Effect of Dental Amalgam Silver Filling on Salivary Amylase Enzyme

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Abstract
The largest source of mercury located in dental amalgam fillings people. In this study, mercury level in saliva of amalgam and non-amalgam human were compared as well as the level of α amylase has been detected.

Materials and methods:
The concentration of mercury, silver, and activity of amylase in saliva were measured in 55 adult study subjects. The mercury contents were determined by cold–vapor atomic absorption spectrometry.

Results:
The concentration of mercury in the serum was significantly higher in subjects with dental amalgam fillings (n = 25) compared to the non-amalgam group (n = 30) (p<0.01). The activity of α-amylase has significantly decreased in amalgam group.

Conclusion:
Higher amount of amalgam fillings synchronized with a lower activity of α-amylase.

Keywords:-Mercury, silver, α-amylase

Introduction
Dental amalgam is considered as amixture of 50% liquid mercury that is unstable and referred to the main component of amalgam. Mercury is the major toxic substance, as well as, silver (35%), copper (6%), tin (9%) and trace amount of zinc are also present in dental amalgam(1, 2). Since mercury continuously vaporizes at room temperature from the amalgam mixture, high levels of mercury in the oral air and saliva will be produced (1). This will lead to pump mercury in galvanic currents and other toxic metals into the gums and oral mucosa, from which it is transferred and carried throughout the body through the blood and nerves. Therefore, dental amalgam filling people have high mercury level (1,3). Mercury can be methylated by bacteria or yeasts and other microorganisms, which perhaps is the reason that amalgam fillings is the largest source of methyl mercury in oral cavity (4).The mercury in people who have the amalgam fillings will targets and accumulates in the most &large body organs like the liver, brain, heart, kidneys and stomach, which receive a large amount of blood that causing block or damage the metabolism or the hormonal processes in these organs even at low concentration of exposure (5).

Mercury is neurotoxic, cytotoxic, immunotoxic, inflammatory, endocrine disrupting and reproductive toxin substance. It causes many abnormal functions such as hormonal, cardiovascular, oral, immune and reproductive disorders (1). It also associates with some neurodegenerative diseases such as Alzheimer’s disease and Parkinson’s disease. The allowable intake of mercury is lower than or equal to 0.1 microgram/day to each kilogram of the weight of the body (6). People with several amalgam fillings have higher mercury level in saliva and feces about 10 times than people without amalgam. However, replacing the amalgam found to cause 90% decline of mercury level in saliva and feces and about 75% in urine(7). in the present study, the real effect of silver amalgam fillings on the activity of salivary α-amylase enzyme has been investigated.

Materials and methods
Salivary collection:
Preparation and handling of saliva from amalgam fillings patients having amalgam fillings was done according to standard procedure(8). Saliva sample collected from 25 patients (with 1-2 fillings) and from 30 persons without filling who served as control. Saliva collected in non-citric acid plane tubes after gum chewing for 10-15min., then stored in -4°C prior to assay to precipitate the mucins. Next day thaw completely, good vortexes, and centrifuged at 1500 × g (3000 rpm) for 15 minutes. The analysis carried out on a clear sample after separation.

Estimation of serum mercury concentration
Preparation of reagents and samples of this study was performed according to standard procedure(9). The measurement of serum Hg^{2+} concentrations in Mg/L was carried out with a newly developed cold vapor flow-injection mercury system (FIMS 100) with cold-vapor atomic absorption from the shimadzue 6800.

Statistical analysis:
Results are expressed as the mean±SD, using non-paired student t-test. Differences were considered significant if P<0.05 by SPSS version 16 software.
**Results:**

**Table 1:** Enzymes activities of different groups of patients.

<table>
<thead>
<tr>
<th>Enzyme tests</th>
<th>Amalgam Fillings groups</th>
<th>Without amalgam fillings group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±s</td>
<td>No</td>
</tr>
<tr>
<td>α-Amylase</td>
<td>24.87±15.43</td>
<td>25</td>
</tr>
</tbody>
</table>

**Table 2:** Hg^{2+} conc. in ug/l, and Ag^{+1} conc. in ppm in all groups of students.

<table>
<thead>
<tr>
<th></th>
<th>Amalgam Fillings group</th>
<th>Without fillings group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±s</td>
<td>No</td>
</tr>
<tr>
<td>Hg^{2+} Conc.</td>
<td>11.63±3.69*</td>
<td>25</td>
</tr>
<tr>
<td>Ag^{+1} ppm Conc.</td>
<td>16.3<em>10^{-3}±5.3</em>10^{-3}</td>
<td>25</td>
</tr>
</tbody>
</table>

* p<0.01

Table (1) lists the activity of the amylase enzyme in the tested and controlled groups, where enzyme activities significantly decreased in the amalgam fillings group compared with non-amalgam fillings (p<0.05).

Table (2) compares the levels of serum Hg^{2+} and Ag^{+1} in the target study groups, where there is an elevation in the Hg^{2+} and Ag^{+1} concentrations in amalgam group than that in control group (p<0.01 and p>0.05 respectively).

**Discussion**

The world health organizations have reported that the dose of mercury in dental fillings is safe (10). Previous study found that there is no evidence that mercury has specific effects on the dental patients, although they found that a number of people have true allergies to the components of amalgam(11). Nevertheless, the results of the current study suggest that there is a serious impact on the level of salivary α-amylase.

Fortunately, few countries in Europe have worked in accordance with precautionary principle concerned to dental amalgam.

Hujoel and his colleagues quote, Norway, Germany, Austria, Finland, the United Kingdom, and Switzerland as countries have advised dentists not to install, or must remove amalgam fillings in pregnant women (12). In addition, Bose- o’ Reilly quotes Germany as one of many countries that amalgam fillings are no longer permitted (13).

The activity of salivary amylase was decreased in the amalgam filling group when compared with non-amalgam group, Table 1. It may be related to some factors such as heavy metals and some ions that are present in the amalgam fillings like mercury, silver, copper, tin, etc, which alter the activity of the enzymes and these substances are work as enzyme inhibitors. These inhibitors combine with the enzyme and then affect the binding of the substrate with the active site of the enzyme molecule(14). Some common bacteria in mouth converts the mercury of the amalgam to methyl mercury which is 100 times more toxic than the elemental mercury(15). Previous studies reported that mercury accumulates in human bodies, and inhibits the activity of many enzymes by denaturation of their protein molecules. One of these enzymes is salivary amylase which is inhibited or prevented from working normally because the shape of the enzyme and active site will be changed. The current result consists with Usha and his colleagues who found that the pre-incubation of amylase with mercury ions and aluminum ions cause complete loss in the enzyme activity(16). This effect might occur due to the presence of cysteine residue in the active site of the enzyme molecule,which has been believed to have an impact on the enzyme activity(17). The thiol of a cysteine residue occasionally coordinates with metal ions and impairs the chemical enzymatic reactions(18). Accordingly, low levels of amylase in patients with high levels of mercury and silver(Table 1, and 2) could be attributed to the interaction between the thiol group of cysteine and metal ions, which affected the enzyme activity negatively.

**Conclusion**

An increase in the number of amalgam fillings might cause the significant elevation in mercury levels in sera of amalgam patients, which affected the activity of α-amylase enzyme. The activity has significantly decreased if compare with non-amalgam subjects.

**References**

11- Fung YK, Molvar MP. Toxicity of mercury from dental environment and from amalgam restorations. Clinical Toxicology 1992;30:49-61