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Establishing a protocol for trace element determinations in serum samples from healthy elderly population in São Paulo city, SP, Brazil

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In this study a protocol for blood serum analysis was defined and the concentrations for Br, Ca, Cl, Fe, Na, Rb, Se and Zn were obtained by instrumental neutron activation analysis. Blood samples were collected from healthy elderly volunteers who were selected based on the SENIEUR protocol. Contamination of blood by the collection procedure was also evaluated and found negligible. The serum was separated by centrifugation, then freeze-dried and analyzed. Most of results obtained were within the acceptable value ranges used by physicians for normal population. The certified reference material, NIST SRM 1566b Oyster Tissue was analyzed for quality control.

Introduction

There is an increasing interest of trace element determinations in serum samples to investigate their vital role in human metabolism, as well as, to provide an important basis for clinical disorders and intoxication diagnosis. Trace element determinations in serum samples are increasing and becoming an important test in medicine, due to the fact that levels of certain elements are related to various pathological conditions in human beings.[1–5]

However, data of reference values or reference intervals of elemental concentrations in sera are very scarce since these determinations require the establishment of an adequate protocol for sampling, reference population selection, control of possible sample contamination and quality control of the analytical results.[6–9] Recent studies have been concerned with reliability of the results and their usability in clinical chemistry and medical investigations.

The objective of this study was to develop a reliable protocol to determine trace elements in blood serum of a healthy elderly population included in a program for "Successful Ageing" of the Hospital das Clínicas, São Paulo University Medical School. This study of the analysis of blood sera from an elderly population can contribute to the future monitoring of aging pathologies, treatment of particular diseases and to detect micronutrient deficiencies. Besides, reference values for trace elements in serum are generally obtained using an adult population, which usually does not include the elderly. The availability of trace element data in serum may represent an important indicator of elderly health status that is of fundamental importance in geriatric medicine.

For the determination of trace elements in serum samples, several techniques, such as hydride generation and flame atomic absorption spectrometry,[10,11] inductively coupled plasma spectrometry, atomic emission spectrometry,[12,13] neutron activation analysis,[14,15] inductively coupled plasma mass spectrometry,[16,17] particle induced X-ray emission[2] and total reflection X-ray fluorescence[18] have been applied. In this study instrumental neutron activation analysis (INAA) was applied to the analysis of Br, Ca, Cl, Fe, Na, Rb, Se and Zn. INAA constitutes an advantageous method for serum analysis, which enables simultaneous multi-element analysis and does not require the step of dissolution.

Experimental

Cleaning procedures

The labware was cleaned first by soaking it in the diluted Extran solution and rinsed with de-ionized water. It was then soaked overnight in a diluted nitric acid solution bath and finally thoroughly rinsed with purified Milli-Q water. All precautions to eliminate metal contamination during serum handling and storage were taken.

Contamination estimates and definition of blood serum sampling protocol

In order to evaluate possible contaminants that could be released from the stainless-steel needles, the Teflon cannula and the plastic tips of pipettors to be used for collecting and storing the samples, the composition of these devices was analyzed by neutron activation analysis.

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The elements released from these devices were also determined by analyzing aliquots of 0.065% nitric acid solution submitted to test in the place of total blood or blood serum. The use of nitric acid solution was more practical than serum sample. The nitric acid solution was drawn up into the cannula, needles and tips and then these devices were immersed in 5 ml of nitric acid solution for 15 hours. In the case of Nalgene polypropylene flasks and vacuum tubes, nitric acid solutions kept in these containers for 15 hours were analyzed to determine the released elements from their walls. The elements released in the nitric acid solution were determined by neutron activation analysis. The nitric acid solutions were previously freeze-dried to reduce the volumes and then they were dried in the clean polyethylene capsule from Vrije Univesiteit Amsterdam. Analysis of the blank value was also evaluated by analyzing the capsule without the sample.

The blood was also collected using, both, intravenous cannula BD Angiocath™ gauge 20 (1.1 mm) and 30.0 mm long (Becton, Dickinson and Company) and stainless steel needles gauge 21 (0.8 mm) and 25 mm long (Becton, Dickinson and Company) to verify the contamination problem during the sample collection. Blood was drawn from three individuals of the laboratory. These preliminary experiments showed that the contamination problem of the sample could be considered negligible for the elements determined in this study.

Collection of blood samples

Both Ethics Committees, of the Hospital das Clínicas of the São Paulo University Medical School (HC-FMUSP) and of the Instituto de Pesquisas Energéticas e Nucleares (IPEN) approved this research. The approval numbers of this project by these committees are: 565/03 from HC-FMUSP and 101/CEP-IPEN/SP from IPEN. The blood donors were elderly subjects considered healthy and attended at the Hospital das Clínicas of the São Paulo University, Medical School for medical checkup. The participants were informed about the aim of our study and invited for medical examinations, biochemical exams and were selected based on the SENIEUR protocol.[19] The following exclusion parameters were used to select the individuals: (a) without alcoholism and smoking (b) without hepatitis, human immune virus (HIV) and chronic disease, diabetes, hypertension (c) atypical dietary habits, (d) previous 6 months blood transfusion, anemia, (e) supplement intakes and (f) mental disorders.

A written consent was obtained from the donors prior to the collection of blood samples that were collected after a 12 hours fast from 28 subjects (7 male and 21 female) aged 60 to 87 years, using sterile metallic needles. About 10 ml of whole blood was collected in heparin free BD Vacutainer™ tube, royal blue Hemogard™ closure, without additive (Becton-Dickinson and Company) for trace element determinations. To avoid metal contamination no anticoagulants were used. The blood was centrifuged after completely clotted and 3.0 or 4.0 ml of serum were pipetted into a clean Nalgene polyethylene vial and frozen to transport to Neutron Activation Analysis Laboratory, where they were kept at –20 °C until the analysis. Haemolyzed samples were excluded for the analyses. For INAA analysis the serum samples were freeze-dried. The weight loss during this freeze-drying process was about 90.8%.

Preparation of the standards

The 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 pipettors and volumetric flasks were calibrated before the use. These filter sheets were dried at room temperature inside a desiccator and placed into clean polyethylene involucres, which were sealed. In these standards the quantities of each element, in µg (in parentheses) were the following: Br (5.2), Ca (1000), Cl (500), Fe (280), Na (500), Rb (4.0), Se (40) and Zn (35.0).

Instrumental neutron activation analysis

Aliquots of about 200 mg of serum weighed in polyethylene involucres 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 under a thermal neutron flux of $1.4\cdot10^{12}$ $n\cdot cm^{-2}\cdot s^{-1}$ were carried out for Cl and Na determinations. Sixteen-hour irradiations under a thermal neutron flux of about $5\cdot10^{12}$ $n\cdot cm^{-2}\cdot s^{-1}$ were performed for the determination of Br, Ca, Fe, Rb, Se and Zn. 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. Each sample and standards were 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 radionuclides considered. The radionuclides measured were identified according to their half-lives and gamma-ray energies. The concentrations of elements were calculated by a comparative method. The radionuclides used in serum analyses were: ^{82}Br, ^{47}Ca, ^{38}Cl, ^{59}Fe, ^{24}Na, ^{86}Rb, and ^{65}Zn.

The quality of the analytical results was evaluated by analyzing the certified reference material (CRM), SRM 1566b Oyster Tissue[20] provided by the National Institute of Standards and Technology (NIST), USA. Although it would have been better to use a serum CRM, at the time of this study, but this material was not available and so the Oyster Tissue was used instead. This reference material was analyzed by applying the same experimental conditions used in serum analyses. The element concentrations of reference material were evaluated on a dry weight basis, as recommended in the certificate. A moisture weight loss of 5.2% was found to correct the final results.

Results and discussion

The results obtained in the analyses of stainless-steel needles, the Teflon cannula and the plastic tips of pippetors presented in Table 1 show the elements that could be released from these devices and cause the sample contamination.

The results presented in Table 2 indicate the element concentrations leached from the devices to the nitric acid solution and blank value. The mass of the elements transferred into 1 ml of the nitric solution is the same magnitude or similar to those obtained for each capsule for the elements detected. As they are very low, the release of the elements analyzed in this study could be considered negligible. A slightly higher level of Cr obtained in the blank analysis indicated that the capsule used for sample irradiation contains this element.

The comparison between the results obtained for blood samples collected using needles and cannula presented in Table 3 shows there is no difference for the elements Br, Ca, Cl, Fe, Na, Rb, Se and Zn analyzed in this study.

Table 1. Elemental composition of stainless steel needles, Teflon cannula and plastic tips

Element	Stainless steel needles	Teflon cannula	Plastic tips
As, $\mu g \cdot g^{-1}$	49.35 ± 0.08	0.0105 ± 0.0002	–
Br, $\mu g \cdot g^{-1}$	–	2.04 ± 0.02	0.09 ± 0.01
Co, $\mu g \cdot g^{-1}$	1371 ± 3	0.463 ± 0.017	0.04 ± 0.01
Cr, %	19.4 ± 0.2	$(0.826 \pm 0.084) \cdot 10^{-4}$	$(0.44 \pm 0.05) \cdot 10^{-4}$
Cu, $\mu g \cdot g^{-1}$	2944 ± 179	–	–
Fe, %	68.8 ± 1.2	$(22 \pm 4) \cdot 10^{-4}$	$(3.9 \pm 3.3) \cdot 10^{-4}$
Mo, $\mu g \cdot g^{-1}$	3275 ± 21	4.08 ± 0.05	
Na, $\mu g \cdot g^{-1}$	–	7.26 ± 0.03	7.8 ± 0.4
Ni, %	8.84 ± 0.04	–	–
Zn, $\mu g \cdot g^{-1}$	67 ± 8	11.8 ± 0.2	–

– Indicates not detected.

Table 2. Trace elements leached into nitric acid solution from sampling and storage devices and blank values

Element	Devices				Blank mass of element per irradiation capsule (unit)
	Polypropylene flask	Cannula	Pippetor tip	Vacuum tube	
As, $ng \cdot ml^{-1}$	0.62 ± 0.04*	0.66 ± 0.05	0.33 ± 0.06	0.52 ± 0.15	0.58 ± 0.06 (ng)
Br, $ng \cdot ml^{-1}$	28.4 ± 0.2	25.7 ± 0.2	25.0 ± 0.2	153.8 ± 0.4	26.4 ± 0.2 (ng)
Ca, $\mu g \cdot ml^{-1}$	2.8 ± 0.3	4.2 ± 0.7	2.7 ± 0.5	4.8 ± 0.6	2.8 ± 0.6 (µg)
Cl, $\mu g \cdot ml^{-1}$	3.7 ± 0.1	3.1 ± 0.1	2.67 ± 0.09	1.95 ± 0.08	≤7.3*** (µg)
Co, $ng \cdot ml^{-1}$	3.63 ± 0.15	1.61 ± 0.09	2.32 ± 0.08	1.90 ± 0.09	1.9 ± 0.7 (ng)
Cr, $ng \cdot ml^{-1}$	472 ± 3	415 ± 2	363 ± 2	334 ± 2	406 ± 3 9 (ng)
Cs, $ng \cdot ml^{-1}$	2.06 ± 0.15	0.82 ± 0.10	1.09 ± 0.08	1.00 ± 0.07	≤0.85 (ng)
Fe, $ng \cdot ml^{-1}$	n.d.**	n.d.	n.d.	n.d.	≤237 (ng)
Mo, $ng \cdot ml^{-1}$	67.7 ± 4.8	58.8 ± 0.7	54.4 ± 0.6	49.3 ± 0.9	47.6 ± 0.8 (ng)
Na, $\mu g \cdot ml^{-1}$	4.2 ± 0.1	3.2 ± 0.2	2.67 ± 0.09	1.95 ± 0.08	7.3 ± 0.2 (µg)
Rb, $ng \cdot ml^{-1}$	n.d.	n.d.	n.d.	n.d.	≤10.4 (ng)
Sb, $ng \cdot ml^{-1}$	n.d.	n.d.	0.11 ± 0.01	0.14 ± 0.02.	0.13 ± 0.02 (ng)
Se, $ng \cdot ml^{-1}$	n.d.	n.d.	n.d.	n.d.	≤7.2 (ng)
Zn, $ng \cdot ml^{-1}$	123 ± 4	102 ± 3	78 ± 5	82 ± 5	60 ± 2 (ng)

* Contribution of the blank was not discounted.
** n.d.: Not detected.
*** Detection limit values estimated according to CURRIE[21] criterion.

Table 3. Elemental concentrations in serum for blood samples collected using stainless steel needles (A) and Teflon cannula (B)

Element	Donor 1		Donor 2		Donor 3	
	A	B	A	B	A	B
Br, $\mu mol \cdot l^{-1}$	32.75 ± 0.09	34.34 ± 0.11	31.61 ± 0.13	32.04 ± 0.13	34.91 ± 0.11	36.72 ± 0.11
Ca, $mmol \cdot l^{-1}$	1.99 ± 0.10	2.11 ± 0.10	2.34 ± 0.15	2.21 ± 0.13	2.41 ± 0.14	2.34 ± 0.08
Cl, $mmol \cdot l^{-1}$	84.2 ± 1.3	92.2 ± 2.2	87.4 ± 1.1	90.5 ± 1.1	95.9 ± 2.2	92.6 ± 2.1
Fe, $\mu mol \cdot l^{-1}$	17.3 ± 1.7	17.3 ± 1.6	20.6 ± 1.2	22.4 ± 1.2	31.5 ± 1.3	25.5 ± 1.1
Na, $mmol \cdot l^{-1}$	144.7 ± 3.3	130.5 ± 1.9	134.8 ± 1.6	139.5 ± 1.6	133.2 ± 2.0	129.6 ± 1.9
Rb, $\mu mol \cdot l^{-1}$	3.31 ± 0.08	3.07 ± 0.05	3.93 ± 0.06	3.66 ± 0.06	2.83 ± 0.05	2.84 ± 0.05
Se, $\mu mol \cdot l^{-1}$	1.06 ± 0.04	1.08 ± 0.03	0.89 ± 0.03	0.88 ± 0.03	0.96 ± 0.03	1.04 ± 0.03
Zn, $\mu mol \cdot l^{-1}$	14.50 ± 0.14	14.79 ± 0.12	16.61 ± 0.14	15.86 ± 0.12	15.43 ± 0.14	15.23 ± 0.15

Table 4. Elemental concentrations obtained for NIST 1566b Oyster Tissue certified reference material

Element	This work			Certified values[20]
	Mean ± SD	RSD, %	RE, %	
Br, $\mu g \cdot g^{-1}$	51 ± 3	5.9	–	–
Ca, $\mu g \cdot g^{-1}$	841 ± 25	3.0	0.36	838 ± 20
Cl, $\mu g \cdot g^{-1}$	4987 ± 114	2.3	3.0	5140 ± 100
Fe, $\mu g \cdot g^{-1}$	196.3 ± 6.3	3.2	4.6	205.8 ± 6.8
Na, $\mu g \cdot g^{-1}$	3155 ± 151	4.8	4.3	3297 ± 53
Rb, $\mu g \cdot g^{-1}$	3.150 ± 0.021	0.7	3.4	3.262 ± 0.145
Se, $\mu g \cdot g^{-1}$	2.08 ± 0.05	2.4	0.97	2.06 ± 0.15
Zn, $\mu g \cdot g^{-1}$	1386 ± 23	1.6	2.7	1424 ± 46

Table 5. Concentration of trace elements in serum of healthy elderly population living in São Paulo city

Element	This work			Reference values used in clinical labs[23]	Literature value
	Mean ± SD	*n*	Range		
Br, $\mu mol \cdot l^{-1}$	41.3 ± 8.7	28	26.3 – 66.3	–	69.2 ± 8.39 (average age = 65.6)[2]
Ca, $mmol \cdot l^{-1}$	2.42 ± 0.12	28	2.0 – 2.7	2.20–2.55	Range of 2.20–2.60[24]
Cl, $mmol \cdot l^{-1}$	88.5 ± 1.9	28	71.7 – 99.1	98–108	
Fe, $\mu mol \cdot l^{-1}$	21.8 ± 2.2	23	11.9 – 32.5	11.6–31.3 (for men) 9.0–30.4 (for women)	21.5 ± 8.1 (average age = 65.6)[2] Range of 13–35 (men)[24] Range of 10–28 (women)[24]
Na, $mmol \cdot l^{-1}$	128.4 ± 12.5	28	111.1 – 150.9	136–145	
Rb, $\mu mol \cdot l^{-1}$	3.9 ± 0.4	28	3.0 – 5.3	0.9–6.5	1.80 (1.00–2.60) (men)[25]
Se, $\mu mol \cdot l^{-1}$	0.981 ± 0.039	28	0.58 – 2.35	0.58–1.81	1.2 ± 0.4 (average age = 65.6)[2] 1.14 ± 0.22 (age 60–90)[26] 0.871 (0.623–1.119) (men)[25] 0.947 (0.749–1.145) (women)[25]
Zn, $\mu mol \cdot l^{-1}$	16.4 ± 4.0	28	12.2 – 21.4	10.7–18.4	15.6 ± 2.6 (average age = 65.6)[2] 14.51 ± 2.07 (men)[26] 13.48 (9.23–17.73) (women)[25]

Results obtained for certified reference material SRM 1566b Oyster Tissue showed good precision and good agreement with the certified values[20] for most elements (Table 4). The relative standard deviations of the results were lower than 5.9% and relative errors from 0.36 to 4.6%. The standardized difference or *Z*-score values[22] obtained for the elements analyzed are presented in Fig. 1, and they were <1, indicating that the results are satisfactory and in agreement with the certified values.

Table 5 shows the results obtained in the analyses of serum samples. To our knowledge, serum 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, sex, etc., which can affect trace element levels in serum, our preliminary findings agree with the data used as reference values in clinical laboratories[23] and those recently published by other authors.[2,24–26]

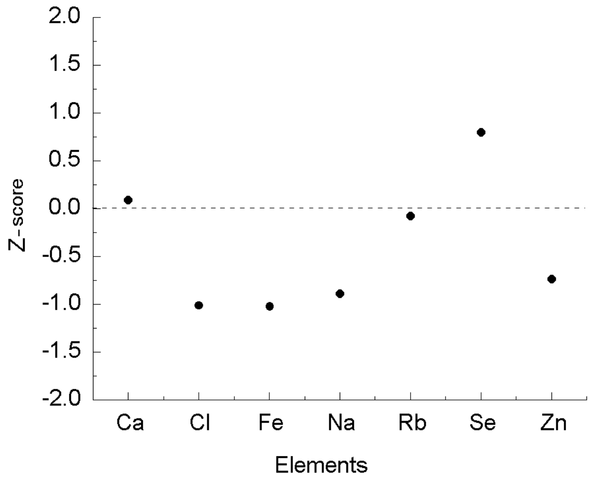

Fig. 1. Z_{score} values for the elements determined in NIST 1566b Oyster Tissue certified reference material

Conclusions

On the basis of the data obtained it can be concluded that the protocol established in this study was adequate since using contamination-free sampling and storage devices and reducing the sample preparation steps before the analysis avoided problems associated with the contamination. The experimental procedure established could be successfully applied in serum element determination for the elements Br, Ca, Cl, Fe, Na, Rb, Se and Zn. The preliminary data encourage further study for a larger population to obtain reference values.

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