

http://www.uem.br/acta ISSN printed: 1679-9291 ISSN on-line: 1807-8648

Doi: 10.4025/actascihealthsci.v34ispec.13428

Assessment of occupational exposure of dental professionals to mercury in dental offices of a public primary health care in Maringá, Paraná State, Brazil

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ABSTRACT. In order to evaluate the occupational exposure of dental professionals to metallic mercury in dental offices of a public primary health care in the city of Maringá, Brazil, samples of blood and urine were collected from 149 dental professionals (group exposed), and 51 healthy adults similar for age and gender of the exposed group (control group) in September and October, 2008. Urinary mercury was determined using atomic absorption spectrophotometry, urea and creatinine in blood and urine by UV/VIS spectrophotometry and analysis of physical, chemical and microbiological characteristics of the urine by reactive bands. The program 'Statistic' version 7.1 and the software R version 2.6.2 were used for the statistical calculations. Urinary mercury was $2.08 \pm 2.11 \,\mu g \, g^{-1}$ creatinine in workers exposed to mercury and $0.36 \pm 0.62 \,\mu g \, g^{-1}$ creatinine in the control group (p < 0.05). Urinary levels of mercury were below the maximum allowed by the biological index established in Brazil (35 $\,\mu g \, g^{-1}$ creatinine); 11% of these professionals (n = 16) had mercury levels above the reference value (5.0 $\,\mu g \, g^{-1}$ creatinine), whereas the maximum value found was 13 $\,\mu g \, g^{-1}$ creatinine. The dental professionals of public primary health care in the city of Maringa was exposed to metallic mercury at levels 5.8 times higher than the non-exposed subjects.

Keywords: mercury poisoning, health evaluation, occupational exposure, odontology, biomonitoring,

Avaliação da exposição ocupacional de profissionais de odontologia ao mercúrio em unidades básicas de saúde de Maringá, Estado do Paraná, Brasil

RESUMO. Para avaliar a exposição ocupacional dos profissionais de odontologia ao mercúrio metálico nas Unidades Básicas de Saúde (UBS) de Maringá, Brasil, foram coletadas amostras de sangue e urina de 149 profissionais de odontologia (grupo exposto) e de 51 adultos saudáveis similares em relação à idade e ao gênero do grupo exposto (grupo controle), no período de setembro e outubro de 2008. Foi determinado o mercúrio urinário por espectrofotometria de absorção atômica, a uréia e creatinina no sangue por espectrofotometria UV/VIS, e análise dos aspectos físicos, químicos e microbiológicos da urina por fitas reativas. Para a análise estatística foi utilizado o programa *Statistic* versão 7.1 e o R versão 2.6.2. O mercúrio urinário foi 2,08 \pm 2,11 μ g g⁻¹ de creatinina nos profissionais expostos ao mercúrio e 0,36 \pm 0,62 μ g g⁻¹ de creatinina no grupo controle (p < 0,05). Os níveis de mercúrio urinário detectados estavam abaixo do Índice Biológico Máximo Permitido estabelecido no Brasil (35 μ g g⁻¹ de creatinina), 11% destes profissionais (n=16) apresentaram níveis de mercúrio urinário acima do valor de referência (5 μ g g⁻¹ de creatinina), sendo que o valor máximo encontrado foi 13 μ g g⁻¹ de creatinina. Os profissionais de odontologia das UBS de Maringá estavam expostos ao mercúrio metálico em níveis 5,8 vezes maior que a população controle.

Palavras-chave: intoxicação por mercúrio, avaliação da saúde, exposição ocupacional, odontologia, biomonitoramento.

Introduction

Mercury is the metal found in liquid form, which emits toxic colourless and inodorous vapours and offers contamination risks during handling, since the main introduction of mercury into the body is through inhalation (GLINA et al., 1997; BOERING, 2000). In addition to industrialuse, mercury has

been used in medical environments and dental offices (PERAZA et al., 1998). In dentistry, the silver amalgam containing 42-52% of mercury is used as restoration material, because it presents some characteristics: easy handling, low cost, inherent mechanical properties such as wear resistance and good marginal blocking/hindrance (FIALHO et al., 2000).

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Exposure to mercury in dental clinics is considered high due to the preparation and use of dental amalgams (SKARE al., et AYYADURAI; KRISHNASHAMY, 1988). The average levels of urinary mercury in dentists of the American Dental Association were 14.2, 5.8 and 7.6 µg·L⁻¹ in samples collected between 1975-1983, 1985 and 1986, respectively (NALEWAY et al., 1985; NALEWAY et al., 1991). The decrease in urinary levels of mercury is due to handling care, use of safe amalgamators, and the decrease of weekly working hours of dental professionals.

The most used biomarkers to evaluate the mercury are the exposure to determination in blood, urine and hair (GOYER; LARKSON, 2001). The determination of mercury in the urine enables to evaluate the total amount of this metal stored in the workers exposed, as well as recent exposure (MASON et al., 2001; NUTTALL, 2004). In Brazil, the reference values and Biological Exposure Indices (BEIs) of 5 and 35 μg g⁻¹ creatinine (BRASIL, 2005), respectively, were established for mercury in the urine. In this study, we evaluated by the analysis of mercury in the urine the occupational exposure of dental professionals to metallic mercury in dental offices of public primary health care units in Maringá, Brazil. In addition, we compared these professionals with a group which was not occupationally exposed to the metal.

Material and methods

Study design

The samples of blood and urine were collected from 149 dental professionals (group exposed), and 51 healthy adults similar for age and gender of the exposed group (control group). A transversal study was performed with a population of dental professionals exposed to mercury (group exposed) and a population not exposed to metal (control group). Visits were made to dental offices of 22 public primary health care units of Maringá, in September and October, 2008.

Study population

The population consisted of 200 subjects, divided into two groups: 51 healthy adults not occupationally exposed to mercury, age and gender similar to the exposed group. The other group consisted of 149 people occupationally exposed to mercury in dental offices of public primary health care units, represented in this study by dentists, dental office assistants (DOAs), and dental hygiene technicians (DHTs).

Ethics

All participants gave their written consent to participate in the study. This study was approved by the Permanent Committee of Ethics in Research Involving Human Beings (COPEP) of the State University of Maringá. The protocol was analyzed in accordance with the Resolution N°. 196/96 of Health National Council of the Health Ministry at the 158th COPEP meeting on August 11th, 2008, register No. 0225.0.093.093-08.

Analytical methods

The variables studied were obtained by means of a self-applied questionnaire about working hours, number of workers and their respective occupations, identification data, occupational record, information about exposure to mercury and data concerning morbidity, related to renal system.

Urine samples were collected in September and October 2008, in a minimal quantity of 60 mL, following the hygiene, the first morning urine, using sterile flasks, out of work place, and the individuals could not consume fish on the three days prior to the collection. The samples were fractionated; 10 mL reserved for partial urine test and creatinine to adjust for sample dilution or concentration, 50 mL for the analysis of the metal by atomic absorption spectrophotometry with cold vapor (Perkin Elmer®, Waltham, USA). This aliquot was acidified with concentrated hydrochloric acid (Merck®, Darmstadt, Germany) to reach pH between 4 and 4.5. The samples were then kept under refrigeration for a maximum of 5 days before analysis.

For determining urea and creatinine, 5 mL of blood were collected in a tube without anticoagulant. After the serum separation by centrifugation at 3,000 rpm for 5 minutes, the analyses were carried out on the same day.

Urine strips Makromed® (Jonnesburg, South Africa) were used to evaluate biochemical parameters such as protein, glucose, urobilinogen, bilirubin, ketones, nitrate and hemoglobin. Physical parameters evaluated were volume, color, aspect and density and, for the sedimentological analyses, microbiological aspects were evaluated by GRAM bacterioscopy, and a Neubauer chamber was used for the total leukocytes and blood cell count.

Mercury was determined by atomic absorption spectrophotometry by cold vapor, according to Friese et al. (1990). The samples were processed in the Samples Preparation System by Microwaves (CEM Co., Matthews, USA), with hydrochloric acid (Merck®, Darmstadt, Germany) and later transferred to the Hydride Generator (sodium

borohydride 0.2%, Merck®, Darmstadt, Germany) and to Atomic Absorption Spectrophotometer FIMS model Analyst 200 (Perkin Elmer®, Waltham, USA). The standard used was 500 μ g L⁻¹ of mercury chloride (Merck®, Darmstadt, Germany). The method quantification limit was 0.03 μ g L⁻¹ and linearity 0.03 a 300.0 μ g L⁻¹.

Serum urea quantification was carried out by using the Uréia UV test (Katal®, Belo Horizonte, Brazil). The urea from the sample was hydrolyzed by urease with production of carbon dioxide and ammonium ions, which take part in the reaction with NADH $_2$ and α -ketoglutarate by glutamic dehydrogenase. The velocity of NADH $_2$ concentration decrease was evaluated at 340 nm (spectrophotometer model BS-300, Mindray®, Shenzhen), being proportional to the concentration of urea in the sample.

The quantification of serum and urinary creatinine was carried out by using Creatinine K test (Labtest[®], Lagoa Santa, Brazil). Creatinine forms a compound with the picrate in alkaline medium, according to Jaffé reaction, which was measured in 510 nm (spectrophotometer model BS-300, Mindray[®], Shenzhen).

Statistical analysis

The program 'Statistic' version 7.1 and the software R version 2.6.2 were used for the statistical calculations. The statistic differences between the groups were evaluated by application of the Student's t-test. The tests with nullity coefficient lower than 5% (p < 0.05) were considered significant.

Results and discussion

We identified 25 public primary health care units in the Maringa with 220 dental professionals. Onehundred-forty-nine participated in the study. Forty professionals refused to be subject of this research for personal reasons, 6 professionals were on holiday, and 25 were professionals from three public primary health care units, which did not authorize the study. The visits to the dental professionals and delivery of the self-applied questionnaire occurred between September and October 2008. Among the 149 professionals, 111 were white (74%), 24 Asian descendants (16%), 13 Afro-descendants (9%) and 1 was a Brazilian indian (1%). Regarding the control group, 47 (92%) were white, 1 (2%) Asian descendants, 3 (6%) Afro-descendants. The distribution according to gender and age for the control group and professionals exposed to mercury is shown in Table 1. In the exposed group, 16% were men and 84% women, whereas in the control group, 10% were men and 90% women. The average age of the exposed group was 38 years old, and in the control group, 36 years old (Table 1).

The dentists work 4 hours daily and the technicians and assistants work 6 hours. The higher values listed in Table 2 refer to work in private offices. The average working-hours was 7.2 ± 1.7 hours, but the most professionals (47%) work 6 hours daily. Concerning the time as professionals, the average was 17 ± 7 years for the dentists, 13 ± 6 years for technicians and 14 ± 8 years for assistants (Table 2).

Among the professionals in this study, 56% handled amalgam and the average use of amalgam in dental restoration was 36% for dentists, 38% for technicians and 42% for assistants. Only 71 (48%) professionals asserted knowing the toxic effects of mercury, 74% were dentists, 55% technicians, and only 27% assistants. None of the professionals reported a case of mercury intoxication.

Forty-four percent of the professionals were periodically tested, and 37% reported some pathology such as diabetes (2%), hypertension (4%), and renal conditions such as hematuria, dysuria or painful discharge of urine (10%), urinary infections (14%), and some associations of these pathologies (7%).

As for the use of individual protection equipment (IPE) all dental professionals assumed to wear gloves, surgical masks for medical procedures, white coat, glasses and bonnet.

Table 1. Distribution by gender and age of the group exposed to mercury (n = 149) and control group (n = 51) in public primary health care units of Maringá, Brazil.

Carrie	Gender	Age(years)										
Group	Gender	19 –24	25 -29	30 -34	35 - 39	40 -44	45 -49	50 - 3	55 –59	60	Total	Frequency
Exposed	Male	0	0	4	3	6	7	3	0	1	24	16%
	Female	4	11	24	38	25	15	5	2	1	125	84%
	Total	4	11	28	41	31	22	8	2	2	149	100%
	(%)	3	7	19	28	21	15	5	1	1	100	
Control	Male	1	0	0	0	2	0	1	0	1	5	10%
	Female	10	8	8	5	5	5	0	2	3	46	90%
	Total	11	8	8	5	7	5	1	2	4	51	100%
	(%)	22	16	16	10	14	10	2	4	8	100	

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Table 2. Distribution of working-hours of dental professionals according to their occupation in the public primary health care units and in private offices.

Occupation		Workin	Total	(%)				
Occupation	4	6	8	10	12	1 otai	(70)	
Assistant	2	53	14	3	2	74	50%	
Dentist	5	4	27	16	1	53	35%	
Technician	0	13	8	0	1	22	15%	
Total	7	70	49	19	4	149	100%	
(%)	5%	47%	33%	13%	3%	100%		

Physical, chemical microbiological and parameters were evaluated in the 200 urine samples analyzed by the partial urine test. In the group of professionals, 86% (n = 128) of the results were within normality, 5% (n = 8) showed infection, and 9% (n = 13) presented some crystals, whereas in the control group, 91% (n = 43) were within normality, 2% (n = 1) showed infection and 6% (n = 3) presented crystals. The chemical parameters measured by urine strip showed the presence of proteins in 1% (n = 2) of the group of professionals, and in 2% (n = 1) of control group. None of the subjects had positive result for bilirubin, glucose, urobilinogen, ketones and nitrate. However, two professionals (1%) had blood cells in their urine, and one in the control group (2%).

In the present study, the average level of urinary mercury of dental professionals of public primary health care units in Maringá, Brazil, was $2.08 \pm 2.11 \,\mu g \, g^{-1}$ creatinine. Only 11% (n = 16) of professionals had urinary mercury levels above 5.0 μ g g⁻¹ creatinine, and the maximum value found was 13 µg g⁻¹ creatinine, therefore, no value was above the BEI of 35 μg g⁻¹ creatinine established in Brazil (BRASIL, 2005). Our findings were lower than the obtained by other authors, such as Akesson et al. (1991) that found average content of 5.2 μ g L⁻¹ mercury in the urine of 244 dentists, 10% had levels above 10.4 μ g L⁻¹, and 1%, level above 33.4 μ g L⁻¹. Similar result was found in Turkey with 20 dentists and 9 controls, the average was $6.3 \pm 3.5 \,\mu g \,g^{-1}$ creatinine and 2.0 \pm 0.9 μ g g⁻¹ creatinine, respectively (KARAHALIL et al., 2005). In Brazil, 41 dentists showed urinary mercury concentrations between 1.97–31.19 μ g L⁻¹ (SILVA et al., 2000).

In the control group, the mean values of mercury were $0.36 \pm 0.62 \,\mu g \,g^{-1}$. The control group presented maximum value of 3.54 μ g g⁻¹ creatinine, but 92% of this group showed value lower than 1.0 $\mu g g^{-1}$ creatinine; 43% of the subjects had mercury content below the detectable limit, 0.01 μ g g⁻¹, and 82% of the professional group had mercury content that ranged from the detectable limit of 0.01 to 5.0 μg g⁻¹ creatinine (Table 3). Besides that, the age range of professionals which presented higher mercury concentration was from 30 to 44 years old. The urinary mercury content of dental professionals did not present significant difference in relation to race (p = 0.20), function (p = 0.57), gender (p = 0.30) or age (p = 0.46). On the other hand, a significant difference was found between the content of urinary mercury in the exposed and non-exposed population $(p = 4 \times 10^{-11}, p < 0.05).$

The average mercury contents found in dental professionals in this study was 5.8 times higher than the average level of the control group. Richie et al. (2004) and Karahalil et al. (2005) recorded lower differences between both groups, the average in the professionals in Scotland was 4 times higher than non-exposed population and, in Turkey, 3 times higher.

Dental professionals have often presented mercury levels above the levels of the population in general. They are exposed to mercury daily due to handling of amalgam, removal of fillings or inhaling vapours of residues inadequately stored (HORSTED-BINSLEV, 2004). In Brazil, 92% of dentists employ amalgam in teeth restorations and the occupational hazards are due to mercury concentration in the working place, inadequately stored residues, and the lack of biological monitoring to evaluate the degree of exposure of these professionals (FUENTES; GIL, 2003).

Table 3. Distribution of number and percentage of group exposed to mercury (n = 149) and control group (n = 51) in public primary health care units of Maringá, Brazil, according to mercury concentration in urine, and age.

Group	Mercury	Age (years)										
	content	19-24	25 -29	30 -34	35 -39	40 - 4	45-49	50 -53	55 -59	> 60	Total	%
	nd	7	5	4	1	2	0	0	2	1	22	43%
	0.01 - 4.99	4	3	4	4	5	5	1	0	3	29	57%
Control	> 5	0	0	0	0	0	0	0	0	0	0	0%
	Total	11	8	8	5	7	5	1	2	4	51	100%
	%	22%	16%	16%	10%	14%	10%	2%	4%	8%	100%	
Exposed	nd	0	2	0	1	4	2	2	0	0	11	7%
	0.01 - 4.99	4	8	26	34	24	18	4	2	2	122	82%
	> 5	0	1	2	6	3	2	2	0	0	16	11%
	Total	4	11	28	41	31	22	8	2	2	149	100%
	%	3%	7%	19%	28%	21%	15%	5%	1%	1%	100%	

 $nd = no\ detected\ (LD = 0.01\ \mu g\ L^{-1}).\ Content\ of\ mercury\ in\ urine\ x\ groups\ (p = 4\cdot10^{-11}, p < 0.05).\ Content\ of\ mercury\ in\ urine\ x\ age\ (p = 0.46, p > 0.05).$

At one of the public primary health care unit studied, all professionals presented urinary mercury levels above $5.0 \,\mu g \, g^{-1}$ creatinine. The place had some structural flaws such as inadequate dimensioning of rooms, windows and doors constantly closed; the amalgamator placed next to a

heat source, thus favouring mercury vapour emissions. In this public primary health care unit the highest level of urinary mercury (13 μ g g⁻¹ creatinine) was verified in one assistant, who has been working for 8 years at this place, and reported to have worked for 2 years at a place where the handling of mercury had been manual.

Mercury contamination depends on individual aspects of susceptibility and safety, since in the same exposure place, 90% of professionals had values of urinary mercury below 5.0 μg g⁻¹ creatinine, and 10% had levels above 5.0 μg g⁻¹ creatinine (CAVALLERI; GOBBA, 1998). Other important factor influencing the metal concentration obtained was the working hours, the longer the exposure, the greater the mercury contamination (p = 2.2·10⁻¹⁶, p < 0.05). Similar results were observed by Cury et al. (1991), examining 69 dentists, which observed higher urinary mercury concentration as a function of working hours and the number of amalgam restorations performed.

Salgado (1987) confirmed that occupational exposure may result in higher levels of urinary mercury compared to the population not occupationally exposed. Younger professionals had higher levels of mercury in the urine than the more experienced professionals. The author suggests that dental schools should improve the training of students and preventive measures against the metal contamination. However, in this study, the 16 professionals that presented higher contents of urinary mercury were experienced professionals, with more than 15 years of profession, in other words, the individuals exposed to mercury for more than 15 years presented urinary mercury more than two-fold higher than the group with less than 5 years of exposure (p = 0). Similar result was reported by Lacerda et al. (2002).

According to a study carried out in Brazil with 204 professionals, 57 dentists and 147 assistants, the latter had the higher risk compared to the time in the occupation (KARAHALIL et al., 2005). In this study, the worker's occupation did not present significant difference (p > 0.05) concerning urinary mercury levels.

Inadequate storage of amalgam waste in dental offices is also an aspect discussed in literature as important source of contamination by mercury vapours (CLARO et al., 2009). Thus, the storage in

hermetically closed containers, which contain a fixing solution or water, as suggested by Magro et al. (1994) may not be considered a definitive solution, but seems practical and accessible in developing countries. The public primary health care units employed this kind of storage in hermetically closed flasks, which contained water, a practice that reduced the work place contamination by mercury vapours.

All subjects, when their blood samples were analyzed, presented urea and creatinine values within normality indicating normal renal function, which was corroborated by partial urine test. There was no significant difference in the results of serum creatinine (p = 0.055) and serum urea (p = 0.09) between the control group and the group exposed to mercury. The serum creatinine value ranged from $0.4 \text{ to } 1.1 \text{ } (0.68 \pm 0.15) \text{ mg dL}^{-1}$, and from 0.3 to 1.0 (0.67 ± 0.16) mg dL⁻¹ for the group of professionals and the control group, respectively. The serum urea ranged from 12 to 48 (26.7 \pm 7.09) mg dL⁻¹, and from 16 to 36 (26.6 \pm 6.3) mg dL⁻¹ for the group exposed to mercury and the control group, respectively. According to morbidity data reported by professionals, we could not to confirm a correlation with mercury contamination, being necessary a clinical evaluation of the professionals.

Conclusion

In conclusion, dental professionals were exposed to mercury at levels significantly higher than the population not exposed occupationally ($p = 4.10^{-11}$); moreover, the professionals who presented higher mercury levels had, on average, more than 15 years of occupational exposure to this metal. This study showed the importance of biological monitoring, the need of training and the awareness of dentistry professionals concerning the toxic effects of mercury.

Acknowledgements

This research was supported by the Unidade Gestora do Fundo Paraná, Secretaria de Ciência, Tecnologia e Ensino Superior do Paraná – SETI-PR (CV N°. 3134/2004). We are grateful to the authorities of public primary health care units and the chemical lab personnel of the State University of Maringa for technical assistance. We thank the dentists, dental office assistants and dental hygiene technicians of public primary health care units of Maringá, Brazil. This study is dedicated to them.

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Received on May 18, 2011. Accepted on June 20, 2011.

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