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RELEASE OF MERCURY FROM DENTAL AMALGAM AND ITS INFLUENCE ON SALIVARY ANTIOXIDANT ACTIVITY

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ABSTRACT

Dental amalgam fillings are known to release significant levels of mercury (Hg) in saliva which could represent a continuous source of oxidative damage to tissues. The present investigation was aimed at verifying this hypothesis by determining a possible correlation between salivary Hg levels and salivary total antioxidant activity (TAA), used as an index of oxidative stress. Samples of saliva from 34 healthy donors were analyzed for Hg content, through vapor atomic absorption spectrometry, and for TAA, by determining the ferric reducing ability ('FRAP' method). A significant correlation between Hg and the number of amalgam restorations or total amalgam surface was evident in both the male and female subjects. A significant negative correlation between TAA and Hg levels or number of amalgam restorations or amalgam surface was evident in females, indicating that small increases in salivary Hg were sufficient to produce a decrease in salivary TAA. On the other hand, no significant correlation was found in the males. The present study provides, for the first time, evidence of a pro-oxidant role of the amalgam Hg chronically released in saliva.

RESUME

Les obturations en amalgame libèrent continuellement des quantités significatives de Hg dans la salive et peuvent donc créer un stress oxydant dans les tissus de la cavité orale. Le but de cette recherche est de vérifier cette hypothèse. Pour cela, nous avons cherché sur 34 patients sains un rapport entre les niveaux salivaires de Hg (déterminés au moyen du spectrophotomètre à absorption atomique) et l'Activité Anti-oxydante Totale (AAT) de la salive (déterminée par FRAP). Nous avons observé une corrélation significative, aussi bien chez les hommes que chez les femmes, entre le numéro des obturations et le Hg salivaire et entre la superficie des obturations et le Hg salivaire. Nous avons relevé une corrélation négative significative entre Hg et AAT chez les femmes, mais pas chez les hommes. L'éventuel rôle pro-oxydant du mercure provenant de l'amalgame est donc remis en discussion.

INTRODUCTION

Mercury (Hg) is the major component (50% by weight) of tooth filling materials and it has been utilized for years because it is stable, inexpensive, easy to manipulate and position, with relatively low cost. Elemental mercury vapor Hg° released from dental amalgam is inhaled (Vimy et al. 1990), absorbed by lung, gastrointestinal and jaw tissues (Hahn et al. 1989), retained mostly in the kidney, liver (Hahn et al.1989; Hahn et al. 1990), and central nervous system, as determined by human autopsy (Nylander et al. 1987; Weiner et al. 1993), and increases in expired air (Svare et al. 1981; Vimy et al. 1985), saliva (Ahmad et al. 1990; Berglund, 1992), urine (Begerow et al. 1994; Berglund, 1990), blood (Mackert et al. 1997; Sandborgh-Englund et al. 1998) and feces (Bjorkman et al. 1997).

The release of mercury from the amalgam surface in the oral cavity may be ascribed to the effect of

biological corrosion due to bacteria, pH of saliva, chewing, brushing, temperature (Brune and Evje, 1985; Berdouses et al. 1995; Marek 1997; Furhof et al. 1998), electrochemical corrosion (Olsson et al. 1994) and different types of amalgam alloys (Berglund, 1993). The toxicological consequences of long-term Hg exposure from old amalgam (Ekstrand et al. 1986; Siblerud et al. 1994), dental profession (Uzzell and Oler, 1986; Langworth et al. 1997) or industrial work (Angotzi et al. 1981) are currently a matter of debate in several countries, and in particular the potential pro-oxidant effects of Hg on tissues and cells. In fact, a number of studies demonstrate the ability of Hg, like other metal ions, to interact with soluble and protein bound -SH groups, resulting in the production of reactive oxygen species (ROS) like superoxide anion, hydrogen peroxide, hydroxyl-radical, capable to produce oxidative injury to tissues through diverse mechanisms (e.g. lipid peroxidation, DNA damage, alterations of calcium homeostasis) (Stohs and Bagchi, 1995).

Living organisms are normally protected against oxidative stress by a number of non-enzymatic compounds endowed with antioxidant activity, such as albumin, GSH, ascorbic acid, a-tocopherol, b-carotene, uric acid, bilirubin, and flavonoids, however the measurements of any individual antioxidant appears less representative of the total antioxidant activity (TAA). Despite several procedures available to determine TAA, to date no data is present in existing literature concerning the correlation between Hg in saliva and salivary TAA. Therefore, the aim of this investigation is to evaluate TAA and Hg levels in the saliva of healthy donors and to correlate them with the number of amalgam restorations and the amalgam surface.

MATERIALS AND METHODS

Thirty-four young healthy donors (17 female, 17 male; mean age = 21 years; range =18-23 years) were chosen to participate in this study and all gave their informed consent. The subjects were asked not to eat or drink for 1 h prior to the collection of a saliva sample, which was performed between 9 and 12 a.m.. All subjects were asked to rinse their mouths five times with distilled water, to swallow the saliva produced during a 5 min interval, to collect the newly produced saliva in the mouth for 5 min and then to deposit the saliva in a test tube. For Hg determination, 1-1.5 ml samples were stored at -80°C until utilized.

0.2 ml of the samples, to which the antioxidant butyl hydroxytoluene (BHT) was added (0.1 mM final concentration), were used to determine salivary TAA with the FRAP assay (Benzie and Strain, 1996). Cold vapor atomic absorption spectrometry (Perkin Elmer FIMS 400) was used to determine the total Hg. Saliva samples (1 ml) were digested with 3 ml of nitric acid (Merck Suprapur) in Teflon vessels under pressure for 8 hours at 120°C (Chien et al. 1996; Drexler and Schaller, 1998).

Each series of analysis was accompanied by concurrent mineralization and identification of Standard Reference Materials (SRMs) n° 1577b "Bovine Liver" from NIST (Gaithersburg, USA). Batches with accompanying SRMs outside the certified range were repeated.

The reliability of Hg determination, expressed as the coefficient of variation on repeated assays of the same samples, was below 5%. An amalgam score was calculated yielding a score of 1 when the amalgam surface had a diameter of 1 mm or less, 2 if it was above 1 and less than 2 mm, 3 if it was 3mm or less and 5 if it was more than 3 mm. The amalgam score is the summation of scores of all the amalgam surfaces on all the teeth of each subject.

A simple regression analysis was used to compare the experimental results, separately for females and males. Only p values < 0.05 were considered as significant.

RESULTS

Table 1 indicates all data referring to donors. As can be seen, higher salivary Hg levels were detected in subjects with a greater number of restorations and higher amalgam scores.

A significant correlation between salivary Hg and the number of amalgam restorations was evident both in male and female subjects (Fig. 1). A similar correlation was observed between salivary Hg and amalgam scores, as reported in Fig. 2.

As shown in Figure 3, no correlation between salivary antioxidant capacity, evaluated by FRAP, and the number of amalgam restorations was observed in the male subjects, while a significant inverse correlation was evident in the females. Similar results were obtained comparing FRAP values and amalgam scores, as reported in Fig. 4. It is interesting to note that in Fig.5 a significant inverse correlation was observed in the female subjects only between the log of salivary Hg levels and the corresponding FRAP values.

N° amalgam restoration	Salivary Hg	Amalgam score	FRAP
0	0	0	300
0	0.42	0	257
0	0	0	285
0	0	0	144
0	0	0	372
0	0	0	192
0	0	0	253
0	0	0	143
0	0	0	266
0	0.73	0	393
0	0	0	278
0	0	0	258
1	0.73	2	363
1	3.93	2	144
1	0.71	2	165
1	0.57	3	363
1	0.15	3	283
1	1.06	2	212
2	1.53	10	347
2	1.47	5	93
2	2.77	5	85
2	1.64	6	456
2	3.17	6	248
2	2.96	5	220
3	1.97	6	303
3	14.76	12	206
5	8.99	21	327
5	2.47	10	190
5	3.12	11	215
6	23.33	19	119
6	4.08	16	134
6	8.67	20	105
10	51.91	34	138
11	47.24	19	379

Table 1: Number of amalgam restoration, Hg levels, amalgam score and FRAP from healthy subjects.

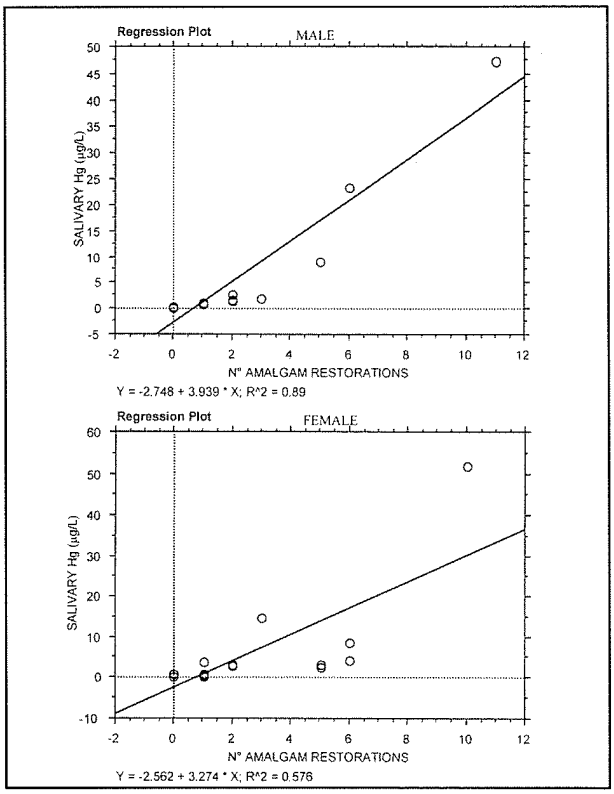

Fig. 1:

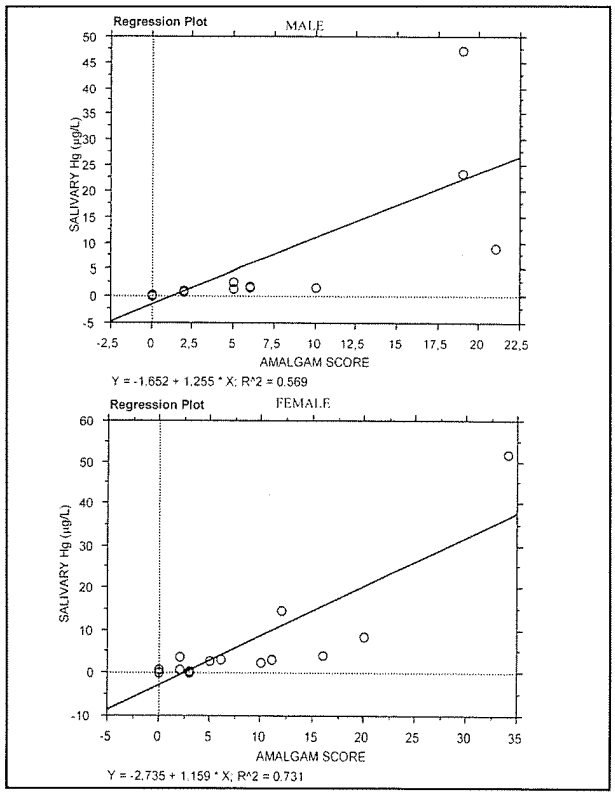

Fig. 2

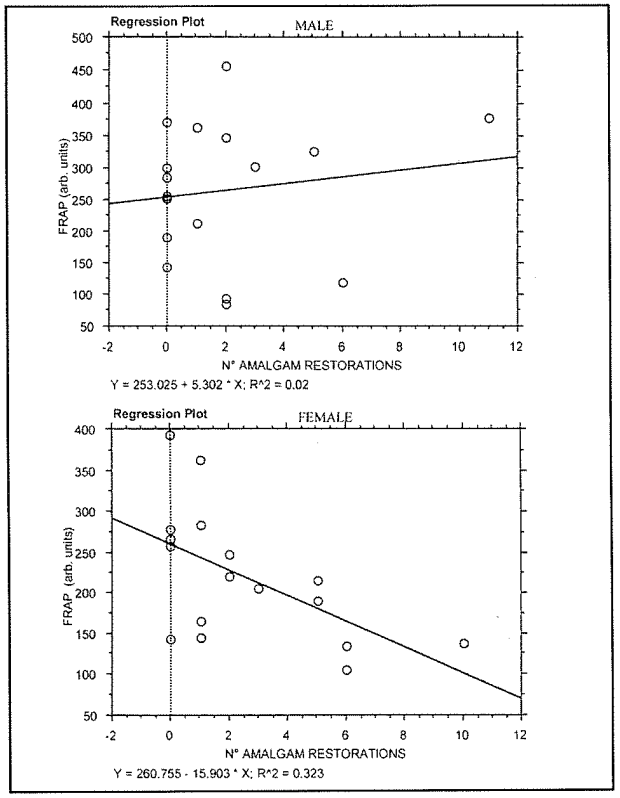

Fig. 3

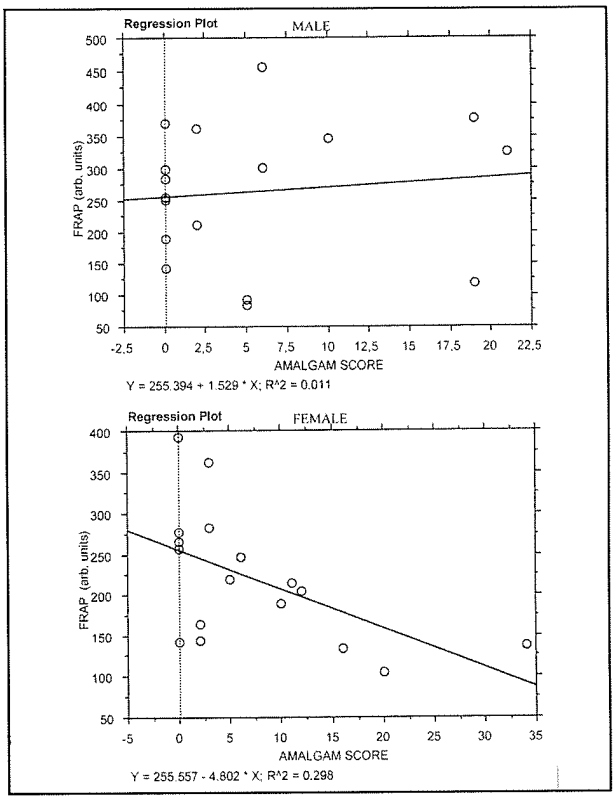

Fig. 4

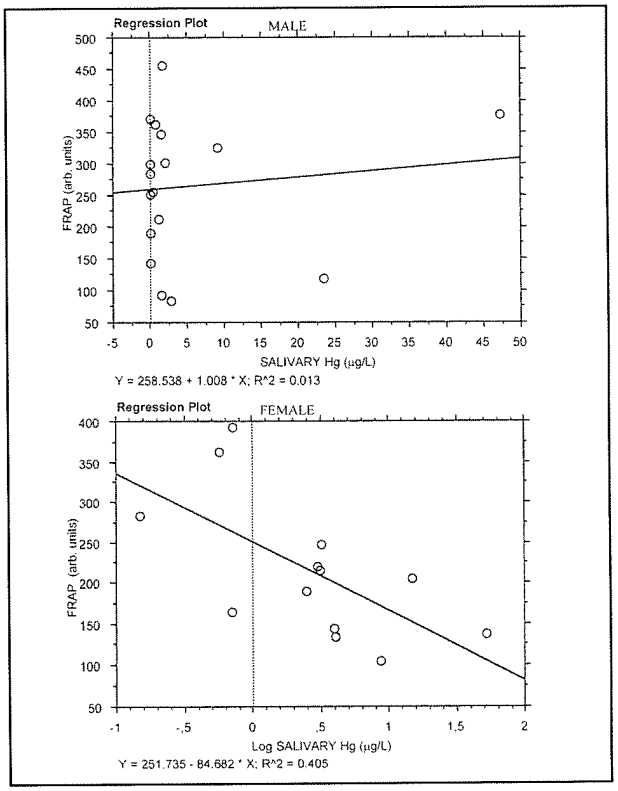

Fig. 5

Fig. 1: Correlation between salivary Hg levels and number of amalgam restorations in all examined subjects ($p<0.0001$ R= 0.943 for male; $p<0.0005$ R= 0.759 for female).

Fig. 2: Correlation between salivary Hg and amalgam score in all examined subjects ($p<0.001$ R= 0.754 for male; $p<0.0001$ R= 0.855 for female).

Fig. 3: Correlation between FRAP and number of amalgam restorations (p not significant R= 0.142 for male, $p<0.05$ R= -0.568 for female).

Fig. 4: Correlation between FRAP and amalgam score in all examined subjects (p not significant R= 0.103 for male, $p<0.05$ R= -0.546 for female).

Fig. 5: Correlation between FRAP and Hg saliva in all examined subjects (p not significant R= 0.113 for male, $p<0.05$ R= -0.637 for female).

DISCUSSION

Saliva possesses antioxidant activity exerted by different compounds like antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase, reductase and transferase) (Moore et al. 1994). Other substances capable to donating an electron (reducing compounds) are present in whole saliva; the major acqueous antioxidant component is uric acid, while ascorbic acid and albumin contribute less (Moore et al. 1994).

Measurements of salivary TAA reveal that it is influenced by many conditions. TAA decreases in old healthy subjects, in those with G6PD deficiency, in subjects treated with amoxycillin, carbamazepine or progesterone (Kohen et al. 1992). A decrease of TAA was reported in patients with periodontal disease (Chapple et al. 1997).

The total antioxidant activity (TAA) can be assayed with several methods, like chemiluminescent procedures (Chapple et al. 1997), cyclic voltametry (Kohen et al. 1992), Randox kit assay (Rice-Evans et al. 1994), ORAC assay (Cao et al. 1993). For the present study, we chose to use the so-called FRAP method (Benzie et al. 1996) because it provides a more global appraisal of oxidative stress and is convenient and simple to perform in routine analyses.

Data reported in the present study clearly indicate that amalgam restorations are a source of Hg in saliva. Salivary Hg levels were, in fact, significantly correlated with both the number of amalgam restorations and the amalgam surface in all subjects studied.

Hg is known to generate reactive oxygen species (ROS) in vivo and in vitro (Stohs et al. 1995; Hussain et al. 1997; Hussain et al. 1999). Studies carried out using transgenic Chinese hamster ovary cell line A552 demonstrated that Pb and Hg disrupt the redox status by enhancing the activity of copper-zinc superoxide dismutase and xanthine oxidase (Ariza et al. 1998). Hg causes oxidative damage to the kidney, inhibiting the antioxidant role of GSH and forming a reactive Hg-thiol complex, which can interact with H2O2 to promote the oxidation of bio-molecules (Miller et al. 1993). The possibility that Hg released in saliva might interfere with salivary antioxidant capacity was therefore investigated. Indeed, although only in female subjects, a significant inverse correlation was observed between salivary FRAP values and both amalgam restoration number and amalgam scores. In addition, the FRAP values in the females showed a significant inverse correlation to the log of salivary Hg levels indicating that, in these subjects, small increases in salivary Hg are already sufficient to produce significant decreases in salivary antioxidant capacity. A lower salivary TAA in females as compared to males was previously reported by Moore et al. (1994), although no statistical analysis or dental examination of subjects was provided.

The present study demonstrates that the different TAA between females and males is strictly correlated with salivary Hg levels and this fact, for the first time provides evidence for a pro-oxidant role of Hg released from amalgam restoration in saliva. TAA variations in saliva might be indicative of susceptibility or resistance to periodontal disease, as reported by Chapple et al. (1997), who suggested that the reduced TAA in saliva of patients with periodontitis may result from the loss of a specific antioxidant produced by gingival crevicular fluid.

Our results confirm the potential toxicity of amalgam Hg, and give clinical dentists an insight into the effects of Hg on general health. However, further studies are required in order to clarify the cause of the evident differences between female and male subjects.

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