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Original Article

Correlation between dental amalgam fillings and urinary mercury levels in the Jordanian population: A comparative population-based study

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Abstract *Background/purpose:* The health risks of mercury-containing dental amalgam fillings remain controversial. Urinary mercury levels are used as a tool to assess mercury exposure toxicity. Therefore, this study aimed to examine the effects of dental amalgam fillings on urinary mercury levels in the Jordanian population.
Materials and methods: This study recruited an experimental group of 108 participants with dental amalgam fillings and control group of 109 participants without dental amalgam fillings. Urinary mercury levels were measured and compared between the two groups.
Results: The results showed a statistically significant increase in urinary mercury concentration in participants with dental amalgam fillings compared to participants with no dental amalgam fillings (6.42 ± 0.37 μg/L vs 1.55 ± 0.05 μg/L, $P < 0.05$). It also showed that this increase in urinary mercury levels among Jordanians with dental amalgam fillings was statistically significant when individuals had more than 6 dental amalgam fillings (9.36 ± 1.68 μg/L) compared to those with fewer than 6 (4.73 ± 0.34 μg/L, $P < 0.05$). Increased urinary mercury levels among Jordanians were also correlated with having dental amalgam fillings for more than 10 years.
Conclusion: Increased urinary mercury levels in the Jordanian population are correlated with both the number of amalgam fillings and the duration of having these fillings. However, this increase in urinary mercury levels observed in this study is below the threshold that would pose significant health risks.

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Introduction

Mercury (Hg) is a heavy metal with toxic effects found in air, water and soil. It exists in 3 different forms. Elemental (or metallic) form (Hg^0); inorganic form (Hg^{+1} and Hg^{+2}, e.g. mercuric chloride, $HgCL_2$); and organic form (e.g., methyl- and ethyl mercury). [1,2]Elemental mercury is liquid at room temperature and can readily evaporate to produce vapor. Mercury vapor is more hazardous than the liquid form.[3]

Mercury exposure has been linked to various deleterious health consequences in humans including neurological disorders,[4] immunological disorders[5] and kidney injury.[6] Humans' sources of exposure to mercury varies and include ingestion of contaminated fish, occupational exposure, thermometers, light bulbs, pharmaceutical products, vaccine, pesticides in addition to dental amalgam fillings.[1,7,8] Urinary mercury concentration is used to assess chronic exposure to elemental mercury. Urinary mercury concentrations are the most accurate and widely used biomarker for assessing the absorbed dose that results from chronic mercury exposure. Urinary mercury concentrations can be expressed as μg Hg per gram creatinine (Cr) or μg Hg per liter of urine (L).

Amalgam is a malleable mixture of elemental mercury (50 %), silver (35 %), tin, zinc and copper (15 %).[9,10] It has been used worldwide for decades to fill cavities in a tooth, restoring their shape and function to its normal condition. Dental amalgam filling may cover one or more of the five surfaces of a tooth.[10] Dental amalgam fillings in humans are considered a source of low-level mercury exposure because they release mercury vapor during chewing, tooth brushing, or when the restorations are removed. Some of this vapor is inhaled, and some may dissolve in saliva and be swallowed.[2] The average daily intake of elemental mercury from dental amalgam restorations is estimated to be 3—17 μg depending on the number of restorations.[11] While most mercury entering the body is excreted, a small amount may accumulate in the kidneys and other tissues.

Dental amalgam has been subject to criticism at various times and the dental amalgam health risk and its contribution to various diseases in humans remain controversial. To the best of our knowledge, no recent studies have evaluated the effect of dental amalgam fillings on mercury exposure and urinary mercury concentration in the Jordanian population. Therefore, this study aims to examine the effects of dental amalgam fillings on urinary mercury levels in the Jordanian population.

Materials and methods

Ethics approval

All procedures in this study were performed in line with the principles of the Declaration of Helsinki. Ethical approval was granted by the relevant Ethics Review Board and is filed under MLS_R_15/01/2023.

Study population

Participants in the current cross-sectional, observational, population-based study were recruited voluntarily from April 2023 to June 2024. Subjects were attending the department of dentistry at various medical centers in the private sector in Jordan. A total of 108 subjects with dental amalgam filling were selected in the experimental group, while 109 subject without dental amalgam filling were randomly selected as healthy controls.

A short face-to-face interview was conducted with each participant after a written informed consent was obtained from all participants involved in the study after providing a comprehensive explanation of the study's purpose, procedures, and significance. Participants details were collected by asking all participants about (a) age (b) number of amalgam fillings (c) duration of amalgam fillings. Participants with dental amalgam fillings were examined by professional dentist and categorized into six groups; 3, 4, 5, 6, 7 and $\geq$ 8 dental amalgam fillings. The duration of having amalgam fillings was categorized as the following: 1—5 years, 6—10 years, 11—15 years, 16—20 years and $\geq$21 years.

Exclusion criteria

The exclusion criteria were patients with bridges, crowns, gold inlays or dentures, those with kidney disease, hypertension, diabetes, and cognitive dysfunction or mental disorders long-term drug users; individuals working in mercury-related workplaces and those with missing data record were excluded. Participants who classified themselves as moderate or frequent fish and seafood consumers were also excluded.

Urine samples collection

Each participant provided a 25 ml urine sample in a sterile urine container. The samples were labelled with participant details and stored in a refrigerator at −20 °C for later

analysis. All urine samples were processed at an accredited private diagnostic laboratory in Irbid city in Jordan.

Determination of urinary mercury levels

For linearity calculations, a stock standard solution of mercury (1000 mg/L) was used to prepare a series of mercury standard solutions of 20, 15, 10, 5, 2 and 1 μg/L by adding a deionized water (PerkinElmer, Norwalk, CT, USA).

The urine sample was homologized by well shaking, a 5 ml HNO3 was added to about 1 ml urine, then 0.5 ml HCl and 5 ml deionized water were added then the solution was heated in microwave, 25 ml deionized water were added to solution, after cooling for 30 min the sample was injected to Inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer).

Statistical analysis

A two-tailed, unpaired Student's t-test assuming unequal variances was used to assess the statistical significance of differences between the experimental group and the control group. Differences in mean ± standard error of the mean (SEM) values among the experimental group were analyzed using one-way ANOVA test followed by Tukey's post hoc test. Correlation analysis was conducted using parametric Pearson's (r) correlation analysis. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0 for windows (SPSS Inc., Chicago, IL., USA) and Microsoft Excel 2010. All results are expressed as the mean ± SEM. $P < 0.05$ was considered to be statistically significant.

Results

Our results showed that the mean age ± SEM of participants in the experimental group (33.39 ± 0.88 years) was statistically significantly higher than that of the controls (24.62 ± 0.37 years, $P < 0.05$, Table 1). The mean urinary mercury concentration of the participants in the experimental group was also statistically significantly higher compared to the controls (6.42 ± 0.37 μg/L vs 1.55 ± 0.05 μg/L, $P < 0.05$, Table 1).

Table 2 presents the distribution of participants with dental amalgam fillings, categorized by the number of dental amalgam fillings, gender, and the duration of exposure to dental amalgam in years.

Investigating the impacts of age, number of dental amalgam fillings and the duration of having dental amalgam fillings on urinary mercury concentration in the participants from the experimental group, Pearson's correlation analysis was conducted. The results revealed evident significant positive correlation coefficients between elevated urinary mercury concentrations and each of these parameters in the participants ($P << 0.0001$, Table 3).

This study also showed that the number of dental amalgam fillings required to cause statistically significant increase in urinary mercury concentration in participants from the experimental group was 7 or more while 3, 4 and 5 dental amalgam fillings showed no significant effect on urinary mercury concentration (Figure, 1). To this regard, the mean urinary mercury concentrations in participants with 3, 4, 5 and 6 dental amalgam fillings were (4.73 ± 0.34, 5.53 ± 0.56, 4.98 ± 0.95, 4.93 ± 0.42 μg/L) respectively. These values were statistically significantly lower than the mean urinary mercury concentration of participant having 7 dental amalgam fillings (9.36 ± 1.68 μg/L, $P < 0.05$). Similarly, participants with 8 or more dental amalgam fillings exhibited a significantly higher mean urinary mercury concentration compared to those having less than 7 dental amalgam fillings (10.90 ± 0.88 μg/L, $P < 0.05$). Additionally, there were no statistically significant differences in urinary mercury concentrations among participants with 3, 4, 5 and 6 dental amalgam filling nor between participants with 7 fillings and those with 8 or more dental amalgam fillings (Fig. 1).

Moreover, our results showed that the statistically significant increase in urinary mercury concentration in participants from the experimental group was observed after 10 years of having dental amalgam fillings (Fig. 2). The mean urinary mercury concentrations were (3.46 ± 0.22 and 5.09 ± 0.29 μg/L) in participants who had dental amalgam fillings for 1–5 years and 6–10 years respectively, indicating no significant increase in urinary mercury concentration before 10 years. The significant increased

Table 1 Age and urinary mercury concentrations in the control group and experimental group.

Characteristic	Control group n = 109	Experimental group n = 108	*P*-values
Age (years)	24.62 ± 0.37	33.39 ± 0.88	<0.0001
Urinary Hg concentration (μg/L)	1.55 ± 0.05	6.42 ± 0.37	<0.0001

Data were analyzed using two-tailed, unpaired student's t-test assuming unequal variances, results are expressed as the mean ± SEM. Statistical significance was considered when $P < 0.05$. n is number of subjects in each group.

Table 2 Distribution of number of amalgam fillings by gender and duration of having dental amalgam fillings in the experimental group.

Duration of having amalgam fillings (Years)	Number of dental amalgam fillings							
	Gender	3	4	5	6	7	≥8	Total
1–5	Male	11	4	2	0	1	0	18
	Female	8	1	0	0	0	0	9
6–10	Male	5	3	1	1	1	3	14
	Female	6	3	1	5	0	0	15
11–15	Male	10	0	1	0	1	5	17
	Female	3	2	0	2	1	2	10
16–20	Male	5	1	0	0	0	4	10
	Female	0	0	0	0	2	1	3
≥21	Male	1	2	0	0	2	3	8
	Female	0	1	0	0	0	3	4
Total		49	17	5	8	8	21	**108**

Table 3 Pearson's correlations analysis.

	Correlation coefficient	Age	Number of amalgams	Duration of amalgams
Urinary Hg concentration (μg/L)	ρ (r)	0.364	0.650	0.724
	P value	<0.0001	<0.0001	<0.0001

Pearson's correlations analysis between urinary mercury concentrations and age, number of dental amalgams and duration of having dental amalgams in the experimental group. Data were analyzed using Pearson's correlation analysis. Statistical significance was considered when $P < 0.05$. ρ (r) is Pearson's correlations coefficient.

urinary mercury concentration was observed only in participants who had dental amalgam fillings for 11–15 years (6.75 ± 0.54 μg/L), 16–20 years (9.77 ± 1.17 μg/L) and 21 yeas or more (11.85 ± 0.89 μg/L, $P < 0.05$, Fig. 2) compared to those who had dental amalgam for 10 years or less.

Discussion

The main finding of the current study is that dental amalgam fillings lead to an increase in urinary mercury concentration in humans. This increase in urinary mercury concentration was observed only in cases where individuals had more than 7 dental amalgam fillings for more than 10 years. However, we found that this increase in urinary mercury concentrations in the Jordanian population resulting from exposure to dental amalgam fillings remains below the threshold urinary mercury level of 20 μg/L, which is associated with mercury toxicity and an increased risk of health hazards.

For the estimation of potential health risks due to the exposure to mercury (biomonitoring), three human biomonitoring categories of exposure have been suggested.[12] Category I: urinary mercury concentration is < 5 μg/L, which is within normal ranges. Category II: Urinary mercury concentration is 5–20 μg/L, indicating an elevated level, and risk to general health is not expected but follow up is suggested. Category III: Urinary mercury concentration is > 20 μg/L, representing a significantly elevated level where a risk to general health is possible. Human exposure to mercury from dental amalgam fillings is considered low-grade exposure.[13] The presence of mercury in the blood indicates recent or current exposure to mercury[14] because mercury has a short half-life of approximately 3 days in blood. In contrast, the presence of mercury in urine indicates long-term exposure to this heavy metal.[15] Urinary mercury concentrations are the most accurate and widely used biomarker for assessing the absorbed dose resulting from chronic exposure to elemental mercury vapor.[16]

Findings from this study on the effect of dental amalgam fillings on increased urinary mercury concentration align with other studies that reported elevated urinary mercury levels in children[17,18] and in young women.[19] Additionally,

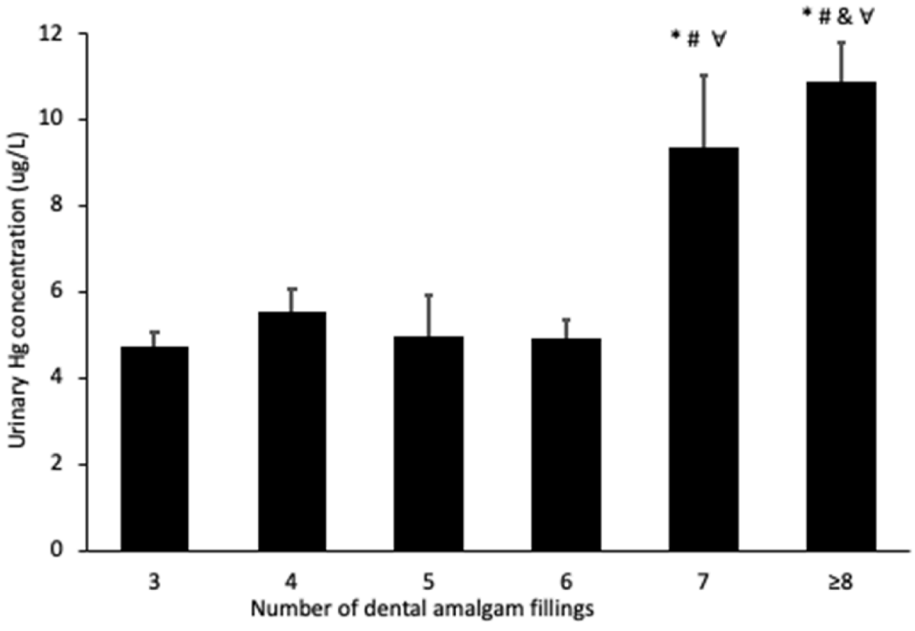

Figure 1 Urinary mercury concentrations in relation to the number of dental amalgam fillings.
The figure shows that 7 or more dental amalgam fillings lead to increased urinary mercury levels in individuals from the experimental group. n = 5–49. *: $P < 0.05$ compared to 3 dental amalgams fillings, #: $P < 0.05$ compared to 4 dental amalgams fillings. &: $P < 0.05$ compared to 5 dental amalgams fillings. ∀: $P < 0.05$ compared to 6 dental amalgams fillings.

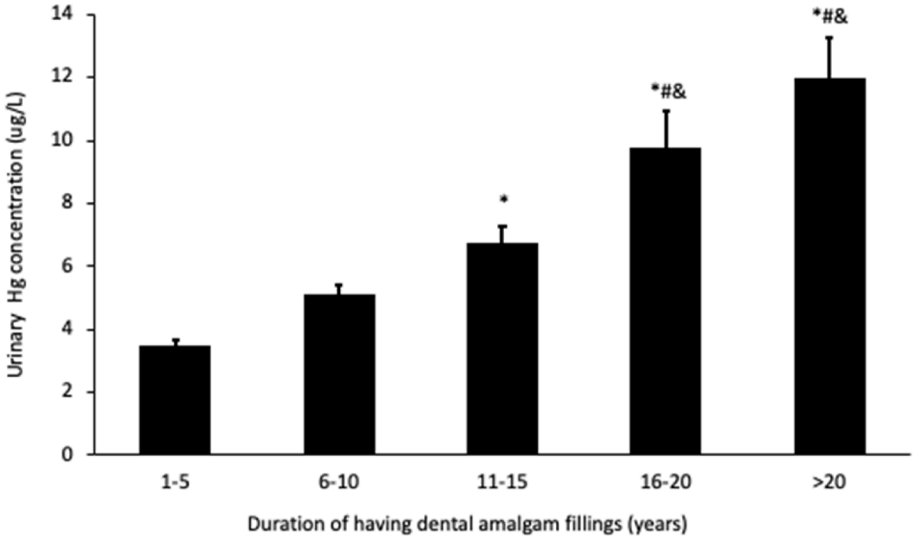

Figure 2 Urinary mercury concentrations in relation to years of having dental amalgam fillings.
The figure shows that it took at least 11 years of having dental amalgam fillings for urinary mercury levels to increase in individuals from the experimental group. n = 13–29. *: $P < 0.05$ compared to 1–5 years, #: $P < 0.05$ compared to 6–10 years, &: $P < 0.05$ compared to 11–15 years.

an increase of 1–1.8 μg Hg/L in urinary mercury concentration for every 10 dental amalgam fillings has been reported.[20,21] It has also been reported that evidence suggests low levels of human exposure to mercury from dental amalgam fillings exerts an effect on kidney tubular functions in children.[22] In this regard, the exact impact of the increase in urinary mercury concentration due to dental amalgam fillings on individuals in the Jordanian population requires further investigation.

Consistent with our findings, it has been reported that dental amalgams did not prove to increase mean urinary mercury levels to a degree that poses health risks in the general Canadian population.[16] Furthermore, a simulation experiment by Berdouses et al. showed that the daily mercury dose ftom a single amalgam filling is 0.03 μg/day, which is well below the calculated threshold-limiting of 82.29 μg/day considered dangerous for occupational exposure.[23]

In the current study, the observed impact of dental amalgam fillings on increased urinary mercury concentration in the Jordanian population was evident when individuals had more than 6 dental amalgam fillings. This aligns with previous report found significant correlation between the number of amalgam surfaces and daily amounts of urinary mercury in the American adult population.[10] Other studies have also reported a correlation between the number of dental amalgam fillings and a significant increase in urinary mercury levels[24] and hair mercury content.[25] Additionally, Guzzi et al. reported a significant increase in total mercury levels in all types of tissue in individuals with a greater number of occlusal amalgam surfaces (>12) compared to those with fewer occlusal amalgams (0–3).[26] In this study, we also observed an increase in urinary mercury concentration in the Jordanian population with the duration of having dental amalgam of more than 10 years in this study. Urinary mercury concentrations required more than 8 dental amalgam fillings for over 20 years to reach approximately 12 μg/L. This increase is clearly a result of prolonged exposure to mercury from dental amalgam fillings.

Mercury is one of the most toxic metals, and the molecular mechanisms underlying its toxicity are still not fully understood. Depending on its chemical form, dose, exposure duration, and pathway, mercury can exert varying degrees of harmful effects on human tissues and organs. Once elementary mercury (Hg^0) in dental amalgam evaporates, inhaled and absorbed into the bloodstream, it undergoes biotransformation predominantly in erythrocytes to mercuric ion (Hg^{2+}), the ultimate mediator of mercury toxicity. This is due to its strong affinity for sulfhydryl groups (–SH), which are functional components of most proteins, enzymes and hormones, leading to interference with intracellular signaling pathways and disruption of various biological systems.[27] Elemental mercury in the dental amalgam can also generate free radicals, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), which induce oxidative stress. This causes a reduction in the activity of antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase (SOD).[28] Furthermore, mercury has the capacity to induce phospholipase D (PLD) activation, which has been implicated in many human cancers and diseases. Studies examining protein phosphatases 1 (PP1) metal inhibitors have shown that Hg^{2+} has inhibitory effects on the PP1 enzyme.[3] Additionally, mercury can promote epigenetic alterations, including DNA methylation and histone modifications.[3]

There is also increasing evidence that the pathophysiological target of mercury is in fact selenium.[29] Selenoproteins are integral components of the thioredoxin system (thioredoxin reductase 1 and thioredoxin reductase 2) and the glutathione-glutaredoxin system (glutathione peroxidase). Mercury binds to the selenium site on these selenoproteins, permanently inhibiting their function and disrupting the intracellular redox environment. The

impairment of the thioredoxin and glutaredoxin systems resulting in the proliferation of intracellular reactive oxygen species which leads to glutamate exocytosis, calcium dyshomeostasis, mitochondrial injury and loss, lipid peroxidation, impairment of protein repair, and apoptosis.[29]

In this study, we were not significantly challenged by the confounding factors related to mercury exposure, such as fish and seafood consumption. This is because Jordan is not a coastal country, and such foods are not very commonly consumed. Alcohol intake confounder is also rare in the study population due to cultural and religious reasons. Additionally, exposure to mercury from environmental pollution in Jordan is unlikely, as the country is not an industrial one. This study has distinct strength, to the best of our knowledge, it is the first to evaluate the correlation between dental amalgam fillings and urinary mercury levels in the Jordanian population. However, our study also has some limitations. We did not stratify urinary mercury concentrations by the sex of participants. Sex may have different effects on the mercury exposure between males and females.

Finally, this study advocates for discouraging dentists and dental industry to use mercury in dental treatment. If the use of amalgam being continued, it is recommended to use pre-encapsulated amalgam rather than mixing their own.

To conclude, this study corroborated that dental amalgam fillings increase the mean urinary mercury concentration among Jordanians; however, this increase remains below the level associated with mercury toxicity. The increase was observed in individuals with more than 7 dental amalgam fillings for over 10 years. The findings of this study suggest that dental amalgam fillings are still safe for use in humans. Nevertheless, they underscore the importance of monitoring mercury exposure in individuals who have had dental amalgam fillings for more than 10 years.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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