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Measurement of trace element content in bone samples using long-lived neutron activation products and high-resolution gamma-ray spectrometry

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Abstract A nondestructive instrumental neutron activation analysis with high-resolution gamma-ray spectrometry of long-lived radionuclides was developed and used for measurement of trace element contents in samples of bone to determine health and diseases. Using this method, the silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), rubidium (Rb), antimony (Sb), selenium (Se), and zinc (Zn) mass fractions were estimated in bone samples from 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 traumas, and from 5 patients with chondroma (2 females and 3 males, 15–42 years old), obtained from open biopsies or after operation. The reliability of the differences in the results between intact bone and bone affected by chondroma was evaluated by a parametric Student's *t* test and a nonparametric Mann–Whitney *U* test. It was found that in the bone affected by chondroma, the mean mass fractions of Co, Cr, Fe, Se, Sb, and Zn were significantly higher than in normal bone tissues. In the neoplastic bone, many correlations between trace elements found in the control group were no longer evident. This work revealed that there is a significant disturbance of the trace element metabolism in bone affected by chondroma.

Keywords Neutron activation analysis · Trace elements · Human bone · Bone tumors

1 Introduction

The measurement of the trace element contents in bone samples is a difficult analytical task because bone is a very highly mineralized tissue. On average, bone tissue contains about 10 %–25 % water, 25 % protein fibers, like collagen, and 50 % mineral hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. All the usual analytical methods for trace element measurement are based on the investigation of processed tissue with a goal to resolve samples or to resolve samples and remove the organic/mineral matrix. 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 [1–3]. Thus, when using destructive analytical methods, it is necessary to control for the loss of trace elements, for complete acid digestion of the sample, and for the contamination by trace elements during sample decomposition, which requires adding some chemicals. It is possible to avoid these difficult procedures using nondestructive nuclear analytical methods, including neutron activation analysis.

Trace elements play a great role in the normal function and pathophysiology of bone. The effects of trace elements are related to content, and recorded observations range from a deficiency state to functioning as biologically essential components to an unbalance when an excess of one element interferes with the function of another to pharmacologically

The study was approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk.

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active doses and, finally, to toxic and even life-threatening levels [1, 4]. The roles of trace elements in the development and inhibition of diseases have given rise to many questions because of their essential and toxic effects on bone health. Thus, in normal environmental and health conditions, there is a range of normal levels of trace elements in bone. An imbalance of trace element contents could be a causative factor for many diseases [1]. On the other hand, pathological conditions can affect the content and relationships of trace elements in bone, and it is possible to use these changes as markers of disease [1].

Bone diseases can derive from all the tissue components of bone (cartilage, osteoid, fibrous tissue, and bone marrow elements). Each tissue can be subject to inflammation and benign or malignant tumors. It is well known that the tissues of human bodies differ greatly in their contents of trace elements. Our previous detailed studies have shown this using a chemical composition analysis of bone tissues [5–31].

Chondroma (chondromas), also called exostosis or osteochondroma, are benign tumors composed of mature hyaline cartilage. They generally have limited growth potential and are not locally aggressive. In the USA, benign cartilage tumors account for 27.5 % of all bone tumors, and international data for chondroma do not differ significantly from the US figure [32]. About 60 % of chondroma occurs 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 is involved more often than the diaphyses [32]. Histological differentiation of chondroma can be extremely difficult [33]. To our knowledge, no data are available for the trace element contents in bone affected by chondroma to permit conclusions about their role in the etiology, pathogenesis, and diagnostics of this disease.

The primary purpose of this study was to investigate the possibilities of nondestructive instrumental neutron activation analysis with high-resolution gamma-ray spectrometry of long-lived radionuclides (INAA-LLR) for trace element measurements in samples of osseous tissue. The second aim was to evaluate the quality of obtained results. The third aim was to compare the trace element mass fractions in intact bone and bone affected by chondroma. The final aim was to estimate the interelement correlations in intact bone and a disturbance of these correlations in the chondroma tissue.

2 Experimental

Thirty-two adolescents and adults were included in this study. The subjects were divided into two groups: reference and chondroma. The reference group consisted of 27 patients with intact bone (12 females and 15 males, aged from 16 to 49 years, $M \pm SD$ 34 ± 11 years) who had died from various non-bone-related causes, mainly unexpected trauma. The intact cortical bone samples of the femur, femoral neck, tibia, and iliac crest were collected at the Department of Pathology, Obninsk City Hospital. Samples from 5 patients with chondroma (2 females and 3 males, 15–42 years old, $M \pm SD$ 32 ± 11 years) 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 scrub samples [2, 34]. All bone and tumor tissue samples were freeze-dried, until constant mass was obtained, and pulverized using a porcelain pestle and mortar. 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.

To determine the contents of the elements by comparison with a known standard, biological synthetic standards (BSSs) 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 previously [35]. Ten certified reference material (CRM) IAEA H-5 (Animal Bone) subsamples weighing about 100 mg were analyzed under the same conditions as the bone and tumor samples to estimate the precision and accuracy of the results.

A vertical channel of the WWR-c research nuclear reactor was used 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, and CRM was soldered, positioned in a transport aluminum container, and exposed to a 100-h neutron irradiation in a vertical channel with a thermal neutron flux of about 10^{13}n/(cm^2 s). Two months after irradiation, the samples were reweighed and repacked in polyethylene ampoules for spectroscopy. The duration of each measurement was from 1 to 10 h. To reduce the high intensity of ^{32}P β-particles ($T_{1/2} = 14.3$d) in the background, a beryllium 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 a 2.9 keV resolution at the ^{60}Co 1332 keV line. Sample–detector distance depended on the intensity of gamma radiation from the sample and varied from 0 to 5 cm. The detector's shielding was 5cm of lead.

A dedicated computer program of INAA mode optimization was used [36]. Using the Microsoft Office Excel program, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and

maximum values, median, and percentiles with 0.025 and 0.975 levels were calculated for different trace element mass fractions. The reliability of the differences in the results between intact bone and chondroma tissue was evaluated by a parametric Student's t test and a nonparametric Mann–Whitney U test. The Microsoft Office Excel program was also used for the estimation of the Pearson correlation coefficient between different pairs of the trace element mass fractions in intact bone and bone affected by chondroma.

3 Results and discussion

It was found that the equipment and parameters used for the nondestructive neutron activation analysis in the study allow a precise measurement of nine trace elements in the samples of osseous tissue: Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn.

Information on used nuclear reactions, radionuclides, gamma energies, and possible interferences is presented in Table 1. ^{203}Hg has the only line of 279.19 keV, which coincides with the 279.54 keV (25 %) line of ^{75}Se. However, ^{75}Se has more intensive lines, 136 (56 %) and 265 keV (60 %) (Table 1). Using information about the 136 and 265 keV ^{75}Se lines, the intensity of the 279.54 keV line was calculated and the interference with the ^{203}Hg 279.19 keV line was under control.

We determined the contents of all 9 trace elements (Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn) that cover the range of 8 elements (Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn) with certified and informative values in CRM IAEA H-5 (Animal Bone). Mean values of the contents ($\pm SEM$) for all eight trace elements (Table 2) measured were in the range of 95 % confidence interval values of the certificate [37]. Good agreement with the certified and informative data of CRM indicates an acceptable accuracy of the results obtained in the study of trace element contents in the samples of osseous tissue presented in Fig. 1 and Tables 3, 4 and 5.

Table 1 Radionuclides and some of their characteristics used for INAA-LLR of bone samples

Element	Radionuclide	Half-life	γ-energy used (keV)
Ag	^{110m}Ag	250.0 days	658, 1384
Co	^{60}Co	5.64 years	1173, 1332
Cr	^{51}Cr	27.8 days	320
Fe	^{59}Fe	45.6 days	1099, 1292
Hg	^{203}Hg	46.91 days	279[a]
Rb	^{86}Rb	18.66 days	1076
Sb	^{124}Sb	60.9 days	603, 1691
Se	^{75}Se	120.4 days	136, 265, 401
Zn	^{65}Zn	245.7 days	1115

[a] Interference with ^{75}Se

In the control group, the mass fractions of Co, Fe, and Zn were measured in all samples. The mass fraction of Rb was measured in 11 samples, and mass fractions of Ag, Cr, Hg, Sb, and Se, in 10 samples. In the chondroma group, the mass fraction of all nine trace elements was determined in all samples. Figure 1 shows the individual data sets for the Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions (mg/kg, dry mass basis) in all samples of intact bone (1) and chondroma tissue (2). Table 3 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 and bone affected by chondroma. This table shows basic statistical parameters valid for normal and abnormal distribution of results. Statistical parameters, such as M, SD, and SEM, are valid only for a normal distribution. However, as a rule, no less a hundred results need to prove a law of distribution. Therefore, Table 3 also includes some parameters valid for an abnormal distribution, such as median and percentiles.

The ratio of means and the reliability of the differences between the mean values of Al, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions in intact bone and bone affected by chondroma is presented in Table 4. It shows that, in the chondroma group, the mean mass fraction of Ag, Co, Cr, Fe, Se, and Zn is higher and, vice versa, the mass fraction of Hg, Rb, and Sb is lower than in the normal bone tissue. The parametric Student's t test shows that, in the chondroma tissue, only the mean mass fraction of Co is significantly ($p \leq 0.0073$) increased when compared with those in normal bone. However, the nonparametric Mann–Whitney U test shows significant differences for the Co, Cr, Fe, Sb, Se, and Zn contents (Table 4).

The data from the intercorrelation calculations (values of r—the Pearson correlation coefficient), including all pairs of the chemical elements identified by us in the intact bone and the bone affected by chondroma, are given in Table 5. In the 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 Rb and Cr ($r = 0.56$, $p \leq 0.05$) mass fractions (Table 5). In the same group, a pronounced inverse correlation was observed between 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.

Table 2 INAA-LLR data of trace elements of CRM IAEA H-5 animal bone (mg/kg, dry mass basis)

Element	CRM IAEA H-5			This work results
	M	95 % confidence interval	Type of values	*M* ± *SEM*
Ag	–	–	–	0.030 ± 0.011
Co	0.25	0.16–0.33	Informative value	0.22 ± 0.08
Cr	2.56	1.80–3.31	Informative value	1.44 ± 0.40
Fe	79 ± 11	76.6–84.8	Certified	84 ± 9
Hg	0.008	0.0012–0.0149	Informative value	<0.01
Rb	1.07	0.25–1.90	Informative value	<1.0
Sb	0.024	0.0127–0.0362	Informative value	0.023 ± 0.006
Se	0.054	0.0335–0.0740	Informative value	0.051 ± 0.009
Zn	89 ± 15	85.2–94.6	Certified	83.4 ± 1.8

M arithmetic mean, *SEM* standard error of mean

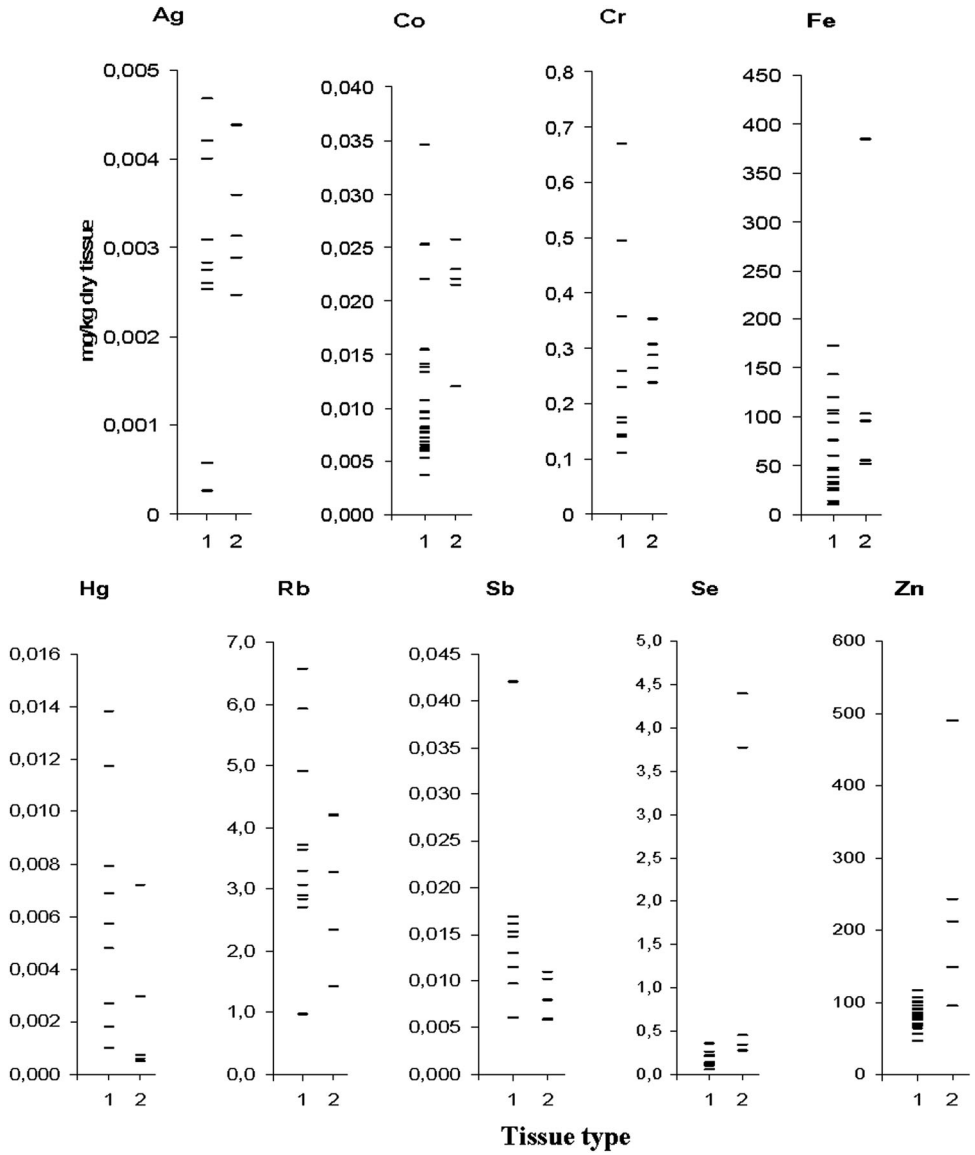

Fig. 1 Individual data sets for Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions (mg/kg dry tissue) in intact bone (*1*) and bone affected by chondroma (*2*)

Table 3 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 bone affected by chondroma

Element	*M*	*SD*	*SEM*	Min	Max	Med	$P_{0.025}$	$P_{0.975}$
Intact bone, $n = 27$								
Ag	0.0027	0.0015	0.0005	0.00026	0.0047	0.0028	0.00032	0.0046
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	0.0053	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, $n = 5$								
Ag	0.0033	0.0007	0.0003	0.0025	0.00444	0.0031	0.0025	0.0043
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	0.0005	0.0072	0.0005	0.0005	0.0068
Rb	3.09	1.21	0.54	1.43	4.21	3.27	1.52	4.21
Sb	0.0082	0.0024	0.0011	0.0058	0.011	0.0079	0.0058	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

M arithmetic mean, *SD* standard deviation, *SEM* standard error of mean, *Min* minimum value, *Max* maximum value, *MED* median, $P_{0.025}$ percentile with 0.025 level, $P_{0.975}$ percentile with 0.975 level

Table 4 Means ($M \pm SEM$, mg/kg, 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 bone affected by chondroma

Element	Intact bone M_1	Chondroma M_2	Ratio M_2/M_1	Student's *t* test *p*	Mann–Whitney *U* test *p*
Ag	0.0027 ± 0.0005	0.0033 ± 0.0003	1.20	0.354	*NS*
Co	0.0107 ± 0.0014	0.0208 ± 0.0023	1.94	0.0073	≤ 0.01
Cr	0.274 ± 0.057	0.289 ± 0.020	1.06	0.81	≤ 0.01
Fe	51.2 ± 9.3	138 ± 62	2.70	0.24	≤ 0.01
Hg	0.0057 ± 0.0014	0.0023 ± 0.0013	0.40	0.10	≤ 0.05
Rb	3.68 ± 0.48	3.09 ± 0.54	0.84	0.43	*NS*
Sb	0.0151 ± 0.0032	0.0082 ± 0.0011	0.54	0.067	≤ 0.01
Se	0.176 ± 0.029	1.84 ± 0.92	10.5	0.14	≤ 0.01
Zn	80.6 ± 3.0	237 ± 68	2.94	0.083	≤ 0.01

M arithmetic mean, *SEM* standard error of mean, *NS* not significant

In the bone affected by chondroma, many significant correlations between trace elements found in the control group are no longer evident, for example direct correlations between Fe and Co (Table 5). However, inverse correlations between Ag and Co ($r = -0.89$, $p \leq 0.05$) and Co and Zn ($r = -0.82$, $p \leq 0.05$) were observed (Table 5). Thus, if we accept the levels and relationships of the trace element mass fractions in the intact bone samples of the control group as a norm, we have to conclude that in chondroma tissue the levels and relationships of trace elements are significantly changed.

The changes in the trace element contents of a bone tumor in comparison with intact bone tissues may be attributed to a cause or effect of neoplastic transformation. 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. Many trace elements are bone-seeking elements, and they are closely associated with hydroxyapatite [26–30]. Chondroma is classified as a benign bone tumor. Our previous findings showed that the means of the

Table 5 Intercorrelations of pairs of the trace element mass fractions in tissue of intact bone and bone affected by chondroma

Tissue	Element	Co	Cr	Fe	Hg	Rb	Sb	Se	Zn
Intact bone $n = 27$	Ag	−0.23	0.51	−0.80[a]	−0.02	0.62[b]	0.31	−0.45	0.38
	Co	–	0.16	0.55[b]	0.79[a]	−0.10	0.08	0.52	0.17
	Cr	0.16	–	−0.48	0.51	0.56[b]	−0.31	−0.08	0.46
	Fe	0.55[b]	−0.48	–	0.09	−0.54	−0.25	0.60[b]	−0.17
	Hg	0.79[a]	0.51	0.09	–	0.18	−0.13	0.35	−0.14
	Rb	−0.10	0.56[b]	−0.54	0.18	–	−0.05	−0.06	0.34
	Sb	0.08	−0.31	−0.25	−0.13	−0.05	–	0.04	0.22
	Se	0.52	−0.08	0.60[b]	0.35	−0.06	0.04	–	0.24
	Zn	0.17	0.46	−0.17	−0.14	0.34	0.22	0.24	–
Chondroma $n = 5$	Ag	−0.89[b]	−0.12	−0.24	0.19	0.55	−0.17	−0.08	0.56
	Co	–	0.10	0.02	−0.29	−0.34	0.46	0.26	−0.82[a]
	Cr	0.10	–	−0.43	−0.60	0.64	−0.34	−0.29	0.38
	Fe	0.02	−0.43	–	−0.21	−0.42	−0.54	0.61	−0.03
	Hg	−0.29	−0.60	−0.21	–	−0.60	0.46	−0.58	0.02
	Rb	−0.34	0.64	−0.42	−0.60	–	−0.28	0.11	0.39
	Sb	0.46	−0.34	−0.54	0.46	−0.28	–	−0.13	−0.74
	Se	0.26	−0.29	0.61	−0.58	0.11	−0.13	–	−0.45
	Zn	−0.82[b]	0.38	−0.03	0.02	0.39	−0.74	−0.45	–

Statistically significant difference: [a] $p \leq 0.05$; [b] $p \leq 0.01$

Ca and P mass fractions in the chondroma tissue are lower than in normal bone, but the mean of the Ca/P ratio is similar [38]. It suggested that chondroma continues to form bone hydroxyapatite, but to a lesser degree than normal bone.

Our findings show that the mean of the Fe mass fraction in chondroma tissue samples was 2.7 times greater ($p \leq 0.01$, U test) than in normal bone tissues (Table 4). It is well known that an Fe mass fraction in the sample depends mainly on the blood volumes in the tissues. Chondroma is considered as a well-vascularized bone tumor [39]. Thus, it is possible to speculate that chondroma is characterized by an increase in the mean value of the Fe mass fraction because the level of tumor vascularization is higher than that of normal bone.

In the chondroma tissue, the mean Se mass fraction is 10.5 times higher ($p \leq 0.01$, U test) than in normal bone (Table 4). The high Se level was reported in malignant tumors of the ovary [40], lung [41], prostate [42], breast [43, 44], intestine [45], and gastric tissue [46]. 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. The literature-based analysis found the association of neoplastic transformation with local oxidative stress. Studies have shown that oxidative stress conditions play an important role in both the initiation and progression of a tumor by regulating molecules such as DNA, enhancers, transcription factors, and cell cycle regulators [47]. However, the cause of increased Se in tumors, particularly in chondroma, is not completely understood and requires further study. The elevated levels of Co, Cr, Sb, and Zn in chondroma tissue also need additional investigation.

The nondestructive INAA-LLR was developed and used in this research study because this method has many definite advantages over other analytical methods, particularly in clinical chemistry. For example, after nondestructive INAA-LLR, there is a possibility to check the results for some trace elements and to receive additional information about other trace element contents by destructive analytical methods, such as atomic absorption spectrometry, inductively coupled plasma atomic emission spectrometry, and inductively coupled plasma mass spectrometry, using the same bone samples. Moreover, if a deep-cooled channel of the nuclear reactor is available, the nondestructive INAA-LLR allows determining trace element contents in the fresh bone/tumor samples and combining a trace element study with a histological investigation. It is also necessary to keep in mind that the nondestructive methods are the current gold-standard solution to control destructive analytical techniques [1].

4 Conclusion

A nondestructive instrumental neutron activation analysis with high-resolution spectrometry of long-lived radionuclides was developed and used in the study of trace

element contents in samples of osseous tissue. It was shown that INAA-LLR is an adequate analytical tool for the nondestructive, precise determination of the mass fraction of 9 chemical elements (Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn) in human intact bone samples and samples of intraosseous lesions weighing about 100 mg. The method developed in the study has many definite advantages over other analytical methods, particularly in clinical chemistry.

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