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Mercury Exposure in Dental Practice

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Clinical Relevance

This study failed to find a correlation between blood mercury levels and cytogenic damage in dentists exposed to mercury vapor below 0.1mg/m^3.

SUMMARY

Since elemental mercury is absorbed by dental professionals through direct skin contact or inhalation, the use of mercury in dental amalgam continues to be a controversial issue. In this study, the authors address the possible health risk of occupational exposure to mercury vapor in the dental office. The cytogenetic examination of leukocytes with alkaline comet assay and blood mercury levels with Atomic Absorption Spectrometer of dentists exposed to mercury vapor below 0.1mg/m^3 concentrations failed to find cytogenetic damage and related correlation. However, higher cytogenetic damage and blood mercury levels evaluated in controls from mercury intake by seafood consumption justifies additional study.

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INTRODUCTION

Dental amalgam has been used extensively as a restorative material in teeth for more than 150 years. Amalgams are alloys of various metals with mercury; therefore, in the broadest sense, the material used in the early 1800s in France—D'Arcets Mineral Cement—could be considered the first dental amalgam.[1] Dental amalgams contain approximately 50% metallic mercury, the biologic effects of which have long been regarded as hazardous.[2] Exposure to elemental mercury in the form of vapor has been regarded as a potential occupational hazard to dental professionals, as it is taken up by direct skin contact and through inhalation.[3] Although mercury can accumulate in many organs, the target organs in the human body are the kidneys and the brain.

The genotoxicity of mercury has usually been attributed to the ability of the metal to bind with tubulin SH, which impairs spindle function and results in genomic mutations of numerical chromosome aberration. Mercury is also believed to induce DNA damage through oxidative mechanisms.[4] Despite its well known neurotoxicity and teratogenicity, the performed genotoxicity studies in subjects occupationally exposed to mercury are not completely defined. Also, no correlations have been reported between mercury levels in

blood/urine and cytogenetic analyses. Therefore, this study was conducted to evaluate: i) blood mercury levels, ii) the degree of genetic damage by alkaline comet assay and iii) mercury concentration in the expired air among a small group of dentists who were exposed to mercury from dental amalgam while in the university clinic.

METHODS AND MATERIALS

Study Population

The study population included 10 dentists who were occupationally exposed to mercury in the dental department of the Faculty of Dentistry. The mean age of the exposed dentists was 25.6 ± 5.95, and the 10 amalgam-free controls were office employees with the mean age of 30.1 ± 5.74. The duration of exposure in the dentists was between 5 and 9 years. For each individual, a full, detailed questionnaire was completed, providing information about age; seafood consumption; life habits, such as smoking and alcohol and occupational exposure and health, which could be confounding factors for cytogenetic analysis according to the criteria developed by Carrano and Natarajan.[5] Non-smoking and amalgam-free individuals were chosen for both study groups.

To determine blood Hg levels and comet assay, blood samples were collected from all subjects and processed on the same day. All peripheral blood samples were collected in mercury-free heparinized vacutainer glass tubes.

Blood Mercury Levels

An ATI-UNICAM model 939 Atomic Absorption Spectrometer (Cambridge, UK) equipped with deuterium background corrector was used. A Unicam coded Hg hollow cathode lamp was employed at 253.7 nm, 5 mA, with a spectral band pass of 0.5 nm.[6-7]

For digesting blood samples, the procedure reported by Bourcier and Sharma[8] was adopted. A 3 ml volume of each sample was transferred to longneck digestion flasks and 4.0 ml of H_2SO_4:HNO_3 (4:1) solution was added. The temperature of the digestion block was increased to 58°C for 2 hours. The solutions were cooled to room temperature, then 3 ml of $KMnO_4$ (5% w/v) and 3 ml $Na_2S_2O_8$ were added, respectively. The solutions were heated to 95°C for 2 hours. Finally, the solutions were cooled to room temperature and diluted to 25 ml. A known volume of mercury standard or the sample solution was introduced to the reaction vessel—2.5 ml of 5% v/v HNO_3—and 0.8 ml of 10% w/v $SnCl_2$ were added, and the reduction vessel was closed immediately. The stirrer was turned on for 70 seconds; during this time, no carrier gas (N_2) was allowed to pass through the reduction vessel. At the end of stirring period, the carrier gas was allowed to pass through the reduction vessel to transfer mercury vapor to the absorption cell. The peak height of the signal was measured.

Comet Assay

With minor modifications, the procedure described for the alkaline comet technique by Singh and others[9] was followed. Individual microscopic slides were covered with 110 µl of 0.5% normal melting agarose (NMA) at about 45°C in Ca^{2+} and Mg^{2+} free PBS. The slides were immediately covered with a large cover slip and kept at room temperature for about 5 minutes to allow the agarose to solidify. This layer was used to promote attachment of the second layer of 0.5% NMA. A quantity of 10 µl of fresh whole blood was mixed with 75 µl of 0.5% LMA to form a cell suspension. After gently removing the cover slip, the cell suspension was rapidly pipetted onto the first agarose layer, spread out with a cover slip and maintained on an ice-cold flat tray for 5 minutes to solidify. After removal of the cover slip, the slides were immersed in cold lysing solution for at least 1 hour at 4°C (2.5M NaCl, 100mM Na_2EDTA, 10mM Tris, 1% sodium sarcosinate, pH 10) with 1% Triton X-100 and 10% DMSO added just before use. The slides were removed from the lysing solution, drained and placed in a horizontal gel electrophoresis tank near the anode. The tank was filled with fresh electrophoresis solution (1mM Na_2EDTA and 300mM NaOH, pH 13) to a level approximately 0.25 cm above the slides. Before electrophoresis, the slides were left in the solution for 20 minutes to allow for the unwinding of the DNA and the expression of alkali-labile damage. Electrophoresis was conducted at 1.6 V/cm for 20 minutes (300 mA) at room temperature. The slides from all subjects were electrophoresed on the same day. These steps were conducted under dimmed light (the tank was covered with a black cloth) to prevent the occurrence of additional DNA damage. After electrophoresis, the slides were taken out of the tank. Tris buffer (0.4M Tris, pH 7.5) was gently added drop-wise to neutralize the excess alkali and the slides were allowed to sit for 5 minutes. The neutralizing procedure was repeated 3 times. To each slide, 65 µl ethidium bromide (EtBr-20 µg/ml) was added. The slides were covered with a cover slip, placed in a humidified, air-tight container to prevent drying of the gel and analyzed within 3 to 4 hours.

Slide Scoring

One hundred individual cells were analyzed from duplicate slides at 200x magnification under a fluorescent microscope (Zeiss, Germany) equipped with an excitation filter of 546 nm and a barrier filter of 590 nm. Comets were formed upon the principle of releasing damaged DNA from the core of the nucleus during electrophoresis. At low damage levels, the stretching of attached strands of DNA is likely to occur. With an increasing numbers of breaks, DNA pieces migrate freely and form the tail of the comet. The degree of damage is assessed by grading the cells by eye into 3 categories: no migration (NM), low migration (LM) and

high migration (HM), depending on the fraction of DNA pulled out into the tail under the influence of the electric field. The number of comets in each sample was calculated and expressed as the total number of cells in order to summarize the damage frequencies. The analyses were performed by a single slide reader, thus minimizing variability due to subjective scoring.

Statistical Analysis

The results were expressed as the mean ± standard deviation (SD). Differences between the means were analyzed for statistical significance using unpaired *t*-test. A *p*-value of 0.01 was taken to denote significance.

RESULTS

Table 1 summarizes the mean blood mercury levels and total number of cells from dentists and controls with different categories of DNA damage. Figure 1 shows a comparison between the high and no migrated cells of a dentist. In this study, the total number of low migrated and highly migrated cells, as evaluated by comet assay in dentists, were 56 and 15, respectively, and were found to not be statistically different from the controls, which were 56 and 18, respectively ($p>0.05$). The median mercury concentration in the blood was 3.57 ± 0.95 µg/dl for dentists and 5.21 ± 5.52 µg/dl for the controls and showed non-significant differences. However, a statistical correlation was found between blood mercury levels and the duration of time working in dental offices (r=0.01); this ranged from 5 to 9 years.

DISCUSSION

The possibility of cytogenetic damage in various occupations exposed to mercury has been discussed in several papers.[2,10-11] Some studies have found evidence of a genotoxic effect in workers exposed to mercury in various occupations;[9,12-14] whereas, others did not,[11,15-16] but the possible genotoxicity of mercury found in dentists due to occupational exposure to dental amalgam is unknown.

The National Institute for Occupational Safety and Administration (OSHA) has set the threshold limit value of exposure to mercury vapor at 0.05 mg per cubic meter of air for 8 hours a day, 40 hours a week.[17] In this study, in the dental department, GASTEC detector tubes were used to detect mercury vapor release from amalgam fillings, while the work was carried out and the mercury vapor concentrations were all below 0.1 mg/m^3. This level is currently used as the MAK limit in Turkish regulations for mercury exposure in work places.

CONCLUSIONS

Mercury levels in whole blood were not high in exposed dentists, although it was reported that dentists have almost twice the concentration of mercury in their blood as non-dentists.[18-19] In fact, the methyl mercury that was detected in the blood specimens of the controls was higher than that of exposed dentists, and it was interesting to see from the questionnaires that seafood consumption was higher among the control subjects than the dentists. It appears that fish consumption was probably a greater source of mercury than amalgam exposure, since it is known that about 20% of total mer-

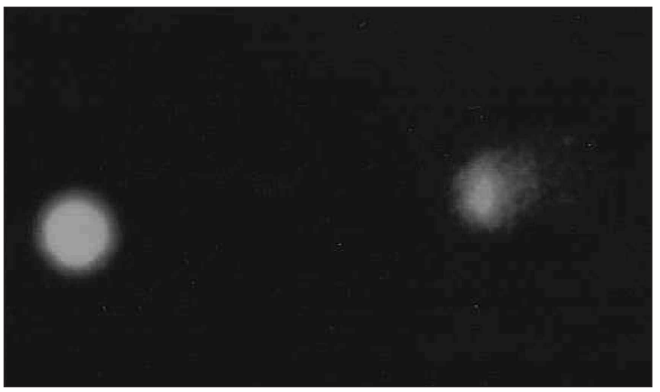

Figure 1. *A demonstration of high and no migrated cells of an exposed dentist using alkaline comet assay.*

Table1: *Total Number of Cells with Different Grades of DNA Damage and Blood Mercury Levels of Dentists Exposed to Mercury from Functioning Dental Amalgam and Controls*

			Grades of DNA Damage							
Groups	**Mean Age**	**Duration of Exposure**	**No Migration**		**Low Migration**		**High Migration**		**Total #**	**Blood Mercury Level, µ/dl (Mean ± SD)**
			Number	**%**	**Number**	**%**	**Number**	**%**		
Dentists (n=10)	23-42	5-9	926	92.6	59	5.9	15	1.5	1000	3.57 ± 0.95
Mean	25.6 ± 5.95		92.6 ± 2.99		5.9 ± 2.33		1.5 ± 1.12			
Controls (n=10)	23-43	-	925	92.5	56	5.6	18	1.8	1000	5.21 ± 5.52
mean	30.1 ± 5.74		92.6 ± 3.47		5.6 ± 2.72		1.8 ± 1.14			

p>*0.05 for all compared parameters*

cury from fish products is in the form of inorganic mercury and 80% is in the form of methyl mercury.[20] Observations by the authors of this study are consistent with the findings of Franci and others,[10] who reported higher DNA damage in a group of 51 fishermen exposed to mercury as a result of eating seafood. When considered together, the limited studies conducted to date do not give any strong indications of cytogenetic effects in blood at low or moderate exposure to mercury vapor. In order to make any positive associations, future investigations with a large number of subjects who have high and/or prolonged exposure to mercury have to be correlated with epidemiologic studies examining health problems. Also, the dietary contribution of mercury intake from fish and seafood consumption must not be overlooked.

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