NEUTRON ACTIVATION ANALYSIS OF Ca, Cl, Mg, Na, AND P CONTENTS IN THE HUMAN OSTEOGENIC SARCOMA

S. Zaichick[1,2], V. Zaichick[1]

[1] *Medical Radiological Research Centre of RAMS,*
Koroleva str., 4, Obninsk, 249020, Russia,
e-mail: vezai@obninsk.com

[2] *Current address: Northwestern University, Chicago, IL, 60611, USA*

Introduction

Bone tumors are a heterogeneous group of tumors that all arise from bone tissue, which consists of cartilaginous, osteoid and fibrous tissue, and bone marrow elements. Each tissue can give rise to benign or malignant tumors. The differentiation of benign and malignant intraosseous lesions can often be accomplished by means of conventional roentgenology, CT, and MRI. All of these methods of introscopy are very important, particularly for the assessment of tumor location, form, size, and infiltration of the adjacent tissue. However, the radiographic appearance of many lesions is indeterminate, and final diagnosis must be achieved using biopsy and histopathologic evaluation.

It is well known that tissues of human body differ greatly in their contents of chemical elements. Thus, it can be expected that bone tumors of a different origin would have specific elemental composition. In vivo neutron activation analysis (*in vivo* NAA) allows determination of some chemical element contents (Ca, Cl, and Na) in tumor tissue and has a potential to become a useful tool in oncology diagnostics [1,2].

To our knowledge, no data are available about the chemical element contents of bone tumors with respect to different origin of disease. Therefore, we determined the Ca, Cl, Mg, Na, and P contents in the osteogenic sarcoma and intact bone tissue using instrumental neutron-activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR).

Experimental

Samples of osteogenic sarcoma tissue were obtained from 61 patients (18 females and 43 males from 6 to 71 years old). All patients were hospitalized at the Medical Radiological Research Centre. In all cases the diagnosis has been confirmed by clinical and morphological data. The tumor samples for NAA were received from biopsy and resected specimens. The control group consisted of 27 patients with intact bone (7 females and 20 males from 6 to 50 years old) who died from different deceases. The intact cortical bone samples of femur and tibia were collected at the Department of Pathology, Obninsk City Hospital. All bone samples were freeze dried until constant mass was obtained. Then samples were sealed separately in thin polyethylene films washed with acetone and rectified alcohol. The sealed samples were placed in labeled polyethylene ampoules.

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol–formaldehyde resins were used [3]. Corrected certified values of BSS element contents were reported by us before [4]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. Ten certified reference material CRM IAEA H-5 (Animal Bone) and standard reference material SRM NIST 1486 (Bone Meal) sub-samples weighing about 50–100 mg were analyzed in the same conditions as bone samples to estimate the precision and accuracy of results.

The contents of Ca, Cl, Mg, Na, and P were determined by INAA-SLR using a horizontal channel equipped with the pneumatic rabbit system of the WWR-c research nuclear reactor. The neutron flux in the channel was 1.7×10^{13} n cm^{-2} s^{-1}. Ampoules with bone samples, BSS, intralaboratory-made standards, CRM and SRM were put into polyethylene rabbits and then irradiated separately for 60 s. Copper foils were used to assess neutron flux. The measurement of each sample was made 1 min after irradiation. The duration of the measurements was 10 min. A coaxial 98 cm^3 Ge (Li) detector and a spectrometric unit (NUC 8100), including a PC-coupled multichannel analyzer, were used for measurements. The spectrometric unit provided 2.9 keV resolution at the ^{60}Co 1332 keV line. The information of used nuclear reactions, radionuclides, gamma-energies, and other details of the analysis including the quality control of results were reported by us before [5].

A dedicated computer program of NAA mode optimization was used [6]. Using standard programs, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels were calculated for different chemical element mass fractions. The reliability of difference in the results between intact bone and osteogenic sarcoma tissue was evaluated by Student's t-test.

Results and discussion

Table 1 represents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Ca, Cl, Mg, Na, and P mass fractions in intact cortical bone samples and osteogenic sarcoma tissue.

The information of the effect of cancerous transformation on the chemical element contents in bone tissue is presented in Table 2. From Tables 2, it is observed that in osteogenic sarcoma tissue the mass fractions of Cl and Na are higher ($p \leq 0.001$) and the mass fraction of Ca is lower ($p \leq 0.001$) than in normal tissues. Different directions of changes suggest potential of mass fraction ratios of these elements as osteogenic sarcoma markers.

Table 3 depicts our data for some ratios of Ca, Cl, Mg, Na, and P mass fractions in intact cortical bone samples and osteogenic sarcoma tissue. It was shown that higher Cl/Ca and Cl/Na mass fraction ratios as well as lower Ca/P, Ca/Mg, and Ca/Na mass fraction ratios were typical of osteogenic sarcoma tissue compared with intact cortical bone (Table 3).

Fig.1 shows the histograms of Ca/Na, Cl/Ca, and Cl/Na ratios in all samples of intact bone (1) and osteogenic sarcoma tissue (2). Ca/Na, Cl/Ca, and Cl/Na ratios were chosen among others because it is possible to determine these ratios by *in vivo* NAA [1]. Using ratios of chemical elements mass fractions instead of the absolute values of mass fraction is better for making a specific diagnosis because of at least two reasons: 1) relations of elements do not depend on the moisture content (water) in the tissue; 2) defining relations of elements is more convenient for *in vivo* analysis.

Table 1. Some statistical parameters of Ca, Cl, Mg, Na, and P mass fractions in intact cortical bone and osteogenic sarcoma tissue ($g \cdot kg^{-1}$, dry weight basis)

Tissue	Element	M	SD	SEM	Min	Max	Med	P0.025	P0.975
Intact	Ca	222	43.6	9.3	166	369	212	174	317
cortical	Cl	1.52	1.42	0.30	0.40	6.80	1.10	0.455	5.04
bone	Mg	2.94	0.79	0.17	0.90	5.04	3.00	1.51	4.36
n=27	Na	6.40	1.74	0.36	3.80	11.7	6.00	4.41	10.9
	P	112	29.5	6.1	66.0	174	107	66.1	168
Osteogenic	Ca	136	70	10	20.4	287	141	22.4	279
sarcoma	Cl	8.68	6.81	0.99	1.60	35.4	6.60	1.73	28.8
n=61	Mg	2.84	1.14	0.17	0.298	5.10	2.55	1.30	4.89
	Na	8.73	3.43	0.51	2.90	15.5	8.30	3.40	15.3
	P	117	57	8.5	34	306	103	40.0	202

M - arithmetic mean; SD – standard deviation; SEM – standard error of mean; Min – minimum value; Max – maximum value; Per. 0.025 – percentile with 0.025 level; Per. 0.975 – percentile with 0.975 level

Table 2. Comparison between mean values (M±SEM) of Ca, Cl, Mg, Na, and P mass fraction in intact cortical bone and osteogenic sarcoma tissue ($g \cdot kg^{-1}$, dry weight basis)

Element	Intact cortical bone (I) n=27	Osteogenic sarcoma (II) n=61	Ratio II to I p (Student's t-test)
Ca	222±9	137±10	0.62^{c}
Cl	1.52±0.30	8.7±1.0	5.72^{c}
Mg	2.45±0.37	2.9±0.2	1.18
Na	6.40±0.36	8.7±0.5	1.36^{c}
P	112±6	117±9	1.04

M - arithmetic mean, SEM – standard error of mean, n – number of samples, ^{c} – $p \leq 0{,}001$

Table 3. Comparison between mean values (M±SEM) of Ca/P, Ca/Mg, Ca/Na, Cl/Ca, and Cl/Na mass fraction ratios in intact cortical bone and osteogenic sarcoma tissue

Element	Intact cortical bone (I) n=27	Osteogenic sarcoma (II) n=61	Ratio II to I p (Student's t-test)
Ca/P	2.06±0.07	1.31±0.09	0.64^{c}
(Ca/Mg)·0.01	0.81±0.07	0.49±0.03	0.60^{c}
(Ca/Na)·0.01	0.36±0.02	0.20±0.02	0.56^{c}
(Cl/Ca)·100	0.59±0.10	14±4	23.7^{b}
Cl/Na	0.22±0.03	0.84±0.04	3.82^{c}

M - arithmetic mean, SEM – standard error of mean, n – number of samples,
^{b} – $p \leq 0{,}01$, ^{c} – $p \leq 0{,}001$

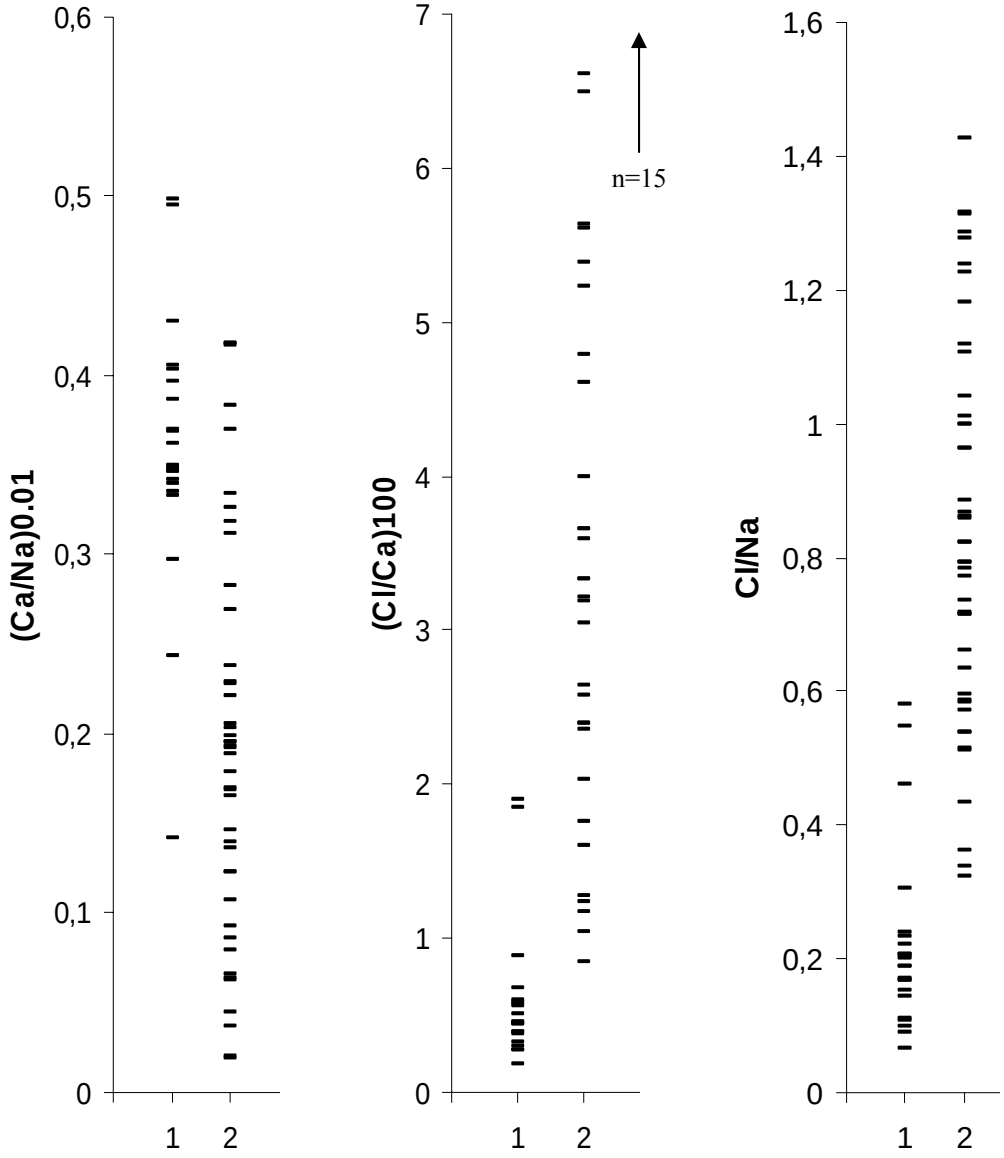

Fig. 1. Histograms of Ca/Na, Cl/Ca, and Cl/Na ratios in all samples of intact bone (1) and osteogenic sarcoma tissue (2)

As evident from the histograms, the Cl/Ca ration is the most information for the differential diagnostics. If (Cl/Ca)×100 ratio level of 1.0 (M±SD) was assumed to be an upper limit of intact bone tissue (Fig.1), the results of estimation "osteogenic sarcoma or intact bone" are the following:

Sensitivity = {correct positive test (CPT)/[CPT + false negative test (FNT)]}×100% = 98±2%;

Specificity = {correct negative test (CNT)/[CNT + false positive test (FPT)]} ×100% = 92±5%;

Accuracy = [(CPT+CNT)/(CPT+FNT+CNT+FPT)] ×100% = 96±2%.

The confidential intervals of these calculations with taking account of the number of the examined samples were taken from the Statistical tables by Genes [7]. In other words, if the analysis showed that (Cl/Ca)×100 ratio does not below 1.0 in tissue of bone examined site, one can diagnose a osteogenic sarcoma with accuracy of 96±2%. Using Cl/Ca -test makes it possible to find the 98±2% osteogenic sarcoma cases (sensitivity).

Conclusions

INAA- SLR is the adequate analytical tools for the non-destructive determination of Ca, Cl, Mg, Na, and P contents in the human bone samples and samples of intraosseous lesions weighing about 50 mg. It needs no more than 15 min (1 min irradiation by neutrons + 1 min exposure + 10 min spectrometric measurement) for analysis. It was found that in osteogenic sarcoma tissue the mass fractions of Cl and Na are significantly higher and the mass fraction of Ca is lower than in normal tissues. Moreover, it was shown that higher Cl/Ca and Cl/Na mass fraction ratios as well as lower Ca/P, Ca/Mg, and Ca/Na mass fraction ratios were typical of osteogenic sarcoma tissue compared with intact cortical bone. Differences between Cl/Ca ratio can be used as an additional test for differential diagnosis of normal bone and osteogenic sarcoma.

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