J Radioanal Nucl Chem (2009) 282:81–84
DOI 10.1007/s10967-009-0272-7

Analysis of elements in human blood of patients with chronic kidney disease using neutron activation analysis

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Received: 1 July 2009 / Published online: 25 July 2009
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Abstract Neutron activation analysis has been used to determine Br, Ca, Cl, K, Mg and Na concentrations in whole blood of patients with chronic kidney disease (CKD) as well as in whole blood of normal individuals (control group). The dependence of the elements concentration in function of sex, age, time and type of treatment were investigated. The similarities and differences between healthy individuals and CKD are discussed.

Keywords NAA · Whole Blood · Biological material · Reference values

Introduction

Recently we have performed investigations in biochemistry using the Neutron Activation Analysis technique (NAA) resulting in an efficient tool for clinical practice [1–4]. Success in these medical applications has motivated us study the chronic kidney disease (CKD) which has high prevalence in Brazil: according to SBF ("Nephrology Brazilian Society") a percentile variation (2000/2008) increase of 86% [5] shows CKD has become an increasing public health problem.

Patients with CKD suffer from gradual and usually permanent loss of kidney function and need to have the blood filtered by machine (dialysis) or have to have a kidney transplant to stay alive. According to Table 1 five stages of increasing severity can be identified [6]. All individuals with a Glomerular filtration rate (GFR) < 60 mL/min/1.73 m^2 for 3 months are classified as having CKD, irrespective of the presence or absence of kidney damage. The treatment also depends on the GFR. For stages 1 to 3 the treatment is made by medication and alimentary control, because patients still have satisfactory residual renal function but for stages 4 and 5 it is necessary to submit the patient to the dialysis.

There are two types of dialysis: the Peritoneal Dialysis (PD) and Hemodialysis (HD). In the first, a sterile solution containing minerals and glucose is run through a tube into the abdominal body cavity, where the peritoneal membrane acts as a semi permeable membrane. In Hemodialysis treatment, the patient's blood is pumped through the blood compartment of a dialyzer, exposing it to an artificial semi permeable membrane. Because survival and quality of life are similar with both peritoneal and hemodialysis treatment, the selection of modality by the patient should be dictated by the life style that each therapy offers.

For CKD diagnoses the result of serum creatinine test is used to calculate GFR. Creatinine is a waste product in blood that comes from muscle activity. It is normally removed from blood by kidneys. When kidney function slows down, the creatinine level rises. This disease can also be identified by an image test (such as contrast X-ray) and by monitoring urea in serum [6].

According to National Health Surveillance Agency (ANVISA) Resolution—RDC N° 154 – 15 June 2004 [7] the Ca, Fe, K and Na levels in blood must be monitored monthly in these patients to check the efficiency of the treatment. Yet, according to this resolution the K levels in blood emphasize adequate clinical status of the patients [7].

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Table 1 Stages of chronic kidney disease [6]

Stage	Description	GFR* mL/min/1.73 m^2
1	Slight kidney damage with normal or increased filtration	More than 90
2	Mild decrease in kidney function	60–89
3	Moderate decrease in kidney function	30–59
4	Severe decrease in kidney function	15–29
5	Kidney failure requiring dialysis or transplantation	<15

*GFR is glomerular filtration rate, a measurement of the kidney's function

To better understand this disease first we investigated the behavior of Fe and P in whole blood of patients with CKD [8, 9]. These results revealed significant variations in the normal levels, mainly for Fe, in patients submitted for many years of treatment (>5 years). Now we intend to complement this investigation studying the behavior of the Br, Ca, Cl, K, Mg and Na elements that are relevant for checking the clinical status of patients in different stages of this kidney disease. Although the Br, Cl and Mg levels, usually, are not monitored in kidney diseases, but recent investigations, involving patients submitted to HD, have indicated several variations of those (Br, Cl and Mg) elements in blood [10].

Bromine is not majoritary in blood, but its measured could be useful for checking toxicology occurrence by medicine intake usually prepared with potassium bromide and/or sodium bromide. Furthermore, in clinical practice specifically in biochemistry analyses of blood, chlorine and magnesium are used by checked at the same time as a blood test for sodium: the correlation between them can be used for clinical diagnostics.

In this study Neutron Activation Analysis (NAA) has been used to determine Br, Ca, Cl, K, Mg and Na concentrations in whole blood of patients with CKD as well as in whole blood of "normal" individual (control). A comparison between these groups permits a discussion about behavior of these elements in blood of the CKD patients.

Experimental

Sample collection

To perform these measurements 136 health volunteers (control group), as well as, 24 patients with CKD were evaluated.

The biological samples of the volunteers came from HEMOPE bank blood of Recife city (Brazil). This control group (70 male and 66 female blood donors), age between 25 to 60 years old at 50 to 85 kg, were selected from this blood bank following the procedure conventionally established [4]. The patients with CKD were selected at the Pernambuco University (UPFE)—Hospital das Clinicas, also at Recife city. The patients were arranged in four groups, in function of type and time of treatment. The first group, submitted to conservative treatment (denominated GI), was composed of 6 patients. This group still has some satisfactory residual renal function. The treatment in this case is made by medicine and alimentary control. For these patients the whole blood collection was performed before the medical examination that has the monthly frequency. The GII group with 6 patients, undergoing Peritoneal Dialysis for 2 years, has the whole blood collection performed monthly before each treatment, as does the GIII group with 6 patients, submitted to Hemodialysis weekly for 2 years. The fourth group (GIV) was composed of 6 patients submitted to Hemodialysis for 5–11 years. For these patients the whole blood collection was performed after the Hemodialysis treatment. In Table 2 is presented the profile of these groups.

Sample preparation

The whole blood sample was collected in a vacuum plastic tube attached to the donor's/patient's arm (about 0.5 mL) and immediately after the collection exactly 100 µL of blood was transferred to filter paper (Whatman - N° 41). Details of this procedure are presented in reference [4]. All the samples were prepared in duplicate.

To determine the concentration of the elements, each biological sample was sealed into individual polyethylene bags and irradiated under a thermal neutrons flux

Table 2 Profile of the patients

Patient (n = 6)	Age* years	Sex (F) (%)	Type of treatment	Time of treatment
GI	60	60	Conservative	17 months
GII	42	70	PD	~2 years
GIII	52	83	HD	~2 year
GIV	43	100	HD	5–11 years

n Number of patients, *Mean value (±2), *F* Female, *PD* Peritoneal Dialysis, *HD* Hemodialysis

of $\sim 3.10^{12}$ n $cm^{-2}s^{-1}$, in the nuclear reactor IEA-R1 (2 MW, pool type) at IPEN. The irradiation time of 4 minutes was used and the counting time was 15 min for ^{80}Br, ^{49}Ca, ^{42}K and ^{56}Mg determination and 10 min for ^{24}Na and ^{38}Cl. The measurements were performed using an ORTEC Model GEM-60195 and ORTEC 671 amplifier (in pile-up rejection mode) coupled to a MCA ORTEC Model 919E. An iron shield [11] was employed to reduce the background radiation. The data was analyzed using in—house software.

Results and discussion

The quality of analytical results was evaluated by analyzing the NIST 8414 bovine muscle powder. The *Z*-score values (Table 3) indicate that the results are in agreement with certified values.

The Br, Ca, Cl, K, Mg and Na concentrations determined in whole blood samples of the four groups are presented in Table 4. The range of the control group is also presented for comparison using a confidence interval of 68% (±1 Standard Deviations).

All the concentration results of the four groups for Br, Ca, Cl, K, Mg and Na were included in the confidence interval of the control. Yet, according to ANVISA (Resolution – RDC N° 154)[7] these comparative analysis suggest that although these patients have kidney disorders the clinical status of them are satisfactory (93% of patients have the potassium concentration in adequate level). Yet, according to the *t*-test these cases are altered at significance ($p < 0.05$). However for Na the data revealed high concentration: for GIV (2.08 ± 0.50 gL^{-1}) is near of the upper limit for a confidence interval of 68% (1.48–2.06 gL^{-1}) and for GI (2.36 ± 0.21 gL^{-1}), GII (2.34 ± 0.23 gL^{-1}) and GIII (2.36 ± 0.50 gL^{-1}) they are near of the upper limit since a confidence interval of 95% (1.19–2.35 gL^{-1}) is adopted. Considering that the main way of entrances of sodium in the organism is by the diet and, also considering that individuals living in a coastal city have a greater intake of sea food rich in salt (mainly Na and Br), the alimentary habit could be responsible for the Na increase. Yet, considering that Na is the main extracellular electrolyte and Cl is the main anion in the blood, small variation in one of them could be reflected in both, consequently Cl evaluation in blood is also very useful. According to Table 4 there is correlation between the concentration values for Cl and Na: for GI, GII and GIII, both are over of the upper limit (3.48 gL^{-1} for Cl and 2.06 gL^{-1} for Na) while for GIV both present small reductions.

For Br, patients in the conservative treatment have high concentration near of upper limit (27 mgL^{-1}) while in those submitted to HD (GIV), where the medicine ingestion is severely controlled, the concentration is reduced. According to Canavese et al. [10] the Br levels could be monitored in patients submitted to HD due to the high incidence of insomnia (that reduce the Br levels in Blood) and the high consuming of somniferous (rich in Bromides)

Table 3 Element concentrations obtained in the analysis of NIST 8414 bovine muscle powder standard reference material

Element	Mean ± SD	Certified values	RSD (%)	Er (%)	Z-score
Br (mg kg^{-1})	1.3 ± 0.3	1.1 ± 0.5	23.1	18.2	0.4
Ca (mg kg^{-1})	151 ± 15	145 ± 20	9.9	4.1	0.3
Cl (%)	0.190 ± 0.018	0.188 ± 0.015	9.47	1.1	0.13
K (%)	1.506 ± 0.082	1.517 ± 0.037	5.4	−0.73	−0.30
Mg (mg kg^{-1})	933 ± 60	960 ± 95	6.4	−2.8	−0.28
Na (%)	0.211 ± 0.009	0.210 ± 0.008	4.3	0.48	0.13

Table 4 Mean value of the Br, Ca, Cl, K, Mg and Na concentrations in whole blood of GI, GII, GIII and GIV groups

Element *(Range)	GI MV ± 1 SD	GII MV ± 1 SD	GIII MV ± 1 SD	GIV MV ± 1 SD
Br (mg L^{-1}) [9–27]	31 ± 4	25 ± 10	20 ± 9	6 ± 7
Ca (gL^{-1}) [0.150–0.316]	0.286 ± 0.165	0.221 ± 0.082	0.181 ± 0.020	0.205 ± 0.026
Cl (gL^{-1}) [2.52–3.48]	3.50 ± 0.36	3.42 ± 0.41	3.40 ± 0.19	2.87 ± 0.73
K (gL^{-1}) [1.32–1.90]	1.46 ± 0.16	1.34 ± 0.34	1.56 ± 0.33	1.52 ± 0.49
Mg (mg L^{-1}) [40–74]	41 ± 15	41 ± 20	34 ± 11	35 ± 14
Na (gL^{-1}) [1.48–2.06]	2.36 ± 0.21	2.34 ± 0.23	2.36 ± 0.50	2.08 ± 0.50

MV Mean value, *SD* Standard deviations, *Control group

that increase the Br levels in blood. This control in all of the stages, including the conservative treatment, could keep the Br levels in the normal range avoid intoxication that has also high prevalence in Brazil, mainly for patients with CKD [7].

Conclusion

In this study some elements in whole blood of patients with CKD, that are relevant for clinical practice, were investigated by using NAA technique. The results revealed high concentration of Na (for ~40% of the patients). Also significant variations of Br level suggest that this element must also be evaluated in patients with CKD. The factors that may be responsible for the Br and Na behavior could be related to nutritional habits, medicine ingestion as well as the evolution of the CKD.

No serious alteration in the concentration of the Ca, Cl, K and Mg in blood samples of all groups when compared with healthy individuals.

Acknowledgement Financial support: FAPESP, CAPES and FACEPE.

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