Measurement of Mercury Concentration in Saliva of Selected Sample of Children in relation to Amalgam Fillings

Ban Ali Salih B.D.S, M.Sc. (1)
Abdul Wahab T. Shakir B.D.S, M.Sc. (2)

ABSTRACT

Background: Dental amalgam fillings interact in a complex way with the environment in the oral cavity as they are subjected to chemical, biological, mechanical, and thermal forces. These forces change the restoration’s appearance and properties, causing metal ions, amalgam debris, non-metallic corrosion products, and mercury released into the oral cavity. The aims of this study were to measure the concentration of mercury in saliva of children in relation to the number of amalgam fillings before and after chewing and to measure the continuous release of mercury vapor from amalgam fillings in children and its relation to gender and age.

Materials and methods: The sample consists of 51 children between 8-10 years of age and they were divided into three groups according to the number of amalgam fillings they have in their teeth (1st group children didn’t have amalgam fillings in their teeth, 2nd group children had 1-2 amalgam fillings, 3rd group children had ≥ 3 amalgam fillings). Then saliva samples were collected from each child before and after chewing of sugar-free chewing gum in sterilized disposable cups.

Results: The results indicated that minute amount of mercury were continuously released from amalgam restorations and the release is accelerated after stimulation (chewing). Also the results demonstrated a positive correlation between the number of amalgam fillings and mercury concentration in saliva, mercury in saliva of children who didn’t have amalgam fillings in their teeth may come from other sources such as air, water and food. There was no relation between mercury concentration in saliva of children and the gender and age of children.

Key words: children, mercury, saliva, amalgam filling.

INTRODUCTION

Since the 1800s, dental amalgam has been the most commonly used restorative material in dentistry. The mercury content of dental amalgam (approximately 50%) has created an ebb and tide of controversy regarding its safety for patient and dental personnel (1,2).

Frykholm (1957) used radioactive mercury in dental amalgam to demonstrate that systemic mercury levels in patients returned to baseline measurements two weeks after placement of dental amalgam restorations. However, Frykholm’s study did not address long-term accumulation of mercury in brain tissue, more over Svare et al. 1981, demonstrated that minute amounts of mercury vapor are continuously released from dental amalgam restorations in humans and that the release is accelerated 15-folds in expired air immediately after mastication (3).

When γ₂ liberates tin or when γ₆ liberates silver and tin during the corrosion of amalgam, mercury must be released concurrently.

Some of this liberated mercury may react with unconsumed alloy particles, while some may be dissolved into the oral fluids (3,5,7).

Many national dental trade associations still claim that mercury fillings are safe. They base their conviction on the anecdotal facts that mercury fillings had been used for over 150 years, billions of fillings had been placed, and they do not see sickness or death from the mercury exposure. But, the diagnosis of mercury toxicity lies outside the purview of dentistry, falling more appropriately within the jurisdiction of medicine. From the medical perspective, dental amalgam fillings are significant mercury sources; having potential medical consequences (8). The best strategy to reduce the potential risk from dental filling materials is through the prevention of dental decay which is the best way to protect oral health and overall body health. If decay can be kept from starting, there will be no need for filling, and thus helping to protect the environment (9,10).

However, there is increasing use of non amalgam materials in children. Several of the new tooth-colored materials are suitable for use where cavities are small, as they often are in children. Also, it is sometimes possible to treat a child’s tooth with a preventive resin filling that stops existing decay and prevents further decay, rather than inserting an amalgam filling (10).

(1)Professor. Department of Pedodontics and Preventive Dentistry, College of Dentistry, University of Baghdad.
(2)M.Sc Student. Department of Pedodontics and Preventive Dentistry, College of Dentistry, University of Baghdad.
MATERIALS AND METHODS
Materials and equipment
The following material and equipment were used in this study:
1. Examination instruments and sample collection materials Fig. 1.
2. Refrigerator.
4. Flameless Atomic absorption spectrophotometer (Shimadzu-AA-670) with mercury vaporizer unit (MVU-1A).

Methods
1. Children selection:
   In this study children were selected according to the No. of amalgam filling present in their teeth and saliva sample were collected from 51 (21 male and 30 female) Primary school children aged from 8-10 years from different primary school in Baghdad city.
   In the test group the selected children should have at least 1 amalgam filling and more and the age of the fillings should be one year or more.

2. Children grouping:
The 51 child were divided according to:
A. The number of amalgam filling present in their teeth into 3 groups, each group consists of 17 child as follows:
   1. First group had no amalgam filling.
   2. Second group had 1-2 fillings.
   3. Third group had >=3 fillings.
B. The age of children into 3 groups each group consists of 17 child as follows:
   1. First group 8 years old.
   2. Second group 9 years old.
   3. Third group 10 years old.
C. The gender of children into 2 groups (30 female and 21 male).

3. Collection and preparation of saliva samples:
After selection of children, they should be fasting for at least 2 hours before saliva collection, each child get a cup and asked to collect 3-5 ml saliva in the first cup before chewing Fig. 2-A, then the children asked to chew sugar free chewing gum for 10 min Fig. 2-B, and then collect saliva again in the second cup.
Atomic absorption spectrophotometer:

The flameless AAS was used in this study (Shimadzu AA-670) with mercury vaporizer unit (MVU-1A) Fig.3, 4. Parameters for Hg detection are shown in Table (1).

Each saliva sample kept in a volumetric flask where the following reagent added to it

- HNO₃ or H₂SO₄ for oxidation.
- KMnO₄ to help the reduction of mercury.
- SnCl₂ for reduction of mercury ions to metallic mercury vapor as shown in the following equations:

\[
\begin{align*}
Hg + HNO_3 + KMnO_4 & \rightarrow Hg^{2+} \\
SnCl_2 & \leftrightarrow Sn^{2+} + Cl^- \\
Hg^{2+} + Sn^{2+} & \leftrightarrow Sn^{4+} + Hg^0
\end{align*}
\]

The reductant (SnCl₂) is dispensed into the sample solution where it reacts to liberate Cl⁻, and Hg²⁺ ions were reduced to the metallic state Hg⁰, and since mercury has an appreciable volatility even at ambient temperature metallic mercury vapor is driven out of the sample by SnCl₂ and transported to the quartz cell, where the resonance beam passed through the Hg vapor and its atomic absorption is measured Fig. 5.
Table 1: Instrumental parameters for flameless AAS for Hg detection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave length (nm)</td>
<td>196</td>
</tr>
<tr>
<td>Lamp current (mA)</td>
<td>16</td>
</tr>
<tr>
<td>Volume of sample (ml)</td>
<td>10</td>
</tr>
<tr>
<td>Sheathing gas</td>
<td>Argon</td>
</tr>
<tr>
<td>Dry time (min)</td>
<td>30</td>
</tr>
<tr>
<td>Ashing temp. (°c)</td>
<td>1000</td>
</tr>
<tr>
<td>Atomization temp.(°c)</td>
<td>2000</td>
</tr>
<tr>
<td>Atomization time(sec.)</td>
<td>3</td>
</tr>
</tbody>
</table>

RESULTS
Table (2) show that in the 1st group (No. of amalgam fillings 0) there were statistically no significant differences between the means of Hg conc. in saliva before and after stimulation, while in the 2nd group (No. of amalgam fillings 1-2) there were statistically Highly significant differences between the means of Hg conc. in saliva before and after stimulation.

Table 2: Difference in Hg conc. before and after stimulation

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Fillings</th>
<th>Sample Size</th>
<th>MEAN Before</th>
<th>± S.D. Before</th>
<th>MEAN After</th>
<th>± S.D. After</th>
<th>T- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>0</td>
<td>17</td>
<td>0.0010</td>
<td>0.0011</td>
<td>0.000092</td>
<td>0.000086</td>
<td>0.19 N.S.</td>
</tr>
<tr>
<td>2nd</td>
<td>1 - 2</td>
<td>17</td>
<td>0.0027</td>
<td>0.0047</td>
<td>0.00156</td>
<td>0.00239</td>
<td>2.89 **</td>
</tr>
<tr>
<td>3rd</td>
<td>≥ 3</td>
<td>17</td>
<td>0.0034</td>
<td>0.0159</td>
<td>0.00137</td>
<td>0.0253</td>
<td>2.03 *</td>
</tr>
</tbody>
</table>

A comparison for the means of Hg conc. in saliva among the three groups before stimulation Table (3) and after stimulation Table (4), show that there were statistically highly significant differences among the three groups before and after stimulation.

Table 3: Comparison among the three groups before stimulation

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>Sum of square</th>
<th>D.F</th>
<th>Mean square</th>
<th>F-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>0.0000509</td>
<td>2</td>
<td>0.0000255</td>
<td>17.60</td>
<td>***</td>
</tr>
<tr>
<td>Within groups</td>
<td>0.000694</td>
<td>48</td>
<td>0.000014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.001203</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Comparison among the three groups after stimulation

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>Sum of square</th>
<th>D.F</th>
<th>Mean square</th>
<th>F-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>0.02033</td>
<td>2</td>
<td>0.001017</td>
<td>4.705</td>
<td>*</td>
</tr>
<tr>
<td>Within groups</td>
<td>0.010366</td>
<td>48</td>
<td>0.000216</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.012399</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Comparison between each two groups before and after stimulation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before Stimulation</th>
<th>After Stimulation</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean differences</td>
<td>P-value</td>
<td>Mean differences</td>
</tr>
<tr>
<td>1st group</td>
<td>0.00167</td>
<td>0.000</td>
<td>0.00366</td>
</tr>
<tr>
<td>2nd group</td>
<td>0.00238</td>
<td>0.000</td>
<td>0.01484</td>
</tr>
<tr>
<td>3rd group</td>
<td>0.00070</td>
<td>0.000</td>
<td>0.0117</td>
</tr>
</tbody>
</table>
The results showed that there were statistically no significant differences between Hg conc. in saliva of males and females before and after stimulation Table (6).

Table 6: Difference in Hg conc. between Male and Female before and after stimulation

<table>
<thead>
<tr>
<th>Gender</th>
<th>Sample size</th>
<th>Mean</th>
<th>± S.D.</th>
<th>T-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>0.0028</td>
<td>0.0020</td>
<td>1.11</td>
<td>N.S.</td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
<td>0.0023</td>
<td>0.0011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>0.0043</td>
<td>0.0029</td>
<td>1.25</td>
<td>N.S.</td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
<td>0.0043</td>
<td>0.0028</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7: Comparison among the age groups before and after stimulation

<table>
<thead>
<tr>
<th>D.f.</th>
<th>Between groups</th>
<th>Within groups</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.0000032</td>
<td>0.0000024</td>
<td>1.34 N.S.</td>
</tr>
<tr>
<td>After</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>0.000251</td>
<td>0.000248</td>
<td>1.01 N.S.</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Saliva of some children could be attributed to the non dental sources. In this study saliva samples were used to determine the amount of Hg released from amalgam fillings before and after stimulation (chewing). Hg had been found dissolved in saliva in which amalgam was immersed or coated; particles of amalgam material were also released into oral cavity due to corrosion or abrasive stress (1). Salivary content of the mercury may be considered a good indicator of a possible leakage from amalgam dental fillings, while urine, feces, and blood have to be considered a reliable indicator for Hg intake, their content can be affected also by other sources i.e. air, water, food specially fish, and some medical preparations (13). This study concerned with the measurement of the total Hg in saliva because of bidirectional conversion between inorganic and organic Hg in humans (3).

Result of this study demonstrated a positive correlation between the No. of amalgam fillings and Hg conc. in saliva. The results indicated that minute amounts of Hg were continuously released from dental amalgam restorations due to abrasive effect of chewing gum. Many researchers demonstrated that Hg release is accelerated after mastication, breathing and other stimulations such as temperature changes, pH and access Hg in amalgam fillings which may influence the evaporation (3,12).

In the 1st group there were no significant differences between Hg conc. before stimulation and after stimulation, Table (2); because they have no amalgam fillings and the little increased Hg conc. in saliva of exposure to Hg such as eating fish, while the 2nd group show a statistically highly significant difference between Hg conc. in saliva before and after stimulation and the 3rd group show a statistically significant difference between Hg conc. in saliva before and after stimulation and this result is due to the presence of amalgam fillings which are continuously release Hg and their release increased after stimulation because of the increase in corrosion effect of chewing on the surface of amalgam.

Table (3) shows that there was statistically highly significant difference in Hg conc. in saliva among the three groups before stimulation, and Table (4) shows that there was statistically significant difference in Hg conc. in saliva among the three groups after stimulation. To determine where the significance does occur the L.S.D test, Table (5) show that there was statistically highly significant difference between 1st and 2nd groups and between 1st and 3rd groups before and after stimulation and this is due to that the 1st group had no amalgam fillings while the 2nd and 3rd groups had amalgam fillings ranged from 1-7 fillings which result in more Hg released from them than from the 1st group.

The 3rd group had more amalgam fillings than the 2nd group resulted in significant difference between them and this indicate that the release of Hg from amalgam fillings increased with the increase in the No. of fillings. The set amalgam is a dynamic material that undergoes many micro structural changes; these changes are related to the nature of amalgam matrix and the corrosive environment of the mouth (13).
The Hg matrix phase is a major phase in any set amalgam and therefore represents a potential for continuous and some times prolonged Hg release, which may increased by chewing or exposure to heat and certain acids.4,14,15.

The reduction in the conc. of dissolved Hg in saliva after insertion of new filling could be explained by the fact that corrosion in many metal environment systems follows the same dissolution rate decreasing with time. The phenomenon usually responsible for this behavior is the film formation of the corrosive products on the surface of the metal that limited the dissolution of elements from the surface into the environment (13,16,17).

The result of this study could be explain that the stimulation or chewing may degenerate the protective surface film of corrosion products and exposed the silver-Hg matrix, which is the main source of Hg. The unprotected surface is further oxidized leading to chemical dissolution of the Hg phase and diffusion of available Hg to the outer surface and then into saliva (6,18). Results from Table (6) shows that there was statistically no significant difference in Hg conc. in saliva before and after stimulation between boys and girls. This could be explained that most of the Hg in saliva come from amalgam filling and its level does not affected by variables exist between boys and girls (ex. Hormones), but there may be difference in Hg conc. in body organs and it’s toxic effect.

The studied sample divided into three age groups (8 years, 9 years and 10 years) and the results demonstrated that there was statistically no significant difference in Hg conc. in saliva before and after stimulation among the three age groups Table (7) this indicate that the age of children doesn’t affect the Hg conc. in saliva but only the No. of fillings present.

The result in this study showed (means of Hg conc. before stimulation range from 0.0010 to 0.004 ppm and means of Hg conc. after stimulation range from 0.0011 to 0.015 ppm) and these values can be considered to be negligible, but in the age of 8-10 years old apparently the lower No. of fillings is may be sufficient to exceed the (PTWI) frequently, because of the lower body weight (18). The findings of this study and many other studies which concern about Hg release from amalgam fillings in the teeth of children and it’s possible toxic effect on the general health of the children suggest that it will be better to use alternative filling materials that doesn’t contain Hg such as composite, glassionomer cement and resin modified glassionomer cement (19-21). It’s clearly obvious that the best way to protect children oral and over all body health from the possible toxic effect of mercury from amalgam fillings and the effect of other materials used to fill the teeth is by preventing decay so that there will be no need for fillings and thus helping to protect the environment.

Prevention of dental caries could be achieved by the application of preventive measures which include:-

1. Reduction in the frequency and amount of intake of so-called ‘added sugars’ or free sugars.
2. Systemic fluoridation (ex: water fluoridation).
3. Professionally applied topical fluoride.
5. School programs for preventing caries which will provide a group activity not only on caries prevention but also on effective oral health.
6. The majority of carious cavities now occur in pits and fissures, thus the combination of fluoride therapy and fissure sealing is very attractive.

REFERENCES
1. Richardson GM. Assessment of mercury exposure and risks from dental amalgam, final report. Medical Devices Bureau, EHD, Canada;1995
12. Hedegard L. Amalgam related illness FAQ 2.9.1:1-44.1998. Strindbergsgatan 44, S-115 31 Stockholm, SWEDEN, E-mail: leif@algonet.se