



Scalp hair and saliva as biomarkers in determination of mercury levels in Iranian women: Amalgam as a determinant of exposure

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ABSTRACT

The aim of this study was to determine the relationship between mercury concentrations in saliva and hair in women with amalgam fillings and its relation with age and number of amalgam fillings. Eighty-two hair and saliva samples were collected randomly from Iranian women who have the same fish consumption pattern and free from occupational exposures. The mean \pm SD age of these women was 29.37 ± 8.12 (ranged from 20 to 56). The determination of Hg level in hair samples was carried out by the LECO, AMA 254, Advanced Mercury Analyzer according to ASTM, standard No. D-6722. Mercury concentration in saliva samples was analyzed by PERKIN-ELMER 3030 Cold Vapor Atomic Absorption Spectrophotometer. The mean \pm SD mercury level in the women was $1.28 \pm 1.38 \mu\text{g/g}$ in hair and $4.14 \pm 4.08 \mu\text{g/l}$ in saliva; and there were positive correlation among them. A significant correlation was also observed between Hg level of saliva (Spearman's $\rho = 0.93$, $P < 0.001$) and hair (Spearman's $\rho = 0.92$, $P < 0.001$) with number of amalgam fillings. According to the results, we can conclude that amalgam fillings may be an effective source for high Hg concentration in hair and releasing the mercury to the saliva samples.

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1. Introduction

Mercury (Hg) is a toxic element that exists in the environment and accumulates in the food chain [1]. It evaporates at 20°C , increasing its volatility with the increase of the temperature; increasing up to 8 times case the temperature reaches 50°C [2]. Around the world, about 65% of the mercury contamination, in daily life is due to vaporization. Because mercury is known as a neurotoxin element, concerns about the health effects of exposure to this chemical are widespread [3]. Also, its systematic acute and chronic effects on different systems of body including central nervous system, digestive system, skin and oral tissues have been described [4–6].

Mercury gives rise to different effects especially on the nervous, reproductive and immune systems [7]. Among different mercury exposure sources, dental amalgam fillings have special importance. Amalgam is an alloy containing approximately 50% mercury and other metals, including silver, copper, or tin [8]. For people with dental amalgam fillings, these fillings constitute the major source of inorganic mercury, because an amalgam filling contains elemental mercury as a major component, and the possible health risks

have been debated for a long time [9,10]. Amalgam is the most widely used restorative material for dental fillings, which has been used in dentistry for more than 150 years [6–11]. During the past two decades, this material has come under increasing scrutiny with regard to its safety as it is known that amalgam restorations continuously discharge metallic mercury into the oral cavity, mostly in vapor form [12–17]. Hg vapor (Hg^0) is well absorbed from the lung and exposure to high concentrations can cause pneumonitis, bronchitis, chest pain, dyspnea, cough, stomatitis, gingivitis, excessive salivation and diarrhea [18]. In fact, in general population, the major source of Hg exposure is derived from dental amalgam restorations by inhalation of Hg vapor. In addition to this, some Hg may be dissolved in saliva or swallowed as amalgam particles [19].

There are some reports suggesting that amalgam fillings may induce oral lichen planus or oral lichenoid lesions and decreasing the antioxidant activity of saliva [5,6,20]. The release of mercury from dental amalgam in the oral cavity may be attributed to the effect of chewing, brushing, temperature, pH of saliva, biological corrosion due to bacteria [5,21–23], and electrochemical corrosion [24,25].

The potential risk of health hazards of humans via mercury exposure has been estimated by examining the metal contents in breast milk, blood, hair, nail, adipose tissues, and various organs [26]. In this research, we aim to determine the mercury level in the hair and saliva of Iranian women and evaluate the relationship between mercury concentrations in saliva and hair with number of amalgam fillings and age of these women.

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2. Materials and methods

2.1. Study sample

Eighty-two healthy women (with mean age of 29 years; ranged from 20 to 56 years), with absence of known occupational and/or environmental exposure to mercury were randomly selected and recruited to the study. The informed consent was obtained from all participants at the beginning of the study. Except participants who had no amalgam fillings, all of the other individuals had amalgams at least from 1 year ago. The participants were asked to fill out a questionnaire (a copy of this questionnaire is available upon request from the corresponding author) in order to collect data about the age, job, food habits and number of dental amalgam fillings.

2.2. Sample collection

A lock of scalp hair approximately 3 cm long was obtained from the root in the occipital region of each participant. It means longer hairs were cut down to 3 cm and only the 3 cm on the root side were analyzed. All participants should have two important conditions: firstly, since mercury releasing from amalgam fillings is more intensive in subjects who have recently used amalgam fillings as dental material and such mercury is more inconstant in first month of fillings, a time period of several months should be passed to mercury releasing would reach to constant limit. Furthermore, according to some studies, there is 6 months or in some cases slightly more long to half life of mercury in biological issues get to constant limit, so just individuals that their amalgam fillings belonged to at least 1 year ago could participate in this study. Secondly, since this study investigates amalgam as an exposure variable, in order to omit fish consumption factor (as one of the mercury resources), all participants had the same fish consumption patterns (<3 times a month). After cutting down the hair, samples were coded and stored in plastic bags until analysis. For collecting saliva, the participants were asked not to eat or drink for 1 h prior to the collection of a saliva sample (the samples were collected between 09.00 and 11.00 AM). Then, all subjects were asked to rinse their mouth 5 times with distilled water, to swallow the saliva produced during a 5 min interval, then collect the newly produced saliva in the mouth for 5 min and deposit the saliva in a test tube. Saliva samples were collected in a special prepared tube, and kept in freezer at -18 to -20°C .

2.3. Analytical procedure

For measurement of mercury concentration, hair samples were washed 3 times with nonionic detergent (1% (v/v), Triton X-100) and rinsed 3 times with the deionized water. Then the samples were dried in an electric oven at 60°C [27]. The determination of Hg level was carried out by the LECO, AMA 254, Advanced Mercury Analyzer (USA) according to ASTM, standard No. D-6722. Mercury concentration in saliva samples was determined using atomic absorption spectrophotometer (cold vapor method, apparatuses: PERKIN-ELMER 3030 and MHS-10). For analyzing Hg concentration in saliva samples, 9 ml nitric acid (95% BDH) was added to 1 ml of saliva, and then 5 drops of potassium permanganate were added to the solution. Afterward, mercury ions were reduced to elemental mercury by a sodium tetrahydridoborate-solution (0.2%).

2.4. Quality control

The accuracy of total Hg analysis via Advanced Mercury Analyzer (USA) according to ASTM, standard No. D-6722 was checked by running 3 samples of Standard Reference Material (SRM). Recovery varied between 98.5% and 103%. There was a good agreement

Table 1

Results of quality assurance procedure for mercury ($\mu\text{g/g}$).

Standard reference materials	N	Certified value	Obtained mean	SD ^a	R ^b
NIST-1633	3	0.141	0.139	0.015	98.5
NIST-2709	3	1.400	1.434	0.146	102
NIST-2711	2	6.250	6.437	0.419	103

^a Standard deviation.

^b Recovery (%).

^c National Institute of Standards and Technology.

between the obtained mean and the certified value. In order to check the reproducibility of the analysis, 10% of the samples were analyzed 3 times. The coefficient of variation was between 0.05% and 2.5% (Table 1).

In analyzing saliva samples, all reference solutions were prepared in glass volumetric flasks. Before preparing the reference solutions, the flasks were rinsed 3 times with HNO_3 (65%) and 3 times with deionized water. Then calibration solutions with the suitable concentrations were prepared from the reference solution. As the solutions decompose with time, they were freshly prepared for each series of measurements.

One of the standards was measured after every 15 samples as a quality control sample. Otherwise completely new calibration was carried out and the samples since the last successful quality control sample were measured again. The limit of detection is about $0.01 \mu\text{g Hg/l}$. We were able to confirm the statements made by Guo et al. [28] with regard to the precision of the measurements.

2.5. Statistical analysis

Statistical analyses were performed using SPSS (version 16.0, SPSS Inc., Chicago, IL, USA) software. Among questionnaire variables, women's age and number of amalgam filling were considered as potentially effective factors on Hg concentrations in hair and saliva. The data were tested for the assumption of normality using the Kolmogorov–Smirnov test. For analytic purposes, the independent samples *t*-test was used for comparing the means in two independent populations with normal distribution, and the Mann–Whitney test was utilized for other variables with non-normal distribution. In addition, the Wilcoxon test was used for comparing the median of two dependent non-normal populations. We also used the Kruskal–Wallis test for comparing the median of dependent variables in more than two non-normal populations. The Spearman's correlation coefficient and the multiple linear regression analysis were also used for assessing the relationship between dependent and explanatory variables under study. Finally, in order to assess the effect of number of amalgam fillings and age simultaneously on mercury levels in hair and saliva, we used a marginal modeling technique (GEE analysis). *P*-values <0.05 were considered statistically significant.

3. Results

In this study, a random sample of 82 healthy women was recruited. The mean \pm SD age of these women was 29.37 ± 8.12 (ranged from 20 to 56). For this sample, the mean \pm SD of the amalgam fillings was 3.80 ± 3.43 (ranged from 0 to 16). Among them 20 women (24.4%) had no amalgam fillings, 30 women (36.6%) had 1–4 amalgam fillings and 32 women (39.0%) had more than 4 amalgam fillings.

Table 2 shows the descriptive statistics about the Hg concentrations in hair and saliva of these women.

The results of the Kolmogorov–Smirnov test showed that assuming the normal distribution for Hg concentration in hair and

Table 2

Descriptive statistics for Hg concentrations in hair ($\mu\text{g/g}$) and saliva ($\mu\text{g/l}$) of this sample.

Sample	Number	Minimum	Maximum	Mean	SD	SE
Hair	82	0.150	6.320	1.281	1.384	0.152
Saliva	82	0.001	40.500	4.141	7.048	0.778

Table 3

A comparing Hg concentration in hair and saliva in different categorize of amalgam fillings (95% CI).

Sample	No. of amalgam fillings	Number	Mean	SD	SE	<i>P</i> -value [*]
Hair	0	20	0.209	0.040	0.009	<0.001
	1–4	30	0.456	0.138	0.025	
	≥ 5	32	2.725	1.199	0.212	
Saliva	0	20	0.002	0.001	0.000	<0.001
	1–4	30	1.117	1.774	0.323	
	≥ 5	32	9.564	8.752	1.547	

^{*} Kruskal–Wallis *P*-value.

Table 4

Univariate relationship between age and number of amalgam fillings with Hg concentration in hair and saliva.

Hg concentration	Age	No. of amalgam fillings
Hair	0.337 ^a (0.002) [*]	0.936 (<0.001)
Saliva	0.309 (0.005)	0.929 (<0.001)

^a Spearman's correlation coefficient.

^{*} Correlation *P*-value.

saliva is not true ($P < 0.001$). So, we used the non-parametric tests for analyzing these data.

First of all, the Wilcoxon test showed that Hg concentration in saliva samples were significantly higher than hair samples ($P < 0.001$).

In this research, participants were divided into three groups in accordance with the number of amalgam fillings: first group: without amalgam fillings, second group: 1–4 amalgam fillings and third group: more than 4 amalgam fillings. For comparing the mercury levels of hair and saliva in different categories of amalgam fillings, we used the non-parametric Kruskal–Wallis test. Table 3 shows the results.

In the next step, to assess the univariate relationship between women's age and number of amalgam fillings with the Hg concentration in hair and saliva, we used the Spearman's correlation coefficient test. Table 4 shows the obtained results. As we can see, all of the correlations are positive and statistically significant, but it is clear that number of amalgam fillings is severely correlated with the Hg concentration both in hair and saliva of the women under study.

In addition, there was strong positive correlation between the Hg concentration in hair and saliva of the women (Spearman's $\rho = 0.887$, $P < 0.001$) (Fig. 1). A positive correlation was also found between age and number of amalgam fillings ($r = 0.37$, $P < 0.001$).

Table 5

Multiple linear regression results for assessing the concurrent effect of explanatory variables on Hg concentration.

Dependent	Independent	Est ^a	SE ^b	<i>P</i> -value [*]
Hg concentration in hair	Age	0.000	0.011	0.931
	No. of amalgam fillings	0.343	0.026	<0.001
Hg concentration in saliva	Age	−0.18	0.083	0.831
	No. of amalgam fillings	1.277	0.197	<0.001

^a Regression coefficient estimate.

^b Standard error of the estimate.

^{*} Regression *P*-value at 95% significantly level.

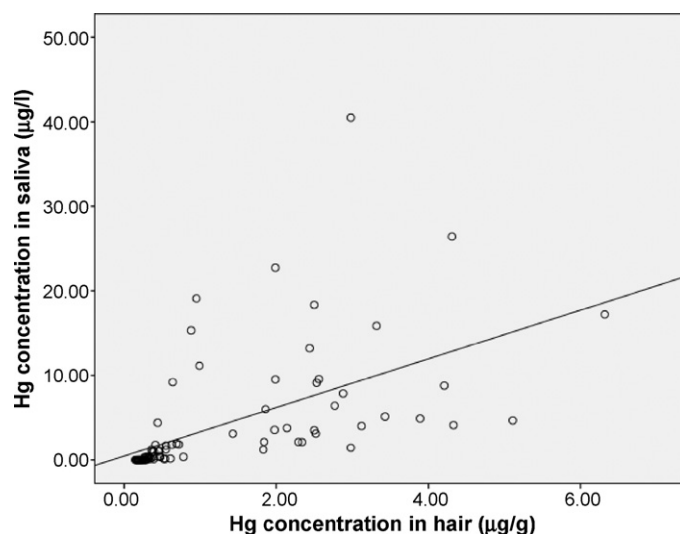


Fig. 1. Correlation between Hg concentrations in hair and saliva samples of women (Spearman's $\rho = 0.887$, $P < 0.001$).

Table 6

GEE results for assessing the effect of age and number of amalgam fillings on mercury concentration.

Parameter	Est ^a	SE ^b	<i>P</i> -value
Age	−0.009	0.049	0.851
No. of amalgam fillings	0.818	0.126	<0.001

^a GEE estimate.

^b Standard error of the estimate.

Afterward, we utilized a multiple linear regression model for evaluating the concurrent effect of number of amalgam fillings and women's age on Hg concentration in hair and saliva. Table 5 shows the estimates. These findings show that when we consider the number of amalgam fillings in the model, the age of the women had no significant effect on mercury concentration.

Finally, we utilized the GEE (Generalized Estimating Equations) analysis with exchangeable correlation structure for evaluating the concurrent effect of age and number of amalgam fillings on Hg concentration of hair and saliva, simultaneously. Table 6 shows the results. Regarding these results, one can conclude that when the number of amalgam fillings is considered in the model, the age of the women shows no significant effect on Hg concentrations.

4. Discussion

In recent years, increasing concern over mercury toxicity has focused especially on the evaporation of mercury from amalgam restorations in the oral cavity. It has been shown that mercury vapor is released from dental amalgam not only when the material is inserted into the cavity and from the newly made restoration, but also afterwards, from the hardened material after filling

[12,13,29,30]. This release is enhanced during activities such as chewing, tooth brushing, drinking hot beverages, or oral breathing [31].

It is well established that subjects with amalgam restorations exhibit significantly higher mercury concentrations in saliva than subjects without such fillings [32,33]. It has been proposed that hair Hg (H-THg) reflects inorganic mercury exposure at low MeHg exposure in populations with no or low fish consumption [34] and dental amalgam fillings are the major source of mercury exposure in the general population [35]. In the present study, eighty-two hair and saliva samples were collected from Iranian women (in Mashhad city) with mean \pm SD age of 29.37 ± 8.12 years old. We only considered the women because many studies showed that gender was unlikely to play a role in determining mercury accumulation [27–36]. The average mercury in saliva and hair was $4.14 \mu\text{g/l}$ and 1.28 mg/kg , respectively. In our study, 28% of subjects had Hg concentrations (average of $3.18 \mu\text{g/g}$) greater than $2.0 \mu\text{g/g}$ in hair (WHO 'normal' level). As the questionnaires show, all of these subjects had more than 4 amalgam fillings. However, the overall mean of hair Hg was $1.28 \mu\text{g/g}$ which was below the WHO 'threshold' level ($5.0 \mu\text{g/g}$) [37]. Also, 37% of all hair samples had Hg concentrations higher than the USEPA-recommended $1 \mu\text{g/g}$ [38]. Many researches have been undertaken for determining the mercury quantity in biomarkers, like blood, urine, saliva, hair and nail. However, unfortunately, the normal level of saliva mercury amount has not been mentioned in guideline principles of the WHO. But it is important to notice that mercury does not have any beneficial function for human body, so, any amount of this toxic element could be harmful. In the present study, the univariate analyses revealed that the age of the women has positive correlation with Hg levels in both hair and saliva. But, when we consider concurrent effect of number of amalgam fillings and age on mercury concentration in hair and saliva, the results showed that the age of the participants had no significant effect on Hg levels. It could be concluded that older women may have more amalgam fillings and amalgam is a potential source of mercury in human body.

As the results showed, in subjects without amalgam fillings, mercury concentration in saliva samples was very low (almost zero), but in hair samples, mercury concentration in subjects without amalgam fillings was $0.208 \mu\text{g/g}$. So it can be concluded that, although occupational exposure and fish consumption (as an effective factors on mercury concentrations) were omitted from the study and all of the participants had the same fish consumption patterns, the mercury level in hair is as a result of other mercury sources like using cosmetic materials or chemical shampoos which potentially effect on Hg levels in hair. In fact, these days cosmetic products (with mercury in their structure) are widely available and frequently used as bleaching agents, skin-lightening cream or other beauty products by women throughout the world [39] and despite the well-known hazards of mercury exposure and the ban against the sale of creams containing mercury in some countries like the United States, these products are widely available in pharmacies, beauty aid stores and cosmetic markets around the world [40].

There are many researches around the world on mercury concentrations in human body. In study of Pizzichini et al. a considerable relation has been shown between the saliva mercury with the quantity of amalgam fillings [4,5]. Mercury concentrations were determined in scalp hair of 233 school children aged 6–16 years by Batista et al. [41]. They reported the mean mercury concentration in hair of these children about $0.77 \mu\text{g/g}$. The influence of the variables, such as place of residence, age, fish and seafood consumption, number of dental amalgam fillings and some other parameters was also examined in their study. The reported mean level of Hg in hair was less than our study. The place of residence and lower mean age may be the reasons for this difference. Another study was carried out by Dakeishi et al. [42] among 327 women at age 24–49

years to determine hair mercury levels. As their results revealed, hair mercury levels in the women was between 0.11 and $6.86 \mu\text{g/g}$ (median 1.63) [42], which is similar to the present study. Dickman et al. demonstrated that mercury concentrations found in the hair of 159 Hong Kong males aged 25–72 (mean age of 37 years) was positively correlated with their age [43], which is not verified in this study, when we consider women's age in presence of amalgam fillings. The difference between the range of women's age in Dickman's study and present study may be the reason of it. Leisteuvuo et al. had taken advantage of the CVAAS technique for analyzing saliva samples like the present study. In their research, the amount of mercury in saliva was significantly higher ($P < 0.001$) in subjects with dental amalgam fillings, compared to the non-amalgam study groups [44].

Regarding these results, it is obviously necessary to do comprehensive research to answer the questions such as, safety of amalgam fillings, determining the normal amount of Hg in saliva, relation of mercury of saliva with systemic absorption in different forms, relation between Hg in saliva and hair with other samples obtained from human body in different periods of time after fillings, and safety of using other dental materials.

5. Conclusion

According to the results of the present study, in Iranian women, dental amalgam fillings may be as an important resource for releasing the mercury to saliva and such mercury can be absorbed systematically upon swallowing and to be concentrated in different body tissues such as hair.

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