

The determination of mercury levels in the blood and hair of rabbits with amalgam fillings

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Abstract

Aim: This study was conducted to investigate the level of mercury transmitted into the blood and hair of rabbits based on the number of amalgam-filled teeth present in the mouth.

Methodology: A total of 18 male rabbits from New Zealand were used in the study. The rabbits were separated into 3 groups: a control group, a dichotomous amalgam-filled group, and a quadruple amalgam-filled group. Blood samples were collected from the animals before fillings and after 24 hours, one week and one month after the fillings were applied. Hair samples were taken before the fillings were applied and at one month after. Blood and hair samples were taken from the rabbits, and the transfer of mercury was measured with the hydride system in the absorption spectrophotometer.

Results: The data obtained from the study were evaluated using the SPSS 21 package program. As compared with the control group at the 24th hour, no statistical difference was seen between the dichotomous and quadruple amalgam filling groups in terms of blood mercury level ($P > 0.05$). However, the levels of blood mercury observed one week and one month later were significantly higher than in the control group observed at the same times ($p < 0.05$). Over each time period measured, the level of blood mercury was found to be similar between dichotomous and quadruple amalgam filling groups ($p > 0.05$).

Conclusion: Mercury could not be detected in hair samples. As a result, it was revealed that amalgam fillings affect the blood mercury level.

Keywords: Mercury, amalgam, rabbit, blood, hair.

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Introduction

Mercury, indicated by the symbol Hg, is a naturally occurring metal. Due to its physical and chemical properties; it is liquid at room temperature. Mercury is

found in three different natural forms: elemental, inorganic and organic. However, only the elemental form is used in dentistry. Amalgam is an alloy filling material used in dentistry that contains approximately 50% mercury; the remaining material is mostly silver (Ag) and trace amounts of copper (Cu), tin (Sn), and

zinc (Zn) (1-3). Although alternative materials are increasingly used for posterior fillings, amalgam is still one of the popular filling materials (4–7).

Amalgam fillings consistently release mercury vapor at low levels. Release rate depends to fill size, chewing habits, food texture, tooth brushing methods, as well as the surface area, composition and age of the amalgam. Correlations have been shown between the number of amalgam fillings a subject has and the concentration of excess mercury found in the urine. Studies show that subjects with amalgam fillings were observed to have more mercury in their saliva and stool. Further, the number of amalgam surfaces was found to be related to the mercury content of autopsied brain and kidney tissue (3, 8).

The presence of mercury in the body has been shown to relate to several immune diseases, primarily allergies, arthritis, eczema, multiple sclerosis, autoimmune thyroiditis and rheumatoid arthritis (9-12). Moreover, mercury in the brain can lead to neurological problems by decreasing the levels of dopamine, serotonin, norepinephrine and acetylcholine (13). Further, in a study of 94 children diagnosed with autism, the level of mercury found in hair samples was 3.63 ppm, while 0.47 ppm were observed in the control group (14). In addition, there are increased incidences of microalbuminuria in children with amalgam fillings (15). It has also been reported that Alzheimer's disease could be related to mercury expressed from amalgam fillings (16).

The aim of this in vivo study was to investigate how the presence and number of amalgam fillings affected the passage of mercury into the blood and hair.

Materials and Methods

The animals used in this study were obtained from Firat University Experimental Research Unit. A total of 18 New Zealand rabbits were used, aged 6-8 weeks and weighing 1660-2150 g each. Approval for the study was granted by the Animal Experiments Ethics Committee of Firat University (2013/112), and the study was conducted in accordance with ethical regulations.

Study Groups

The study was conducted on a total of 18 rabbits with a mean age of 6-8 weeks. The rabbits were randomly separated into the following 3 groups of 6 animals:

Group 1 (control group): No procedure was applied to this group.

Group 2: Two amalgam fillings were applied to the lower third of the buccal region of the 2 incisor teeth of the upper jaw.

Group 3: Four amalgam fillings were applied to the lower third of the buccal region of the 2 incisor teeth of the upper jaw and the 2 incisor teeth of the lower jaw.

Throughout the study, the animals were kept in standard conditions and fed standard food. Anesthesia was provided using Rompun 0.5mg/kg IM and Ketamine 0.8 mg/kg IM. Capsule amalgam was used in the study (Cavex, Holland). Single dose capsules were prepared by mixing in an amalgamator. In the teeth to which the procedure was to be applied, class V cavities were opened with an aerator with a mesio-distal width of 1mm, an occluso-gingival width of 1mm and a depth of 1mm, in accordance with traditional principles. The prepared amalgam was placed in the cavity and shaped with a spatula.

Sample Collection and Preparation

Blood and hair samples were taken from all groups 24 hours before the operation. Following the application of the fillings, blood samples were taken again at 24 hours, 1 week and 1 month. Hair samples were taken again at 1 month after the procedure. The blood was withdrawn with a syringe into heparin tubes. The samples were centrifuged at 3500 rpm for 10 minutes, and then the separated serum was withdrawn with a pipette. The obtained samples were placed in glass tubes, coded, and stored at -80°C until assay.

The hair samples were collected by shaving the animals at 24 hours before and 1 month after the filling procedure. The hairs were kept for 24 hours in a homogenous mixture of 2ml sulphuric acid (H₂SO₄) and 1ml hydrogen peroxide (H₂O₂). The mixture was passed through filter paper into glass tubes, which were then coded and stored at -80°C until assay.

The level of mercury in the blood samples taken from the subjects was determined by an absorption spectrophotometer, as described by Batur et al. Solvent was added to the serum samples, and they were left for 8 hours. Next, hydroxyl amine hydrochloride solvent was added to each sample; deionized water was added to a level of 10 ml; and the mercury level was determined.

At the end of the study, the rabbits were not sacrificed and continued to live their natural lives.

Statistical Analysis

Statistical analyses of the data obtained in the study were made using SPSS 21 software. Conformity of the data to normal distribution was assessed with the Shapiro-Wilk test. In the group comparisons in which the data did not conform to normal distribution, the Mann Whitney U-test was used. Results were stated as mean±standard deviation values. A value of $p < 0.05$ was accepted as statistically significant.

Results

When the mercury values of the subjects were examined before and after the procedure, no mercury was observed in the blood of any of the animals. The measurements of the control group at the beginning and the end of the study were determined to be zero.

These zero values, as well as the preoperative zero values of the study group, are of vital importance for the study because they show that the mercury could not have been acquired through any route other than the teeth and that no measurement error was made from any external effect (Table 1).

Table 1. The effects of the different applications of fillings on the levels of mercury in the blood (mean \pm standard deviation)

Filling (ppm)	Measurement at 24 hours after the application of amalgam filling (ppm)	Measurement at 1 week after the application of amalgam filling (ppm)	Measurement at 1 month after the application of amalgam filling (ppm)
Control group	0.000 \pm 0.000	0.000 \pm 0.000 ^b	0.000 \pm 0.000 ^b
2-filling group	0.729 \pm 0.461	15.558 \pm 2.244 ^a	3.780 \pm 0.993 ^a
4-filling group	3.089 \pm 2.026	17.680 \pm 1.952 ^a	3.103 \pm 0.478 ^a
p value: statistical significance	0.155	0.003	0.003

*Data are stated as mean \pm standard deviation.

p < 0.005 indicates statistical significance

^a, ^b : The difference between the values shown with different letters in the same column is significant.

Discussion

Amalgam is a filling material which has been used for many years in dental treatments. Although current use is relatively lower than in the past, amalgam is still being used at a substantial rate. The advantages of amalgam are that it can remain in the mouth for many years, is a resistant material, can tolerate a humid environment and can be used simply and inexpensively in clinical settings. However, since amalgam includes mercury—a toxic metal—there has been a debate about whether amalgam fillings are harmful to human health. The current general opinion, which has emerged from the findings of several studies, is that amalgam does not constitute a serious problem to human health (1,3).

Studies conducted on the effects of amalgam fillings on human health have sought to measure the passage and accumulation of mercury from the filling material into the body, especially in the vital organs. Using various methods, these studies have attempted to determine the level of mercury which could pass into the body from the amalgam filling (17-19). However, there is a need for more information about the methods of these studies.

A previous study states that as the number of amalgam fillings increases, the level of mercury in the brain, blood and urine increases (20). This study investigated at specific time periods whether or not mercury passed into the blood and hair of rabbits applied with 2 and 4 amalgam fillings, respectively, and determined that a significant amount of mercury passed into the blood of these rabbits (p < 0.05, Table 1). It was further observed that the passage of mercury into the blood was greatest in the first week, and due to rapid accumulation in the tissues, a reduction in this amount was seen after 1 month. Significantly, the study showed that as the number of teeth with amalgam fillings increased, an increase in the passage of mercury to the blood was also determined. Since laboratory animals were used and provided with a standard diet throughout the experiment, mercury was not detected in the blood and hair samples of the rabbits in the control group.

In rat study found that the level of mercury concentration increases in the brain, liver and kidney as the number of amalgam fillings increases (21). In our study determined that there was an increase in the mercury level in the blood up to 1 week after the amalgam filling application.

An additional study found that the mercury levels in the brains of subjects with 12 or more amalgam

fillings were more than 10 times greater than in those with fewer than 3 fillings (22). Likewise, the current study determined that a greater level of mercury was detected in the blood of the rabbits with 4 amalgam fillings compared to those with 2 fillings. Thus, this study supports prior findings that the increase in the number of amalgam fillings correlates with an increase in the passage of mercury into the body.

In another study, blood, urine and hair samples were collected on the 1st, 3rd and 12th days after the application of amalgam fillings, and the mercury concentrations from the samples of these individuals were reported to be related to age, weight, fish consumption and number of amalgam fillings. The mercury levels in the blood and urine samples of the study group were measured as 6-8-fold higher than those of the control group, and the mercury level in the hair samples was determined to be low (23). Likewise, the current study found that mercury was not detected in the subjects' hair samples, which may be due to the longer amount of time it takes for mercury to pass into keratinized tissue.

Conclusions

Despite studies that have shown that amalgam does not negatively affect human health, the findings obtained in this study (similar to those of the above-mentioned studies) demonstrated that there is in fact a transfer of the mercury in amalgam fillings into the body. There are many studies related to the toxic effects of mercury, and although there are opinions that mercury causes damage to the nervous system in particular, there has not been sufficient research on this subject with an extensive population and a control group.

Nevertheless, these findings suggest that to reduce the harmful effects of mercury to a minimal level, amalgam should be used with caution, especially on children, those who are pregnant and those with chronic disorders. The risk-benefit balance of alternatives to amalgam should be evaluated, and there is a need for additional, more detailed studies related to amalgam fillings.

In summation, the results of this study indicate the following conclusions:

- There is a transfer of mercury from amalgam fillings to the blood.
- The increase in the number of amalgam fillings has led to an increase in the mercury passing through the body.
- The level of mercury in the blood decreased one month after the application of the filling.
- The greatest increase in the mercury level in the blood was determined to be in the first week after the application of the filling.
- Mercury was not determined in the hair samples.

Ethical Approval: Ethics committee approval was received for this study from Animal Experiments Ethics Committee of Firat University (2013/112).

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