The Content of Silver, Cobalt, Chromium, Iron, Mercury, Rubidium, Antimony, Selenium, and Zinc in Malignant Giant Cell Tumor of Bone

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Abstract

Background and Purpose: The changes of trace element contents and trace element relationships in malignant giant cell tumor of bone (GCTB) in a comparison with normal bone tissue were investigated using a non-destructive neutron activation analysis with high resolution spectrometry of long-lived radionuclides.

Materials and Methods: The silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), rubidium (Rb), antimony (Sb), selenium (Se), and zinc (Zn) content and Co/Ag, Cr/Ag, Fe/Ag, Hg/Ag, Sb/Ag, Se/Ag, Co/Rb, Cr/Rb, Fe/Rb, Hg/Rb, Sb/Rb, Se/Rb, Co/Zn, Cr/Zn, Fe/Zn, Hg/Zn, Sb/Zn, and Se/Zn ratios were estimated in normal bone samples from 27 patients with intact bone (12 females and 15 males, aged from 16 to 49 years) and in tumor samples, obtained from open biopsies or after operation of 8 patients with malignant GCTB (3 females and 5 males, 14 to 56 years old). The reliability of difference in the results between intact bone and malignant GCTB was evaluated by Student's t-test.

Results: It was found that in malignant GCTB tissue the mass fractions of Co, Fe, Sb, and Se are significantly higher and the mass fraction of Rb is lower than in normal bone tissues. Moreover, it was shown that significantly higher Fe/Ag, Se/Ag, Se/Rb, Co/Zn, Fe/Zn, Hg/Zn, Sb/Zn, Se/Zn mass fraction ratios are typical of the malignant GCTB tissue compared to intact bone. In malignant GCTB tissue many correlations between trace elements found in the control group was no longer evident.

Conclusions: Finally, it was concluded that in malignant transformed bone tissues a trace element metabolism is significantly disturbed. The studies on the role of trace elements in the etiology of malignant GCTB and the usefulness of trace elements as bone tumor markers have to be continued.

Keywords: Trace elements, Human bone, Malignant giant cell tumor of bone, Neutron activation analysis

Introduction

The roles of trace elements in the development and inhibition of cancer have a complex character and have raised many questions because of their essential and toxic effects on human health. The effects of trace elements are related to content and recorded observations range from a deficiency state, to function as biologically essential components, to an unbalance when excess of one element interferes with the function of another, to pharmacologically active doses, and finally to toxic and even life-threatening levels [1,2]. Thus, there is a trace element homeostasis in tissues and fluids of human body in normal

environmental and health conditions and an unbalance of trace element contents could be a causative factor for many diseases, including cancer [2]. On the other hand pathological condition can effect on contents and relationships of trace elements in tissues and fluids. It puts a question about using these changes as markers of disease [2].

It is well known that tissues of human body differ greatly in their contents of trace elements. Particularly it concerns the chemical composition of bone tissue investigated by us in details in our previous studies [3-29]. Bone tumors are a heterogeneous group of tumors that all arise from bone tissue, which consists of cartilaginous, osteoid, osseous mineralized and fibrous tissue, and bone marrow elements. Each tissue can be subject to inflammation, benign or malignant tumors. Giant cell tumor of the bone (GCTB) is a relatively uncommon tumor [30]. It is a heterogeneous tumor composed of three different cell populations and characterized by the presence of multinucleated giant cells (osteoclast-like cells). GCTB is normally benign with unpredictable behavior including malignant transformation [31]. The World Health Organization classifies GCTB as "an aggressive, potentially malignant lesion" [32]. Identification of the malignant GCTB is a great challenge [30]. GCTB of bone was first described in 1818 and historically the lesion has been referred to by numerous terms, including *myeloid sarcoma*, *tumor of myeloplaxus*, *osteoclastoma*, and *osteoblastoclastoma* [33-37]. GCTB is a tumor, accounting for 4%–9.5% of all primary osseous neoplasm and 18%–23% of benign bone neoplasm [37,38]. GCTB predominately arise in long tubular bones (75-95%) with most cases (50%–65%) occurring near the knee. The next most common site is the distal radius (~10%). The epicenter of giant cell tumors is in the epiphysis [39,40]. GCTB is typically seen in early adulthood, with 80% of cases reported between the ages of 20 and 50, with a peak incidence between 20 and 30 [37]. GCTB may have aggressive features, including cortical expansion or destruction with a soft-tissue component [41]. The prevalence of malignant GCTB is controversial, although a figure of 5%-10% of all GCTB appears to be the most frequent consensus [37]. Because malignant GCTB is a very rare tumor entity, which is little examined, the exact etiology of this disease is still unknown [42]. To our knowledge, no data are available for the trace element contents of GCTB, to permit conclusion about their role in malignant transformation or pathogenesis. Due to a gap in knowledge about the trace elements of malignant GCTB we screened silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), rubidium (Rb), antimony (Sb), selenium (Se), and zinc (Zn), which are accessible by a non-destructive instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR).

This work had four aims. The first was to assess the Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn contents in two groups of bone tissue samples-intact bone and malignant GCTB using INAA-LLR. The second aim was to compare the trace element contents in different groups of samples and to choose trace elements which contents change in different directions. The third aim was to calculate the ratios of trace element that have been chosen and to compare these ratios in intact bone and malignant GCTB. The last aim was to evaluate the inter-correlations of trace element contents in normal and GCTB tissue.

All studies were approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk.

Materials and Methods

Patient's population

Thirty-five adolescents and adults were included in this study. The subjects were divided into two groups: reference (1) and malignant GCTB (2). The reference group consisted of 27 patients with intact bone (12 females and 15 males, aged from 16 to 49 years) who had died from various non bone related causes, mainly unexpected from trauma. The intact cortical bone samples of femur, femoral neck, tibia and iliac crest were collected at the Department of Pathology, Obninsk City Hospital. Samples from 8 patients with malignant GCTB (3 females and 5 males, 14 to 56 years old) were obtained from open biopsies or after operation from resected specimens. All patients with bone diseases were hospitalized at the Medical Radiological Research Centre. In all cases the diagnosis was confirmed by clinical and histological data.

Sample preparation

A titanium tool was used to cut and to scrub samples [43,44]. All bone and tumor tissue samples were freeze dried, until constant mass was obtained, and homogenized. Then samples weighing about 100 mg were wrapped separately in high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

Quality control

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins and aliquots of commercial, chemically pure compounds were used. Corrected certified values of BSS element contents were reported by us before [45]. Ten certified reference material (CRM) IAEA H-5 (Animal Bone) and standard reference material (SRM) NIST 1486 (Bone Meal) sub-samples weighing about 100 mg were analyzed in the same conditions as bone and tumor samples to estimate the precision and accuracy of results.

Neutron activation analysis

A vertical channel of the WWR-c research nuclear reactor was applied to determine the mass fraction of Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn-all trace elements which could be quantitative measured using INAA-LLR with available equipments and conditions. The quartz ampoule with bone samples, tumor samples, standards, CRM, and SRM was soldered, positioned in a transport aluminum container and exposed to a 100-hour neutron irradiation in a vertical channel with a thermal neutron flux about 10^{13} $n \cdot cm^{-2} \cdot s^{-1}$. Two months after irradiation samples were reweighed and repacked. The duration of each measurement was from 1 to 10 hours. To reduce the high intensity of ^{32}P β-particles ($T_{1/2}$=14.3 d) background, a berillium filter was used. 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 [24,45,46].

Statistical methods

A dedicated computer program of INAA mode optimization was used [47]. Using the Microsoft Office Excel 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 trace element mass fractions and their selected ratios. The reliability of difference in the results between intact bone and malignant GCTB tissue was evaluated by Student's t-test. For the estimation of the Pearson correlation coefficient between different pairs of the trace element mass fractions in intact bone and malignant GCTB tissue the Microsoft Office Excel program was also used.

Results

Table 1 depicts the basic statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) for the Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fraction in intact bone (age-matched control group) and malignant GCTB tissue.

The ratio of means and the reliability of difference between mean values of Al, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions in tissue of intact bone and malignant GCTB are presented in **Table 2**.

Table 3 represents the basic statistical parameters of Co/Ag, Cr/Ag, Fe/Ag, Hg/Ag, Sb/Ag, Se/Ag, Co/Rb, Cr/Rb, Fe/Rb, Hg/Rb, Sb/Rb, Se/Rb, Co/Zn, Cr/Zn, Fe/Zn, Hg/Zn, Sb/Zn, and Se/Zn mass fractions ratios in the samples of intact bone and malignant GCTB.

The ratio of means and the reliability of difference between mean values of Co/Ag, Cr/Ag, Fe/Ag, Hg/Ag, Sb/Ag, Se/Ag, Co/Rb, Cr/Rb, Fe/Rb, Hg/Rb, Sb/Rb, Se/Rb, Co/Zn, Cr/Zn, Fe/Zn, Hg/Zn, Sb/Zn, and Se/Zn mass fractions ratios in tissue of intact bone and GCTB are presented in **Table 4**.

The data of inter-correlation calculations (values of *r*-coefficient of correlation) including all pairs of the chemical elements identified by us in intact bone and malignant GCTB tissue are shown in **Table 5**.

Discussion

The non-destructive instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides was used in this research work because this method has many definite advantages over other analytical methods, particularly, in the clinical chemistry. Moreover, the non-destructive methods are the current gold-standard solution to control destructive analytical techniques [2]. The destructive analytical methods are based on measurements of processed tissue. In such studies tissue samples are ashed and/or acid digested before analysis. There is evidence that certain quantities of chemical elements are lost as a result of such treatment [2,44,48]. There is no doubt that every method available for the measurement of trace element

contents in bone and tumor samples can be used. However, when using destructive analytical methods it is necessary to control for the losses of trace elements, for complete acid digestion of the sample, and for the contaminations by trace elements during sample decomposition, which needs adding some chemicals.

In our previous study it was shown that the results of mean values for all representative elements of CRM IAEA H-5 (Animal Bone) and SRM NIST1486 (Bone Meal) were in the range of 95% confidence interval (M ± 2SD) of the certificates' values [24,45,46]. Good agreement with the certified data of CRM and SRM indicate an acceptable accuracy for the trace element mass fractions obtained in the study of intact bone and malignant GCTB tissue presented in **Tables 1**-**5**.

The mean values and all selected statistical parameters were calculated for 9 trace element (Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn) mass fractions (**Table 1**) and 18 trace element mass fraction ratios (Co/Ag, Cr/Ag, Fe/Ag, Hg/Ag, Sb/Ag, Se/Ag, Co/Rb, Cr/Rb, Fe/Rb, Hg/Rb, Sb/Rb, Se/Rb, Co/Zn, Cr/Zn, Fe/Zn, Hg/Zn, Sb/Zn, and Se/Zn) (**Table 3**). In control group the mass fractions of Co, Fe and Zn were measured in all samples, but the mass fraction of Rb – in 11 samples and mass fractions of Ag, Cr, Hg, Sb, and Se – in 10 samples. In malignant GCTB group the mass fraction of all nine trace elements were determined in all samples.

From **Table 2** it is observed that in the malignant GCTB tissue the mean mass fraction of Co, Cr, Fe, Hg, Sb, and Se is higher as well as the mean mass fraction of Ag, Rb, and Zn is lower than in normal bone tissues. However, in malignant GCTB only the mean mass fractions of Co ($p \leq 0.0050$), Fe ($p \leq 0.0047$), Sb ($p \leq 0.034$), and Se ($p \leq 0.0010$) are significantly increased and the mean mass fraction of Rb ($p \leq 0.050$) is significantly decreased when compared with those in normal bone. Different directions of mass fraction changes suggest potential use of mass fraction ratios of these trace elements as malignant GCTB markers. This conclusion was the main reason for calculating Co/Ag, Cr/Ag, Fe/Ag, Hg/Ag, Sb/Ag, Se/Ag, Co/Rb, Cr/Rb, Fe/Rb, Hg/Rb, Sb/Rb, Se/Rb, Co/Zn, Cr/Zn, Fe/Zn, Hg/Zn, Sb/Zn, and Se/Zn mass fractions ratios in intact bone and malignant GCTB samples (**Table 3**). It was found that higher mean values of all selected mass fraction ratios were typical of malignant GCT tissue compared with intact bone (**Table 4**). However, a statistically significant differences were only shown for the means of Fe/Ag ($p \leq 0.015$), Se/Ag ($p \leq 0.026$), Se/Rb ($p \leq 0.036$), Co/Zn ($p \leq 0.0029$), Fe/Zn ($p \leq 0.032$), Hg/Zn ($p \leq 0.042$), Sb/Zn ($p \leq 0.0043$), and Se/Zn ($p \leq 0.040$) mass fraction ratios (**Table 4**).

In control group a statistically significant direct correlation was found, for example, between the Fe and Se ($r=0.60$, $p \leq 0.05$), Fe and Co ($r=0.55$, $p \leq 0.01$), Co and Hg ($r=0.79$, $p \leq 0.01$), Rb and Ag ($r=0.62$, $p \leq 0.05$), and between Rb and Cr ($r=0.56$, $p \leq 0.05$) mass fractions (**Table 5**). In the same group a pronounced ($p \leq 0.01$) inverse correlation was observed between the Fe and Ag ($r=- 0.80$, $p \leq 0.05$). If some positive correlations between the trace elements were predictable (e.g., Fe-Co), the interpretation of other observed relationships requires further study for a more complete understanding.

In malignant GCTB tissue many significant correlations between

Table 1 Basic statistical parameters for Al, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions (mg/kg, dry mass basis) in tissue of intact bone and malignant giant cell tumor of bone .

Element	M	SD	SEM	Min	Max	Med	P0.025	P0.975
				Intact bone, n=27				
Ag	0.00274	0.00152	0.00051	0.000256	0.00468	0.00282	0.000320	0.00458
Co	0.0107	0.0070	0.0014	0.00370	0.0345	0.00785	0.00464	0.0288
Cr	0.274	0.182	0.057	0.110	0.669	0.202	0.117	0.629
Fe	51.2	46.3	9.3	9.20	173	30.2	9.68	155
Hg	0.0057	0.0044	0.0014	0.00100	0.0138	0.00525	0.00100	0.0133
Rb	3.68	1.58	0.48	0.970	6.57	3.30	1.40	6.41
Sb	0.0151	0.0102	0.0032	0.00600	0.0420	0.0139	0.00600	0.0364
Se	0.176	0.092	0.029	0.0550	0.358	0.169	0.0633	0.336
Zn	80.6	15.4	3.0	45.4	115	82.1	51.7	109
				Malignant giant cell tumor of bone, n=8				
Ag	0.00178	0.00189	0.00067	0.000200	0.00500	0.000785	0.000219	0.00484
Co	0.0365	0.0184	0.0065	0.0200	0.0780	0.0328	0.0202	0.0714
Cr	0.420	0.283	0.100	0.114	0.914	0.387	0.120	0.864
Fe	640	410	145	118	1362	559	148	1291
Hg	0.0077	0.0046	0.0017	0.00180	0.0167	0.00745	0.00225	0.0155
Rb	2.17	1.52	0.54	0.170	4.06	2.06	0.170	4.04
Sb	0.0328	0.0184	0.0065	0.0160	0.0620	0.0259	0.0164	0.0617
Se	1.84	0.88	0.31	0.730	3.14	1.77	0.784	3.11
Zn	65.6	38.0	13.4	20.6	127	59.6	22.8	124

M: Arithmetic mean, SD: Standard deviation, SEM: Standard error of mean, Min: minimum value, Max: maximum value, Med: median, P0.025: percentile with 0.025 level, P0.975: percentile with 0.975 level

Table 2 Means (M ± SEM, mg/kg on dry mass basis), ratio of means and the reliability of difference between mean values of Al, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions in tissue of intact bone and malignant giant cell tumor of bone.

Element	Intact bone	mGCTB	Student's t-test	Ratio
	M_1	M_2	$p \leq$	M_2 / M_1
Ag	0.00274 ± 0.00051	0.00178 ± 0.00067	0.254 (NS)	0.65
Co	0.0107 ± 0.0014	0.0365 ± 0.0065	0.0050	3.41
Cr	0.274 ± 0.057	0.420 ± 0.100	0.249 (NS)	1.53
Fe	51.2 ± 9.3	640 ± 145	0.0047	12.5
Hg	0.0057 ± 0.0014	0.0077 ± 0.0017	0.352 (NS)	1.35
Rb	3.68 ± 0.48	2.17 ± 0.54	0.050	0.59
Sb	0.0151 ± 0.0032	0.0328 ± 0.0065	0.034	2.17
Se	0.176 ± 0.029	1.84 ± 0.31	0.0010	10.5
Zn	80.6 ± 3.0	65.6 ± 13.4	0.309 (NS)	0.81

M: arithmetic mean, SEM: standard error of mean, mGCTB: malignant giant cell tumor of bone, NS: not significant

trace elements found in the control group are no longer evident, for example, direct correlation between Fe and Se, etc. (**Table 5**). However, many other significant correlations between trace elements were arisen, for example, direct correlation between Zn and Co (r=0.67, $p \leq$0.05), Zn and Hg (r=0.57, $p \leq$0.05), Zn and Sb (r=0.74, $p \leq$0.05), Se and Co (r=0.56, $p \leq$0.05), etc. (**Table 5**) Thus, if we accept the levels and relationships of trace element mass fraction in the intact bone samples of control group as a norm, we have to conclude that with a malignant transformation the levels and relationships of trace elements in bone significantly changed. No published data referring to correlations between trace elements mass fractions in malignant GCTB tissue were found.

The changes in trace element contents of cancerous tissues in comparison with non-cancerous tissues may be attributed to a cause or effect of malignant transformation. Bone is a mineralized connective tissue. Many trace elements are bone-seeking elements and closely associated with bone hydroxyapatite [24-28]. GCTB is classified as a bone tumor. Our previous findings showed that the means of the Ca and P mass fraction in the malignant GCTB tissue are lower than in normal bone, but the mean of Ca/P ratio is similar [49]. It suggested that GCTB tissues continue to form bone hydroxyapatite but in a less degree than normal bone. A decrease in content of hydroxyapatite may be a cause of low level of Zn mass fraction in the malignant GCTB (**Table 4**), because Zn is a bone-seeking element [27,28].

Our findings show that the mean of the Fe mass fraction in the malignant GCTB tissue samples was 12.5 times greater than in normal bone tissues (**Table 2**). It is well known that Fe mass fraction in sample depends mainly from the blood volumes in tissues.

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Table 3 Basic statistical parameters for Co/Ag, Cr/Ag, Fe/Ag, Hg/Ag, Sb/Ag, Se/Ag, Co/Rb, Cr/Rb, Fe/Rb, Hg/Rb, Sb/Rb, Se/Rb, Co/Zn, Cr/Zn, Fe/Zn, Hg/Zn, Sb/Zn, and Se/Zn mass fractions ratios in tissue of intact bone and malignant giant cell tumor of bone.

Ratio	M	SD	SEM	Min	Max	Med	P0.025	P0.975
Intact bone, n=27								
Co/Ag	12.7	19.3	6.1	1.46	60.0	4.13	1.59	54.3
Cr/Ag	162	183	58	41.7	645	88.0	42.1	555
$(Fe/Ag)\times10^{-2}$	824	1443	457	21.9	4023	144	27.9	3795
Hg/Ag	3.81	5.94	1.88	0.325	20.3	2.32	0.338	16.6
Sb/Ag	11.4	15.9	5.0	2.18	50.8	4.81	2.22	45.7
Se/Ag	19.8	34.1	10.8	2.12	102	4.55	2.36	92.7
$(Co/Rb)\times10^{2}$	0.61	0.99	0.30	0.128	3.56	0.252	0.145	2.87
$(Cr/Rb)\times10$	0.79	0.41	0.13	0.237	1.47	0.735	0.269	1.45
Fe/Rb	29	51	15	2.49	178	10.8	2.75	142
$(Hg/Rb)\times10^{3}$	2.3	3.5	1.1	0.303	12.1	1.23	0.308	9.84
$(Sb/Rb)\times10^{3}$	5.2	4.9	1.6	1.48	16.6	3.52	1.52	15.4
$(Se/Rb)\times10^{2}$	7.1	10.6	3.3	2.04	36.9	3.48	2.28	30.4
$(Co/Zn)\times10^{3}$	0.136	0.088	0.017	0.053	0.404	0.0995	0.0593	0.364
$(Cr/Zn)\times10^{3}$	2.94	1.78	0.56	1.57	6.37	2.07	1.59	6.27
Fe/Zn	0.692	0.745	0.149	0.0984	3.15	0.329	0.120	2.48
$(Hg/Zn)\times10^{4}$	0.649	0.479	0.152	0.0990	1.37	0.592	0.0995	1.36
$(Sb/Zn)\times10^{3}$	0.165	0.108	0.034	0.0723	0.449	0.133	0.0753	0.393
$(Se/Zn)\times10^{2}$	0.193	0.104	0.033	0.0647	0.420	0.169	0.0735	0.388
Malignant giant cell tumor of bone, n=8								
Co/Ag	60	59	21	4.22	183	49.6	5.15	167
Cr/Ag	603	595	210	41.8	1839	453	45.3	1704
$(Fe/Ag)\times10^{-2}$	7967	6317	2234	904	16220	6841	1125	15902
Hg/Ag	14.0	13.8	4.9	0.667	38.6	11.5	0.820	36.9
Sb/Ag	65	101	36	3.98	310	37.9	4.48	266
Se/Ag	352	335	118	17.9	920	290	18.8	883
$(Co/Rb)\times10^{2}$	5.5	7.9	2.8	0.520	22.9	2.03	0.585	21.0
$(Cr/Rb)\times10$	4.7	5.0	1.8	0.291	14.9	2.94	0.330	13.8
Fe/Rb	1159	1795	635	36.8	5206	477	43.2	4701
$(Hg/Rb)\times10^{3}$	11.3	15.9	5.6	1.06	43.8	3.32	1.21	41.1
$(Sb/Rb)\times10^{3}$	44	57	20	4.90	165	15.1	5.03	152
$(Se/Rb)\times10^{2}$	199	210	74	28.1	612	115	31.4	580
$(Co/Zn)\times10^{3}$	0.66	0.33	0.12	0.287	1.41	0.594	0.317	1.28
$(Cr/Zn)\times10^{3}$	9.01	9.35	3.31	0.898	30.6	5.45	1.47	27.4
Fe/Zn	14.0	14.0	5.0	1.89	46.5	11.6	1.95	41.1
$(Hg/Zn)\times10^{4}$	1.49	0.91	0.32	0.234	3.16	1.39	0.299	3.01
$(Sb/Zn)\times10^{3}$	0.57	0.28	0.10	0.288	1.15	0.491	0.292	1.09
$(Se/Zn)\times10^{2}$	4.00	4.29	1.52	1.29	14.4	2.85	1.32	12.5

M: arithmetic mean, SD: standard deviation, SEM: standard error of mean, Min: minimum value, Max: maximum value, Med: median, P0.025: percentile with 0.025 level, P0.975: percentile with 0.975 level

Giant-cell tumors are predominantly hypervascular lesions [50]. Thus, it is possible to speculate that GCTB is characterized by an increase of the mean value of the Fe mass fraction because the level of tumor vascularization is higher than that in normal bone. As was found by us, there is a direct correlation between Fe and Co mass fractions (**Table 5**). Therefore an increased level of Co in the malignant GCTB may be closely connected with a high Fe content in tumor tissue (**Table 2**).

In the malignant GCTB tissue the mean Se mass fractions is 10 times higher ($p \leq 0.001$) than in normal tissues (**Table 2**). The high Se level was reported in malignant tumors of ovary [51], lung [52], prostate [53], breast [54,55], intestine [56], and in gastric cancer tissue [57]. The role played by Se in those tumors remains unknown, but in general it is accepted that certain proteins containing Se can mediate in the protective effects. The literature-based analysis found the association of malignant tissue transformation with local oxidative stress. Studies have shown that oxidative stress conditions play an important role in both the initiation and the progression of cancer by regulating molecules such as DNA, enhancers, transcription factors, and cell cycle regulators [58]. Sancak et al. [59] have confirmed the association between oxidative stress and Se levels in patients with breast cancer at different clinical stages. Increased glutathione peroxidase enzyme activity in erythrocytes, closely related to

Table 4 Means (M ± SEM), ratio of means and the reliability of difference between mean values of Co/Ag, Cr/Ag, Fe/Ag, Hg/Ag, Sb/Ag, Se/Ag, Co/Rb, Cr/Rb, Fe/Rb, Hg/Rb, Sb/Rb, Se/Rb, Co/Zn, Cr/Zn, Fe/Zn, Hg/Zn, Sb/Zn, and Se/Zn mass fractions ratios in tissue of intact bone and malignant giant cell tumor of bone

Ratio	Intact bone $M_1(R)$	mGCTB $M_2(R)$	Student's t-test $p \leq$	Ratio $M_2(R) / M_1(R)$
Co/Ag	12.7 ± 6.1	60 ± 21	0.059 (NS)	4.72
Cr/Ag	162 ± 58	603 ± 210	0.078 (NS)	3.72
$(Fe/Ag) \times 10^{-2}$	824 ± 457	7967 ± 2234	0.015	9.67
Hg/Ag	3.81 ± 1.88	14.0 ± 4.9	0.083 (NS)	3.67
Sb/Ag	11.4 ± 5.0	65 ± 36	0.181 (NS)	5.70
Se/Ag	19.8 ± 10.8	352 ± 118	0.026	17.8
$(Co/Rb) \times 10^2$	0.61 ± 0.30	5.5 ± 2.8	0.122 (NS)	9.02
$(Cr/Rb) \times 10$	0.79 ± 0.13	4.7 ± 1.8	0.062 (NS)	5.95
Fe/Rb	29 ± 15	1159 ± 635	0.118 (NS)	40.0
$(Hg/Rb) \times 10^3$	2.3 ± 1.1	11.3 ± 5.6	0.156 (NS)	4.91
$(Sb/Rb) \times 10^3$	5.2 ± 1.6	44 ± 20	0.099 (NS)	8.46
$(Se/Rb) \times 10^2$	7.1 ± 3.3	199 ± 74	0.036	28.0
$(Co/Zn) \times 10^3$	0.136 ± 0.017	0.66 ± 0.12	0.0029	4.85
$(Cr/Zn) \times 10^3$	2.94 ± 0.56	9.01 ± 3.31	0.111 (NS)	3.06
Fe/Zn	0.692 ± 0.149	14.0 ± 5.0	0.032	20.2
$(Hg/Zn) \times 10^4$	0.649 ± 0.152	1.49 ± 0.32	0.042	2.30
$(Sb/Zn) \times 10^3$	0.165 ± 0.034	0.57 ± 0.10	0.0043	3.45
$(Se/Zn) \times 10^2$	0.193 ± 0.033	4.00 ± 1.52	0.040	20.7

M: arithmetic mean, SEM: standard error of mean, mGCTB: malignant giant cell tumor of bone, NS: not significant

Table 5 Intercorrelations of pairs of the trace element mass fractions in tissue of intact bone and malignant giant cell tumor of bone.

Tissue	Element	Co	Cr	Fe	Hg	Rb	Sb	Se	Zn
Intact bone n=27	Ag	-0.23	0.51	-0.80[b]	-0.02	0.62[a]	0.31	-0.45	0.38
	Co	**1.00**	0.16	0.55[b]	0.79[b]	-0.10	0.08	0.52	0.17
	Cr	0.16	**1.00**	-0.48	0.51	0.56[a]	-0.31	-0.08	0.46
	Fe	0.55[b]	-0.48	**1.00**	0.09	-0.54	-0.25	0.60[a]	-0.17
	Hg	0.79[b]	0.51	0.09	**1.00**	0.18	-0.13	0.35	-0.14
	Rb	-0.10	0.56[a]	-0.54	0.18	**1.00**	-0.05	-0.06	0.34
	Sb	0.08	-0.31	-0.25	-0.13	-0.05	**1.00**	0.04	0.22
	Se	0.52	-0.08	0.60[a]	0.35	-0.06	0.04	**1.00**	0.24
	Zn	0.46	0.46	-0.17	-0.14	0.34	0.22	0.24	**1.00**
mGCTB n=8	Ag	-0.13	-0.08	0.13	-0.18	-0.01	-0.08	-0.57[a]	-0.30
	Co	**1.00**	0.30	0.75[a]	0.73[a]	0.02	0.35	0.56[a]	0.67[a]
	Cr	0.30	**1.00**	0.31	-0.16	-0.07	0.24	0.52	-0.03
	Fe	0.75[a]	0.31	**1.00**	0.52	-0.34	0.04	0.46	0.10
	Hg	0.73[a]	-0.16	0.52	**1.00**	0.33	-0.23	0.54	0.57[a]
	Rb	0.02	-0.07	-0.34	0.33	**1.00**	0.24	0.29	0.37
	Sb	0.35	0.24	0.04	-0.23	0.24	**1.00**	0.10	0.74[a]
	Se	0.56[a]	0.52	0.46	0.54	0.29	0.10	**1.00**	0.27
	Zn	0.67[a]	-0.03	0.10	0.57[a]	0.37	0.74[a]	0.27	**1.00**

mGCTB: malignant Giant Cell Tumor of Bone, Statistically significant difference: [a] – p 0.05, [b] – p 0.01.

Se level, may result from a protective mechanism developed by breast cancer patients against free radical damage. Thus, we can partially explain high Se level in malignant GCTB tissues via hypervascularity of these lesions. However the cause of increased Se in cancerous tissue and particularly in the malignant GCTB is not completely understood and requires further studies.

The low number of malignant GCTB samples (8 patients, 3 females and 5 males, 14 to 56 years old) examined in this study does not allow statistical comparisons of trace element accumulation or dynamics between different age and gender groups. Therefore it cannot be fully excluded that the presented results for malignant GCTB samples include effects linked to age and gender. Moreover, there are many other trace elements associated with the levels of oxidative stress in tissue. Thus, further studies are needed to increase the number of bone samples affected by malignant GCTB and to extend the list of trace elements investigated.

Conclusion

Instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides is a satisfactory analytical tool to determine non-destructively the elemental content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn in human bone samples and samples of intraosseous lesions weighing about 100 mg. In malignant GCTB tissue the mass fractions of Co, Fe, Sb, and Se are significantly higher and the mass fraction of Rb is lower than in normal bone tissues. Moreover, significantly higher Fe/Ag, Se/Ag, Se/Rb, Co/Zn, Fe/Zn, Hg/Zn, Sb/Zn, Se/Zn mass fraction ratios are typical of the GCT tissue compared to intact bone. In malignant GCTB tissue many correlations between trace elements found in the control group are no longer evident. Thus, if we accept the levels and relationships of trace element mass fraction in the intact bone as a norm, we have to conclude that in malignant transformed bone tissues a trace element homeostasis is significantly disturbed. The studies on the role of trace elements in the etiology of malignant GCTB and the usefulness of trace elements as bone tumor markers have to be continued.

Acknowledgements

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