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Mercury exposure among dental staff in the legal Amazon

Exposição a mercúrio entre profissionais da área odontológica na Amazônia legal

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ABSTRACT

Elemental mercury is highly toxic and may be absorbed by dental professionals through direct skin contact or inhalation. The use of mercury in dental amalgam has been a concern of the academic community for years, for its incorporation is likely to affect vital organ systems. Several studies have been conducted to address the possible risks of occupational exposure to mercury vapor in dental offices. The present study aimed to present evidences that mercury is assimilated by exposed workers through the determination of urinary mercury (HgU) from dental professionals (n = 91) of public offices in Araguaína (Tocantins, Brazil). This uptake was verified against samples from unexposed individuals (n = 43), which activities are not dentistry related. Cold vapor atomic absorption spectrometry (CV-AAS) technique enabled the subjects' biological monitoring. Approximately 44.8% (n = 60) of the 134 participants were aged between 21 and 30 years and were at the beginning of their professional lives; 9.7% (13) of the study participants were men and 90.3% (121) were women. Hg concentrations in all samples analyzed were within the maximum biological limit set by the

World Health Organization (WHO) (<50 μgHg·L^{-1}). HgU concentrations in dental professionals were within the limits proposed by the Brazilian regulatory standard, Regulatory Norm-7 (RN-7) (≤35 μgHg·g^{-1} creatinine). Nevertheless, the average concentration of HgU was approximately 8 times higher in the potentially exposed group (5.61 μgHg·g^{-1} creatinine) than in the unexposed group (0.65 μgHg·g^{-1} creatinine), highlighting the potential risk of occupational exposure to mercury.

Keywords: occupational exposure; mercury; CV-AAS; dentistry, validation.

RESUMO

O mercúrio elementar é altamente tóxico e pode ser absorvido pelos profissionais da área odontológica através do contato direto com a pele ou inalação. O uso de mercúrio em amálgamas dentais tem sido uma preocupação da comunidade acadêmica há anos, pois sua incorporação pode afetar órgãos vitais. Diversos estudos foram realizados para abordar os possíveis riscos da exposição ocupacional ao vapor de mercúrio em consultórios odontológicos. O presente estudo teve por objetivo apresentar evidências de que o mercúrio é assimilado por trabalhadores ocupacionalmente expostos através da determinação do mercúrio urinário (HgU) em profissionais da área odontológica (n = 91) de repartições públicas em Araguaína (Tocantins, Brasil). Essa assimilação foi verificada comparando-se as amostras desses indivíduos com as de indivíduos não expostos (n = 43), isto é, aqueles cujas atividades não estavam relacionadas à odontologia. A técnica de espectrometria de absorção atômica com geração de vapor frio (CV-AAS) possibilitou o monitoramento biológico dos indivíduos estudados. Aproximadamente 44,8% (n = 60) dos 134 participanetes tinham entre 21 e 30 anos e estavam no início de suas carreiras; 9,7% (13) dos participantes do estudo eram homens e 90,3% (121) eram mulheres. As concentrações de mercúrio em todas as amostras analisadas estavam dentro do limite biológico máximo estabelecido pela Organização Mundial de Saúde (OMS) (< 50 μgHg·L^{-1}). As concentrações de HgU nos profissionais da área odontológica estavam dentro dos limites propostos pelo padrão regulatório brasileiro, a Norma Regulamentadora 7 (NR-7) (≤35 μgHg·g^{-1} creatinina). Mesmo assim, a concentração média de HgU foi aproximadamente 8 vezes maior no grupo potencialmente exposto (5.61 μgHg·g^{-1} creatinina) do que no grupo não exposto (0.65 μgHg·g^{-1} creatinina), enfatizando o risco potencial de exposição ocupacional ao mercúrio.

Palavras-chave: exposição ocupacional, mercúrio, CV-AAS, odontologia, validação.

1 INTRODUCTION

Mercury (Hg) is the only metal found in the liquid state at room temperature. It is naturally found in the environment in the elemental (or metallic) form and as inorganic and organic compounds. In dentistry, elemental Hg is known as dental or raw Hg and has a silver color and shiny appearance. It is used to form dental amalgam by preparing an alloy of Hg and other metals (silver, copper, and tin). Dental amalgams have been used as restorative material for posterior teeth for more than two centuries (1).

Exposure of dental professionals to Hg vapors has attracted the attention of the academic community from various countries in the attempt to reduce the amount of amalgam used in dental offices and clinics. Other professionals working closely with dentists and who are potentially more exposed than dentists include dental auxiliary personnel, dental assistants (DAs), and dental technicians (2,3).

In Brazil, the Unified Health System (SUS) adopted by the federal government recommends the use of silver amalgam as the restoration material in public dental offices. In public dental centers of various municipalities of the state of Tocantins located in the Legal Amazon in northern Brazil, the amalgam is prepared manually using a mortar and pestle, exposing the professionals to excessive amounts of Hg vapor.

The sources of occupational exposure to Hg in dentistry include handling of dental amalgam during its preparation, contact with Hg drops spilled accidentally, contact with excess Hg during amalgam removal, and handling of leaking dental amalgamators (2,4-8).

In humans and other mammals, the kidney is the main site of Hg^{+2} accumulation when the body is exposed to high concentrations of Hg (26). To determine the concentration of urinary Hg (HgU), urinary creatinine (CR) and total Hg concentrations are determined separately and the values are later correlated.

The effects of Hg exposure can be assessed using several variables, including its chemical form, route of exposure, dosage, and individual factors (9). The individual factors include age, genetics, environmental aspects, nutritional status, and individual dose–response characteristics (10,11).

Chronic mercury poisoning, also called mercurialism, is a disease resulting from the long-term exposure to low Hg concentrations and is described in the literature as an occupational disease that can affect both dental surgeons (DSs) and DAs (12). In this case, the longer the professionals are exposed to Hg, the higher is the probability of developing the disease.

In Brazil, a few studies have reported the actual Hg-related occupational risks experienced by DAs working for SUS. These studies focused primarily on dentists; however, in public centers, DAs are responsible for the preparation, handling, and disposal of amalgam residues.

According to the World Health Organization (WHO), the concentration of HgU considered normal (unexposed individuals) is $< 4\ \mu g \cdot L^{-1}$. In Brazil, Regulatory Norm-7 (RN-7), which regulates the parameters for the control of occupational exposure to chemical agents, proposes the use of HgU as a biological indicator of exposure (BIE) to Hg and its compounds in dental workers (18). Moreover, it defines a concentration of HgU of 50 μg·L^{-1} as the biological threshold limit and 10 μg·L^{-1} as the reference concentration.

In this context, and considering that Hg amalgams are widely used for the restoration of posterior teeth in dental services provided by SUS in Tocantins, Brazil, according to data from DATASUS, biological monitoring of Hg was performed in dental professionals and Hg concentrations were compared with reference values recommended by RN-7 (13).

2 MATERIALS AND METHODS

2.1 SELECTION OF VOLUNTEERS

This study followed the ethical standards of the Brazilian legislation. In addition, it was approved by the Research Ethics Committee of the Tropical Medicine Foundation of Tocantins, associated with the Brazilian Committee of Research Ethics (CE/FMT-103/2008). The study was performed in accordance with resolution 196/96 of the National Health Council and with the global ethical principles, including the Declaration of Helsinki.

Information on the health of the participants has been achieved using a baseline questionnaire. Urine samples were collected between 2008 and 2010. The volunteers resided in 30 municipalities in the northern region of the state of Tocantins, Brazil. The volunteers included dental professionals (n = 91) and individuals who had never worked in the field of dentistry (n = 43), all of whom signed an informed consent form after receiving clarification about the study.

A questionnaire including variables such as age, sex, weight, and eating habits was provided to the unexposed group (n = 43). Additional questions on biosafety work routines were asked to the potentially exposed group (n = 91).

2.2 SELECTION OF URINE AS A BIE

Urine is considered the best biological indicator of exposure (BIE) and a biomarker of long-term occupational exposure to elemental and inorganic Hg (4,14-16). Quantification of HgU is reliable and simple, allowing quick identification of contaminated individuals (4,15).

Urinary concentrations of inorganic Hg have better correlation with Hg exposure compared with blood concentrations of inorganic Hg after long-term occupational exposure to low concentrations of elemental Hg vapors (14).

To monitor inorganic Hg concentrations, the present study adopted RN-7 as a reference. RN-7 uses Hg concentrations present in early morning urine samples as a biological indicator.

Atomic absorption spectrometry (AAS) was used as the analytical technique for the quantification of total Hg in urine samples. The reference value for normality (a value that can be found in populations not occupationally exposed to Hg), a concentration of ≤5 μgHg·g^{-1} CR was used; for the maximum allowable concentration (MAC) (the maximum concentration of BIE that does not compromise the health of most people who are occupationally exposed), a concentration of ≤35 μgHg·g^{-1} CR in urine was used. Doses above this value indicate excessive exposure to Hg (13).

2.3 COLLECTION AND QUANTIFICATION OF HGU

First morning urine samples were collected in sterile cups at a fixed time to minimize variations in Hg concentrations, according to the methodology proposed by Mason et al. and Trzcinka-Ochocka et al. (14,17). An aliquot was used for the quantification of urinary CR, and 10-mL aliquots of each sample were transferred to sterile conical Falcon tubes (Sarstedt®) and frozen at −20°C for subsequent lyophilization. Assays were performed in triplicates according to the protocol of operating procedures of the Chemical and Environmental Analysis Laboratory in the Chemistry and Environment Center of the Nuclear and Energy Research Institute (IPEN/CNEN-SP) (16,18).

2.4 QUANTIFICATION OF URINARY CR

Using molecular absorption spectrometry, CR was quantified with an automated equipment model Labmax Plenno and the creatinine K quantitation kit. Calibration was performed using NIST SRM 914a (pure crystalline CR), which yields traceable results, according to the CR standardization guidelines established by the National Kidney Disease Education Program.

2.5 ANALYTICAL METHOD FOR THE QUANTIFICATION OF INORGANIC HG

For the quantification of inorganic Hg in urine samples, cold vapor atomic absorption spectroscopy (CV-AAS) was used in compliance to the method proposed by Lopez-Colon and Lozano (19) and validated by Guilhen et al. (16). The parameters evaluated were limit of detection (LOD), limit of quantification (LOQ), selectivity, linearity, accuracy, and precision.

Measurement of LOD and LOQ is particularly important when the concentration of the analyte in the sample is very low. To validate an analytical method, it is sufficient to indicate the analyte concentration at which quantification and detection became problematic. The

LOQ or minimum quantifiable value is expressed as the concentration that corresponds to the desired accuracy (10%), considering the relative standard deviation (RSD%) calculated for a known concentration range. The LOD or minimum detectable value takes into consideration the α (false positive) and β (false negative) errors. LOQ corresponds to the lowest analyte concentration that can be determined with an acceptable level of accuracy and reliability and can be obtained from the analysis of 7 replicates of the sample's blank solution (16).

Each urine sample was analyzed in triplicate, and 3 independent measurements were made for each triplicate. Accuracy and precision were determined using certified reference materials provided by the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA): standard reference material 1641d—Hg in water (acidified with 2% nitric acid), with a mass fraction of 1.59 ± 0.018 $mg \cdot kg^{-1}$, and standard reference material Ɍ 2670a—toxic elements in urine (freeze dried) at 95.1 ± 0.98 $\mu gHg \cdot L^{-1}$.

LOD and LOQ established for the quantification of Hg in demineralized urine using CV-AAS were 0.07 $\mu gHg \cdot L^{-1}$ and 0.3 $\mu gHg \cdot L^{-1}$, respectively (16).

In addition, the method proved to be able to provide results with accuracy and precision throughout the range of study.

3 RESULTS

3.1 ANALYSIS OF THE QUESTIONNAIRE

The data obtained were converted into a database, which was processed and statistically analyzed using Epi Info® and Statistica® software.

With regard to the place of residence, 64.9% (n = 87) of the participants resided in Araguaína, Tocantins, and 35.1% resided in 33 smaller municipalities in the northern region of Tocantins.

Approximately 44.8% (n = 60) of the 134 respondents were aged 21–30 years and were at the beginning of their reproductive lives, 33.6% (n = 45) respondents were aged 31–40 years, and 16.4% (n = 22) were aged >40 years.

In the exposed group (n = 91), 91.2% (n = 83) of the respondents worked as DAs and 8.8% (n = 8) worked as DSs. In addition, 62.6% (n = 57) were involved in the "Family Health Program" team, which is a part of a strategy to reorganize primary health care in Brazil. However, 35.2% (n = 32) of the respondents were not included in this program, and 2 participants did not answer the questionnaire.

With regard to gender, 9.7% (n = 13) of the study participants were men and 90.3% (n = 121) were women. According to Nazar et al. (20), the predominance of female DAs can be explained by the fact that these professionals also perform secretarial duties, which are culturally attributed to women in Brazil.

In the present study, 81.32% (n = 74) of the interviewed professionals in the “exposed” group had been in the profession for at least 10 years.

Nazar et al. (20) evaluated the profile of DAs from 54 dental offices in Belo Horizonte. Among these, 68.5% (n = 37) were employed for 5–10 years.

3.2 BIOLOGICAL MONITORING OF HG

Seventy-nine samples from dental professionals potentially exposed to Hg were collected and analyzed, and each sample belonged to a study participant. Table 1 shows the frequency distribution of participants potentially exposed as a function of the length of employment (in years).

Table 1. Frequency distribution of potentially exposed workers as a function of the length of employment (years).

Length of employment (years)	Number of participants (N)	Representativeness (%)
1–10	74	81.32
11–20	13	14.28
21–30	1	1.10
31–40	1	1.10
Did not answer	2	2.20
Total	91	100

Tables 2 and 3 show the means of 3 independent measurements of total Hg, CR, and HgU in urine and the respective standard deviations.

Table 2. Mean values of total Hg, CR and HgU in 79 urine samples from potentially exposed workers evaluated between November 2008 and July 2010.

Number of Samples	Concentration		
N = 79	Hg* ($\mu g.L^{-1}$)	CR ($g.L^{-1}$)	HgU* ($\mu gHg.g^{-1}$ CR)
Mean	5.40	1.19	5.61
SD	5.14	0.65	4.92
Minimum	0.13	0.19	<0.30
Maximum	29.80	3.57	24.40

NOTE: (*) Mean of 3 independent measurements.

Of the 79 samples analyzed, the highest values for total Hg and HgU in the potentially exposed group were 29.80 $\mu g \cdot L^{-1}$ and 24.40 $\mu gHg \cdot g^{-1}$, respectively, whereas the mean HgU concentration was 5.61 $\mu gHg \cdot g^{-1}$ CR (Table 2).

Table 3. Total Hg concentration in urine samples in the unexposed group evaluated between November 2008 and July 2010.

Number of Samples	Concentration		
N = 31	Hg* ($\mu g.L^{-1}$)	CR ($g.L^{-1}$)	HgU* ($\mu gHg.g^{-1}$ CR)
Mean	1.10	1.44	0.65
SD	1.74	0.90	0.91
Minimum	8.70	3.57	4.24
Maximum	<0.30	0.19	<0.30

NOTE: (*) Mean of 3 independent measurements

Table 3 shows the total Hg concentrations in the urine samples in the unexposed group.

On considering the urine samples from 31 unexposed participants, in 38.70% (n = 12) samples, the total Hg concentration was inferior to the LOQ (0.3 $\mu g \cdot L^{-1}$). The mean HgU concentration in the unexposed group was 0.65 $\mu gHg \cdot g^{-1}$ CR.

In the study by Chaari et al. (21), 61% subjects at risk for Hg exposure were workers at dental offices, and HgU concentrations were significantly higher in subjects working in dental offices than in the control group. In our study, we observed that HgU concentrations were significantly higher in the exposed group than in the unexposed group.

Table 4 shows the distribution of the potentially exposed group as a function of the total Hg and HgU concentration in urine. 60.77% (n = 48) workers potentially exposed to Hg had total Hg concentrations of ≤ 5 $\mu g \cdot L^{-1}$ (Table 4).

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Table 4. Distribution of potentially exposed workers as a function of the total Hg concentration in urine (μgHg·L^{-1} urine) and as a function of HgU concentration (μgHg·g^{-1} CR).

Hg concentration in urine (μgHg·L^{-1})	Number of participants (n)	Representativeness (%)
$0 < x \leq 5$	48	60.77
$5 < x \leq 10$	19	24.05
$10 < x \leq 15$	8	10.13
$15 < x \leq 20$	2	2.53
$20 < x \leq 25$	1	1.26
$25 < x \leq 30$	1	1.26
Total	79	100
HgU (μgHg·g^{-1} CR)	**Number of participants (n)**	**Representativeness (%)**
$0 < x \leq 5$	50	63.3
$5 < x \leq 10$	15	19.0
$10 < x \leq 15$	8	10.1
$15 < x \leq 20$	5	6.3
$20 < x \leq 25$	1	1.3
Total	79	100

NOTE: normal HgU concentration recommended by WHO: 10 μgHg·L^{-1} urine; Maximum tolerable limit recommended by WHO: 50 μgHg·L^{-1} urine.

Only 1 participant exhibited a total Hg concentration between 25 and 30 μgHg·L^{-1}. When considering the limit of normality for HgU established by WHO (10 μgHg·L^{-1}), Hg concentrations in 84.81% (n = 67) participants were within the recommended limits, whereas the concentrations in 15.19% (n = 12) participants were above this limit. Hg concentrations in all samples analyzed were within the maximum biological limit set by WHO (<50 μgHg·L^{-1}).

Lung damage, high blood pressure, eye irritation, nausea, vomiting, diarrhea, chest pain, dyspnea, cough, gingivitis, liver problems, and hypersalivation can occur in Hg poisoning. If the brain is affected, neuropsychiatric disorders can occur, such as vision and hearing distortions, irritability, shyness, tremors, and memory loss (22). However, the association between HgU and health status was not investigated because physical and neurological tests were not performed in the present study and only questionnaires were used.

None of the 79 urine samples belonging to the potentially exposed workers exceeded the MAC value established by RN-7. Moreover, most of these workers (63.3%) had HgU of ≤5 μgHg·g^{-1} CR, which corresponds to the acceptable concentration for the unexposed group (Table 4).

The mean HgU values of the exposed and unexposed groups were compared using Student's t-test, with a confidence interval of 95%, $t = 5.32$, $df = 108$, $p < 0.01$; the mean values obtained for these groups were 5.61 μgHg·g^{-1} CR and 0.65 μgHg·g^{-1} CR, respectively (Fig. 1).

Figure 1

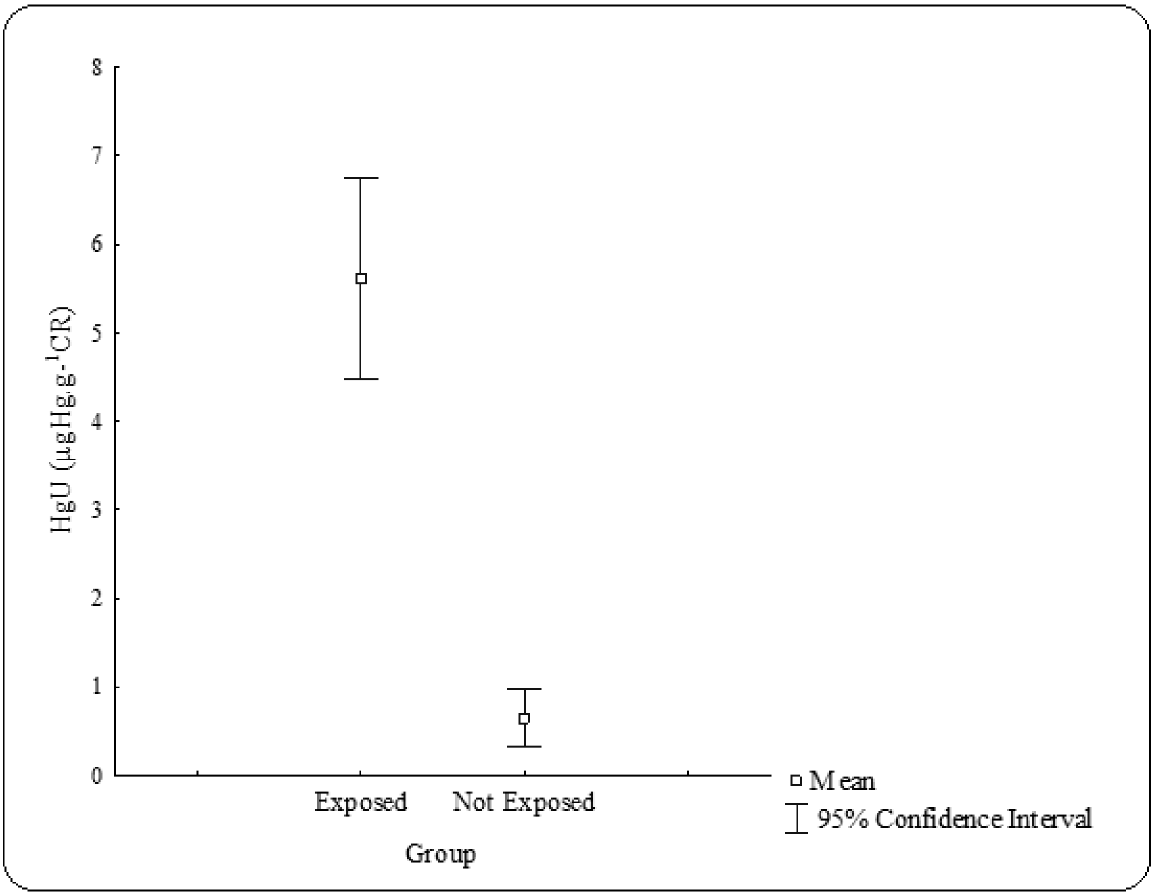


Fig. 1 Graphical representation of the mean HgU concentration in the exposed and unexposed groups.

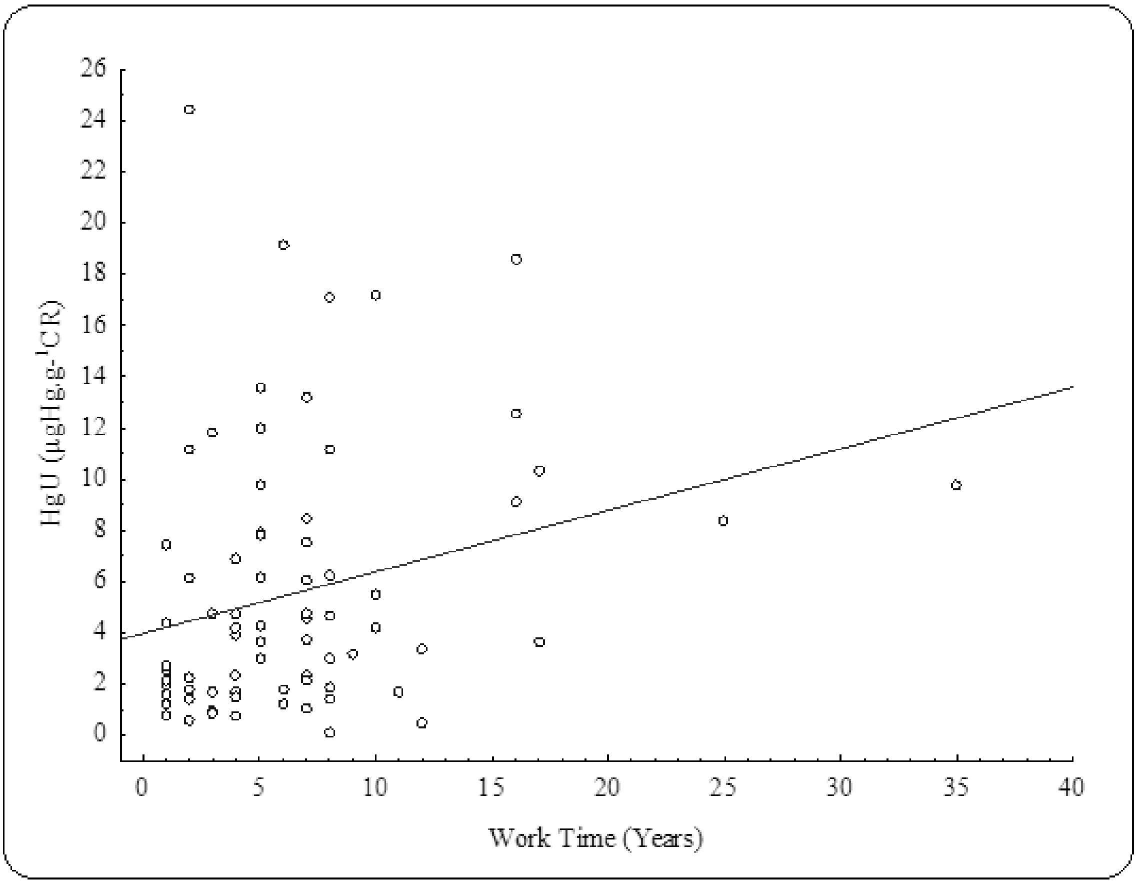
The blue bars shown in Fig. 1 indicate 95% confidence intervals. Notwithstanding the sample size difference between the groups (n = 79 and n = 31 for the exposed and unexposed groups, respectively), the mean HgU concentration in the exposed group was approximately 8 times higher than that in the unexposed group, indicating the occupational exposure to Hg among professionals working in dental offices.

The results of Student's t-test indicate that although Hg concentrations in the potentially exposed group were normal and acceptable in accordance with RN-7, this group can be much more susceptible to diseases caused by contact with Hg than the unexposed group. Therefore, annual biological monitoring of these professionals should be established as a routine practice during periodic occupational examinations.

These results are consistent with other studies in the literature (15,23,24,25,27) in which the professionals present higher concentrations of mercury in the urine.

With regard to the period of employment and HgU and considering that a longer period of employment would imply increased contact with Hg amalgam and consequently lead to higher HgU concentrations, simple linear regression analysis was performed to test this hypothesis. In this study, $r = 0.2644$, $r^2 = 0.0699$, $p = 0.0210$, and the regression equation was $y = 3.9783 + 0.2402*x$, where y = HgU concentration and x = length of employment. A significant positive correlation was observed, confirming this hypothesis, as seen in Fig. 2.

Figure 2



NOTE: $r^2 = 0.0699$, $r = 0.2644$, $p = 0.0210$, $y = 3.9783 + 0.2402*x$

Fig. 2 Linear regression between the length of employment (years) and HgU concentration ($\mu gHg \cdot g^{-1}$ CR).

4 DISCUSSION

On considering the results on Hg contamination, the mean Hg concentrations in the exposed group (n = 91) during the study period were approximately 8 times higher than those in the unexposed control group (n = 43). The concentrations of Hg found in the urine of dental workers were significantly higher than those in the unexposed group.

Collectively, these results reveal the increased occupational exposure to Hg among professionals working in public dental clinics. Although considered low, these values were higher than the mean values reported in the literature. Therefore, medical and psychological evaluation of dental professionals is needed to check for the signs and symptoms of Hg intoxication. It is necessary to develop methods to protect patients and dental staff from excess exposure to dental amalgam, to protect staff from high momentary exposures, and to isolate Hg-laden wastewater from the sewage stream.

5 SUMMARY IN PORTUGUESE

O mercúrio elementar é altamente tóxico e pode ser absorvido pelos profissionais da área odontológica através do contato direto com a pele ou inalação. O uso de mercúrio em amálgamas dentais tem sido uma preocupação da comunidade acadêmica há anos, pois sua incorporação pode afetar órgãos vitais. Diversos estudos foram realizados para abordar os possíveis riscos da exposição ocupacional ao vapor de mercúrio em consultórios odontológicos. O presente estudo teve por objetivo apresentar evidências de que o mercúrio é assimilado por trabalhadores ocupacionalmente expostos através da determinação do mercúrio urinário (HgU) em profissionais da área odontológica (n = 91) de repartições públicas em Araguaína (Tocantins, Brasil). Essa assimilação foi verificada comparando-se as amostras desses indivíduos com as de indivíduos não expostos (n = 43), isto é, aqueles cujas atividades não estavam relacionadas à odontologia. A técnica de espectrometria de absorção atômica com geração de vapor frio (CV-AAS) possibilitou o monitoramento biológico dos indivíduos estudados. Aproximadamente 44,8% (n = 60) dos 134 participanetes tinham entre 21 e 30 anos e estavam no início de suas carreiras; 9,7% (13) dos participantes do estudo eram homens e 90,3% (121) eram mulheres. As concentrações de mercúrio em todas as amostras analisadas estavam dentro do limite biológico máximo estabelecido pela Organização Mundial de Saúde (OMS) ($< 50\ \mu gHg \cdot L^{-1}$). As concentrações de HgU nos profissionais da área odontológica estavam dentro dos limites propostos pelo padrão regulatório brasileiro, a Norma Regulamentadora 7 (NR-7) ($\leq 35\ \mu gHg \cdot g^{-1}$ creatinina). Mesmo assim, a concentração média de HgU foi aproximadamente 8 vezes maior no grupo potencialmente exposto (5.61 $\mu gHg \cdot g^{-1}$ creatinina) do que no grupo não exposto (0.65 $\mu gHg \cdot g^{-1}$ creatinina), enfatizando o risco potencial de exposição ocupacional ao mercúrio.

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