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Determination of trace elements in human head hair by neutron activation analysis

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Instrumental neutron activation analysis was used to measure concentrations of elements in hair samples from a group of patients of a medical clinic and from a control group. Elements Al, As, Br, Ca, Cd, Cl, Co, Cu, Fe, Hg, K, Mg, Mn, Na, Sb, Sc, Se, V and Zn were analyzed and comparisons were made between the results obtained for these two groups of individuals. Normal ranges for elemental hair by commercial laboratories are also presented, for comparison, with those results obtained for the control group of individuals living in São Paulo, Brazil. Precision and accuracy of the results were evaluated by analyzing NIES No. 5 Human Hair and SHINR GBW09101 Human Hair reference materials.

Introduction*

It is well-known that the trace element deficiency or excess may be harmful to the organism and there is an evidence that nutritional status and some diseases are accompanied by specific changes in hair composition.[1]

Recently in Brazil, the determination of trace element levels in hair has become very useful for routine clinical diagnosis of toxic and essential elements in the body mainly in the orthomolecular medicine field. Changes in the elemental composition of hair are believed to depend on alterations of the external and internal media of the human body[2] and it is considered that the hair of normal and healthy individuals contain each element within a well defined range of concentrations.[3] The basis of clinical testing is a comparison of one test with a normal range, so called reference value.

Therefore, for routine analysis of hair, it is of great interest to measure the well defined normal range of elemental concentrations. For the Brazilian population, these data are not available.

Besides, it is important to evaluate the precision, the accuracy and the detection limit of the method in order to demonstrate the validation of the results. Hair analyses present a series of advantages when compared to the analyses of biological fluids (blood, urine) or biopsies from tissues and they have been performed by a great number of laboratories on a commercial scale. Information concerning the analytical quality of their results are not presented, though.

In the present work, instrumental neutron activation analysis (INAA) was used to determine trace elements in hair samples from a group of patients of a medical clinic and from a control group of individuals living in São Paulo, Brazil. The quality assurance of the analytical results was evaluated by analyzing the certified reference materials: No. 5 Human Hair from National Institute for Environmental Studies (NIES), Japan and GBW09101 Human Hair from Shanghai Institute of Nuclear Research, China.

Experimental

Hair sample collection and preparation

Hair samples from patients (10 males and 30 females) were collected in collaboration with physicians of a medical clinic and the samples for a control group were collected from healthy people (25 males and 10 females) composed mainly of university students residing in São Paulo, Brazil. For the control group, an interview was carried out to collect general information and to evaluate the health condition of the individuals. The age of the individuals from the control group varied from 21 to 45 years. Hair strands were cut close to the scalp of the occipital region, and the length did not exceed 5 cm. About 500 mg of each sample were collected and placed in polyethylene bags. In the laboratory, hair filaments were cut in lengths smaller than 2 mm using a pair of stainless steel scissors. Then, each hair sample was placed in a beaker and washed four times using each one of the solutions: 2% non-ionic detergent Triton X100, acetone and water.[4] The water used in this work was distilled in a quartz apparatus. The washed samples were placed on Whatman filter paper and dried at room temperature inside a class 100 laminar flow hood. In the case of certified reference materials of hair, they were analyzed without any treatment.

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Table 1. Elemental concentrations in hair samples (in μg/g unless otherwise indicated) from control and patient groups of Brazilians obtained by INAA

Element	Controls				Patients			
	$x_G \times\div s_G$	Range	Median	n	$x_G \times\div s_G$	Range	Median	n
Al	14.0 ×÷ 1.8	1.6 – 37.4	13.7	35	14.3 ×÷ 2.07	0.60 – 46.0	14.6	34
As, μg/kg	22.2 ×÷ 1.8	6.7 – 126	22.0	35	22.9 ×÷ 2.5	4.0 – 386	19.6	36
Br	2.5 ×÷ 3.1	0.42 – 85.4	2.6	28	2.85 ×÷ 2.54	0.17 – 15.6	3.2	40
Ca	470 ×÷ 2.1	118 – 1788	457	33	485 ×÷ 2.2	87 – 2654	405	39
Cd, μg/kg	193 ×÷ 2.6	43.6 – 1220	172	17	188.6 ×÷ 2.0	70 – 1160	153	19
Cl	248 ×÷ 2.9	40.7 – 1339	257	33	420 ×÷ 3.0	18.5 – 2080	464	40
Co, μg/kg	32.4 ×÷ 2.5	8.1 – 325	25.9	33	35.6 ×÷ 2.1	10.1 – 160	34.0	40
Cr, μg/kg	172.9 ×÷ 1.8	68.2 – 753	163.5	32	143.7 ×÷ 2.0	43 – 970	141.5	40
Cu	16.4 ×÷ 2.1	4.0 – 56.1	14.9	35	15.4 ×÷ 2.6	0.38 – 168	14.9	37
Fe	14.5 ×÷ 1.5	7.2 – 36.8	14.0	33	14.6 ×÷ 1.7	6.5 – 51	14.0	41
Hg	1.05 ×÷ 2.51	0.08 – 4.75	1.16	35	0.91 ×÷ 2.2	0.17 – 4.37	1.06	40
K	3.76 ×÷ 2.3	0.53 – 25.7	3.50	30	4.1 ×÷ 3.2	0.12 – 45	3.95	39
Mg	57.1 ×÷ 2.4	7.7 – 267	54.5	29	68.0 ×÷ 2.3	16.8 – 231	76.0	23
Mn, μg/kg	393 ×÷ 2.2	105 – 2500	359	36	407 ×÷ 2.0	110 – 1900	420	41
Na	4.27 ×÷ 1.8	1.50 – 29.7	4.1	36	4.94 ×÷ 2.5	1.0 – 72.5	4.1	37
Sb, μg/kg	30.5 ×÷ 2.9	3.1 – 848	25.6	33	30.2 ×÷ 2.3	6.3 – 380	28.2	41
Sc, μg/kg	1.92 ×÷ 1.6	1.18 – 5.70	1.53	14	1.61 ×÷ 1.74	0.71 – 4.9	1.42	11
Se, μg/kg	351 ×÷ 2.3	9.1 – 869	425	31	363.8 ×÷ 1.3	200 – 652	385	38
V, μg/kg	19.5 ×÷ 3.7	1.5 – 54	54.2	9	34.2 ×÷ 2.0	12.2 – 86	35.5	6
Zn	159 ×÷ 1.3	106 – 264	157.8	32	156.5 ×÷ 1.3	59.1 – 262	159.4	42

$x_G \times\div s_G$ – geometric mean and standard deviation.
n – number of individuals.

Table 2. Normal ranges or tolerated limit values (in μg/g) for trace elements in human head hair

Element	Doctor's data[8]	MineraLab[8]	Clinice – Centro de Medicina Avançada[9]	Range for control (this work)
Al	2.9–82.5	20–40	17.00	1.60–37.4
As	0.4*	2.0–3.0	1.100	0.0067–0.126
Ca	204–712	200–600	220–1600	118–1788
Cd	1.6*	1.0–2.0	0.750	0.044–1.22
Co	not established	0.20–1.0	0.0040–0.3000	0.008–0.325
Cr	0.80–1.25	0.50–1.50	0.011–0.370	0.068–0.753
Cu	17–67	12–35	5.48–40.00	4.0–56
Fe	21–50	20–50	5.46–13.70	7.2–37
Hg	3.0*	2.5–5.0	1.30	0.08–4.75
K	42–40	75–180	5–40	0.53–26
Mg	29–137	25–75	20–130	7.7–267
Mn	0.62–1.97	1.0–10	0.070–1.000	0.105–2.50
Mo	0.59–2.55	0.10–1	0.040–0.320	
Na	346–1080	150–350	10–130	1.50–30
Se	0.08–0.64	3.0–6.0	0.20–5.46	0.009–0.869
V	–	0.50–1.0	0.070–0.300	0.0015–0.054
Zn	104–288	160–240	142.0–248.0	105–264

* One standard deviation above mean that represents approximately 68% of this selected healthy population. This limit was established by Doctor's data.

Table 3. Trace elements in certified reference materials (in μg/g unless otherwise indicated) of human hair

Element	NIES No.5 Human Hair		GBW0901 Human Hair	
	This work	Ref. 10	This work	Ref. 11
Al	295 ± 45	(240)*	16.4 ± 2.8	13.3 ± 2.3
As, μg/kg	88 ± 13		537 ± 45	590 ± 71
Br	102 ± 1	(90)	0.664 ± 0.085	(0.602)*
Ca	820 ± 44	728 ± 30	984 ± 73	1090 ± 72
Cd, μg/kg		200 ± 30	90.2 ± 17	95 ± 12
Cl	251.6 ± 9.5	(250)	151 ± 19	(152)
Co, μg/kg	108 ± 2	(100)	127 ± 13	135 ± 8
Cr	0.92 ± 0.09	1.4 ± 0.2	4.07 ± 0.45	4.77 ± 0.38
Cu	13.4 ± 1.9	16.3 ± 1.2	22.1 ± 3.0	23.0 ± 1.4
Fe	197 ± 19	225 ± 9	77.0 ± 4.8	71.2 ± 6.6
Hg	4.25 + 0.24	4.4 + 0.4	2.02 ± 0.10	2.16 ± 0.21
K	30 ± 3	34 ± 3	16.7 ± 3.9	(11.8)
Mg	308 ± 36	208 ± 10	96.4 ± 10.4	105 ± 6
Mn	5.00 ± 0.02	5.2 ± 0.3	2.68 ± 0.16	2.94 ± 0.20
Na	24 ± 4	26 ± 1	269 ± 19	266 ± 12
Sb, μg/kg	66 ± 3	(70)	227 ± 40	(210)
Sc, μg/kg	49.5 ± 0.5	(50)	2.79 ± 0.15	(2.87)
Se, μg/kg	1470 ± 160	(1400)	574 ± 78	580 ± 50
V	0.55 ± 0.07		63.7 ± 7.2	(69)
Zn	162 ± 5	169 ± 10	180 ± 18	189 ± 8

* Numbers in parentheses are information values.

Table 4. Determination limit values (in μg/g) obtained in hair analysis by INAA

Element	Radiosotope and γ-ray energy, keV	Determination limit	Element	Radiosotope and γ-ray energy, keV	Determination limit
Al	^{28}Al (1779.0)	1.6	Hg	^{197}Hg (77.3)	0.18
As	^{76}As (559.1)	0.0073	K	^{42}K (1524.6)	9.2
Br	^{82}Br (776.5)	0.03	Mn	^{56}Mn (846.8)	0.13
Ca	^{47}Ca (159.4)	126	Mg	^{27}Mg (1014.4)	155
Cd	^{115}Cd (336.3)	0.23	Na	^{24}Na (1368.6)	11.4
Cl	^{38}Cl (1642.7)	25.9	Sb	^{122}Sb (564.2)	0.0033
Co	^{60}Co (1173.2)	0.0076	Sc	^{46}Sc (889.4)	0.0017
Cr	^{51}Cr (320.1)	0.16	Se	^{75}Se (264.7)	0.17
Cu	^{66}Cu (1039.2)	6.6	V	^{52}V (1434.4)	0.10
Fe	^{59}Fe (1099.2)	9.1	Zn	^{65}Zn (1115.6)	0.70

Preparation of synthetic standards of elements

Stock solutions of standards were prepared by dissolving high-purity metals, oxides or salts of elements with appropriate reagents. In the case of the standards of Al, As, Ca and Cu, Titrisol solutions of these elements from Merck were used. Diluted solutions containing one or more elements were prepared from these stock solutions. Aliquots of these diluted solutions were pipetted onto a small sheet of Whatman No. 41 filter paper using an Eppendorf pipette. After drying at room temperature, these sheets were placed into clean polyethylene bags. In the case of the Hg standard for short irradiation of 1 h, the standard solution of this element was pipetted together with thioacetamide solution according to NOGUCHI et al[5] to avoid losses of Hg during irradiations.

Instrumental neutron activation analysis

Aliquots of 100–200 mg of hair weighed and heat-sealed in clean polyethylene bags were irradiated together with the standards. Irradiations were carried out at the IEA-R1 swimming pool-type reactor of IPEN-CNEN/SP. Short and long irradiations were performed in order to determine as many elements as possible using the following conditions:

(a) short irradiations of 5 min using pneumatic system facility, in a thermal neutron flux of $5 \cdot 10^{11}$ n·cm^{-2}·s^{-1} for Al, Cl, Cu, Mg, Mn and V determinations;
(b) short irradiations of 1 h at a thermal neutron flux of $2 \cdot 10^{12}$ n·cm^{-2}·s^{-1} for As, Cu, Hg, K and Na determinations, and
(c) long irradiations of 16 h at a thermal neutron flux of $1 \cdot 10^{13}$ n·cm^{-2}·s^{-1} for the determination of As, Br, Ca, Cd, Co, Cr, Fe, Sb, Sc, Se and Zn.

After adequate decay times, samples and standards were counted using EG & G Ortec Model GMX20190 Ge detector coupled to an ADCAM 918A multichannel buffer connected to a microcomputer. Samples and standards were measured at least twice and the sample-to-detector distances of 3.0 and 0.5 cm were used for first and second countings, respectively. The counting times varied from 200 s to 16 h and they were adequately chosen depending on the half-lives and gamma-ray activities of the radioisotopes measured. The detector used had a resolution (FWHM) of 1.0 keV for 122 keV gamma-rays of ^{57}Co and 2.0 keV for 1332 keV gamma-rays of ^{60}Co. The gamma-ray spectra were processed using VISPECT computer program[6] that evaluates peak area (counting rate) and gamma-ray energies. The standard comparator method was used for calculating the content of the respective elements.

Results and discussion

The concentrations of the analyzed elements in hair samples from the group of patients and from the control group of Brazilians living in the city of São Paulo are presented in Table 1. A comparison of geometric means indicates no significant difference between the two groups for concentration of the elements Al, As, Ca, Cd, Co, Cu, Fe, Hg, Mn, Sb and Zn. Nevertheless, the highest concentrations of Al, As, Ca, Cl, Cr, Cu, Fe, Hg, K, Na and V as well as the highest variability of the results for Al, As, Cu, Fe, Hg, K and Na were found in the patient group. The level of essential element Zn for two groups are within the same magnitude, however its lowest concentration was in the patient group.

Table 2 presents normal range values compiled by commercial laboratories and range of concentrations obtained in this control group residing in São Paulo, Brazil. These samples from control group were not the same ones analyzed to compile the normal range values. For some elements, these ranges or limit values show a significant difference among the laboratories. This fact indicates a very cautious use of these reference ranges and a revision of these values is suggested since normally healthy population groups in distinct regions of the world may have variations in nutritional and environmental conditions. Besides, the accumulation mechanism of elements present in hair may depend on the factors such as sex and age.[7]

Table 3 shows the results obtained for certified reference materials. These results were evaluated on a dry weight basis and the percentages of weight loss were determined as recommended in their respective certificates.[9,10] The following values (in percent) of the weight loss were found and used for correcting the final results: 6.03 for NIES No. 5 Human Hair and 8.35 for GBW09101 Human Hair. Our results are in good agreement with the respective certified or information values. The relative errors, generally, were lower than 15%. Also a good precision, expressed as relative standard deviations varying from 0.2% to 15% was obtained. For Mg in NIES No. 5 Human Hair a higher concentration than the certified value was obtained. Its determination is interfered by the presence of a high level of Al in this material. ^{27}Al forms by the (n,p) nuclear reaction ^{27}Mg, the same radioisotope used for Mg determination.

The determination limit values evaluated according to CURRIE[12] for trace element analyses in hair are presented in Table 4. High sensitivities, varying from 0.0017 to 155 μg/g, were attained by the INAA method.

It can be concluded from the above results that INAA, because of its precision and accuracy, can successfully be applied for hair analysis. Besides the multielement capability of this technique is important because there are several elements with potential toxicity and this approach permits the evaluation of antagonistic and synergystic aspects of these elements.

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