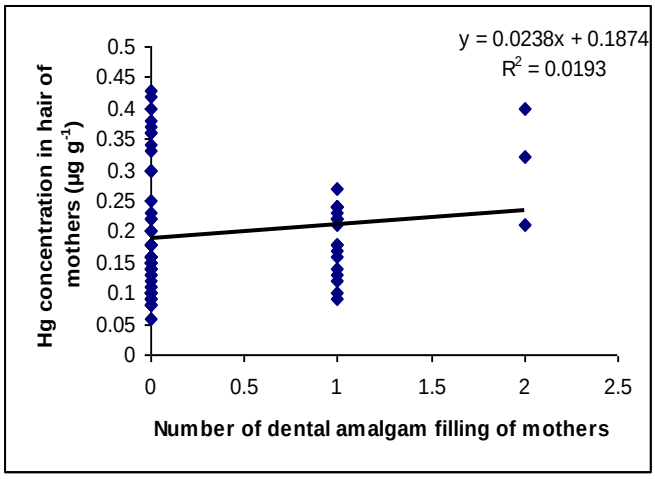


**fig.4 .** The relationship between Hg in the hair of mothers and the number of their dental amalgam fillings:

0=0 dental, 1= 1-4 dental , 2= >4 dental.

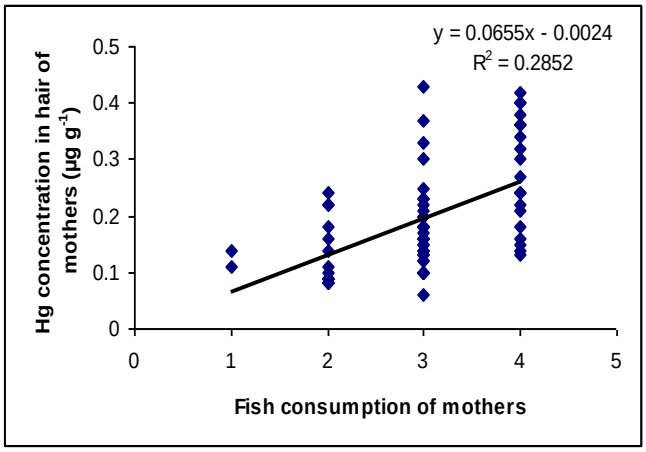


**fig.5 .** The relationship between Hg in the hair of mothers and fish consumption levels: 1= <1 portion per month 2= 1-2 portions per month, 3= 1-2 portions per week, 4= >2 portions per week.

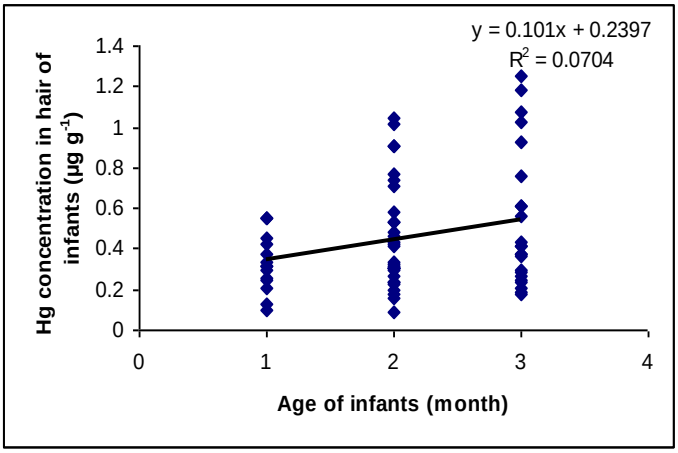


**fig. 6T**he relationship between Hg in the hair of infants and their age: 1= 0-2 month , 2= 3-5 month, 3= >5 month.

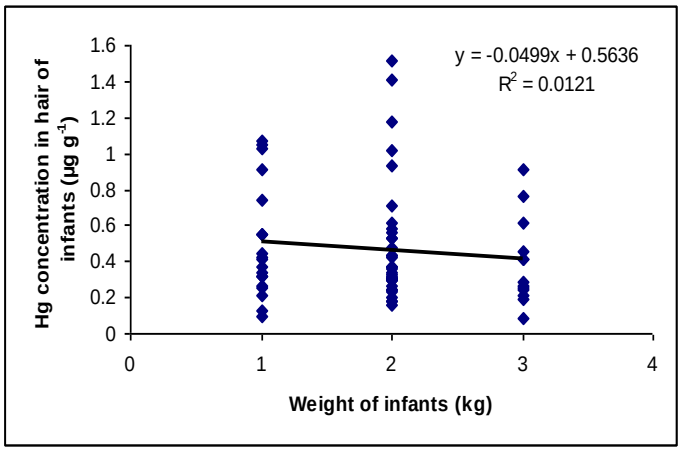


**fig. 7.** The relationship between Hg in the hair of infants and their weight: 1=3-5 kg, 2=6-8 kg , 3= >8 kg

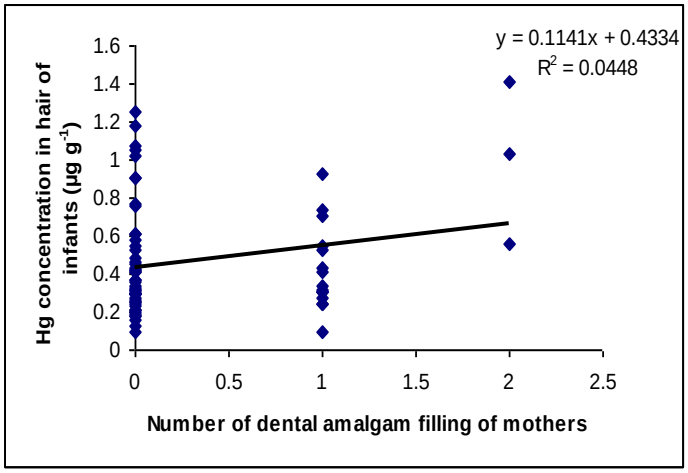


**fig. 8.** The relationship between Hg in the hair of infants and the number of dental amalgam fillings of their mothers: 0=0 dental , 1= 1-4 dental, 2= >4 dental

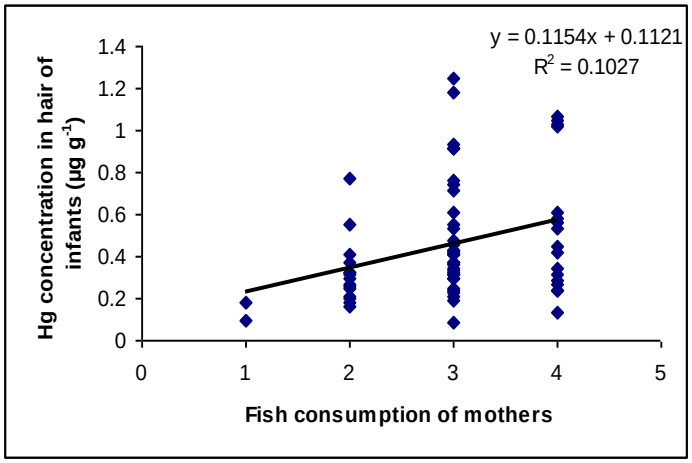


**fig . 9.** The relationship between Hg in the hair of infants and the amount of fish consumption by their mothers: 1= <1 portion per month, 2= 1-2 per month, 3= 1-2 portions per week, 4= >2 portions per week

In our study, as Sikorski showed ,there was a significant correlation (r=0.371 p=0.002) between mercury concentration in mothers and infants hair (Table 2) (30). Barbosa and Dorea (1998) also reported correlation (r=0.555, P<0.001) between Hg concentration in the hair of mothers and their breastfed infants (<2 years age) in non-Indian woman of Amazon Basin (33).

Table 5 are shows the association between mercury concentration in the hair of mothers and other factors. Mothers were classified to 4 age groups, 4 groups for fish consumption, 3 groups concerning the number of dental amalgam fillings, and 5 groups in relation to living location. The number of dental amalgam fillings of mothers and their age had no significant correlation with Hg concentration in the mothers' hair; this suggests that these factors might affect the transfer process of MeHg from mothers to babies. Fish consumption (p<0.001) and the living location (p=0.002) influenced Hg levels in the hair of mothers and with more fish consumption, mercury concentration increased. These results is in accordance with some of the previous studies (34,35,36,37) but Sleno ( 38) and Harakeh (39) reports did not agree with

that. The amount and type of consumed fish could be responsible for significant differences between these studies. Based on our questionnaires, people living in south of the Caspian Sea often consume fishes such as Mullet, Kuttum, and Perch although we did not examine the effects different fishes on Hg contamination. Chamestan is a tiny town and very similar to an agricultural village; therefore, the mean fish consumption is lower in this area. This finding might be responsible for the significant difference between Hg levels in Chamestan and the surrounding villages as compared to Nur and Nowshahr cities.

Table 4, shows the association between mercury concentration in the hair of infants and other factors. Based on sex, age, and living location, infant were classified to 2 , 3, and 5 groups respectively. Among in these factors, the age of infants (p=0.02) and their place of living (p=0.02) had significant influence on Hg level in their hair. Although Boischio ( 40) described a negative correlation between mercury levels in hair samples and the age of 0-1 years old babies, in our study, this correlation was positive for infants(<6 months of age). Other studies reported no significant relationship between mercury content and different ranges of age in both adults and children ( 41,42). We did not observe any significant difference between girls and boys, although the mean mercury level in hair samples in boys (0.55± 0.34 µg / g) were higher than girls (0.41-0.28 µg / g). Pinheiro also did not find any statistically significant difference in mercury levels in hair samples but Barreiras and Sâo luis do Tapajós detected differences based on gender(43). We noticed significant differences (p=0.02) between living locations and Hg level of infants which was due to dissimilarities between the diets in Chamestan and Nur and Nowshahr.

The amount of fish consumption by mothers (p=0.015) and the number of dental amalgam fillings (p=0.016) affected the Hg concentration in the hair of infants. Those with more than 4 amalgam fillings had higher levels of contamination (Table 6). Drexler and Schaller stated that compared to maternal fish consumption, exposure to mercury from maternal amalgam fillings is a minor factor in Hg contamination of breastfed infants (44). Because in this study the number of mothers with more than 4 amalgam fillings was small, it was difficult to evaluate its influencing on Hg levels in hair of mothers and infants.

**Table 6.** Dental amalgam as dependent variable: concentration in the hair of infants

Tukey HSD

| (I) number of dental amalgam filling | (J) number of dental amalgam filling | Mean Difference (I-J) | S.E | P value |
|---|---|---|---|---|
| 0 | 1-4 | -.0318 | .08706 | .929 |
| | >4 | -.5452(*) | .18395 | **.012** |
| 1-4 | 0 | .0318 | .08706 | .929 |
| | >4 | -.5134(*) | .19368 | **.027** |
| >4 | 0 | .5452(*) | .18395 | **.012** |
| | 1-4 | .5134(*) | .19368 | **.027** |

*The mean difference is significant at the .05 level.

**Table 7.** Fish diet as dependent variable: concentration in hair of infant

Tukey HSD

| (I) Fish consumption | (J) Fish consumption | Mean Difference (I-J) | S.E | P value |
|---|---|---|---|---|
| <1 month | 1-2 per month | -.1828 | .22919 | .855 |
| | 1-2per week | -.3467 | .22153 | .406 |
| | >2 per week | -.4930 | .22633 | .140 |
| 1-2 per month | <1 month | .1828 | .22919 | .855 |
| | 1-2per week | -.1638 | .09437 | .314 |
| | >2 per week | -.3102(*) | .10516 | **.022** |
| 1-2per week | <1 month | .3467 | .22153 | .406 |
| | 1-2 per month | .1638 | .09437 | .314 |
| | >2 per week | -.1464 | .08721 | .343 |
| >2 per week | <1 month | .4930 | .22633 | .140 |
| | 1-2 per month | .3102(*) | .10516 | **.022** |
| | 1-2per week | .1464 | .08721 | .343 |

*The mean difference is significant at the .05 level.

In conclusion, elevated levels of mercury in the hair of mothers and infants was mainly due to dietary habits (Table 7); however, further research is mandatory to establish Hg levels in different types of fish of the Caspian Sea. Fish that concentrate mercury also contain long-chain polyunsaturated fatty acids that benefit both mothers and their infants. In order to decrease the risk of perinatal mercury exposure, mothers should be advised to consume appropriate amounts of fishes that are low in Hg.

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