Journal of Radioanalytical and Nuclear Chemistry, Vol. 249, No. 2 (2001) 413–416

Determination of trace elements in human nail clippings by neutron activation analysis

A. R. Aguiar,* M. Saiki

Radiochemistry Division, IPEN-CNEN/SP, Caixa Postal 11 049, CEP 05422- 970, São Paulo, SP, Brazil

(Received December 13, 2000)

In this work instrumental neutron activation analysis was applied to determine trace elements in nail clippings and to make a comparison between the results obtained from samples from healthy children and those with cystic fibrosis (CF) disease. The findings indicated that fingernails from the CF group present higher concentrations of Cl, Cr, K and Na than those found in the control group. On the other hand, the lowest concentrations for Cr were found in the CF group. For the Al, As, Co, Fe, Mg, Mn, Se and Zn elements there were no differences between the results obtained for the CF and control groups. The quality control of the results was evaluated by analysing NIST 1577b Bovine Liver and NIST 1566a Oyster Tissue standard reference materials.

Introduction

Trace elements analyses in human nails have been performed for a series of applications including occupational, environmental, nutritional and medical diagnostic studies.[1–4]

Although the trace elements are present in very low quantities in these biological tissues, their influence on the normal metabolic process can be considerable and often increased through interaction with or incorporation into proteins, particularly enzymes.[5] Elemental concentrations in nail samples have been evaluated to study cystic fibrosis (CF) disease.[6]

Cystic fibrosis is an hereditary disease characterised by malfunctions in endocrine glands of the human body that affect the pancreas and the lung mucous, causing pancreatic insufficiency, chronic pulmonary disease, and high electrolytes levels in the sweat.[7] These effects produced in the organism by CF may cause death in childhood or adolescence.

The analysis of nail presents advantages over biological fluids like sweat, urine and blood because the nails can be collected easily and stored at room temperature; moreover, the nail tissue is easier to handle and also accumulates a large number of trace elements in relatively high concentrations.

The purpose of this work was to establish adequate conditions for the determination of trace elements in human finger and toe nails by instrumental neutron activation analysis. Preliminary data of elemental concentration ranges of reference values for healthy individuals were obtained. These reference values can be used as a basis of comparison for abnormal subjects. Comparisons were made between the results obtained for nails of healthy children and those with cystic fibrosis.

Experimental

Collection and preparation of nail samples

Initially, a protocol for collecting and cleaning the samples was defined. Toe and fingernail clippings were collected from a control group of healthy children living in São Paulo City, SP. Samples from a cystic fibrosis (CF) group were collected by researchers at Medicine School of São Paulo University from patients of the Instituto da Criança, SP. Nail samples were collected using a clipper or a pair of scissors, and were placed in polyethylene bags. The ages of children in both groups varied from 1 to 12 years old.

In the laboratory, each sample was placed in a scintillation flask with 10 ml of 2% non-ionic detergent Triton X100 solution and mechanical shaking was applied for a 30-minute period. The sample was then transferred to a beaker and washed with distilled water until the detergent was completely removed. Finally, the sample was washed with p.a. Merck acetone. The washed samples were placed on Whatman filter paper and dried at room temperature inside a class 100 laminar flow hood.

About 60 to 100 mg of each sample were weighed in clean polyethylene bags and heat-sealed. These polyethylene bags were previously cleaned using diluted nitric acid solution and distilled water.

Preparation of synthetic standards of elements

Standard stock solutions of elements were obtained by dissolving high-purity metals, oxides, or salts of elements with appropriate reagents. For Al, Ca, Co and Mg, certified standard solutions of these elements, provided by Merck, Spex Chemical or J. T. Baker Inc., were utilised. Diluted solutions containing one or more elements were prepared from these stock solutions.

* E-mail: amilton@curiango.ipen.br

0236–5731/2001/USD 17.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

Aliquots of these diluted solutions were pipetted on to small sheets of Whatman No. 41 filter paper, and after drying at room temperature, these sheets were placed in clean polyethylene bags and heat-sealed.

Instrumental neutron activation analysis

Samples and standards were irradiated together at the IEA-R1m nuclear reactor at the 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 minutes using a pneumatic system facility, under a thermal neutron flux of $4.25 \cdot 10^{11}$ n·cm^{-2}·s^{-1} for the determinations of Al, Cl, Cu, K, Mg and Mn, and (b) long irradiations of 16 hours at a thermal neutron flux of 10^{13} n·cm^{-2}·s^{-1} for As, Br, Ca, Cd, Co, Cr, Fe, K, Na, Se and Zn determinations.

The measurements of the gamma-induced activity of the samples and standards were carried out after adequate decay times using an EG&G Ortec Model GMX20190 hyperpure Ge detector coupled to an ACE8K card connected to a microcomputer and electronic devices. The counting system had a resolution (FWHM) of 1.98 keV for the 1332 gamma-ray of ^{60}Co and 1.01 keV for the 122 keV ^{57}Co. The samples and standards were counted at least twice after irradiation to optimize conditions to count radioisotopes with different half-lives. Counting times of 200 seconds were used in the case of short irradiations and times of 1.5 and 10 hours were used in samples with long irradiations. The gamma-ray spectra were processed using a VISPECT computer program[8] that evaluates peak area (counting rates) and gamma ray energies. The radioisotopes measured were identified according to their half-lives and gamma-ray energies.

The comparative method was used for calculating the content of the respective elements.

The areas under the photopeaks corresponding to the gamma-rays of ^{28}Al at 1778 keV, ^{76}As at 559 keV, ^{82}Br at 776 keV, ^{47}Ca at 159 and 1296 keV, ^{115}Cd at 336 and 528 keV, ^{38}Cl at 1642 keV, ^{60}Co at 1773 and 1332 keV, ^{51}Cr at 320 keV, ^{66}Cu at 1039 keV, ^{59}Fe at 1099 and 1291 keV, ^{42}K at 1524 keV, ^{27}Mg at 844 and 1014 keV, ^{56}Mn at 847 and 1810 keV, ^{24}Na at 1368 keV, ^{75}Se at 264 keV and ^{65}Zn at 1115 keV were used.

The precision and accuracy of the results were checked by analysing NIST 1577b Bovine Liver and NIST 1566a Oyster Tissue.

Results and discussion

Since the samples were measured in the same polyethylene bags used during irradiations, the presence of impurities in these involucres was examined. The elements Br, Cr and Na were found but they could be considered negligible in comparison with the amounts of elements present in the samples.

Table 1 presents the results obtained in the analyses of reference materials (Bovine Liver and Oyster Tissue) together with their certified values.[9,10] Most of the element results obtained in these reference materials are in good agreement with their respective certified values.

Table 1. Analysis of certified reference materials NIST 1577b Bovine Liver and NIST 1566a Oyster Tissue

Elements	Bovine Liver		Oyster Tissue	
	This work	Certified values (9)	This work	Certified values (10)
Al, μg·g^{-1}	N.D.[a]	(3)[b]	220 ± 52[c]	202.5 ± 12.5
As, μg·kg^{-1}	N.D.	(50)	13778 ± 1778	14000 ± 1200
Br, μg·g^{-1}	10.9 ± 0.8	(9.7)	65.0 ± 7.7	–
Ca, μg·g^{-1}	95 ± 59	116 ± 4	2095 ± 156	1960 ± 190
Cd, μg·kg^{-1}	580 ± 130	500 ± 30	4299 ± 452	4150 ± 380
Cl, μg·g^{-1}	2510 ± 243	2780 ± 60	7609 ± 585	8290 ± 140
Co, μg·g^{-1}	0.22 ± 0.03	(0.25)	0.488 ± 0.070	0.57 ± 0.11
Cr, μg·g^{-1}	273 ± 69	—	1.62 ± 0.37	1.43 ± 0.46
Cu, μg·g^{-1}	173 ± 25	160 ± 8	81 ± 12	66.3 ± 4.3
Fe, μg·g^{-1}	175 ± 35	184 ± 15	545 ± 22	539 ± 15
K, μg·g^{-1}	10328 ± 1120	9940 ± 20	8241 ± 840	7900 ± 470
Mg, μg·g^{-1}	628 ± 83	601 ± 28	1334 ± 210	1180 ± 170
Mn, μg·g^{-1}	10.1 ± 0.9	10.5 ± 1.7	11.9 ± 1.0	12.3 ± 1.5
Na, μg·g^{-1}	2383 ± 193	2420 ± 60	4013 ± 227	4170 ± 130
Se, μg·kg^{-1}	803 ± 42	730 ± 60	2299 ± 75	2210 ± 240
Zn, μg·g^{-1}	132.7 ± 6.9	127 ± 16	899 ± 54	830 ± 57

[a] Element not detected or not determined.
[b] Numbers in parentheses are information values.
[c] Arithmetic mean and standard deviation obtained from 4 to 12 determinations.

Table 2. Elemental concentrations in toenail samples from CF and control groups

Element	CF (n=22)			Control (n=10)			F
	Range	X_G ×÷ s_G	Median	Range	X_G ×÷ s_G	Median	(p=0.05)
Al, μg·g^{-1}	5.1 – 1696	208.2×÷3.8	236.0	90 – 1084	261.9×÷2.2	257.9	0.0855
As, μg·kg^{-1}	38 – 756	128.7×÷2.1	130.6	26.4 – 165.3	82.7×÷1.8	94.5	1.24
Br, μg·g^{-1}	0.9 – 6.9	3.0×÷1.6	3.0	0.57 – 3.61	1.2×÷1.7	1.1	14.9
Ca, μg·g^{-1}	609 – 5212	1311.0×÷1.6	1273.5	548 – 2562	1205.1×÷1.6	1275.0	0.240
Cd, μg·kg^{-1}	210 – 1953	786.7×÷2.4	1050.0	275 – 2486	583.8×÷2.3	405.0	1.10
Cl, μg·g^{-1}	511 – 10147	1229.3×÷2.5	1003.0	160 – 1162	335.3×÷1.8	299.8	3.35
Co, μg·kg^{-1}	14 – 695	86.3×÷2.6	86.7	54.2 – 151.6	81.7×÷1.4	79.5	1.22
Cr, μg·kg^{-1}	165 – 2276	773.8×÷2.1	784.0	406 – 8407	1416.3×÷2.9	1363.0	5.48
Cu, μg·g^{-1}	0.45 – 31	3.8×÷3.8	4.7	4.1 – 39.4	10.0×÷2.1	7.2	1.08
Fe, μg·g^{-1}	13 – 664	101.5×÷3.3	127.3	57.2 – 582.1	155.6×÷2.1	163.6	0.0484
K, μg·g^{-1}	27 – 996	201.8×÷2.2	197.0	82 – 1339	284.1×÷2.6	315.0	1.65
Mg, μg·g^{-1}	4 – 791	134.4×÷3.5	159.5	111 – 426	187.4×÷1.6	175.0	0.0472
Mn, μg·g^{-1}	0.12 – 8.6	1.9×÷2.1	1.6	0.19 – 3.2	1.2×÷2.3	1.3	0.840
Na, μg·g^{-1}	214 – 885	442.4×÷1.6	438.4	73 – 1304	240.1×÷2.7	195.1	2.80
Se, μg·kg^{-1}	300 – 810	489.1×÷1.3	510.5	394 – 654	499.8×÷1.2	492.5	0.277
Zn, μg·g^{-1}	75 – 495	136.1×÷1.7	118.5	83 – 526	150.3×÷1.8	125.5	0.553

n – Number of samples analysed in each group.

X_G ×÷s_G – Geometric mean and standard deviation.

Table 3. Elemental concentrations in fingernail samples from CF and control groups

Element	CF (n=22)			Control (n=10)			F
	Range	X_G ×÷ s_G	Median	Range	X_G ×÷ s_G	Median	(p=0.05)
Al, μg·g^{-1}	48 – 735	154.61×÷2.4	127.5	79 – 354	198.0×÷1.6	223.3	0.00714
As, μg·kg^{-1}	36 – 250	87.3×÷1.8	75.4	44 – 1397	109.5×÷2.7	77.5	0.0111
Br, μg·g^{-1}	3.5 – 13.7	4.7×÷1.8	4.5	1.81 – 9.14	3.6×÷1.6	3.6	1.58
Ca, μg·g^{-1}	608 – 3858	1168.1×÷1.6	939.5	343 – 1926	1054.8×÷1.6	1114.0	2.85
Cd, μg·kg^{-1}	27 – 4566	693.8×÷4.1	902.0	129 – 3157	583.3×÷3.0	572.5	0.960
Cl, μg·g^{-1}	699 – 2608	1450.0×÷1.5	1540.5	361 – 1508	770.9×÷1.5	796.0	7.97
Co, μg·kg^{-1}	9.9 – 524	93.8×÷2.5	88.7	66 – 1078	115.5×÷2.3	91.0	0.423
Cr, μg·kg^{-1}	211 – 5015	1141.8×÷2.2	1241.5	523 – 14455	1279.9×÷2.7	1132.0	10.30
Cu, μg·g^{-1}	9.1 – 260	13.7×÷4.8	9.6	3.1 – 55.0	15.4×÷2.6	20.9	1.11
Fe, μg·g^{-1}	102 –1015	134.6×÷2.4	123.9	50.7 – 246.1	133.0×÷1.7	137.1	0.477
K, μg·g^{-1}	24 – 351	118.8×÷2.4	146.1	25 – 1963	223.1×÷3.9	271.0	4.61
Mg, μg·g^{-1}	0.18 – 460	50.9×÷8.5	109.0	51 – 298	135.4×÷1.6	156.0	0.0436
Mn, μg·g^{-1}	0.75 – 6.3	1.1×÷2.5	0.98	0.503 – 2.954	1.3×÷1.9	1.7	0.242
Na, μg·g^{-1}	30 – 1078	307.6×÷2.6	410.5	78.9 – 478.6	170.6×÷2.0	125.0	5.62
Se, μg·kg^{-1}	348 – 1024	561.9×÷1.3	688.5	359 – 809	514.0×÷1.3	520.0	0.794
Zn, μg·g^{-1}	55 – 221	134.3×÷1.4	136.1	99 – 566	161.4×÷1.7	143.6	2.22

The relative errors obtained were lower than 13% and the results also presented good precision with relative standard deviations lower than 10%. The precision and the accuracy of the results for the element Ca in Bovine Liver and Cu in Oyster Tissue were poor, because the induced activities of ^{47}Ca and ^{66}Cu were not high, resulting in poor counting statistics.

Analytical results obtained for toenails and fingernails from both groups of children are presented in Tables 2 and 3, respectively. The ranges of concentrations, geometric means and medians of the results are shown. It is seen in these tables that the analyzed elements exhibit considerable intersubject variability. This intersubject variability has been also presented in literature data obtained in the nail analyses.[6] These variations within a same group of individuals may be attributed to the differences of their nutritional or environmental conditions.

The analysis of variance (ANOVA)[11] was applied to compare results obtained for CF and control groups. The parameters F obtained from F-test, at the significance level of 5%, were also included in the Tables 2 and 3. This test indicated that the concentrations of Br and Cr obtained in toenails from CF and control groups present significant differences (F>4). The elements Cl, Cr, K and Na obtained in fingernails from CF group also presented significant difference from those found for controls. The higher concentrations of Cl and Na were obtained from CF group samples, as compared to those from the control group.

For the elements Al, As, Co, Fe, Mg, Mn, Se, and Zn, there were no differences between the results obtained for CF and control groups.

The concentration levels of the trace elements in toenails are, in general, of the same order of magnitude as the fingernails. One exception was Cr, for which a lower value was found for toenails from the CF group.

Results obtained in this work indicated the viability of using neutron activation analysis method in the analysis of nail samples because of its precision and accuracy, as well as its ability to determine a large number of elements of interest from a nutritional and toxicological point of view. Also the results found indicate that CF may be diagnosed by the analysis of Cl and Na in the fingernails.

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FAPESP and CNPq from Brazil for financial support and Medicine School of São Paulo University for the samples are acknowledged.

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