Journal of Trace Elements in Medicine and Biology 35 (2016) 61–65

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Trace metal levels in serum and urine of a population in southern Brazil

G.H.O. Rocha[a], C. Steinbach[a], J.R. Munhoz[a], M.A.O. Madia[a], J.K. Faria[a], D. Hoeltgebaum[a], F. Barbosa Jr.[b], B.L. Batista[b,c], V.C.O. Souza[b], S.B. Nerilo[d], E. Bando[d], S.A.G. Mossini[d], P. Nishiyama[d,*]

[a] *Laboratory of Toxicology, Course of Pharmacy, State University of Maringá, Maringá, Paraná, Brazil*
[b] *Laboratory of Metal Toxicology and Essentiality, Department of Clinical, Toxicological and Bromatological Analyses, Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil*
[c] *Natural and Human Sciences Centre, Federal University of the ABC, Santo André, São Paulo, Brazil*
[d] *Laboratory of Toxicology, Department of Basic Health Sciences, State University of Maringá, Maringá, Paraná, Brazil*

ARTICLE INFO

Article history:
Received 25 August 2015
Received in revised form 1 December 2015
Accepted 18 December 2015

Keywords:
Fluid analyses
Dynamic reaction cell inductively coupled plasma mass spectrometry
Non-occupational exposure
Population studies

ABSTRACT

This study aimed to evaluate serum and urine concentrations of several trace metals of a non-directly exposed population in southern Brazil and establish reference values. Serum and urine samples were obtained from 240 volunteers (175 males and 65 females, age ranging from 18 to 74 years old). Levels of arsenic, chromium, cobalt, copper, lead, nickel, manganese and zinc were determined by means of dynamic reaction cell inductively coupled plasma mass spectrometry (DRC-ICP-MS). Comparison between genders resulted in no significant difference for all metals but serum copper, as concentrations are higher in females than males. For most metals assessed, a negative correlation between serum concentrations and age was found, but no significant correlation was found between urine concentrations and age.

1. Introduction

Many metals are vital to human health in trace amounts, but can be harmful in greater levels. Other metals, including some metalloids and most heavy metals, have no role in human physiology and can cause intoxications. Everyday exposure to pollution and ingestion of contaminated food/water can cause accumulation of such metals in the body; chronic exposure to metals can lead to impairment of human health, being a serious concern for health agencies [1]. Large scale studies have been conducted in different countries in order to establish reference values for trace metal concentrations of different populations, which serve as a basis for further exposure assessments and other toxicological studies [2,3]. While many such surveys have been conducted worldwide, Brazil falls short on the amount of studies attempting the same.

Surveillance of trace metal concentrations by means of biomonitoring is effective in assessing the exposure profile of a specific population, and different analytical techniques can be used. While flame atomic absorption spectroscopy (FAAS) has been the analytical technique of choice for trace metal determination for quite some time, nowadays more powerful methods are being employed. One such method refers to inductively coupled plasma mass spectrometry (DRC-ICP-MS), a much more robust and sensitive technique than FAAS, which has been extensively used in the last years for analysis of trace metals. DRC-ICP-MS not only has better performance, but it also provides consistent results in different matrices, be them serum, plasma, whole blood or urine [4,5].

This work aimed to evaluate trace concentrations of several metals of a population in southern Brazil by means of a simple "dilute and shoot" procedure using DRC-ICP-MS, in order to determine reference values in different matrices.

2. Material and methods

The studied population was selected from a local blood center in the city of Maringá, located in northwestern Paraná, one of Brazil's southern states. Samples were collected within a 6 month period (January–June, 2013). Blood donors were briefed about the study and those that agreed on participating were further interviewed

Abbreviations: FAAS, flame atomic absorption spectroscopy; DRC-ICP-MS, dynamic reaction cell inductively coupled plasma mass spectrometry.

* Corresponding author. Fax: +55 4430116029.
E-mail address: pnishiyama@uem.br (P. Nishiyama).

http://dx.doi.org/10.1016/j.jtemb.2015.12.005
0946-672X/© 2016 Published by Elsevier GmbH.

Table 1
Serum trace metal reference levels.

Metal analyzed	GM	95% CI GM	P25	P50	P75	P95
Arsenic						
Male	1.153	[1.093, 1.217]	0.981	1.134	1.592	1.904
Female	1.195	[1.099, 1.299]	1.014	1.206	1.598	1.85
Cobalt						
Male	0.1501	[0.1360, 0.1657]	0.1123	0.164	0.239	0.336
Female	0.1584	[0.1348, 0.1861]	0.1115	0.17	0.253	0.496
Copper						
Male	891.4	[855.5, 928.8]	735.7	909.7*	1070	1419
Female	1401	[1270,1545]	1027	1493*	1814	2801
Chromium						
Male	1.951	[1.866, 2.041]	1.702	1.96	2.471	3.005
Female	1.965	[1.831, 2.108]	1.625	1.866	2.555	2.998
Manganese						
Male	0.5295	[0.4759, 0.5892]	0.396	0.584	0.817	1.237
Female	0.5593	[0.4882, 0.6407]	0.3675	0.646	0.837	1.199
Nickel						
Male	0.6993	[0.6084, 0.8037]	0.4075	0.722	1.55	2.069
Female	0.8378	[0.6680, 1.051]	0.3838	0.982	1.75	2.526
Zinc						
Male	738.9	[708.5, 770.7]	630.9	741.7	845.2	1228
Female	700.9	[649.1, 756.7]	600.4	721.5	809.3	1002

Serum values are represented as μg/L.
* Statistically different according to Mann–Whitney *U* test when compared to its gender counter part, assuming $p < 0.05$.

so that data concerning age, sex, occupation and habits that might influence metal concentrations could be gathered. All volunteers signed an informed consent form in accordance with the Brazilian Ethics Committee (registered under the number 0375.0.093.000-11). A total of 240 volunteers participated in this study. The selected blood donors consisted only of adults of age ranging from 18 to 74 years old; 175 were male and 65 were female. A small amount of subjects were smokers and thus were not included in the study, as it is known that cigarette consumption does affect metal levels in body fluids [6]; also, the small number of smokers would not amount a sample group of sufficient size to allow proper analysis.

Blood and urine samples were obtained from each subject. Blood samples were collected by standard venous puncture and urine samples were collected by the volunteers themselves while they were urinating. Both types of samples were collected and stored in metal-free glass collection recipients that had been previously decontaminated. Decontaminating was carried out with all recipients washed with running water and bathed in a 10% nitric acid solution for 48 h; later, recipients were again washed in ultrapure MilliQ water and dried out at 40 °C. Finally, all recipients were stored until moment of use without making any contact with metallic surfaces or any kind of dust. Following blood collection, samples were centrifuged at 10,000 rpm for 10 min in order to obtain serum from each sample, which were then stored at −20 °C. Since samples would later be screened for metals in a different laboratory, storage of whole blood was not possible, and therefore serum was preferred as a matrix. Urine samples underwent no treatment after collection, and were stored at −20 °C until moment of analysis as well.

Serum and urine samples were fractioned into three different tubes at a volume of 100 μL and sent to colleagues at the Laboratory of Metal Essentiality and Toxicology at the University of São Paulo (Ribeirão Preto, Brazil). Prior to sample analysis, all samples (urine and serum) were diluted 1 + 19 in a solution containing 0.5% of sub-distilled HNO_3 (DST-100, Savillex, USA) and 0.005% (v/v) of Triton® X-100 (Sigma, USA) and analyzed according to Batista et al. [7] for serum and Batista et al. [8] for urine. The quality control of all analyzed samples was performed by using standard reference materials from the National Institute of Public Health from Canada (ICP04U04 for urine) and from the New York Department of Health from USA (NYSDOH 05S07 for serum). Certified standard reference materials were analyzed in each batch and were in agreement with reference values. The following isotopes were analyzed: ^{75}As (reaction gas H_2/Ar 5/95% used for urine during analysis), ^{60}Ni, ^{59}Co, ^{55}Mn, ^{64}Zn, ^{63}Cu and ^{52}Cr (NH_3 99.999% used for serum during analysis). Rhodium at 10 ng ml^{-1} was used as internal standard. All analyses took place in the period of June–July, 2013.

Data were analyzed using the software GraphPad Prism 5.0®. Mann–Whitney tests were conducted in order to compare metal levels by sex (only creatinine corrected results were analyzed this manner) and Spearman correlation coefficients were determined to assess how metal concentrations correlated with age. Results were considered significant assuming $p < 0.05$.

3. Results

Overall metal values (Tables 1–3) did not follow a normal distribution as determined by D'Agostino and Pearson normality test; proposed reference values for each metal analyzed are presented as percentiles and geometric means with their respective 95% upper and lower confidence intervals.

Results for serum and urine metal levels found for male and female subjects are shown in the following order: arsenic, cobalt, copper, chromium, manganese, nickel and zinc. Male serum concentrations (μg/L): 1.153; 0.1501; 891.4; 1.951; 0.5295; 0.6993 and 738.9. Female serum concentrations (μg/L): 1.195; 0.1584; 1401.0; 1.965; 0.5593; 0.8378 and 700.9. Male urine concentrations (μg/L): 15.17; 0.2563; 29.69; 2.936; 1.160; 1.996; 278.9. Female urine concentrations (μg/L): 11.74; 0.2593; 27.97; 2.143; 1.169; 1.654; 193.6. Male urine concentrations (μg/g creatinine): 12.48; 0.2047; 24.53; 2.217; 0.9352; 1.523 and 228.9 μg/g creatinine. Female urine concentrations (μg/g creatinine): 11.34; 0.2505; 27.01; 1.990; 1.104; 1.475 and 186.9 μg/g creatinine.

Mann–Whitney tests comparing results between genders resulted in no significant statistical difference for all metals but serum copper, as concentrations are higher in females than males.

Spearman's test correlating age and metal concentrations showed a negative correlation between serum concentrations and age for most metals assessed, with the exception of nickel and zinc