Acta Scientific Cancer Biology

Volume 2 Issue 2 February 2018

Research Article

Significance of Trace Element Quantities in Chondroma and Chondrosarcoma

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Received: February 04 2018; **Published:** February 13, 2018

Abstract

To clarify the role of trace elements in the etiology and the pathogenesis of chondroma (ChO) and chondrosarcoma (ChS) of bone, a nondestructive neutron activation analysis with high resolution spectrometry of long-lived radionuclides were performed. The silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), rubidium (Rb), antimony (Sb), selenium (Se), and zinc (Zn) mass fraction were measured in three groups of samples: normal bone samples from 27 patients with intact bone (12 females and 15 males), who had died from various non-bone related causes, mainly unexpectedly from trauma, and also in samples, obtained from open biopsies or after operation of 5 patients with chondroma (2 females and 3 males) and 16 patients with chondrosarcoma (3 females and 13 males). The difference in the results between trace element contents in the three groups was evaluated by the parametric Student's t-test and non-parametric Wilcoxon-Mann-Whitney U-test. In the bone affected by ChO the mean mass fractions of Co, Se, and Zn were significantly higher while the mean mass fraction of Sb was lower than in normal bone tissues. In ChS tissue the mean mass fractions of Co, Fe, and Se were higher while the mean mass fraction of Rb was lower than in normal bone tissues. In ChS tissue the mean mass fractions of Co, Hg and Sb were higher and the mean mass fractions of Rb and Zn were lower than in ChO tissue. Moreover, many correlations between trace elements found in the control group were no longer evident in the neoplastic bone. Thus, considerable changes in trace element content and their relationships were found in chondroma and chondrosarcoma and possible causes and effects of these alterations are discussed.

Keywords: Trace Elements; Human Bone; Chondroma; Chondrosarcoma; Neutron Activation Analysis

Abbreviations

ChO: Chondroma; ChS: Chondrosarcoma; TE: Trace Elements; INAA: Instrumental Neutron Activation Analysis; INAA-LLR: INAA with High Resolution Spectrometry of Long-Lived Radionuclides; BSS: Biological Synthetic Standards; CRM: Certified Reference Material; SRM: Standard Reference Material

Introduction

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, and/or benign or malignant tumors. Bone neoplasms are often difficult to detect in their early stages because the associated signs and symptoms can be nonspecific, insidious in onset, and mimic more common disorders [1]. One of the most important differential diagnoses is between a benign and a malignant neoplasm with similar histology, for example, such as chondroma (ChO) and chondrosarcoma (ChS). In addition between ChOs and ChSs there is no significant difference in clinical symptoms and both tumors present as slowly growing, painful, and fixed structures [2,3].

ChOs, also called exostosis or osteochondromas, are benign tumors composed of mature hyaline cartilage. They generally have limited growth potential and are not locally aggressive. In the United States benign cartilage tumors account for 27.5% of all bone tumors and international data for ChO do not differ significantly from US figure [4]. About 60% of ChO occur in the small bones of the hands and feet. The next most common sites are the long tubular bones. Of the long bones, the femur is the most commonly involved (17%). The proximal and distal metaphysis of the femur are involved more often than the diaphyses [4].

ChS is the second most common primary bone cancer, whose tumor cells produce a pure hyaline cartilage that results in abnormal bone and/or cartilage growth. About one fourth of malignant bone cancers are ChS. Although any bone can be affected, the long bones such as legs, arms, fingers, toes are most commonly involved. ChS is typically seen in adulthood between the ages of late 20 to 60 and occurs more commonly in men than women [2,4].

Thus, ChO is a benign bone tumor, but ChS is a sarcoma, or malignant tumor of connective tissue. It is important to understand the difference between a benign and malignant cartilage tumor, because accurate preoperative diagnosis is important in ensur-

ing appropriate treatment of the patient. Differentiating involves assimilation and interpretation of clinicopathological, radiological, histopathological, and biochemical features [2,3,5-10]. All imaging methods such as conventional roentgenography, functional nuclear medicine including scintigraphy and positron emission tomography, computed tomography, and magnetic resonance imaging are very important for the assessment of tumor location, shape, size, structure and infiltration of the adjacent tissue [5,7,8,10]. However, the review of the specific clinical, radiological, molecular, and histologic criteria currently used clinically to differentiate between ChO and ChS show that a definite distinction is almost impossible, and the histological differentiation can be extremely difficult [2,9]. Moreover, it is obvious that clinical imaging and histopathologic evaluation of biopsy samples is not useful or practical as a routine examination which can be easily used to diagnose ChO and ChS, therefore, special methods should be developed and used to make a distinction between these benign and malignant tumors [3]. Thus, the goals of many investigations are to assist the clinician in making an appropriate diagnosis by providing a rational method of selecting non-traumatic diagnostic tests that maximize specificity and minimize costs.

It is well known that the tissues of human body differ greatly in their proportions of chemical elements and that there is the homeostasis of both bulk and trace element (TE) contents [11]. Our detailed previous studies have confirmed this using a chemical composition analysis of bone tissue [12-38]. Thus, it can be expected that normal bone and bone tumors, possessing very different properties, have specific and different TE compositions. Moreover, as was shown by us in previous studies in vivo neutron activation analysis allows determination of some chemical element contents in intact bone, benign and malignant lesions of bone and has a potential to become a valuable diagnostic tool [14,15,27,39].

To our knowledge, no data are available for the TE contents of ChO and ChS, to permit distinction between benign and malignant tumor.

This work had three aims. The first was to obtain reliable data for silver (Ag), cobalt (Co), chromium (Cr), iron(Fe), mercury (Hg), rubidium (Rb), antimony (Sb), selenium (Se), and zinc (Zn) contents in three groups of bone tissue samples – intact bone, ChO and ChS using non-destructive instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR). The second aim was to compare the TE contents in the different groups of samples and the third was to calculate inter-correlations between TE contents in each group of bone tissue samples.

All studies were approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Material and Methods

Sample Preparation

Forty-eight children, adolescents and adults were included in this study. The subjects were divided into three groups: control (1), chondroma (2) and chondrosarcoma (3). The reference/control group consisted of 27 persons 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 bone samples mainly of femur and tibia were collected at the Department of Pathology, Obninsk City Hospital. Samples from 5 patients with ChO (2 females and 3 males aged between 14 and 42 years) and 16 patients with ChS (3 females and 13 males, from 8 to 65 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.

A titanium tool was used to cut and to scrape samples [40,41]. All bone and tumor tissue samples were freeze dried, until constant mass was obtained, and homogenized. Then samples weighing about 50 - 100 mg were wrapped separately in high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

Instrumentation and Method

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 earlier [42,43]. Ten certified reference material (CRM) IAEA H-5 (Animal Bone) subsamples and ten 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 the results

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 by INAA-LLR. 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 the 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 cm3 Ge (Li) detector and a spectrometric unit (NUC 8100, Hungary), 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. Information concerning the nuclear reactions, radionuclides and gamma-energies employed, together with other details of the analysis including the quality control of results were reported by us previously [31,33,34,43].

Computer Programs and Statistic

A dedicated computer program of INAA mode optimization was used [44]. Using the Microsoft Office Excel software, the following quantities 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 the TE mass fractions. The differences in the results between intact bone, benign ChO and malignant ChS were evaluated using the parametric Student's t-test and non-parametric Wilcoxon-Mann-Whitney U-test. For the estimation of the Pearson correlation coefficient between different pairs of the TE mass fractions in each group of bone and tumor tissue samples the Microsoft Office Excel software was also used.

Results

Table 1 depicts our data for nine TE mass fractions determined by INAA-LLR in ten sub-samples of CRM IAEA H-5 Animal Bone and SRM NIST 1486 Bone Meal reference material and the certified values of this material.

Table 2 presents 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 Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions in the samples of intact bone, benign ChO and malignant ChS.

Element	CRM IAEA H-5		This work results	SRM NIST 1486		This work results
	Mean	Type	Mean ± SD	Mean	Type	Mean ± SD
Ag	-	-	< 0.02 DL	-	-	< 0.02 DL
Co	0.25	N	0.56 ± 0.25	-	-	0.11 ± 0.02
Cr	2.56	N	< 0.8 DL	-	-	≤ 0.9
Fe	79 ± 11	C	85 ± 17	99±8	C	93 ± 11
Hg	0.008	N	≤ 0.01	-	-	≤ 0.01
Rb	1.07	N	≤ 1.0	-	-	≤ 0.9
Sb	0.024	N	≤ 0.02	-	-	≤ 0.02
Se	0.054	N	≤ 0.05	0.13	N	≤ 0.05
Zn	89 ± 15	C	86 ± 7	147 ± 16	C	153 ± 29

Table 1: INAA data of trace elements of CRM IAEA H-5 Animal Bone and SRM NIST 1486 Bone Meal (mg/kg on dry weight basis)

M: Arithmetic Mean; SD: Standard Deviation; C: Certified Values; N: Non-Certified Values.

E	M	SD	SEM	Min	Max	Med	P0.025	P0.975
Intact bone (N), 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
Chondroma (ChO), n = 5								
Ag	0.00329	0.00073	0.00033	0.00246	0.00437	0.00313	0.00250	0.00429
Co	0.0208	0.0052	0.0023	0.0119	0.0257	0.0220	0.0129	0.0254
Cr	0.289	0.045	0.020	0.236	0.353	0.288	0.239	0.348
Fe	138	140	62	51.4	384	95.0	51.8	356
Hg	0.0023	0.0029	0.0013	0.00050	0.00718	0.00050	0.00050	0.00676
Rb	3.09	1.21	0.54	1.43	4.21	3.27	1.52	4.21
Sb	0.0082	0.0024	0.0011	0.00582	0.0109	0.00788	0.00583	0.0109
Se	1.84	2.05	0.92	0.274	4.39	0.450	0.280	4.33
Zn	237	152	68	94.1	489	211	99.5	464
Chondrosarcoma (ChS), n = 16								
Ag	0.00296	0.00286	0.00072	0.000430	0.0122	0.00249	0.000509	0.00961
Co	0.0365	0.0236	0.0061	0.00950	0.0998	0.0282	0.0111	0.0876
Cr	0.483	0.386	0.097	0.0980	1.51	0.416	0.116	1.41
Fe	178	126	34	16.5	413	129	29.8	402
Hg	0.0172	0.0192	0.0048	0.00007	0.0571	0.00796	0.000505	0.0537
Rb	1.56	1.58	0.40	0.170	6.15	1.33	0.170	5.11
Sb	0.0171	0.0122	0.0031	0.00510	0.0437	0.0130	0.00518	0.0418
Se	1.78	1.19	0.31	0.203	4.32	1.70	0.251	3.81
Zn	118	56	14	41.7	226	108	47.4	225

Table 2: 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 (N), chondroma (ChO) and chondrosarcoma (ChS).

E: Element; 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.

Information concerning the effect of benign or malignant transformation on the TE mass fractions in bone is presented in table 3.

The data for inter-correlation calculations (values of r – coefficient of correlation) including all pairs of the TE identified by us in the samples of intact bone, benign ChO and malignant ChS are shown in table 4.

Groups of samples	E	Norm (N)	Chondroma (ChO)	*t*-test $p \leq$	U-test *p*	Rati ChO/N
Chondroma and intact bone	Ag	0.00274 ± .00051	0.00329 ± 0.00033	0.354	> 0.05	1.20
	Co	0.0107 ± 0.0014	0.0208 ± 0.0023	**0.007**	**≤ 0.01**	1.94
	Cr	0.274 ± 0.057	0.289 ± 0.020	0.807	> 0.05	1.06
	Fe	51.2 ± 9.3	138 ± 62	0.240	> 0.05	2.70
	Hg	0.0057 ± 0.0014	0.0023 ± 0.0013	0.105	> 0.05	0.40
	Rb	3.68 ± 0.48	3.09 ± 0.54	0.428	> 0.05	0.84
	Sb	0.0151 ± 0.0032	0.0082 ± 0.0011	0.067	**≤ 0.05**	0.54
	Se	0.176 ± 0.029	1.84 ± 0.92	0.143	**≤ 0.05**	10.5
	Zn	80.6 ± 3.0	237 ± 68	0.083	**≤ 0.01**	2.94
Groups of samples	E	Norm (N)	Chondrosarcoma (ChS)	*t*-test $p \leq$	U-test *p*	Ratio ChS/N
Chondro-sarcoma and intact bone	Ag	0.00274 ± .00051	0.00296 ± 0.00072	0.850	> 0.05	1.08
	Co	0.0107 ± 0.0014	0.0365 ± 0.0061	**0.002**	**≤ 0.01**	3.41
	Cr	0.274 ± 0.057	0.483 ± 0.097	0.156	>0.05	1.76
	Fe	51.2 ± 9.3	178 ± 34	**0.005**	**≤ 0.01**	3.48
	Hg	0.0057 ± 0.0014	0.0172 ± 0.0048	0.128	> 0.05	3.02
	Rb	3.68 ± 0.48	1.56 ± 0.40	**0.001**	**≤ 0.01**	0.42
	Sb	0.0151 ± 0.0032	0.0171 ± 0.0031	0.575	>0.05	1.13
	Se	0.176 ± 0.029	1.78 ± 0.31	**0.001**	**≤ 0.01**	10.1
	Zn	80.6 ± 3.0	118 ± 14	0.078	> 0.05	1.46
Groups of samples	E	Chondroma (ChO)	Chondrosarcoma (ChS)	*t*-test $p \leq$	U-test *p*	Ratio ChS/ChO
Chondro-sarcoma and chondroma	Ag	0.00329 ± 0.00033	0.00296 ± 0.00072	0.685	> 0.05	0.90
	Co	0.0208 ± 0.0023	0.0365 ± 0.0061	**0.019**	**≤ 0.01**	1.76
	Cr	0.289 ± 0.020	0.483 ± 0.097	0.067	> 0.05	1.67
	Fe	138 ± 62	178 ± 34	0.584	> 0.05	1.29
	Hg	0.0023 ± 0.0013	0.0172 ± 0.0048	**0.008**	**≤ 0.01**	7.48
	Rb	3.09 ± 0.54	1.56 ± 0.40	**0.048**	**≤ 0.01**	0.51
	Sb	0.0082 ± 0.0011	0.0171 ± 0.0031	**0.013**	**≤ 0.01**	2.09
	Se	1.84 ± 0.92	1.78 ± 0.31	0.945	> 0.05	0.97
	Zn	237 ± 68	118 ± 14	0.157	**≤ 0.05**	0.50

Table 3: Differences between mean values (M ± SEM) of Al, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions (mg/kg, dry mass basis) in tissue of intact bone (N), chondroma (ChO) and chondrosarcoma (ChS).

E: Element; M: Arithmetic Mean; SEM: Standard Error of Mean; t-test: Parametric Student's t-Test; U-test: Non-Parametric Wilcoxon-Mann-Whitney Test; Statistically significant values are in **bold.**

Tissue	Element	Co	Cr	Fe	Hg	Rb	Sb	Se	Zn
Intact bone	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[a]	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[a]	-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	.17	0.46	-0.17	-0.14	0.34	0.22	0.24	**1.00**
Chondroma	Ag	-0.89[a]	-0.12	-0.24	0.19	0.55	-0.17	-0.08	0.56
	Co	**1.00**	0.10	0.02	-0.29	-0.34	0.46	0.26	-0.82[a]
	Cr	0.10	**1.00**	-0.43	-0.60	0.64	-0.34	-0.29	0.38
	Fe	0.02	-0.43	**1.00**	-0.21	-0.42	-0.54	0.61	-0.03
	Hg	-0.29	-0.60	-0.21	**1.00**	-0.60	0.46	-0.58	0.02
	Rb	-0.34	0.64	-0.42	-0.60	**1.00**	-0.28	0.11	0.39
	Sb	0.46	-0.34	-0.54	0.46	-0.28	**1.00**	-0.13	-0.74[a]
	Se	0.26	-0.29	0.61	-0.58	0.11	-0.13	**1.00**	-0.45
	Zn	-0.82[a]	0.38	-0.03	0.02	0.39	-0.74[a]	-0.45	**1.00**
Chondrosarcoma	Ag	0.14	0.08	0.13	0.12	0.38	-0.24	-0.36	0.20
	Co	**1.00**	0.61[a]	0.38	0.04	0.23	0.34	0.28	-0.08
	Cr	0.61[a]	**1.00**	0.11	-0.03	0.06	0.26	0.12	0.11
	Fe	0.38	0.11	**1.00**	-0.16	-0.01	0.16	0.07	-0.10
	Hg	0.04	-0.03	-0.16	**1.00**	0.75[b]	-0.11	0.19	0.64[b]
	Rb	0.23	0.06	-0.01	0.75[b]	**1.00**	0.01	-0.09	0.39
	Sb	0.34	0.26	0.16	-0.11	0.01	**1.00**	0.34	0.07
	Se	0.28	0.12	0.07	0.19	-0.09	0.34	**1.00**	-0.10
	Zn	-0.08	0.11	-0.10	0.64[b]	0.39	0.07	-0.10	**1.00**

Table 4: Intercorrelations of pairs of the trace element mass fractions in tissue of intact bone, chondroma and chondrosarcoma. Statistically significant difference: [a]: $p \leq 0.05$, [b]: $p \leq 0.01$, [c]: $p \leq 0.001$.

Discussion

The non-destructive INAA-LLR was used in this research study because this method has many definite advantages over other analytical methods, particularly, in the clinical chemistry. For example, after non-destructive INAA-LLR there is a possibility to check the results for some TE and to receive additional information about other TE contents by destructive analytical methods such as atomic absorption spectrometry, inductively coupled plasma atomic emission spectrometry, inductively coupled plasma mass spectrometry and so on, using the same bone samples. Moreover, if a deep-cooled channel of nuclear reactor is available, the non-destructive INAA-LLR allows determining TE contents in the fresh bone/tumor samples and combining TE study with histological investigation. It is also necessary to keep in mind that the non-destructive methods are the current gold-standard solution to control destructive analytical techniques [11]. 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 TE are lost as a result of such treatment [11,41,45]. There is no doubt that every method available for the measurement of TE contents in bone and tumor samples can be used. However, when using destructive analytical methods it is necessary to control for the losses of TE, for complete acid digestion of the sample, and for the contaminations by TE during sample decomposition, which needs adding some chemicals.

The results of mean values for Fe and Zn - two representative TE 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 (Table 1). Good agreement with the certified data of CRM and SRM for Fe and Zn mass fractions determined by INAA-LLR indicate an acceptable accuracy and for other TE mass fractions obtained in the study of intact bone and tumor tissue samples presented in tables 2-4.

The mean values and all selected statistical parameters were calculated for nine TE (Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn) mass fractions in normal bone, benign ChO and malignant ChS samples (Table 2). From Table 3 it is observed that in the ChO tissue the mean mass fractions of Co, Se, and Zn are respectively 1.2, 10.5, and 2.9 times higher while the mean mass fraction of Sb is almost

2 times lower than in normal bone tissues. In the ChS tissue the mean mass fractions of Co, Fe, and Se are respectively 3.4, 3.5, and 10.1 times higher while the mean mass fraction of Rb is more than 2 lower than in normal bone tissues. In malignant ChS tissue the mean mass fractions of Co, Hg, and Sb are significantly higher (about 1.8, 7.5, and 2.1 times, respectively) and the mean mass fractions of Rb and Zn are almost 2 times lower than in benign ChO tissue.

In the control group a statistically significant direct correlation was found between the Ag and Rb ($r = 0.62$, $p \leq 0.05$), Co and Fe ($r = 0.55$, $p \leq 0.05$), Co and Hg ($r = 0.79$, $p \leq 0.01$), Cr and Rb ($r = 0.56$, $p \leq 0.05$), and between Fe and Se ($r = 0.60$, $p \leq 0.05$) mass fractions (Table 4). In the same group a pronounced inverse correlation was observed between the Fe and Ag ($r = -0.80$, $p \leq 0.01$). If some positive correlations between the TE were predictable (e.g., Fe–Co), the interpretation of other observed relationships requires further study for a more complete understanding.

In the ChO tissue many significant correlations between TE found in the control group are no longer evident, for example, direct correlation between Fe and Co or between Fe and Se, etc. (Table 4). However, other inverse correlations between Co and Ag ($r = -0.89$, $p \leq 0.05$), Co and Zn($r = -0.82$, $p \leq 0.05$), and also between Sb and Zn ($r = -0.74$, $p \leq 0.05$) were observed (Table 4).

Similarly, in the malignant ChS tissue many significant correlations between TE found in the control group are also no longer evident, for example, direct correlation between Fe and Co or between Fe and Se, etc. (Table 4). However, direct correlations between Co and Cr ($r = 0.61$, $p \leq 0.05$), Hg and Rb ($r = 0.75$, $p \leq 0.01$), and also Hg and Zn ($r = 0.64$, $p \leq 0.01$) were observed (Table 4).

Thus, if we accept the levels and relationships of TE mass fraction in the intact bone samples of control group as a norm, we have to conclude that with neoplasms the levels and relationships of TE in bone significantly change. No published data referring to contents of TE or correlations between TE mass fractions in the ChO and ChS of bone were found.

Characteristically, elevated or reduced levels of TE observed in neoplasms tissues are discussed in terms of their potential role in the initiation and promotion of tumor. In other words, using the low or high levels of the TE in tumors researchers try to determine the carcinogenic role of the deficiency or excess of each TE in investigated organ. In our opinion, abnormal levels of many TE in tumor could be and cause, and also effect of benign or malignant transformation. From the results of such kind studies, it is not always possible to decide whether the measured decrease or increase in TE level in pathologically altered tissue is the reason for alterations or vice versa.

Bone is a mineralized connective tissue. It is formed by osteoblasts, that deposit collagen and release Ca, Mg, and phosphate ions that combine chemically within the collagenous matrix into a crystalline mineral, known as bone hydroxyapatite. On average, bone tissue contains about 10 - 25% water, 25% protein fibers like collagen, and 50% hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$. Many TE are bone-seeking elements and they are closely associated with hydroxyapatite [33,34,37]. ChO and ChS are classified as a bone tumor. Our previous findings showed that the means of the Ca and P mass fraction in the ChO and ChS of bone tissue are lower than in normal bone, but the mean of Ca/P ratio is similar [46,47]. It suggested that ChO and ChS continues to form bone hydroxyapatite but to a lesser degree than normal bone.

Health effects of high exposure to Co, whether resulting from occupational, environmental, dietary and medical contact are characterized by a complex clinical syndrome, including mainly neurological, cardiovascular and endocrine deficits [48,49]. Co is genotoxic and carcinogenic. This is mainly caused by oxidative DNA damage by reactive oxygen species (ROS), perhaps combined with inhibition of DNA repair [50]. Indeed, Co ions affect osteoblast proliferation, size, and shape. Co ions also promote secretion of cytokines from osteoblasts, which leads to inflammation and osteoclast differentiation, maturation, and stimulation [51]. Thus, a neoplastic effect of elevated Co level in ChO and ChS tissue may be assumed. It was found in the present study, that there is a direct correlation between Fe and Co levels in normal bone (Table 4). Therefore an increased level of Co in both ChO and ChS is closely connected to a very high Fe content in tumor tissue (Table 3). Anyway, the accumulation of Co in ChO and ChS tissue could possibly be explored as a diagnostic marker for these tumors.

Our findings show that the mean of the Fe mass fractions in the ChO and ChS tissue samples were respectively 2,7 and 3.5 times greater than in normal bone tissues (Table 3). It is well known that the Fe mass fraction in a tissue sample depends mainly on the blood volume in that tissue. Thus, one can speculate that ChO and ChS are characterized by an increase of the mean values of the Fe mass fractions because its levels of tumor vascularization are higher than that of normal bone. Moreover, one can deduce that the level of malignant ChS vascularization is higher than that of benign ChO. Thus, this difference could possibly be explored to aid the diagnosis of tumor malignancy.

Hg is one of the most dangerous environmental pollutants. Metallic and inorganic Hg is released into the air and then deposited in the form of methyl mercury. Hg damages the central nervous system and causes neurological symptoms (e.g. Minamata disease). Moreover, this metal has been classified as certain or probable carcinogen by the International Agency for Research on Cancer [52]. Mercury may be involved in four main processes that lead to genotoxicity: generation of free radicals and oxidative stress, action on microtubules, influence on DNA repair mechanisms and direct interaction with DNA molecules [53]. Although no adverse effects of Hg on bone metabolism have been reported, in the light of present study, it would certainly be interesting to further investigate the possible effects of Hg on tumor transformation of bone. Anyway, the great difference between accumulation of Hg in ChO and ChS tissue could possibly be explored to aid the diagnosis of tumor malignancy.

There is very little information about the effects of Rb in organisms. No negative environmental effects have been reported. Rb is only slightly toxic on an acute toxicological basis and would pose an acute health hazard only when ingested in large quantities [54]. Rb has some function in immune responce [55], probably by supporting cell differentiation [56]. The reason for a lower level of Rb in both ChO and ChS tissue than that in normal bone is not completely understood and requires further studies. However, a significantly lower Rb level in ChS in comparison with that in ChO could possibly be explored to aid the diagnosis of tumor malignancy.

Animal carcinogenicity data were concluded sufficient for Sb [57]. Possible mechanisms of Sb's action include its potential to produce active ROS and to interfere with the DNA repair system [57]. The cause of Sb accumulation by ChS tissue is not completely understood and requires further studies. An elevated level of Sb in ChS in comparison with normal bone and ChO could possibly be explored for aid in making the differential diagnosis between these benign and malignant tumors.

In the both ChO and ChS tissue the mean Se mass fractions were more than 10 times higher than in normal bone (Table 3). A high Se level was reported in malignant tumors of the ovary [58], lung [59], prostate [60-68], breast [69,70], gastro intestinal tract [71], and also in cancers of the stomach [72] and thyroid [73]. Moreover, in our previous study elevated levels of Se were found in such malignant tumors of bone as osteogenic sarcoma [74], chondrosarcoma [75], and Ewing's sarcoma [76]. The role played by Se in those tumors remains unknown, but in general it is accepted that certain proteins containing Se can mediate the protective effects against oxidative stress. A 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 [77]. However the cause of increased Se in tumors and particularly in ChO and ChS of bone is not completely understood and requires further studies. Anyway, the great accumulation of Se in ChO and ChS tissue could possibly be explored to aid the diagnosis of these tumors.

Zn is active in more than 300 proteins and over 100 DNA-binding proteins, including the tumor suppressor protein p53, a Zn-binding transcription factor acting as a key regulator of cell growth and survival after various forms of cellular stress. p53 is mutated in half of human tumors and its activity is tightly regulated by metals and redox mechanisms. Zn ions are cofactors of the superoxide dismutase enzymes, which prevent the onset and progression of tumors through cell protection against substances that cause the formation of free radicals and reactive oxygen species. The role of zinc is to act as a membrane stabilizer and to participate in antioxidative protection and oxidative stress inhibition.

A low level of Zn was reported in malignant tumors of liver [78,79,80], kidney [78], uterus [81], lung [80,82], prostate [60-67,80,83-86], stomach [87], testis [87], thyroid [72,80,86,87] and in esophageal squamous cell cancer [88]. These facts imply that reduced Zn content in tumors is probably one of the factors in the etiology of malignant transformation of different tissues, because Zn deficiency has been linked to severe deficiency in immune function and disruption in T-Cell function. Zn deficiency also causes inactivation of p53, a tumor suppressor protein, which has been associated with many cancers [87]. But, ChO and ChS tissues are characterized by higher level of Zn than that in normal bone. The cause of Zn accumulation by ChO and ChS tissue is not completely understood and requires further studies. However, it is well known that not only Zn deficiency but also excess of this metal may be deleterious and involved in the etiology of malignant transformation of different tissues [89]. Thus, it seems that the window of optimal Zn level in tissues is quite narrow. Anyway, a reduced level of Zn in ChS in comparison with ChO could possibly be explored as a means of differential diagnosis between these benign and malignant tumors.

Trace element inter-correlations: Each of the TE is distinct in its primary mode of action. Moreover, there are several forms of synergistic action of the TE as a part of intracellular metabolism, during which several reactive intermediates and byproducts are created [90-92]. These reactive species are capable of potent and surprisingly selective activation of stress-signaling pathways, inhibition of DNA metabolism, repair, and formation of DNA crosslinks, which are known to contribute to the development of human cancers [91,93]. Thus, in addition to TE contents changes of TE relationships (inter-correlations) might be involved in etiology and pathophysiology of bone tumors.

This study has several limitations. Firstly, analytical techniques employed in this study measure only nine TE (Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn) mass fractions. There are many other TE associated with different levels of oxidative stress and carcinogenesis. Thus, future studies should be directed toward using other analytical methods which will extend the list of TE investigated in normal bone as well as in the ChO and ChS of bone. Secondly, the sample size of ChO and ChS groups was relatively small. Despite these limitations, this study provides evidence that the levels of Co, Fe, Hg, Rb, Sb, Se, and Zn mass fractions have altered in ChO and ChS tissue and shows the necessity to continue TE research of these tumors of bone.

Conclusion

INAA-LLR is a most satisfactory analytical tool to determine non-destructively the elemental content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn in samples of human intact bone and also in samples of ChO and ChS. In the bone affected by ChO the mean mass fractions of Co, Se, and Zn were significantly higher while the mean mass fraction of Sb was lower than in normal bone tissues. In ChS tissue the mean mass fractions of Co, Fe, and Se were higher while the mean mass fraction of Rb was lower than in normal bone tissues. In ChS tissue the mean mass fractions of Co, Hg and Sb were higher and the mean mass fractions of Rb and Zn were lower than in ChO tissue. In addition, many inter-correlations between TE contents found in the control group were no longer evident in the tumor transformed bone. Thus, if we accept the levels and relationships of TE mass fraction in the intact bone as a norm, we have to conclude that in ChO and ChS tissues the TE homeostasis was significantly disturbed. The studies on the role of TE in the etiology and pathogenesis of ChO and ChS should be continued, because of the limitations of numbers of different TEs studied in this work and to determine relevant mechanisms which may explain the findings. This paper has only considered two specific bone neoplasms. However the value of this approach to the determination of the malignant or benign nature of a tumor using TE analysis has been confirmed. It is likely to have many other useful applications and deserves to be included in the diagnostician's armamentarium after appropriate experimental confirmation.

Acknowledgements

We are grateful to the late Prof. V.A. Bizer, Medical Radiological Research Center, Obninsk, for supplying GCT tissue samples.

Conflict of Interest

Authors have declared that no competing interests exist.

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