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Relationship between blood metals and inflammation in taxi drivers

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ABSTRACT

Background: Cardiovascular disease is a cause of concern in public health worldwide, reinforcing the need for studies related to the identification of potential agents that contribute to the inflammation process and atherosclerosis. This study aimed to evaluate whether metals are associated with inflammatory and kidney damage and could contribute to the atherosclerosis process.

Methods: Blood metals, inflammatory markers, homocysteine, antioxidants and renal markers were measured in 42 taxi drivers and 27 controls (non-occupationally exposed).

Results: Taxi drivers had increased Hg, As, Pb and Cd levels, however Cu and Zn levels were decreased compared to controls ($p < 0.05$). Hg, As and Pb levels were positively associated with pro-inflammatory cytokines, nitric oxide and negatively associated with glutathione peroxidase. Moreover, Hg, As and Pb presented positive associations with homocysteine, an independent risk factor for atherosclerosis. Regarding markers of kidney function, N-acetyl-beta-D-glucosaminidase levels were increased in taxi drivers and correlated to inflammation markers.

Conclusion: Hg levels were found above the recommended limits in taxi drivers and both Hg and As levels showed associations with inflammatory process, oxidative status and homocysteine. Thus, chemical substances as Hg and As can be considered as additional contributors to the development of cardiovascular diseases.

1. Introduction

According to the World Health Organization (WHO), cardiovascular diseases are the leading cause of deaths, responsible for 17.3 million deaths worldwide in 2008, which accounts for 30% of the number of deaths [1]. Recent studies have shown that, in addition to risk factors, environmental factors are able to impact on the development of cardiovascular disorders [2–4]. Therefore, the investigation of potential agents that could contribute to the inflammation process and atherosclerosis is of great importance.

In general, individuals may be exposed to hazardous substances such as toxic metals present in the environment through multiple routes, including the respiratory tract by inhalation of air pollution or orally by the ingestion contaminated food and water [5–7]. Furthermore, growing evidence indicates that environmental exposures to metals rarely occur in an isolated manner [7,8], since metals are ubiquitous and remain in the environment for long periods of time, thus human exposure to metals can occur from different sources [9,10].

There are reports in the literature demonstrating that some metals are essential to health [5,11], others have no known physiological importance and are able to induce toxicity to humans by disrupting the homeostasis and often promoting oxidative stress and inflammation in biological systems [12–16].

Environmental pollution may present various toxicological properties, according to the geographical area and human socio-economic activities [17]. The major anthropogenic sources of toxic elemental pollution in urban areas are fossil fuel combustion and motor vehicular emission [18]. Traffic congestion increases vehicle emissions and degrades environmental air quality in urban areas from Brazil, considering the increasing number of automotive vehicles circulating in Brazilian roads [19].

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Environmental exposures to contaminants represent a health risk for categories of workers under frequent and prolonged exposure to vehicular emission such as taxi drivers, as well as lifestyle habits caused by irregular work schedule and work about 12 h per day, due to the exposure to several harmful environments [16,20,21]. According to Bakheet et al. [12], environmental pollution increases the risk of exposure to toxic metals and these authors also showed that subjects living near polluted areas present adverse effects at relatively low concentrations.

However, few studies are available on the biological monitoring for assessment of health effects related with harmful environment on populations exposed to urban pollutants in the workplace. Therefore, considering that metals are widely distributed in the environment and that several metals may induce toxic effects, biological monitoring studies are important to assess occupational risk factors associated with the exposure to environmental pollutants [21,22].

2. Materials and methods

2.1. Study population

Recruitment of taxi drivers was achieved by invitation and advertisement in radio stations and in taxi driver syndicates. For recruitment of subjects not occupationally exposed to traffic exhaust, advertisements were distributed at the Federal University of Rio Grande do Sul requesting volunteers with administrative activities. Subjects were excluded based on smoking, presence of any cardiovascular disease, diabetes mellitus, chronic diseases and utilization of food supplements and/or vitamins.

Based on these exclusion criteria, the study included 69 non-smoker men from Porto Alegre, Rio Grande do Sul, Brazil. The occupationally exposed group consisted of 42 male taxi drivers under occupational exposure to urban pollutants from the city traffic. The control group consisted of 27 subjects not occupationally exposed to traffic exhaust. No significant differences were found regarding the age between groups and the subjects evaluated in this study were non-smokers, and both groups simultaneously underwent equivalent examinations and procedures.

All eligible participants provided answers to a questionnaire interview in order to obtain information about lifestyle habits, history of previous and current diseases and general information regarding the work shift (years of service and time spent inside the car).

This study was approved by the Ethics Committee for Research of the Federal University of Rio Grande do Sul (No. 20322/11). All subjects were informed about the examinations to be conducted and provided written informed consent upon acceptance to participate in the study, according to the guidelines of the local committee.

2.2. Blood sampling

Pre-shift blood samples were collected from all participants by the established venipuncture technique into Vacutainer tubes. Blood–heparin tube were collected and stored at −20 °C until analysis to determine the toxic metals lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As), as well as glutathione peroxidase activity. The tube without anticoagulant was centrifuged at 1500 ×g for 10 min at room temperature and the serum obtained was immediately used to determine glucose, lipid profile and the remaining serum samples were frozen and kept under −80 °C for subsequent determination of micronutrients zinc (Zn) and copper (Cu), nitric oxide (NO) and inflammatory cytokines. EDTA–blood tube was immediately centrifuged at 1500 ×g for 10 min at 4 °C and plasma was stored at −80 °C until analysis of non-enzymatic antioxidants. Pre-shift urine samples were also collected and immediately after collection creatinine levels were analyzed; urine samples were then stored under −80 °C until further determination of microalbuminuria (mALB) and N-acetyl-beta-D-glucosaminidase (NAG).

2.3. Analysis of blood metals

For the measurement of elements in blood and serum samples, 1 ml of 65% ultrapure nitric acid was added to 500 μl of sample in a polypropylene digestion tube. The mixture was digested by heating at 95 °C for 4 h. Extracts were cooled at room temperature and the volume was made up to 10 ml with ultrapure water. Afterwards, whole blood metal concentrations of Hg, As, Pb and Cd as well as serum micronutrients concentrations of Cu and Zn were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS; PerkinElmer-Sciex) [23]. The following isotopes were chosen considering the abundances and interferences: ^{75}As, ^{114}Cd, ^{63}Cu, ^{202}Hg, ^{208}Pb and ^{66}Zn. In relation to Zn, the most abundant isotopes are subject to severe interferences (i.e., $ArMg^+$ and ArC_2^+ interfere in $^{64}Zn^+$), then less abundant isotopes were selected. The correction equations suggested by the equipment's software were always employed. The calibration curve ranged from 5 to 80 μg/l and the internal standard was Rh (400 μg l^{-1}) prepared in acidified aqueous solution (1% HNO_3). Calibration solutions were prepared using the stock solution (Perkin Elmer 29) at 10,000 μg/l. The limits of detection (LOD) and quantification (LOQ) were calculated based upon the standard deviation of the calibration blanks ($n = 10$): 3 times the SD for the LOD (or 10 times for the LOQ), divided by the slope of the calibration curve. The precision and accuracy of the instruments were monitored through the use of reference standards analyzed every 15 samples. Differences > 10% determined if the curve should be examined again. Accuracy of the method was evaluated by analysis of the certified reference material DORM-3 (National Research Council—Canada), performed by the same procedure employed for the samples. The concentration results were in agreement with the certified values, at a 95% confidence level, according to Student t test. The average ($n = 3$) measured and certified concentrations (in parenthesis) were, in mg/kg, as follows: As: 6.82 ± 0.78 (6.88 ± 0.30); Cd: 0.32 ± 0.04 (0.290 ± 0.020), Cu: 14.4 ± 0.9 (15.5 ± 0.63), Hg: 0.38 ± 0.04 (0.382 ± 0.060), Pb: 0.370 ± 0.030 (0.395 ± 0.050) and Zn: 50.4 ± 5.1 (51.3 ± 3.1).

2.4. Biochemical analyses

Total cholesterol, triglycerides and glucose levels in serum were analyzed using Cobas Integra 400 Plus® (Roche Diagnostics). Serum homocysteine was analyzed by an automated chemiluminescent enzyme immunoassay kit (Immulite 2000).

2.5. Inflammation markers

Interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) were measured in serum on Immulite® by ELISA according to the manufacturer's instructions (eBIOSCIENCE). Cytokine levels were expressed as pg/ml. Assay sensitivity was 2.0 pg/ml for both IL-1β and IL-6, and 4.0 pg/ml for TNF-α.

2.6. Nitric oxide analysis

Serum nitric oxide (NO) levels, indicated by nitrites/nitrates (NOx) concentration, were measured in serum on Cobas MIRA® [24]. Results were expressed as mmol/l.

2.7. Glutathione peroxidase activity

Enzymatic activity of glutathione peroxidase (GPx) was determined in blood [25]. This method is based on the oxidation of NADPH, which can be measured as the decrease in absorbance at 340 nm. Results were expressed in μmol NADPH/min ∗ mg protein.

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