Mercury in dental restoration: Is there a risk of nephrotoxicity?

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ABSTRACT: Background: Concern has been voiced about exposure to mercury (Hg) from dental amalgam fillings, and there is a need to assess whether this leads to signs of nephrotoxicity.
Methods: A total of 101 healthy adults (80 males and 21 females) were included in this study. The population as grouped into those having amalgam fillings (39 males and 10 females) and those without (41 males and 11 females). Hg was determined in blood, urine, hair and nails to assess exposure. Urinary excretion of β2-microglobulin (β2M), N-acetyl-β-D-glucosaminidase (NAG), γ-glutamyltransferase (γ-GT) and alkaline phosphatase (ALP) were determined as markers of tubular damage. Albuminuria was assayed as an early indicator of glomerular dysfunction. Serum creatinine, β2M and blood urea nitrogen (BUN) were determined to assess glomerular filtration.
Results: Hg levels in blood and urine were significantly higher in persons with dental amalgam than those without; in the dental amalgam group, blood and urine levels of Hg significantly correlated with the number of amalgams. Urinary excretion of NAG, γ-GT and albumin was significantly higher in persons with dental amalgam than those without. In the amalgam group, urinary excretion of NAG and albumin significantly correlated with the number of fillings. Albuminuria significantly correlated with blood and urine Hg.
Conclusion: From the nephrotoxicity point of view, dental amalgam is an unsuitable filling material, as it may give rise to Hg toxicity. Hg levels in blood and urine are good markers of such toxicity. In these exposure conditions, renal damage is possible and may be assessed by urinary excretions of albumin, NAG, and γ-GT.

Key words: Dental amalgam, Mercury, Nephrotoxicity

Introduction

Dental amalgam fillings, that contain 50% Hg by weight (1), contribute to the Hg exposure of the general population. Hg in the form of either vapor or inorganic salts is released during insertion or
replacement of the fillings and during their functional life (2). After absorption, Hg vapor is rapidly oxidized in erythrocytes or tissues to inorganic Hg, so the tissue distribution and toxic effects of Hg vapor and inorganic Hg are the same (3). The current threshold for long-term occupational exposure to Hg vapor recommended by WHO is 25 μg/m³ air (1). The average exposure, based on expired air measurements, in individuals with dental amalgam fillings is in the range of 0.29-29.1 μg/m³ air depending on the number of fillings, toothbrushing, number of meals and their duration, oral breathing habits and swallowing (4). The kidneys are the primary target for accumulation of inorganic Hg, and the proximal tubule is the initial site of toxicity (5). At least two mechanisms are involved in the proximal tubular uptake of Hg. One appears to involve the apical activity of γ-glutamyltranspeptidase, and the other is the basolateral organic anion transport system (6). Chronic low dose exposure to inorganic Hg salts or Hg vapor may also induce an immunological glomerular nephritis (3). Although there have been some investigations, there is still uncertainty about the nephrotoxicity of dental amalgam. Therefore we studied the exposure to Hg from dental amalgam and its impact on renal integrity.

Subjects and Methods

Subjects

Two groups were studied in the present work. The group of 49 persons with dental amalgam fillings (39 males and 10 females, mean age 31 years, range 26-36 years) had an average of 4.4 dental amalgam fillings, range 1 to 8. The amalgam contained approximately 50% Hg, and varying amounts of silver (30%), tin, zinc, and copper. The time since the amalgam had been introduced ranged from 3 to 60 months. The control group comprised 52 healthy volunteers (41 males and 11 females, mean age 31.8 years, range 25-38 years), with no known exposure to Hg. Inclusion criteria, based on a questionnaire for both groups, were the absence of drug intake, cigarette smoking, diabetes, hypertension, or hepatic diseases. Persons with renal or urological diseases, assessed by microscopic urine examination, ultrasound examination and clinical investigation, were excluded. The two groups were matched for residence area of and socio-economic status to avoid any effect of environmental exposure and nutrition on the body burden of Hg.

Sampling

Blood for Hg analysis was drawn into polyethylene tubes containing heparin as anticoagulant and stored at -20°C. Serum samples obtained after centrifugation (3000 rpm, 10 min) were stored at -20°C until analysis for creatinine, β2 M, and BUN. Early morning urine samples were collected in 50 mL polypropylene tubes and centrifuged at 1500 rpm for 10 min to clear them of particulate matter. To prevent degradation of β2M in acidic urine (pH < 5.5), each person took 4 grams of sodium bicarbonate dissolved in water the night before urine was collected. The pH of urine was checked and adjusted to 5.5 - 7.5 using 1 N NaOH and stored at -20°C. For Hg analysis, 10 mL of urine were stored in acid-washed polypropylene tubes at -20°C. For urinary enzyme analysis, ethylene glycol was added, to a final concentration of 30% (v/v). The pH was adjusted to 7 with 1 N NaOH and samples were stored at -20°C (7). Urine for analysis of albumin and creatinine was mixed with sodium azide (0.1% w/v) and stored at 4°C. Hair and nail samples for Hg analysis were obtained using stainless steel scissors and nail clippers. The samples were cut into the smallest pieces possible and washed three times with 1% w/v Triton x 100 then rinsed three times with de-ionized water. The hair and nail samples were then dried in an oven at 80°C and protected in polyethylene bags until analysis.
**Analytical methods**

For analysis of Hg, samples were digested as previously described (8). A sample (1 ml whole blood, 5 mL urine or 0.1-0.3 g hair or nails) was transferred into a 15 mL Pyrex tube with a screw cap. The stoppered tubes were kept overnight at room temperature, placed in an electric oven for about 5 hours at 80°C, then brought back to room temperature. After cooling, 5 mL of supersaturated solution of KMnO₄ were added. The tubes were stoppered, shaken and set again at 80-90°C for about 30 min. The digest was cooled to room temperature and a supersaturated solution of NH₂OH. HCl was added dropwise, to reduce the excess KMnO₄, until a colorless, clear digest was obtained. This was diluted to 25 mL with de-ionized water. Losses during digestion were checked by calculating recovery, which was close to 100%; within and between-run imprecision was <10%.

Hg was determined on a Perkin Elmer atomic absorption spectrometer model 2380 equipped with an MHS-10 hydride generation system (Perkin Elmer Corp., USA). The instrumental conditions were as indicated by the manufacturers. We used matrix-matched calibration curves (standard addition calibration technique) to overcome the matrix interferences. Data on the analytical performance for the determination of Hg in blood, urine, hair and nails was evaluated in terms of limit of detection (mean blank + 3SD), limit of quantification (mean blank + 10 SD) and sensitivity (the amount of the element yielding 0.0044 absorbance unit). The results are summarized in Table I. The accuracy of the method was determined by analysis of Hg in three different standard reference materials, namely LIES No. 13 human hair (Dr. Yoshinaga J, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305, Japan), CONTOX heavy metal blood control level III (Kaulson Laboratories Inc., USA), and urine control level II (Sigma Chemical Co., USA). The results, reported in Table II, were in close agreement with the certified values.

Calibration and quality controls were checked periodically during analysis. Creatinine was determined in serum and urine by a modified Jaffé method (9) on a Synchron CX₇ system (Beckman Instruments, Inc. USA). BUN was measured by an enzymatic conductivity rate method (10) on a Synchron CX₇ system. For enzyme activities in urine, samples were gel filtered on Sephadex-G 50 (fine) (Pharmacia, Sweden), as previously described (11). ALP was measured using p-nitrophenyl phosphate substrate in 2-amino-2-methyl-1-propanol buffer, pH 10.3 (12) on a Synchron CX₇ system. *-GT was measured using *-glutamyl-p-nitroaniline substrate (13) on a Synchron CX₇ system. NAG was measured by colorimetry at 37°C using 4-nitrophenyl-N-acetyl-β-D-glucosaminide, 10 mmol/L in citrate buffer, pH 4.15, as substrate (14). Albumin in urine was measured by an immunoturbidimetric method (15). Albuminuria kits were purchased from Bayer (NY, USA). β₂M in serum and urine was measured by the microparticle enzyme immunoassay on the IMx system (Abbott Laboratories, USA).

**TABLE I - ANALYTICAL PERFORMANCE FOR THE DETERMINATION OF Hg IN BLOOD, URINE, HAIR AND NAILS**

<table>
<thead>
<tr>
<th></th>
<th>Blood</th>
<th>Urine</th>
<th>Hair</th>
<th>Nails</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of detection</td>
<td>0.95</td>
<td>0.89</td>
<td>0.037</td>
<td>0.033</td>
</tr>
<tr>
<td>Limit of quantification</td>
<td>1.77</td>
<td>1.66</td>
<td>0.082</td>
<td>0.069</td>
</tr>
<tr>
<td>Sensitivity (mg)</td>
<td>0.42</td>
<td>0.39</td>
<td>0.48</td>
<td>0.24</td>
</tr>
</tbody>
</table>

* Limit of detection and limit of quantification were calculated as μg/L for blood and urine, and as μg/g for hair and nails (for 500-mg samples)

**TABLE II - ACCURACY OF THE DETERMINATION OF Hg**

<table>
<thead>
<tr>
<th>Value found</th>
<th>Certified value</th>
</tr>
</thead>
</table>


<table>
<thead>
<tr>
<th></th>
<th>Controls (52)</th>
<th>Dental amalgams (49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Hg (μg/L)</td>
<td>4.27 ± 1.84</td>
<td>13.7 ± 3.88**</td>
</tr>
<tr>
<td>Urine Hg (μg/ g Ucr)</td>
<td>0.48 ± 0.22</td>
<td>1.79 ± 0.53**</td>
</tr>
<tr>
<td>Hair Hg (μg/g)</td>
<td>0.23 ± 0.06</td>
<td>0.24 ± 0.05</td>
</tr>
<tr>
<td>Nails Hg (μg/g)</td>
<td>0.92 ± 0.27</td>
<td>0.87 ± 0.30</td>
</tr>
</tbody>
</table>

**p < 0.01**

**Indicators of exposure to Hg and the number of dental amalgams**

The correlation coefficients between indicators of exposure to Hg and the number of dental amalgam fillings show that blood and urine Hg were significantly correlated with the number of fillings (r= 0.412, p< 0.01; r= 0.469, p< 0.001, respectively). Hg levels in hair and nails were not correlated with the number of fillings (r=0.109, p>0.05; r=0.121, p>0.05, respectively).
Markers of kidney damage

Table IV shows the comparison between the Hg exposed group and the control group in relation to markers of kidney damage. Urinary excretion of NAG, *-GT and albumin were significantly higher in the Hg exposed group. The differences between the other markers of kidney damage in the two groups were insignificant.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Controls</th>
<th>Dental amalgams</th>
</tr>
</thead>
<tbody>
<tr>
<td>A- Markers of glomerular filtration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.8±0.07</td>
<td>0.88±0.09</td>
</tr>
<tr>
<td>Serum β2M (µg/L)</td>
<td>1.62±0.33</td>
<td>1.8±0.16</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>11.8±2.39</td>
<td>12.82±1.66</td>
</tr>
<tr>
<td>B- Markers of tubular damage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine β2M (µg/g U cr)</td>
<td>33.85±20.3</td>
<td>37.6±20.98</td>
</tr>
<tr>
<td>NAG (U/g U cr)</td>
<td>2.2±0.54</td>
<td>3.01±1.15***</td>
</tr>
<tr>
<td>ALP (U/g U cr)</td>
<td>5.94±1.82</td>
<td>5.33±1.66</td>
</tr>
<tr>
<td>*-GT (U/g U cr)</td>
<td>24±6.96</td>
<td>32.94±9.94*</td>
</tr>
<tr>
<td>C- Marker of glomerular damage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albuminuria (µg/g U cr)</td>
<td>3.24±2.21</td>
<td>6.66±1.74**</td>
</tr>
</tbody>
</table>

* p<0.05; ** p<0.01; ***p<0.001

Markers of kidney damage and the number of dental amalgams

Correlation coefficients between the markers of kidney damage and the number of dental amalgams show that urinary excretion of NAG and albumin were positively correlated with the number of filling (r=0.323, p<0.05; r=0.419, p<0.05, respectively).

Markers of kidney damage and indicators of exposure to Hg

Correlation coefficients between markers of kidney damage and indicators of exposure to Hg show that only urinary albumin was positively correlated with both blood and urinary Hg (r=0.354, p<0.05; r=0.361, p<0.05, respectively).

Effect of the life of the dental amalgam

No significant correlations were found between the time at which the dental amalgam had been introduced and the markers of Hg exposure or the markers of kidney damage.

Discussion
Dental amalgam releases significant amounts of Hg which is absorbed by the body. Available data is not sufficient to indicate whether this causes nephrotoxicity among the general population. Only few studies have assessed the nephrotoxic effects of Hg from dental amalgam but these studies are limited, due to the small population, lack of healthy controls, short surveillance period (17), poor interpretation of the results (18), or lack of assessment of exposure indices (19). The results are anyway contradictory.

In the present study, Hg was measured in blood, urine, hair and nails as indices of exposure to Hg. Blood Hg is a good marker of recent exposure, while urinary Hg reflects average long-term exposure (20). The levels of Hg in hair and nails reflect exposure during their growth (21). Elevated Hg levels in hair and nails have been found in dental personnel handling Hg amalgam (22).

Hg levels in blood and urine were significantly higher in the Hg exposed group than the control group, and were significantly correlated with the number of dental amalgams. These results, which agree with other reports (23), indicate that persons with dental restorations are at risk of Hg toxicity and measurements of Hg in blood and urine are good markers of such toxicity. In contrast, Hg levels in hair and nails were neither elevated in the Hg exposed group nor correlated with the number of dental amalgams, indicating that exposure to Hg from dental amalgams is not enough to accumulate in hair and nails. High levels of Hg in hair and nails are always suspect in individuals exposed externally to Hg (22).

Sandborg-Englund et al (17) investigated renal function in 10 healthy volunteers, with old dental amalgam fillings (> 10 years), before and after removal. One week before and 60 days after removal, the glomerular filtration rate was determined by $^{51}$Cr-EDTA clearance. Blood and urinary Hg, and urinary excretion of $\beta_2$M, NAG and albumin were determined 1 week before and 1, 2 and 60 days after removal of the amalgam. This study suggested there were no signs of renal toxicity in conjunction with Hg released from amalgam fillings. The study is limited by the small number of cases studied and a lack of healthy controls. Renal function was followed for only 60 days after removal of fillings; this may not be long enough to detect renal toxicity. Molin et al (24) found that albumin levels rose in human subjects 12 months after amalgam removal compared with 4 months before.

The markers of renal dysfunction used in the present study are considered the most sensitive available indices of nephrotoxicity. An effect on glomerular filtration would have been detected from the serum levels of creatinine, $\beta_2$M and BUN, tubular damage from increased urinary excretion of $\beta_2$M, NAG, ALP and $\ast$-GT (25,26), and increased glomerular permeability from albuminuria (27). Disturbances in urinary excretion of these markers might point to the development of chronic renal disease in a later phase (28). Our results show that none of the markers of glomerular filtration significantly differed between the exposed group and controls. Moreover, there were no significant correlations with indices of Hg exposure in the group with amalgam fillings. These measurements are therefore presumably of limited value in the early detection of nephrotoxicity from dental amalgam.

The tubular indicators NAG and $\ast$-GT were significantly elevated in the urine of the group with amalgam fillings. Urinary excretion of NAG positively correlated with the number of dental amalgams. Our findings of increased urinary NAG activity are comparable with the study by Eti et al (18). However, they made no adjustment of the urinary analytes for urinary creatinine, so the urinary concentration of NAG and Hg may be affected by urine flow rate (16) making interpretation of the results difficult. This may explain the absence of any significant correlation between urinary excretion of NAG and Hg in their study.

The increased urinary excretion of albumin in our Hg exposed group is an indication of disturbed glomerular permeability. These findings confirm the report by Anneroeth et al, (19) who removed one amalgam filling from each of ten subjects and compared urine samples before, and two or three days after removal. Unfortunately, urinary Hg was not reported so the degree of Hg exposure is not known. The significant correlations we found between albuminuria and blood and urine Hg and the number of dental amalgams support the usefulness of albuminuria in detecting nephrotoxicity from dental amalgams.

In a meta-analysis, Dodes (29) used an evidence-based approach in analyzing data both for and against the continued use of amalgam. He reviewed the articles and evaluated their relevance, research design and statistical analysis, as well as whether the conclusions follow from the data.
He suggested there were numerous logical and methodological errors in the anti-amalgam literature, and supported the safety of dental amalgam. Our study disagrees with this view, and implies that dental amalgam restoration is a source of Hg toxicity and may be a factor in various diseases.

In a well-performed experimental study (30), Hg vapor released from amalgam restoration in pregnant rats, and Hg concentrations in rat maternal and fetal tissues were studied. The placement of a single amalgam filling increased the levels of Hg 3 to 6 times in the maternal brain, liver, placenta and 20 times in the kidneys. Also, high levels of Hg in fetal organs such as the liver, kidneys and brain. The accumulation of high levels of Hg in both maternal and fetal kidneys supports the results of our study that Hg release from dental fillings may cause kidney damage.

In conclusion, dental amalgam could be a source of Hg nephrotoxicity. The determination of NAG, *-GT and albumin in urine of persons with amalgam fillings could be helpful for early detection of such toxicity. Other non-toxic materials should be used for dental fillings or at any rate the use of amalgam should be minimal, especially in persons with or at risk of renal diseases.

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References (when available, each reference has been linked to PubMed)


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