SODIUM AND CHLORINE DETERMINATIONS IN NAILS OF HEALTHY AND CYSTIC FIBROSIS CHILDREN

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SUMMARY

In this paper instrumental neutron activation analysis was applied to the determinations of Na and Cl in finger and toenails to make a comparison between the results obtained for samples from control group of healthy children and those with cystic fibrosis (CF) disease. Firstly, protocols for collection and washing of the samples were established. The analytical procedure consisted of irradiating samples and standards of Na and Cl in the IEA-R1 nuclear reactor for further gamma ray spectrometry using a HGe detector. The highest concentrations of Na and Cl in nails from CF patients indicated the viability of using these analyses in the investigations and diagnosis of this disease. Comparisons were also made between the results obtained for toe and fingernails as well as between the results obtained for control group of children and adults. The quality control of the results were performed by analysing certified biological reference materials.

Key words: Nails; Neutron activation analysis; Cystic fibrosis

INTRODUCTION

Cystic fibrosis (CF) is a genetic disease characterised by obstruction and infection of the pulmonary tract, pancreatic insufficiency and increased levels of salt in sweat, saliva and nails. In 1953 it was recognised that both sodium and chlorine levels are elevated in the sweat of almost all CF patients[1]. In 1989, the so called cystic fibrosis transmembrane conductance regulator gene (CFTR) was discovered[2] and this gene is responsible for the synthesis of a protein that acts as an ion gate channelling chloride across the membranes into cells. A mutation in this gene causes the formation of a protein that is not capable of normal transport of ion chloride. The blockage of the channel that conduits Cl into the cells causes abnormal metabolism and Cl, blocked from entering cells, is eliminated by the CF individuals in perspiration[3].

Therefore, analyses of sodium and chlorine from CF individuals and control group (C) in different kinds of biological materials such as nails, hair, sweat and saliva have been subject of several researches[4-7].

The nail analyses present advantages over biological fluids like sweat and saliva due to its ease of sample collection, storage and shipment. Besides the nails are easier to handle and they accumulate a number of trace elements in relatively high concentrations.

However the analyses of nails require a limiting factor in obtaining an accurate elemental composition due to environmental contamination of the sample. Before analyses, it is necessary the removal of environmental contamination without causing loss of intrinsic elements of the nails.

In this paper instrumental neutron activation analysis was applied to the determination of Na and Cl in finger and toenails to make a comparison between the results obtained for samples from control group of healthy children and those with cystic fibrosis (CF) disease. The quality assurance of the analytical results was evaluated by analysing the certified reference materials NIST 1577b Bovine Liver and Nist 1566a Oyster Tissue, both from National Institute of Standards and Technology, USA.

MATERIALS AND METHOD

Nail sample collection and preparation for analysis

Finger and toe nail samples from CF children were collected at the Instituto de Criança of Medicine School of São Paulo University, SP. The nail samples from control group were collected from healthy adults and children living in São Paulo, SP. The age of these children varied from one month to 12 years.

Firstly, a protocol for sample washing was defined[8]. Using the reagent acetone p.a. for cleaning the samples, the removal of the elements was practically negligible. On the other hand when a 12.5 M NH_4OH solution was used, the endogenous contents of elements (Sb, Zn) which are supposed to be intrinsic of the nail tissue were removed

partially. A step of washing using 2 % non-ionic detergent Triton X100 solution showed more adequate for cleaning the nails with the removal of external contaminants (Na, K, Br) without causing changing its elemental composition.

Then, each nail sample was placed in a scintillation flask with 5 ml of 2 % Triton X100 and a mechanical shaking was applied for a 30 min period. The sample was then transferred to a beaker and washed with distilled water until the detergent was completely removed. Then, the sample was washed with acetone p.a. Merck. The washed sample was placed on a 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 a clean polyethylene envelopes and heat-sealed for irradiation with the standard of Na and Cl.

Preparation of synthetic standard of Cl and Na

Standard solution of Cl and Na was prepared by dissolving the reagent NaCl p.a., Merck with distilled water. The NaCl reagent was previously heated for two hours at 800 °C in order to eliminate eventual ions carbonate. A standard solution containing 2.6 mg mL^{-1} of Na and 4.0 mL^{-1} of Cl was prepared. 50 microliters of this solution were pipetted onto small sheets of Whatman No. 41 filter paper, and after drying at room temperature in a desicator, these sheets were folded and placed in clean polyethylene envelopes and heat-sealed for irradiation with the samples.

Instrumental neutron activation analysis

Nail samples and the standards were irradiated together with thermal neutron flux of 4.5 10^{11} n cm^{-2} s^{-1}, for a period of 5 minutes and using pneumatic system facility. After adequate times, sample and standard were measured using an EG & G Ortec Model GMX20190 Ge detector coupled to an ADCAM 918^{A} multichannel Buffer that is connected to a microcomputer and an electronic system associated. 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 counting times used were of 300 or 600 s. The gamma-ray spectra were processed using VISPECT computer program that evaluates peak area (counting rates) and gamma ray energies. The radioisotope of ^{38}Cl with half life of 37.24 min and gamma ray energy of 1642.7 keV and the radioisotope of ^{24}Na with 14.96 h half life and gamma ray energy of 1368.0 keV were used in the analyses. The concentrations of elements were evaluated by comparative method.

The biological reference materials NIST 1577b Bovine Liver and NIST 1566a Oyster Tissue were analysed using the same experimental conditions utilised in the analyses of the nail samples.

RESULTS AND DISCUSSION

The concentrations of the Cl and Na in nail samples from the group CF patients and control group of children and from control group of adults are presented in Table 1. To make comparison between the sets of results obtained, the analysis of variance (ANOVA) and F test at the significance level of 5 % were used. In the comparison of the finger and toe nails results of CF group, there were no significant differences. However in the case of children control group, finger nails presented higher concentrations of Cl than those presented by toe nails. For the control group of adults, concentrations of Cl were also higher in fingernail samples. There were not differences between the Na concentrations obtained in finger and toe nail samples from groups of individuals studied in this work.

Table 1. Concentrations of Cl and Na in finger and toenail samples obtained for different groups of individuals

Group of individuals	Sample	n	Elements	X ± s(*)	Range	Median	X_G ×÷ s_G(**)
CF children	Toenails	21	Cl	2216 ± 3095	511 - 10147	1003.0	1229.3 ×÷2.5
			Na	490 ± 218	214 - 885	438.4	442.4 ×÷ 1.6
	Fingernails	22	Cl	1566 ± 602	699 - 2608	1540.5	1450.0 ×÷ 1.5
			Na	416 ± 262	30 - 1078	410.5	307.6 ×÷ 2.6
Control children	Toenails	10	Cl	404 ± 305	160 - 1162	299.8	335.3 ×÷ 1.8
			Na	398 ± 467	73 - 1304	195.1	240.1 ×÷ 2.7
	Fingernails	10	Cl	826 ± 323	361 - 1508	796.0	770.9 ×÷ 1.5
			Na	215 ± 160	78.9 – 478.6	125.0	170.6 ×÷ 2.0
Control adults	Toenails	19	Cl	352 ± 147	137 - 633	320.0	323.1 ×÷ 1.5
			Na	172 ± 106	52 - 500	589.0	554.0 ×÷ 1.2
	Fingernails	28	Cl	605 ± 341	121 - 1424	541.0	512.8 ×÷ 1.8
			Na	212 ± 128	36.3 – 553.0	168.4	177.2 ×÷ 1.9

* X ± s - Arithmetic mean and its respective standard deviation; ** X_G ×÷ s_G - Geometric mean and its respective standard deviation; n = number of the samples

Concentrations of Cl and Na found in the fingernail sample from control group of children were significantly different ($p = 0.05$) from those obtained for finger nails of CF group, indicating the possibility of using analyses of nails in the diagnose of cystic fibrosis disease. Results obtained for finger nails of children were also plotted in graphics of number of individuals as function of elemental concentrations (range) and presented in Fig 1 and 2 for Cl and Na data, respectively. In these Figures it can be observed that the CF group presents higher concentrations of Cl and Na than those of control group, however there were overlappings of concentrations for both group ranges.

The comparison between the results of Cl and Na obtained for healthy children and control group of adults showed that they are the same magnitude.

Results of biological reference materials Bovine Liver and Oyster Tissue are presented in Table 2 together certified reference values and standardised difference or Z values[11]. The relative standard deviations of the results obtained in these analyses varied from 5.3 to 5.7 % and the data obtained are in good agreement with the certified values, with percentage of relative error lower than 3.7 %. The IZI values calculated were lower then 3, which means that the results are within the range of certified values at the significance level of 1%.

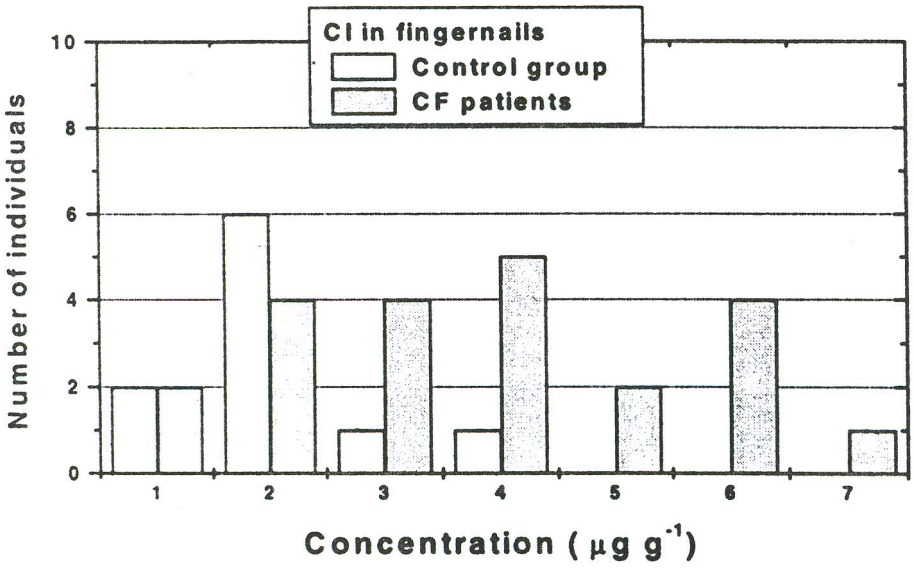

Fig 1. Number of individuals as function of Cl concentrations (range) in fingernails from children

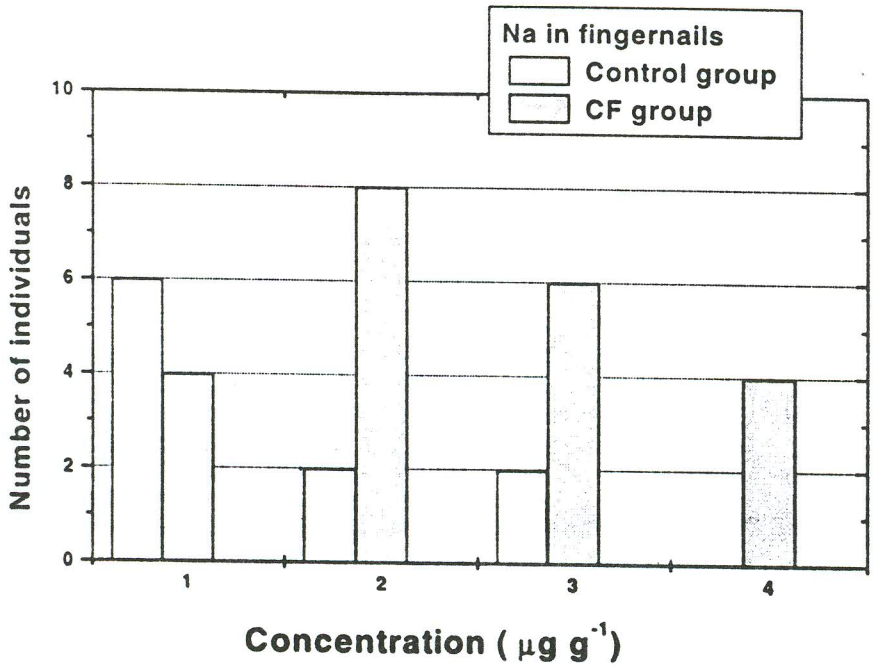

Fig 2. Number of individuals as function of Na concentrations (range) in fingernails from children

Table 2. Concentrations of Cl and Na obtained in the biological reference materials

Elements	1577b Bovine Liver			1566 a Oyster Tissue		
	This work		Ref (9)	This work		Ref (10)
	X ± s*	Z		X ± s	Z	
Cl, $\mu g\ g^{-1}$	2677 ± 145	-0.65	2780 ±60	8016 ± 431	-0.60	8290 ± 140
Na, $\mu g\ g^{-1}$	2390 ± 137	-0.20	2420 ± 60	4041 ± 214	-0.63	4170 ± 130

* X ± s - Arithmetic mean and its respective standard deviation

CONCLUSIONS

It was shown that instrumental neutron activation analysis may be used for a simultaneous determination of Cl and Na and the short time irradiation required in this analysis allows a rapid determination of these elements.

The statistical analysis applied to the results indicated that concentrations of Cl and Na in nails of CF group were higher than those of the control group, however there were overlappings of both groups and the results presented intersubject variability indicating that other factors such as nutritional conditions or environmental conditions might be affecting in the results these two elements.

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