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# Mercury and silver in saliva from subjects with symptoms self-related to amalgam fillings

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Abstract The amount of mercury released into saliva from dental amalgam fillings is currently being debated. Mercury enters saliva as vapor, ions and particles of amalgam. The aim of the present study was to determine mercury and silver concentrations in saliva of persons with amalgam fillings. Moreover, it was the aim to investigate whether amalgam particles were present in samples of stimulated saliva in control subjects. In that case, we also wanted to determine the influence of these particles on the mercury concentrations found. Fifty-three patients with a wide range of complaints selfrelated to their amalgam fillings were examined by the Dental Biomaterials Adverse Reaction Unit of Norway. Among other tests, stimulated saliva was collected from each patient and analyzed for mercury and silver. Mercury and silver correlated with the amount of amalgam present. There was a strong correlation between mercury and silver concentrations. Amalgam particles were found by energy dispersive X-ray analysis. It appears that a considerable part of the mercury and silver were present as amalgam particles. The present study shows that amalgam particles in saliva have to be controlled for when analyzing mercury in saliva from subjects with amalgam fillings.

Key words Mercury  $\cdot$  Silver  $\cdot$  Adverse reactions  $\cdot$  Dental materials  $\cdot$  Saliva

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## Introduction

During the past few decades several reviews have been presented concerning the possible influence of mercury from the amalgam fillings on general health [8, 11, 12]. Mercury from dental amalgam restorations enters saliva as vapor, ions and in particles of amalgam [3, 5]. The amount of mercury released depends on the amount of exposed amalgam and several other factors [7, 8, 10].

Most studies have focused on mercury in blood and urine [1, 2]. Some investigators have presented results for intraoral mercury vapor [3] and mercury in feces [4, 6].

In a debated study from Tübingen, Germany, published only on the Internet [9], mercury released from amalgam restorations into saliva was measured in about 20,000 persons. The authors claim that there is a correlation between mercury concentration in saliva and subjective symptoms.

The aim of the present study was to measure the content of mercury and silver in saliva. We wanted to determine whether amalgam particles were present in samples of stimulated whole saliva from control subjects and, if this was the case, also determine whether such particles might influence the mercury concentrations found.

### **Materials and methods**

During 1997, 53 patients with self-related complaints to dental amalgam were referred to the Dental Biomaterials Adverse Reaction Unit at the University of Bergen, Norway, for examination regarding amalgam-related illness. There were 39 women and 14 men with a median age of 46.5 years. The patients had from 0 to 66 amalgam surfaces (median 21 surfaces). Three patients were lacking mercury values.

The patients chewed paraffin wax for 5 min and the stimulated saliva (0.2–1.7 ml) was collected continuously into test-tubes. Each sample was mixed with 2 ml

nitric acid and 1 ml of 30%  $H_2O_2$  in PTFE tubes, which were then closed. The contents were digested in a microwave oven (Milestone 1200 MEGA, Sorisole, Italy).

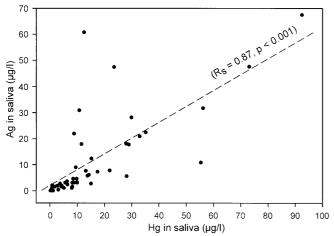
Mercury was measured by cold-vapor atomic absorption spectrophotometry (Perkin Elmer FIMS 100, Germany). The detection limit, defined as three times the standard deviation in one blank solution measured 10 times, was 0.1  $\mu$ g/l. Silver was measured by graphite furnace atomic absorption spectrophotometry, with Zeeman background correction (Perkin Elmer 5000). The detection limit for silver analysis was 0.5  $\mu$ g/l

Stimulated whole saliva from six control subjects with amalgam fillings, but without complaints against dental amalgam, was analyzed for mercury before and after centrifugation at  $3400 \times g$  for 30 min to facilitate detection of potential amalgam particles by electron microscopy. The sediments of the control samples were enzymatically digested by a mixture of the enzymes lysozyme and pronase (50 µg/ml in 50 mM tris buffer, pH 8). Five milliliters of this solution was added to the sediments and incubated for 30 min at 37°C. The samples were shaken continuously.

The partially digested samples were filtered (Millipore HA, pore size 0.45 µm, Bedford, Mass., USA), carbon coated, and subjected to back-scattered electron microscopy (JEOL SEM 6400, Tokyo, Japan) and energy dispersive X-ray analysis (Series 2 X-Ray Analyzer Tracor Northern, Middelton, Wisc., USA). The statistical analyses were performed by a statistical software package (SPSS 6, Chicago, Ill., USA).

#### **Results**

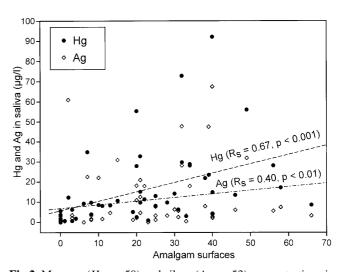
We found large variations in the amounts of mercury and silver in the saliva samples (Table 1). The correlation between mercury and silver concentrations was statistically significant (Fig. 1). Mercury and silver concentrations correlated with the amount of amalgam present (Fig. 2).



**Fig 1** Mercury (*Hg*) and silver (*Ag*) concentrations in stimulated saliva from patients with self-related symptoms associated with amalgam fillings (n=49 data pairs )

**Table 1** Concentrations of mercury and silver measured in stimulated whole saliva from patients with self-related symptoms to amalgam fillings. All measurements in  $\mu g/l$ . (*n* number of patients, *Min* minimum, *Med* median, *Max* maximum, *Q1* lower quartile, *Q3* upper quartile)

Element	n	Min	Q1	Med	Q3	Max
Mercury Silver	50 53	<0.1 <0.5	3.9 1.6	9.5 3.2	22.2 15.1	92.2 67.6



**Fig 2** Mercury (Hg, n=50) and silver (Ag, n=53) concentrations in stimulated saliva from patients with self-related symptoms associated with amalgam fillings related to number of amalgam surfaces

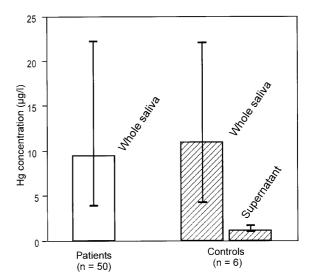
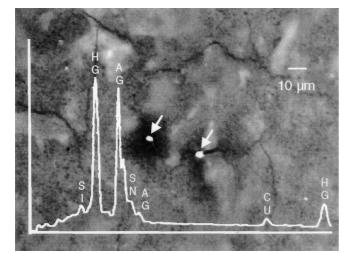


Fig 3 Median mercury ( $H_g$ ) concentrations in stimulated whole saliva from patients and control subjects. The whiskers indicate upper ( $Q_3$ ) and lower ( $Q_1$ ) quartiles



**Fig 4** Back-scattered electron microscopic picture of amalgam particles (*arrows*) in saliva from controls. Results from energy-dispersive X-ray analysis overlaid

#### Discussion

The large inter-individual variations in concentrations as well as the high correlation between mercury and silver indicate the presence of amalgam particles in the saliva samples. This assumption is furthermore supported by the reduction in mercury concentrations by a factor of ten in the centrifuged control samples. Electron microscopy of the sediments deposited on the filter confirmed that there were amalgam particles present in the saliva samples. The centrifugation had probably transferred the relatively heavy particles away from the supernatant.

There are different methods for determining the level of mercury derived from amalgam fillings [7]. We have demonstrated that amalgam particles originating from amalgam fillings are present in stimulated saliva.

In the Tübingen study, which emphasized the release of mercury into saliva [9], the analyses used did not discriminate between mercury dissolved in saliva and mercury bound in amalgam particles. The authors maintained that potential particles pass the analytical system without affecting the results. This assumption is not documented in their presentation. Our results are based on digested samples which include the metals present in the particles. Taking into consideration the relatively high concentration that could result from the presence of particles, it is of major importance to control the level of these.

#### Conclusions

The present study shows that the presence of amalgam particles in saliva have to be controlled when analyzing mercury in saliva from subjects with amalgam fillings.

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