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Determination of trace elements in scalp hair of an elderly population by neutron activation analysis

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Neutron activation analysis was applied to assess trace elements concentrations in head hair from healthy elderly people living in the São Paulo metropolitan area. Concentrations of As, Br, Ca, Cl, Co, Cr, Cu, Fe, K, La, Mn, Na, Sb, Se and, Zn were determined. Comparisons were made between the results obtained for dyed and non-dyed hair as well as for hair from females and males of two different age groups. The results were also compared with range values established by clinical laboratories and published data.

Introduction

Over the past three decades determinations of trace element concentrations in human hair have become increasingly popular for biomonitoring environmental exposure, evaluating systematic intoxication, assessing nutritional status and diagnosing diseases. The advantages of analyzing hair when compared with other tissues such as blood or urine, are: (1) hair samples can be collected painlessly and easily; (2) it accumulates elements over an extended period of time providing a retrospective index of trace elements and (3) special storage conditions for samples are not needed for transportation and preservation. Hair has the capacity to accumulate elements during extended periods which allows the determination of high element concentrations and easier analyses.[1,2]

Although hair is the most convenient tissue for trace element determinations there is considerable controversy over using hair to evaluate nutritional status and clinical symptoms. There is much literature discussing the interpretation and reliability of the results of hair analysis[3–5] and about correlation of element concentrations in hair to those in biological fluids and internal organs.[6–8]

The primary reason for the divergent opinions concerning the validity of hair analysis for disease diagnosis and nutritional status assessment is the difficulty in establishing normal or reference ranges for trace elements in hair. Establishment of normal reference ranges for element concentrations in hair of presumed healthy population has been a subject of a number of studies.[9–11] However, these values have shown wide variation due to natural variance of hair composition as a possible consequence of age, gender, ethnicity, geographic location, dietary habits, hair type, hair treatment and exogenous exposures.

Furthermore, there is also difficulty in distinguishing between exogenous and endogenous contamination. Hair sample preparation and cleaning procedures vary greatly leading to a wide range of analytical results.[12] There is no standardized washing procedure at present available capable of reliably removing external contaminants without affecting endogenously deposited elements.[2,13–16] Often, results from commercial laboratories can be of poor quality. However, reference materials are now available for quality control and the problem of analytical laboratory errors has been overcome.

In the present study scalp hair samples were analyzed by instrumental neutron activation analysis to evaluate trace elements of a selected healthy elderly population living in the São Paulo Metropolitan region. At present the fastest growing population group in the world is that over 60 years old and data on hair analysis for this group are very scarce. Therefore, this study may contribute to the establishment of the range of normal values for trace elements in hair for the elderly population.

Experimental

Sampling

A physician or trained employee performed the collection of hair in accordance with a defined protocol in order to obtain a representative sample and reliable data. Hair was collected from a healthy elderly population aged 50–87 years by using a pair of stainless steel scissors. The participants lived in the São Paulo Metropolitan region and visited the Hospital das

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Clínicas of the São Paulo University Medical School for medical checkups. The participants were informed about the purpose of our study and invited to receive medical and biochemical examinations. All were interviewed to collect information about their state of health, physical activities, and, in particular about hair treatment. An information form was filled out with personal and collection data. The following exclusion parameters were used to select the individuals: (a) alcoholism and smoking (b) hepatitis, human immune virus (HIV) and chronic disease, diabetes, hypertension (c) atypical dietary habits, (d) blood transfusion within the previous 6 months, anemia, (e) supplement intakes and (f) mental disorders.

The Ethics Committee of the Hospital das Clínicas of the São Paulo University Medical School approved the study. For all donors, about 500 mg of hair were cut close to the scalp of the occipital region of the head, placed in polyethylene bags and labeled with the donor's name. The length of hair did not exceed 7 cm.

Sample treatment

In the laboratory, hair filaments were cut in lengths smaller than 2 mm using a pair of stainless steel scissors. Next, each hair sample was placed in a beaker and washed four times using each one of the following solutions: 2% non-ionic detergent Triton X100, acetone p.a. Merck and purified, water purified by using a Millipore system. BORELLA et al.[13] carried out a systematic study on washing procedure for trace element determination in hair and proposed Triton X100 as reliable for removal of external contamination. The washed samples were placed on Whatman filter paper and dried at room temperature inside a class 100 laminar flow hood. Certified reference materials were analyzed (without any treatment) for quality control.

Preparation of synthetic standards of elements

Synthetic standards were prepared by pipetting 50 μl of the elemental standard solutions onto sheets of Whatman No. 40 filter paper. These solutions containing one or more elements were prepared using certified standard solutions provided by Spex Certiprep Chemical, USA. All the pipetors and volumetric flasks were calibrated before use. These filter sheets were dried at room temperature inside a desiccator and then placed into clean polyethylene bags and sealed. In these standards the quantities of each element, in μg (in parentheses) were the following: As (1.5), Br (5.0), Ca (500.1), Cl (200.0), Co (0.150), Cr (2.0), Cu (100.0), Fe (350), K (601.6), La (0.597), Mn (1.5), Na (100.0), Rb (10.0), Sb (0.6), Se (8.0) and Zn (35.0).

Neutron activation analysis procedure

Aliquots of about 180 mg of hair weighed in polyethylene bags were irradiated in the IEA-R1 nuclear reactor along with the synthetic standards of the elements. Two separate irradiations were used to determine elements having short and long-lived radioisotopes. Fifteen-second irradiations with a thermal neutron fluence rate $1.4 \cdot 10^{12}$ n·cm^{-2}·s^{-1} were carried out for Cl, Mn and Na determinations. Sixteen-hour irradiations with a thermal neutron fluence rate of about $5 \cdot 10^{12}$ n·cm^{-2}·s^{-1} were performed for As, Br, Ca, Co, Cr, Cu, Fe, K, La, Na, Rb, Sb, Se and Zn determinations. After adequate decay times, the irradiated samples and standards were measured by a hyperpure Ge detector Model GX2020 coupled to Model 1510 Integrated Signal Processor, both from Canberra. The resolution (FWHM) of the system was 0.90 keV for 122 keV gamma-ray peak of ^{57}Co and 1.87 keV for 1332 keV gamma-ray of ^{60}Co. Samples and standards were each measured at least twice for different decay times. Counting times from 200 to 50,000 seconds were used, depending on the half-lives or activities of the radioisotopes considered. The radioisotopes measured were identified according to their half-lives and gamma-ray energies. The concentrations of elements were calculated by the comparative method. Radioisotopes used in hair analyses were: ^{76}As, ^{82}Br, ^{47}Ca, ^{38}Cl, ^{60}Co, ^{51}Cr, ^{64}Cu, ^{59}Fe, ^{42}K, ^{140}La, ^{56}Mn, ^{24}Na, ^{86}Rb, ^{122}Sb, ^{76}Se and ^{65}Zn.

Quality control was performed by analyzing NIST 1566b Oyster Tissue and NIST 2976 Mussel Tissue provided by the National Institute of Standards and Technology (NIST), USA IAEA-085 Human Hair from International Atomic Energy Agency, Austria and INCT-MPH-2 Mixed Polish Herbs from Institute of Nuclear Chemistry and Technology, Poland. Since there are no certified hair reference materials with certified values for all elements analyzed in this study, these matrices were analyzed by applying the same experimental conditions used for hair analyses and were evaluated on a dry weight basis, as recommended in their certificates.

Results and discussion

For quality control of the analytical results *Z*-score or standardized difference values[17] were calculated for the CRMs. The *Z*-score values presented in Fig. 1 indicate $|Z\text{-score}|<3$ which means the analytical results obtained are within the ranges of certified data at the 99% confidence level. The reproducibility of the results was also evaluated by the analyses of a hair sample in triplicates. The relative standard deviations of the results

were lower than 5% for the elements As, Br, Ca, Cl, Co, Fe, K, Mn, Se and Zn. For the elements Cu, Cr, La, Na and Sb the relative standard deviations ranged from 5.8 to 7.0%.

Since most individuals of the elderly female group selected in this study had dyed hair, the influence of this parameter was also evaluated. Comparisons were made between the results obtained for dyed and non dyed hair by applying Student's *t*-test. These results presented in Table 1 indicate no significant difference between dyed and non-dyed hair for the elements As, Ca, Cl, Co, Cr, Cu, Fe, La, Mn, Sb and Zn (for $\alpha = 0.05$). In the dyed hair, Br and Se concentrations were lower but K and Na were higher than those found for non-dyed hair. The qualitative analyses of three different brands of hair dyes indicated high content of Na.

Concentrations of elements in hair of males and females presented in Table 2 indicate significant difference only for the elements Ca and Sb ($\alpha = 0.05$). Differences in the element concentrations between female and male hair have been also reported.[10,18] The overall order of decrease in mean concentration found in hair of female individuals was: Ca > Cl > Zn > Na > K > Fe > Cu > Br > Mn > Se > Cr > Co > As > Sb > La, while for the male individuals the order was: Cl > Ca > Zn > Fe > Cu > K > Na > Br > Se > Mn > Cr > As > Co > La.

A comparative study based on two different age groups of healthy elderly population was also carried out (Table 3). Student's *t*-test applied indicated that the element concentrations of hair from elderly group aged 50 to 70 years did not present significant difference from those found for the group of 71 to 87 years. KHALINQUE et al.[19] also observed no appreciable change in metal concentrations as a function of age.

Table 4 shows the results obtained for the analyses of hair samples. To our knowledge, hair reference values in elderly healthy populations are very scarce. Consequently, an adequate comparison with the data of elderly population was not possible. In spite of the several individual characteristics such as age, gender, ethnicity and geographic location that might affect trace element levels in hair, our findings agree with data used as reference values in clinical laboratories[20,21] and, those recently published data by other authors.[1,22,23] The element concentrations measured in hair for an elderly group are of a similar order of magnitude or they are within the wide range of published reference levels.

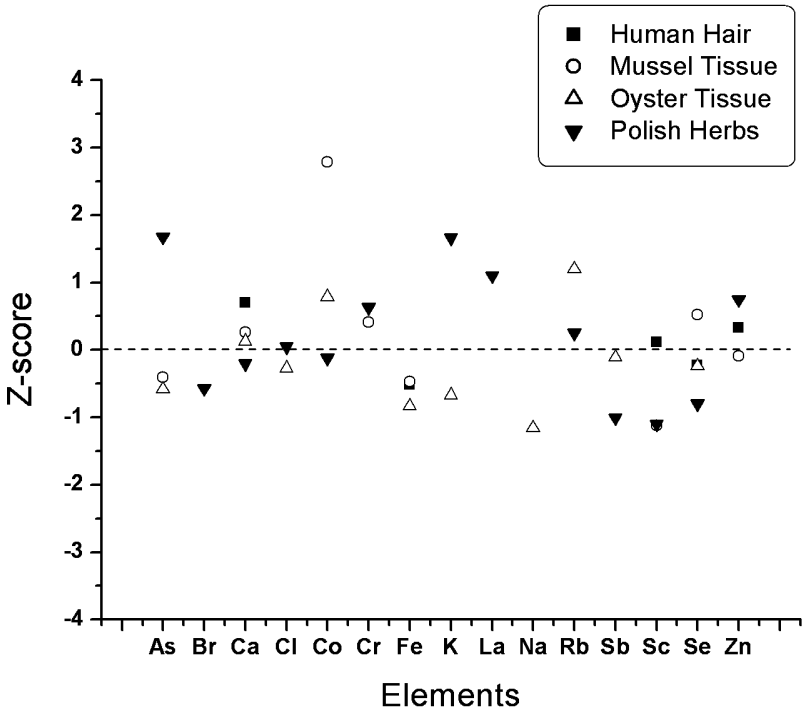

Fig. 1. Z-score values for elements determined in certified reference materials: NIST 1566b Oyster, NIST 2976 Mussel Tissue, IAEA-085 Human Hair and INCT-MPH-2 Mixed Polish Herbs

Table 1. Concentrations of elements in dyed and non-dyed hair from female subjects

Element	Dyed hair[a]			Non–dyed hair[b]		
	Mean ± SD[c]	Median	Range	Mean ± SD	Median	Range
As, μg·kg^{-1}	21.4 ± 24.7	9.71	1.6–85.4	31.4 ± 20.3	26.7	9.7 – 79.7
Br, μg·kg^{-1}	1112 ± 1137	816	106–4096	2086 ± 1500	1649	532 – 4975
Ca, mg·kg^{-1}	924 ± 782	545	72–2349	518 ± 417	364	176 – 1918
Cl, mg·kg^{-1}	524 ± 832	72.6	7.4–3187	763 ± 767	594	19.5 – 2421
Co, μg·kg^{-1}	21.7 ± 9.4	21.7	7.1–42.8	38.1 ± 68.1	19.2	7.6 – 277.0
Cr, μg·kg^{-1}	74 ± 46	66.6	27–216	92 ± 76	57	18 – 242
Cu, mg·kg^{-1}	11.5 ± 5.3	9.5	5.7–23.4	13.0 ± 9.0	9.1	6.13 – 40.9
Fe, mg·kg^{-1}	14.8 ± 15.2	9.9	5.9–73.4	13.7 ± 6.6	10.3	6.6 – 13.7
K, mg·kg^{-1}	31.1 ± 85.1	3.64	0.86–345.3	4.5 ± 7.8	4.90	0.48 – 31.1
La, μg·kg^{-1}	12.8 ± 14.4	6.7	3.04–58.0	7.8 ± 4.4	7.0	2.5 – 17.5
Mn, μg·kg^{-1}	428 ± 305	345	120–1341	345 ± 297	274	80 – 1278
Na, mg·kg^{-1}	35.3 ± 96.4	3.68	1.37–391.7	4.0 ± 3.4	2.94	1.1 – 13.8
Sb, μg·kg^{-1}	20.5 ± 17.6	13.7	3.0–72.5	26.7 ± 26.6	13.3	2.1 – 88.5
Se, μg·kg^{-1}	301 ± 80	304.6	156–430	393 ± 129	378	249 – 793
Zn, mg·kg^{-1}	114 ± 35	120	30–162	128 ± 44	136	31 – 202

[a] Dyed hair from 20 women aged 61 to 81 years (mean = 73 years).
[b] Non-dyed hair from 15 women aged 50 to 87 years (mean = 71 years).
[c] Arithmetic mean and standard deviation.

Table 2. Concentrations of elements in female and male hair samples

Element	Female hair*			Male hair		
	Mean ± SD	Median	Range	Mean ± SD	Median	Range
As, μg·kg^{-1}	26.6 ± 22.7	20.2	1.59–85.4	42.4 ± 25.1	36.2	11.7 – 110
Br, μg·kg^{-1}	1513 ± 1367	1046	106–4975	1264 ± 663	1135	311 – 2635
Ca, mg·kg^{-1}	750 ± 675	479	71.7–2349	24 ± 146	222	66.7 – 587
Cl, mg·kg^{-1}	630 ± 802	301	7.4–3187	703 ± 744	460	56 – 2777
Co, μg·kg^{-1}	29.0 ± 47.0	19.3	7.1–277	23.4 ± 25.8	16.5	4.3 – 102.8
Cr, μg·kg^{-1}	81 ± 60	62	18–242	126 ± 97	89	20 – 323
Cu, mg·kg^{-1}	12.2 ± 7.1	9.5	5.7–23.4	15.9 ± 27.7	8.0	5.2 – 103.9
Fe, mg·kg^{-1}	14.8 ± 15.2	9.9	5.7–40.9	20.4 ± 22.1	13.8	5.7 – 88.2
K, mg·kg^{-1}	20.1 ± 62.6	4.6	0.48–345.3	5.6 ± 3.9	4.3	2.4 – 15.1
La, μg·kg^{-1}	10.4 ± 10.9	6.8	2.5–58.0	19.3 ± 27.7	9.2	2.5 – 102.3
Mn, μg·kg^{-1}	397 ± 304	317	80–1341	220 ± 124	185	71 – 466
Na, mg·kg^{-1}	20.6 ± 71.1	3.14	1.06–391.7	3.03 ± 1.42	2.55	1.8 – 6.8
Sb, μg·kg^{-1}	23.2 ± 21.9	13.5	2.1–88.5	38.5 ± 23.5	33.7	7.1 – 84.3
Se, μg·kg^{-1}	340 ± 111	336	156–430	383 ± 83	386	244 – 508
Zn, mg·kg^{-1}	121 ± 39	124	30–202	137 ± 27	137	96 – 188

* Number of individuals: 35 females and 12 males.

Table 3. Concentrations of elements in hair from two age groups

Element	Hair from group aged 50 to70 years*			Hair from group aged 71 to 87 years		
	Mean ± SD	Median	Range	Mean ± SD	Median	Range
As, μg·kg^{-1}	28.9 ± 32.9	16.7	1.6–110.0	31.8 ± 20.0	28.21	4.47 – 85.3
Br, μg·kg^{-1}	1363 ± 1434	954	226–4341	1497 ± 1138	1176	106 – 4975
Ca, mg·kg^{-1}	805 ± 701	535	140–535	537 ± 578	340	67 – 2239
Cl, mg·kg^{-1}	394 ± 784	64.2	7.4–3187	784 ± 754	559	38.8 – 2776
Co, μg·kg^{-1}	39.9 ± 66.1	22.1	9.6–276.8	21.5 ± 19.7	17.2	4.27 – 102.8
Cr, μg·kg^{-1}	71 ± 50	61.5	20.0–215.6	104.7 ± 81.1	73.8	17.5 – 322.5
Cu, mg·kg^{-1}	11.5 ± 4.9	9.5	6.7–23.4	14.2 ± 19.2	8.52	5.3 – 103.9
Fe, mg·kg^{-1}	15.2 ± 16.2	10.0	5.9–73.4	16.2 ± 15.0	12.5	5.8 – 88.2
K, mg·kg^{-1}	30.6 ± 90.7	4.9	2.4–345.3	8.9 ± 12.7	4.3	0.48 – 57.4
La, μg·kg^{-1}	9.9 ± 138	6.2	2.6–58.0	14.5 ± 19.1	7.8	2.4 – 102.3
Mn, μg·kg^{-1}	370 ± 324	317	71–1341	339 ± 258	253	117 – 1278
Na, mg·kg^{-1}	29.8 ± 100.1	3.42	1.91–391.7	8.6 ± 22.9	2.75	1.06 – 125.3
Sb, μg·kg^{-1}	23.1 ± 18.9	17.7	2.99–72.5	29.2 ± 24.8	19.8	2.11 – 88.5
Se, μg·kg^{-1}	321 ± 96	326	156–509	366 ± 109	359	173 – 793
Zn, mg·kg^{-1}	125 ± 32	124	45–162	125 ± 39	127	30 – 202

* Number of individuals: 15 for a group aged 50 to70 years and 31 for a group aged 71 to 87 years.

Table 4. Concentration of elements in hair from an elderly healthy population of São Paulo metropolitan region and literature values

Element	This work[a]			Mineral Lab[20]	Doctor's data[21]	SAIKI et al.[22]	SENOFONTE et al.[1]	ZHUK and KIST[23]
	Mean ± SD	Median	Range	Range	Range	Range	Range	Mean ± SD
As, $\mu g \cdot kg^{-1}$	30.9 ± 24.1	27.1	1.6–110.0	2000–3000	400[b]	6.7 – 126	20 – 930	–
Br, $\mu g \cdot kg^{-1}$	1452 ± 1230	1125	106–4975	–[c]	–	420 – 85400	–	5900 ± 700
Ca, $mg \cdot kg^{-1}$	630 ± 629	379	67–2349	200–600		118 – 1788	11 – 1301	420 ± 90
Cl, $mg \cdot kg^{-1}$	649 ± 779	355	7.4–3187	–	–	40.7 – 1339	–	1370 ± 210
Co, $\mu g \cdot kg^{-1}$	28 ± 41	19	4–277	200–1000	–	8.1 – 325	20 – 3830	120 ± 16
Cr, $\mu g \cdot kg^{-1}$	93 ± 73	71	18–323	500–1500	800–1250	68.2 – 753	30 – 19700	590 ± 70
Cu, $mg \cdot kg^{-1}$	13.25 ± 15.81	9.10	5.24–103.9	12–35	17–67	4.0 – 56.1	0.29 – 280	22 ± 2
Fe, $mg \cdot kg^{-1}$	15.9 ± 15.2	10.3	5.8–88.2	20–50	21–50	7.2 – 36.8	0.29 – 216	42 ± 5
K, $mg \cdot kg^{-1}$	16.5 ± 54.4	4.4	0.5–345	75–180	42–40	0.53 – 25.7	–	–
Mn, $\mu g \cdot kg^{-1}$	349 ± 278	262	71–1341	1000–10000	620–1970	105 – 2500	20 – 7590	610 ± 130
Na, $mg \cdot kg^{-1}$	15.8 ± 60.9	2.8	1.06–391.7	150–350	346–1080	1.50 – 29.7	–	290 ± 29
Sb, $\mu g \cdot kg^{-1}$	27.2 ± 23.0	18.3	2.11–88.5	–	–	3.1 – 848	–	280 ± 40
Se, $\mu g \cdot kg^{-1}$	350 ± 106	346	156–793	3000–6000	80–640	9.1 – 869	50 – 17500	870 ± 80
Zn, $mg \cdot kg^{-1}$	125 ± 37	124	30–202	160–240	104–288	106 – 264	24 – 477	200 ± 10

[a] Number of individuals = 47.
[b] One standard deviation above that represents about 68% of this selected healthy population.
[c] Data not published.

Conclusions

Preliminary data on trace element concentration in hair of healthy elderly population living in São Paulo metropolitan area were obtained and the comparisons made between the results indicated that most of the elements determined are within the range values established by clinical laboratories or published data. There are few data on hair analysis for elderly population. More analyses are needed in order to be able to use hair analysis in the study of health status of the elderly. Besides the findings indicate the necessity to establish an adequate procedure to remove the hair dyes without affecting endogenously deposited elements. We verified that there was difference between dyed and non-dyed hair for some element concentrations.

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