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Clinical studies

Correlation between long-term in vivo amalgam restorations and the presence of heavy elements in the dental pulp

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ABSTRACT

Project: To measure the levels of heavy metals (Hg, Sn) in the dental pulp and blood samples of patients with long-term amalgam restorations.
Procedure: 12 amalgam restored and 12 non-restored, sound teeth were chosen and access cavity preparation to the pulp chamber was made. The contents were transferred and dissolved in 5 mL of concentrated nitric acid followed by placement in an oven at 180 °C for tissue digestion. After cooling the tubes each digested sample was transferred to an atomic absorption system to measure the levels of heavy metals. The blood samples of five patients in each group were randomly analyzed to determine the levels of these heavy metals in the blood and if there were a correlation between these levels in blood and pulp. Data were analyzed by *t*-test at a $P < 0.05$ level of significance.
Results: No significant difference was seen between the levels of Hg and Sn in pulp tissues ($P > 0.05$); however, the blood analysis showed higher level of Hg amalgam group ($P = 0.009$). The analysis between the pulp and blood samples showed positive correlations for both Hg and Sn elements in dental pulp and the blood ($P = 1.000$) ($P = 0.900$).
Conclusions: The long-term presence of dental amalgam (at least 5 years) did not result in any remarkable changes in the levels of mercury and tin in the pulp tissue; however, there were increases in the level of mercury in the blood circulation even five years following the placement of the restoration.

Introduction

The long-term use of dental silver amalgam has raised public concerns due to its potential side effects, such as mercury release [1]. Mercury, silver, tin, copper, and zinc are main components of this alloy that are mixed with other constituents such as indium, cadmium, lead and antimony [2]. Evaporation, dissolution, and evaporation/dissolution have been indicated as three mechanisms of mercury release from dental silver amalgam, of which the third mechanism is known to be the best model for evaluation of the impact of Hg release [3–5]. Toxic elements such as mercury are defined as substances that can cause hazardous and adverse influence on body health [6]. The toxic effects of mercury on nervous and renal systems depend on acute contamination to elementary mercury [7]. Other investigators have shown that neurotoxicity of this element is highly relevant to the amount of mercury that is concentrated inside body cells [8].

Tin is another component of dental silver amalgam [2] that may be released from amalgams or that can influence the release of Hg [9,10]. The dissolution of tin inside the dental amalgam, especially in low pH environments, can increase the mercury release from the tin-free γ_1 phase [9]. In the presence of the on-going dissolution of tin; mercury undergoes greater evaporation and ionization into the surrounding environment [10]. However, mercury release from dental amalgam can be decreased due to the production of a

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tin oxide layer on the surface of γ_1 phase of Hg, unless this layer dissolves because of low pH values inside the oral cavity [9].

Further concerns are the clinical side-effects and these side-effects include gingivitis, loss of teeth and alveolar bone, oral galvanism, alteration of taste, mucosal ulceration and bruxism [11,12]. The pulpal uptake of mercury has been identified to play an important role in inflammation of dental pulp, following the placement of amalgam restorations [13]. Studies have shown that mercury penetration was not decreased even with the use of cavity liners such as calcium hydroxide or glass ionomer cements, due to their solubility and water contents [14–16]. Furthermore, the dynamic nature of the oral cavity including biological, mechanical, chemical and thermal stresses makes the whole scenario more complex [17] In this regard, some authors have evaluated the amount of mercury release during chewing stimulations and brushing time [18], while others have studied this phenomenon under different pH values [9].

Set dental amalgam in the oral cavity is considered as a dynamic substance that is predisposed to many clinical stresses such as temperature alteration and corrosive attacks [19] that might increase mercury release to some extent over time [18]. The present study was performed to evaluate the levels of mercury and tin the pulpal tissue of amalgam restored teeth and in the blood of patient possessing amalgam restoration. The hypothesis tested was whether or not the amount of these elements increases inside the pulp tissue and the blood over a long period of time.

Materials and methods

Twenty-four premolar and molar teeth of 24 healthy patients, without any clinical signs and symptoms were extracted for orthodontic purposes and following ultrasonic cleaning were analyzed with stereomicroscope to ensure they were free of defects, such as cracks. Twelve specimens had class I amalgam restorations that were between 5 and 20 years old. The exclusion and inclusion criteria were as following:

Exclusion criteria: any subjects who had systemic problems such as physician-diagnosed psychological, behavioral, neurological, immunosuppressive, or renal disorders.

General inclusion criteria: (1) all subjects were older than 18 years old and agreed to the parameters of this study by signing the consent form based on the Helsinki guidelines. (2) The number of amalgam restorations inside the oral cavity of each patient should not have been more than five restorations. (3) Subjects were non-smokers and had no history of dental implants, other NiTi prosthesis in any part of their body, any piercing, and did not wear steel watches. (4) Diet control was done to ensure that subjects did not consume any sea food for at least one week prior to blood sampling.

Specific inclusion criteria: (1) the amalgam restorations must have be 1.5–2 mm from the pulp. (2) The amalgam restorations must weighed 1 ± 0.02 g. In order to check these criteria, samples were grooved vertically on the buccal and lingual surfaces with a diamond disk without entering the pulp chamber, and split longitudinally with a chisel. The filling materials of experimental samples were extracted from the cavity and weighed by a electronic digital analytical balance (Mettler AE-163, Mettler Toledo, CA, USA) with an accuracy of 0.0001 g and the dentin thickness was measured by a caliper to be 1.5–2 mm. The remaining extracted teeth were sound, did not have any restorations and served as the control group (Fig. 1).

After splitting the teeth in bucco-lingual direction, the pulp tissues of all samples were taken out of the pulp chamber with minimal damage by using plastic instruments and transferred into a clean beaker containing deionized pure water. Ultrapure water of 18 MΩ/cm specific resistivity was obtained from Milli-Q water system purification (Millipore, Milford, MA, USA). 5 mL of concentrated nitric acid was added and samples underwent digestion at temperature of 180 °C in the oven. After cooling the tubes, 3 mL H_2O_2 was added and evaporated to about 0.5 mL. Each digested sample was transferred quantitatively into a 10 mL calibrated tube to which was added a hydrochloric acid solution 1 mol/L.

Serial dilution

1 mL aqueous solution of Triton X-100 containing 40 g of NH_4NO_3 per liter as the matrix modifier was used for the final dilution of all samples, including standards and experimental groups.

Preparation of reagents

Purified water in a high-grade Milli-Ro De-ionizer system was used to prepare all reagents. 1 mL aqueous solution of Triton X-100 (iso-octylphenoxypolyethoxy ethanol), containing 40 g of NH_4NO_3 per liter as the matrix modifier, was used for the final dilution of all samples, including standards and experimental groups.

All chemicals were analytical grade when obtainable and high-purity nitrogen was used as the purging gas. A solution containing 60 g of BSA (Sigma Chemical Co., St. Louis, MO), 140 in mol of NaCl, and 5 mrnol of $KHCO_3$ per liter was used to prepare all working standards.

Preparation standards

A standard solution of 1000 ppm Hg was obtained (Fluka Chemie, Buchs, Switzerland) and for the Sn a standard solution was obtained (PerkinElmer, Pure atomic spectrometry standard, Shelton, CT, USA). The measurement of the levels of Hg and Sn were performed by WFX-1B atomic absorption spectrophotometer (No.2 Optical Instrument Factory of Beijing, China) equipped with a graphite furnace (HGA-400, Perkin-Elmer, CT, USA). The silver hollow-cathode lamp (Photron, Dandenong, Victoria, Australia) with a wavelength of 328.1 urn and a high-density graphite carbon tube (Perkin-Elmer, CT, USA) without platform were used in the assay. The calibration graph is linear up to 1 μg/mL of tin. The limit of detection of this method is 0.002 μg/mL.

Evaluation of blood samples

After teeth removal, five samples from each group were chosen randomly and subjected to blood evaluation of these heavy elements. Blood was collected by a vein puncture needle 25×7 (Vacutainer) in a 5-mL tube with the 5% percentage by weight anticoagulant EDTA.

Statistical analysis

The level of Sn and Hg elements represented non-continuous data that were presented after being analyzed by nonparametric tests and descriptors. Data were tabulated; proportions and 95% confidence intervals (CIs) were calculated. Significance of univariate associations was assessed with chi-square tests. For pulp/pulp and pulp/blood analysis, variables and treatment outcomes, the statistical program of SPSS 9.0 (SPSS Inc., Chicago, IL, USA) was used.

Results

The box plot of the level of heavy metals determined in the experimental groups is shown in Fig. 2. The statistical results indicated that there were no significant differences in the levels of Hg and Sn inside the pulpal tissue of the amalgam and control groups ($P > 0.05$). Blood evaluation identified significant differences