

Assessment of Trace Elements in Scalp Hair of a Young Urban Population in Brazil

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Abstract The aim of this study was to establish background values for toxic and essential elements in hair, interelement correlations and the differences in levels between genders in a healthy young population from Southern Brazil. Hair samples ($n=167$) were collected from healthy students aged 12–18 years. Trace element concentrations in hair were determined by inductively coupled plasma mass spectrometry. The study provided relatively low values for toxic elements and balanced concentrations for the essential elements in the adolescents' hair with reliable reference data. Interestingly, this study also demonstrated statistical correlations considered newfound between the elements in hair. Hair mercury levels were influenced by gender; with males presenting higher values. The overall findings of the present study, with respect to the estimated chemical elements, are of prime importance in the evaluation of reference values for determining environmental effects on children living in urban areas.

Keywords Hair · Trace elements · Reference values · Children · ICP-MS

Introduction

Hair is a biological specimen that is easily and noninvasively collected with minimal cost, and it is easily stored and transported to the laboratory for analysis [1]. These attributes make hair an attractive biomonitoring substrate with a widespread use to assess human exposure to toxic elements and deficiency to essential elements [1–7]. However, there is insufficient epidemiological data providing background values for trace elements in hair in

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non-exposed and healthy populations. These data are even scarcer for children [8]. Taking into account the inherent susceptibility of children to toxic substance exposure, it is important to determine the background levels of toxic and essential elements in hair to differentiate exposed to non-exposed groups. Moreover, the knowledge of trace elements profile in hair matrices from children living in different parts of the world is primary in epidemiological studies using this matrix, since specific environment and ethnic conditions may modify the background levels. It is also important to determine the interaction between toxic and essential elements in hair, mostly because of the lack of such studies and the inconsistency of the results [9, 10].

Trace element analysis in hair samples has improved significantly over the last years. Improvements in instrumentation, such as inductively coupled plasma mass spectrometry (ICP-MS) and/or the use of sample preparation procedures with minimal risk of sample contamination, resulted in improved precision, accuracy, reliability, and detection limits [11–16].

The aim of the present study is to provide background values for toxic and essential elements in hair, the interelement concentration, and the differences in levels between genders in a healthy young population from South Brazil.

Materials and Methods

Sampling and Preparation

In July and August 2009, hair samples ($n=167$) were collected from healthy students from nine public schools in Porto Alegre, the capital of Brazil's southernmost state, Rio Grande do Sul. The city is situated at latitude $30^{\circ}01'59''$ S and longitude $51^{\circ}13'48''$ W, and is approximately 100 km from the Atlantic Ocean. The total population is 1,436,123 inhabitants distributed over an area of 497 km², with a population density of 289 inhabitants/km². Services are the principal economic activity, followed by industry and agriculture [17]. Before the hair samples were collected, the participants filled out a questionnaire with questions about individual and socioeconomic characteristics and medical history. None of the students reported having a chronic disease. The study population was considered to be a typical group of students living in an urban area in Brazil, not exposed to toxic substances and not living in unusual conditions. Hair samples were collected from the nape of the neck, as near as possible to the scalp, using a stainless steel scissors. The samples were sealed separately in labeled polyethylene zip lock bags and were not opened until they were cleaned and processed in the laboratory. Informed consent was obtained from each survey participant, as well as from their parents and school supervisors, following guidelines approved by the Ethics Committee of the Federal University of Health Sciences in Porto Alegre.

Reagents

All reagents used were of analytical-reagent grade except HNO₃, which was previously purified in a quartz sub-boiling still (Kürner) before use. A clean laboratory and laminar flow hood, capable of producing class 100, were used for preparing solutions. High purity deionized water (resistivity 18.2 M Ω -cm) obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used throughout. All solutions were stored in high-density polyethylene bottles. Plastic bottles and glassware were cleaned by soaking in 10% (v/v) HNO₃ for 24 h, rinsed five times with Milli-Q water, and dried in a class 100 laminar flow hood before use. All operations were performed on a clean bench.

Instrumentation

All measurements were made with an ICP-MS (Elan DRC II PerkinElmer, Norwalk, CT, USA) with the use of high-purity argon (99.999%, White Martins, Brazil). A complete description of the instrumentation and apparatus are provided by Rodrigues et al. [18]

Determination of Trace Elements in Hair

Hair samples were washed according to the method proposed by Ohmori [19], with acetone, water, and acetone. After washing, samples were dried in a class 100 laminar flow hood before analysis. Hair samples were cut into 1-cm lengths before analysis. Approximately 20 mg of each 1-cm hair sample was weighed into metal-free polypropylene tubes. Trace element levels in hair were determined in samples according to the method proposed by Rodrigues et al. [18]. Briefly, after the hair samples were accurately weighed into 15-mL conical tubes, 1 ml of 25% m/v tetramethylammonium hydroxide (TMAH) solution was added to the tubes and incubated at room temperature overnight. Following this, the volume was made up to 10 mL, with a solution containing 1% v/v HNO₃. After that, samples were directly analyzed by ICP-MS.

Quality Control of the Results

Quality control (QC) of data was ensured by analyzing reference materials provided by the Institut National de Santé Publique du Québec, Canada (INSP-external quality assessment scheme) (EQAS) for trace elements in hair. Reference samples were analyzed before and after ten ordinary samples. Obtained values together with reference values are shown in Table 1.

Statistical Analysis

Since the data are nonparametric, Spearman's correlation was used to evaluate the correlation between trace element levels in all biomarkers. To evaluate statistical differences in the elemental concentrations between genders, the Mann–Whitney *U* test was used. The

Table 1 Analytical performance for the determination of trace elements in a reference hair sample ICP 03H09, provided by the Institut National de Santé Publique du Québec (INSP)

Analyte	ICP 03H09 hair (<i>n</i> =5)	
	Found value (µg/g)	Target value (µg/g)
As	0.60±0.04	0.64±0.14
Cd	0.82±0.02	0.92±0.20
Co	0.90±0.04	0.96±0.05
Hg	2.31±0.05	2.10±0.80
Mn	5.83±0.15	6.40±0.58
Mo	0.220±0.005	0.20±0.03
Pb	4.66±0.30	4.60±0.69
Sb	0.81±0.03	0.83±0.24
Se	1.05±0.04	0.90±0.39

SPSS statistical package version 15.0 was used for calculations (SPSS, version 15.0, Inc., Chicago, IL, USA). Statistical significance was set at 5%.

Results

The socio-demographic characteristics of the study population are summarized in Table 2. Of the 167 participants, 107 were female, and the mean age of the whole population studied was 13.9 years (range 12–18). Nearly half of the mothers had attended secondary education ($n=79$), others had attended only primary school ($n=72$), and 16 mothers had attended college. Socioeconomically, the population was distributed almost equally between the two ranges presented in the questionnaire.

The descriptive data (mean, range of the concentration, standard deviation, standard error, percentiles- 10, 25, 75, and 90- and median) of trace elements in the scalp hair of participants are summarized in Table 3. Additionally, values of elements obtained in other studies are presented for comparison with those from the present study. Among toxic elements, lead (Pb) had the highest mean concentration in the adolescents' hair, while antimony (Sb) and cadmium (Cd) had the lowest. Although these toxic elements were detected in the samples, the values found here are smaller compared to those found in other literature, evaluating subjects environmental and/or occupationally exposed to trace elements, or even in non-exposed subjects. Among the essential elements, manganese (Mn) (mean 0.31 $\mu\text{g/g}$) had the highest concentration in the hair, followed by selenium (Se) (mean 0.12 $\mu\text{g/g}$). Molybdenum (Mo) and cobalt (Co) had slightly lower values than the others cited above (mean 0.094 and 0.007 $\mu\text{g/g}$, respectively).

The interactions between the elements in hair of the whole population are presented in Table 4. Various interrelated elements were identified. Interestingly, correlations considered to be newfound can be seen in the table (bold type). Significant correlations between the elements, which corroborate current findings in the literature, are also presented in this table (italics).

Table 2 Socio-demographic characteristics of the study population

Total city population (inhab)	1,446,777
Area (km^2)	496.8
Population density (inhab/ km^2)	2,912
GDI per capita (R\$)	23,534
Study population	
N	167
Mean age \pm SD (years)	13.96 \pm 1.11
Range of age (years)	12–18
Gender (% ♂)	36
Mother's educational level (%)	
Primary school	43.15
Secondary education	47.35
College	9.5

Inhab, inhabitants; *Km*², square kilometer; *n*, number of students; *SD*, standard deviation; ♂, male gender; *R* \$, Brazilian currency

Table 3 Descriptive analysis of element concentrations accumulated in the hair of the Brazilian adolescents aged from 12–18 years old and results obtained in studies developed in other countries

Element	Minimum	Maximum	Mean	SD	SE	P10	P25	P75	P90	Median	Country	Range of age (years)	Mean ± SD or median	Reference
As	0.0010	0.0166	0.007	0.005	0.002	0.0011	0.0023	0.0122	0.016	0.006	Italy	10–13	0.08±0.09	Senofonte et al. [32]
											Kuwait	4–8	0.13 (0.11–0.16)	Fido and al Saad [33]
Pb	0.0004	0.7483	0.14	0.16	0.021	0.008	0.034	0.17	0.34	0.09	Korea	3–6	0.11±0.05	Park et al. [31]
											Spain	12–14	0.58±0.68	Ferré-Huguet et al. [34]
											Spain	12–14	0.32±0.3	Torrente et al. [14]
											India	4–12	1.56±0.18	Priya and Geetha [2]
											Kuwait	4–8	3.20 (2.80–4.0)	Fido and al Saad [33]
											Italy	10–15	3.19±2.54	Sanna et al. [35]
Cd											China	12–19	3.98-t-2.56	Meng et al. [36]
											Italy	10–13	7.37±6.32	Senofonte et al. [32]
											German	6–14	1.61	Seifert et al. [37]
											Moroccan	0–1	6.6±4.0	Souad et al. [38]
											Korea	3–6	1.68±1.12	Park et al. [31]
											Spain	12–14	0.02±0.01	Ferré-Huguet et al. [34]
											German	6–14	0.096	Seifert et al. [37]
											Kuwait	4–8	0.16 (0.13–0.18)	Fido and al Saad [33]
											Italy	10–13	0.21±0.27	Senofonte et al. [32]
											Spain	12–14	0.21±0.24	Ferré-Huguet et al. [34]
Mn											Spain	12–14	0.26±0.90	Torrente et al. [14]
											Italy	10–13	0.35±0.67	Senofonte et al. [32]
											Korea	3–6	0.29±0.18	Park et al. [31]

Table 3 (continued)

Element	Minimum	Maximum	Mean	SD	SE	P10	P25	P75	P90	Median	Country	Range of age (years)	Mean \pm SD or median	Reference
Co	0.0001	0.027	0.0074	0.006	0.0005	0.001	0.003	0.011	0.017	0.006	Korea	3–6	0.01 \pm 0.01	Park et al. [31]
Se	0.004	0.299	0.12	0.07	0.006	0.03	0.06	0.16	0.20	0.11	Italy	10–13	0.91 \pm 1.90	Senofonte et al. [32]
											Korea	3–6	0.75 \pm 0.14	Park et al. [31]
											India	4–12	3.37 \pm 0.40	Priya and Geetha [2]
Hg	0.004	0.873	0.14	0.18	0.02	0.009	0.02	0.15	0.42	0.07	Italy	10–13	1.09 \pm 4.08	Senofonte et al. [32]
											Turkey	14–73	0.43 \pm 0.29	Doğan-Sağlamtimur and Kumbur [1]
Mo	0.0007	0.2402	0.09	0.06	0.006	0.024	0.05	0.12	0.19	0.08	Spain	12–14	0.56 \pm 0.53	Ferré-Huguet et al. [34]
											India	4–12	0.37 \pm 0.04	Priya and Geetha [2]
											Kuwait	4–8	0.30 (0.24–0.40)	Fido and al Saad [33]
											Spain	12–14	0.70 \pm 0.48	Torrente et al. [14]
Sb	0.0001	0.0243	0.005	0.006	0.0007	0.0003	0.0008	0.006	0.02	0.002	Korea	3–6	0.47 \pm 0.25	Park et al. [31]
											Korea	3–6	0.07 \pm 0.05	Park et al. [31]
											Italy	10–13	0.35 \pm 0.79	Senofonte et al. [32]
											Kuwait	4–8	0.06 (0.05–0.09)	Fido and al Saad [33]

Data about the elements are expressed in $\mu\text{g g}^{-1}$. SD standard deviation; SE standard error; P10, P25, P75, and P90 correspond to respective percentiles

Table 4 Correlation between elements in hair of Brazilian children aged 12–18 years^a

	Pb	Cd	Mn	Co	Se	Hg	Mo	Sb
As	0.115	-0.067	-0.345	0.036	-0.393	0.309	-0.115	0.309
Pb		0.698*	<i>0.497*</i>	<i>0.402*</i>	0.005	0.301	0.220	0.352*
Cd			0.505*	0.616*	<i>0.266*</i>	0.229	<i>0.463*</i>	0.250
Mn				0.744*	<i>0.467*</i>	0.141	<i>0.439*</i>	0.084
Co					0.464*	<i>0.333*</i>	0.710*	0.283*
Se						0.190	<i>0.346*</i>	-0.136
Hg							<i>0.338*</i>	-0.196
Mo								-0.036

^a Spearman correlation test. *Correlation is significant at the 0.01 level (two-tailed). Entries in *italics* indicate the correlations found in the literature and confirmed by the present study. Entries in **bold face**, correlations are considered newfound

In this study, the elemental content of human scalp hair was also evaluated according to gender (Table 5). Mercury had statistically higher concentrations in male hair than in female hair. There were no statistical differences between the genders for the other elements, although arsenic concentrations tended to be higher in females.

Discussion

Due to their silent accumulation in the body and potential toxic effects, it is urgent to establish critical or baseline levels of trace elements in human beings [20, 21]. This is even more urgent for susceptible populations like children. The exposure to toxic metals, especially in the early stages of life, can produce pronounced and even permanent effects [22].

Table 5 Mean values and standard deviations of concentrations of essential and toxic elements obtained in hair compared by gender

Concentration of elements ($\mu\text{g g}^{-1}$)	Gender				P value
	Female		Male		
	Mean	SD	Mean	SD	
As	0.010	0.005	0.004	0.003	0.057
Pb	0.12	0.16	0.18	0.17	0.140
Cd	0.004	0.004	0.005	0.005	0.286
Mn	0.29	0.28	0.35	0.30	0.222
Co	0.007	0.006	0.008	0.006	0.675
Se	0.12	0.07	0.12	0.06	0.644
Hg	0.09	0.09	0.22	0.25	0.009 ^a
Mo	0.08	0.06	0.10	0.06	0.201
Sb	0.005	0.005	0.005	0.006	0.934

^a Statistically significant. SD standard deviation

The advantages of assessing trace elements in hair are that hair collection is noninvasive, and good response rates can be achieved in population subgroups such as children who are difficult to obtain blood specimens from [23].

Besides exposure to a contaminated environment, the levels of trace metals in hair are influenced by factors, such as hygiene and cosmetic use [24], metabolism [25], and eating habits [26]. Despite their importance, these factors are difficult to correlate with hair element content, mainly due to data collection difficulties, which are worse when dealing with youngsters. Data obtained in the present investigation have sufficient quality to serve as reference values for toxic and essential elements in the hair of young individuals. A comparison of the results of the present study with those from studies carried out in other countries (Table 3) shows that the lowest mean values for toxic elements and balanced concentrations for the essential elements are the ones from this study. Concerning the influence of age and gender, we attempted to compare the concentrations obtained in the present study (Table 3) with those from papers that evaluated similar age ranges and provided whole population data. Additionally, in this comparison, when a disease or occasional environmental exposure was part of a study's hypothesis, the concentrations were taken from the control groups (Table 3).

Moreover, in this study, we found the following statistical correlations between the elements (the correlations are grouped according to magnitude):

- Weak to moderate ($r < 0.5$): Pb×Mn, Co, Sb; CdxSe, Mo; MnxSe, Mo; CoxHg; SexMo, Co; HgxMo; SbxCo.
- Strong ($0.5 < r < 0.7$): Pb×Cd; CdxMn, Co.
- Very strong ($r > 0.7$): MnxCo; CoxMo.

Table 4 shows that some of the correlations between the elements found in hair in the present study strongly corroborate the results from other studies [27–30]. There are several correlations reported in this paper that can be considered newfound, for instance the high-magnitude and statistically significant correlations between CoxMn and CoxMo ($r = 0.744$, $P < 0.01$ and $r = 0.710$, $P < 0.01$, respectively) (Table 4). Further statistical correlations were CdxPb ($r = 0.698$, $P < 0.01$), CdxCo ($r = 0.616$, $P < 0.01$), CdxMn ($r = 0.505$, $P < 0.01$), Pb×Sb ($r = 0.352$, $P < 0.01$), and CoxSb ($r = 0.283$, $P < 0.01$). Other correlations already published in the literature are also given in Table 4 (italic).

The data in Table 5 show gender differences in element distribution. Hg concentrations were found to be statistically higher in the hair of males in this study. Chajacka et al. [27] found the same tendency in their study, except for Rb, whose concentration was higher in female hair. However, neither Park et al. [31] nor Caroli et al. [21] found these male–female differences in their study. Rather, Park et al. [31] found statistically greater Pb concentrations in the hair of males while Caroli et al. [21] established that Cd, Mo, and Pb concentrations were statistically higher in the hair of females.

The overall findings of the present study with respect to estimated chemical elements are of prime importance in evaluating reference values for environmental effects on urban children. Further work in evaluating the elemental content of environmental samples with larger sample sizes in distinct age ranges (including other susceptible populations such as the elderly), using populations living in different areas and collecting information on food intake/individual habits will ensure a better understanding of the critical concentrations of trace elements in normal people.

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