Trace Element Contents in Bone Affected by Osteomyelitis

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Abstract: To clarify the role of trace elements in the etiology and the pathogenesis of the osteomyelitis, a non-destructive 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 estimated in normal 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 from trauma, and in samples, obtained from open biopsies or after operation of 10 patients with osteomyelitis (3 females and 7 males, 9 to 21 years old). The reliability of difference in the results between intact bone and bone affected by osteomyelitis was evaluated by Student's t-test. In the bone affected by osteomyelitis the mass fractions of Co, Cr, Fe, Se, and Zn are significantly higher than in normal bone tissues. In the inflamed bone tissue many correlations between trace elements found in the control group are no longer evident. In bone affected by osteomyelitis the trace element homeostasis is significantly disturbed.

Keywords: Trace elements, human bone, osteomyelitis, neutron activation analysis.

1. INTRODUCTION

The roles of trace elements in the development and inhibition of diseases have a complex character and have risen 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, in normal environmental and health conditions there is a trace element homeostasis in tissues and fluids of human body and an unbalance of trace element contents could be a causative factor for many diseases [2]. On the other hand pathological condition can effect on contents and relationships of trace elements in tissues and fluids and it is possible to use these changes as markers of disease [2].

It is well known that the tissues of human body differ greatly in their contents of trace elements. Our detailed previous studies have shown this using a chemical composition analysis of bone tissue [3-29]. 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, benign or malignant tumors.

Osteomyelitis is a difficult-to-treat bone infection characterized by progressive inflammatory destruction of the bone, with necrosis and new bone formation [30]. Osteomyelitis occur most commonly in children, and the overall prevalence is 1 case per 5000 children [31, 32]. Osteomyelitis typically affects the most rapidly growing ends of long bones and is more common in the lower extremity, the metaphysics of the distal femur and of the proximal tibia being the most common sites of infection [33, 34]. The limbs are affected in 90% of cases, and specifically lower limbs in 70% [35]. There is a male predilection [32] as well as a difference in incidence according to racial origin and the geographic region [35]. The diagnosis of childhood osteomyelitis can be challenging [36]. Moreover, early diagnosis, essential for timely appropriate treatment and reduction of complications, can be very difficult [37]. Although imaging is essential in the diagnostic process, a bone biopsy is necessary to confirm a diagnosis of osteomyelitis. This also helps determine the type of organism, typically bacteria, causing the infection so the right medication can be prescribed. The etiology and pathogenesis of osteomyelitis is not well understood, however significant interest and effort in this bone disease led to the identification of numerous etiologic agents [32, 35]. Osteomyelitis can be either acute or chronic. Factors that favor the development of acute bone infection are those that predispose to bacteremia. The major mechanism for the development of acute osteomyelitis is injuries due to penetrating bites and puncture wounds of the foot which may serve to infect bone directly [38]. People with diabetes, HIV,

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E-ISSN: 2313-0954/16

sickle cell anemia, chronic granulomatous or peripheral vascular disease are more prone to chronic osteomyelitis [38, 39].

To our knowledge, no data are available for the trace element contents in bone affected by osteomyelitis, to permit conclusion about their role in etiology, pathogenesis, and diagnostics of this disease.

The aim of the study was to compare and to correlate the contents of selected trace element in two groups of samples (normal bone and bone affected by osteomyelitis). For this purpose, the silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), rubidium (Rb), antimony (Sb), selenium (Se), and zinc (Zn) contents were determined in the two groups of samples using nondestructive instrumental neutron activation analysis (INAA) with high resolution spectrometry of long-lived radionuclides (INAA-LLR).

2. MATERIAL AND METHODS

2.1. Sample Preparation

Thirty-seven children, adolescents and adults were included in this study. The subjects were divided into two groups: reference and osteomyelitis. 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 10 patients with osteomyelitis (3 females and 7 males, 9 to 21 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 scrub samples [40, 41]. All bone samples were freeze dried, until constant mass was obtained, and homogenized. Then samples weighing about 100mg were wrapped separately in high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

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

2.2. 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 before [42]. Ten certified reference material (CRM) IAEA H-5 (Animal Bone) sub-samples and ten standard reference material (SRM) NIST 1486 (Bone Meal) sub-samples weighing about 100mg were analyzed in the same conditions as bone 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, 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 98cm^3 Ge (Li) detector and a spectrometric unit (NUC 8100), including a PC-coupled multichannel analyzer, were used for measurements. The spectrometric unit provided 2.9keV resolution at the ^{60}Co 1332keV 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 [22, 24, 25, 43].

2.3. Computer Programs and Statistic

A dedicated computer program of INAA mode optimization was used [44]. 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. The reliability of difference in the results between intact bone and bone affected by osteomyelitis 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 bone affected by osteomyelitis the Microsoft Office Excel program was also used.

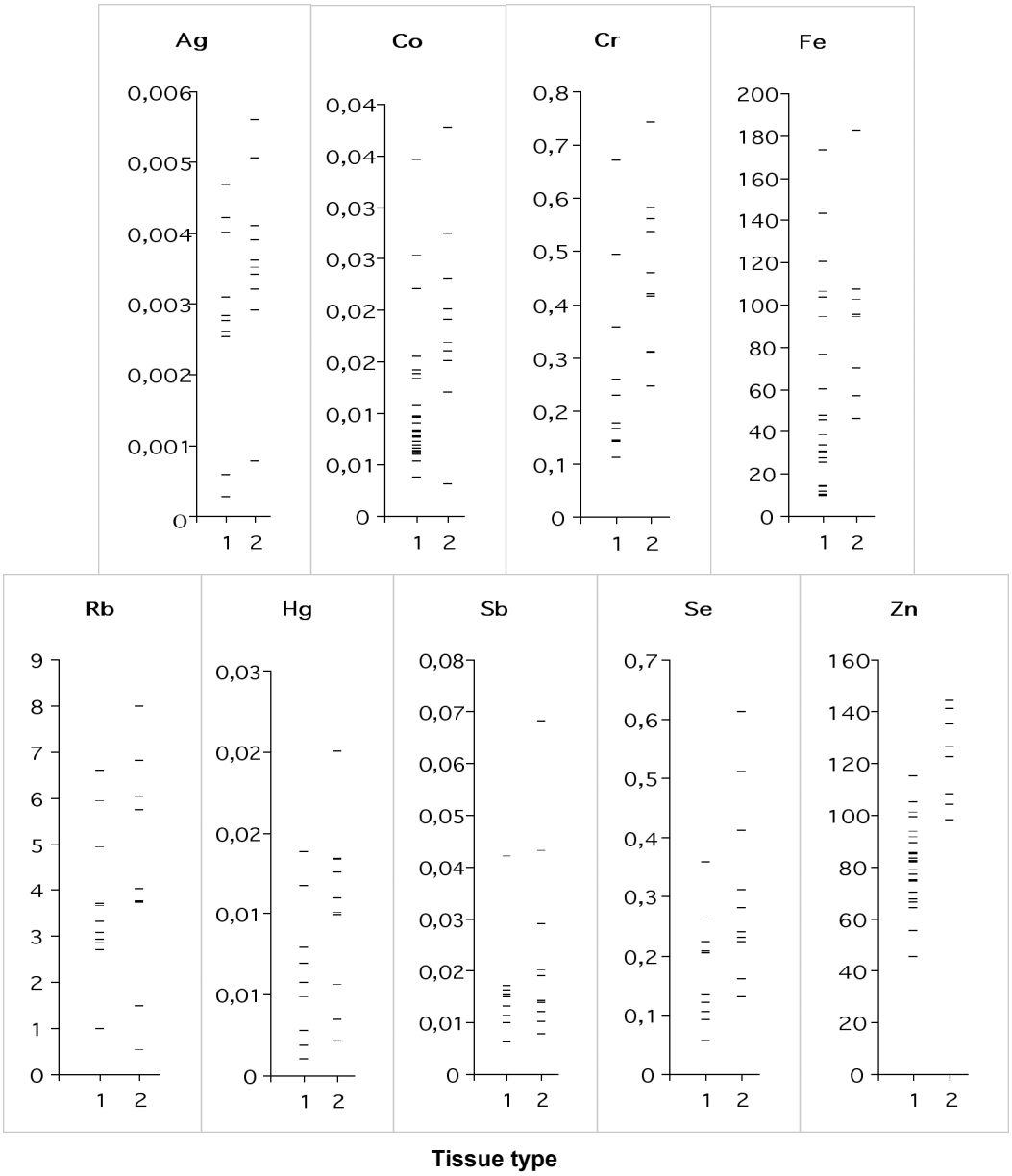

Figure 1: Individual data sets for Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions (mg/kg dry tissue) in intact bone (1) and bone affected by chronic osteomyelitis (2).

3. RESULTS

Figure **1** shows individual data sets for 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 bone affected by osteomyelitis (2).

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 and inflamed bone samples.

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 the inflamed bone samples are presented in Table **2**.

The data of inter-correlation 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 osteomyelitis are shown in Table **3**.

4. 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 nondestruc-

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 Bone Affected by Osteomyelitis

Element	M	SD	SEM	Min	Max	Med	P0.025	P0.975
Intact Bone, n=27								
Ag	0.0027	0.0015	0.00051	0.00026	0.0047	0.0028	0.00032	0.0046
Co	0.0107	0.0070	0.0014	0.00370	0.0345	0.0079	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
Osteomyelitis, n=10								
Ag	0.0036	0.0013	0.0004	0.00076	0.0056	0.0036	0.00124	0.0055
Co	0.0190	0.0092	0.0029	0.00300	0.0376	0.0179	0.00503	0.0353
Cr	0.457	0.151	0.048	0.246	0.741	0.438	0.260	0.705
Fe	94	37	12	45.9	182	94	48.3	165
Hg	0.0101	0.0053	0.0017	0.00210	0.0200	0.0105	0.00239	0.0185
Rb	4.39	2.30	0.73	0.520	7.98	4.22	0.734	7.71
Sb	0.0237	0.0188	0.0059	0.00770	0.0680	0.0166	0.00822	0.0624
Se	0.310	0.155	0.049	0.130	0.610	0.260	0.137	0.588
Zn	122.2	15.4	4.9	97.8	144	122	99.2	143

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, 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 Osteomyelitis

Element	Intact bone M_1	Osteomyelitis M_2	Ratio M_2 / M_1	Student's *t*-test *P* =
Ag	0.0027 ± 0.0005	0.0036 ± 0.0004	1.31	.18
Co	0.0107 ± 0.0014	0.0190 ± 0.0029	1.78	**.023**
Cr	0.274 ± 0.057	0.457 ± 0.048	1.67	**.025**
Fe	51.2 ± 9.3	94 ± 12	1.84	**.0092**
Hg	0.0057 ± 0.0014	0.0101 ± 0.0017	1.77	.062
Rb	3.68 ± 0.48	4.39 ± 0.73	1.19	.44
Sb	0.0151 ± 0.0032	0.0237 ± 0.0059	1.57	.23
Se	0.176 ± 0.029	0.310 ± 0.049	1.76	**0.032**
Zn	80.6 ± 3.0	122 ± 5	1.34	**.0000017**

M arithmetic mean, *SEM* standard error of mean, *bold* statistically significant.

Table 3: Intercorrelations (Values of *r* - the Pearson Correlation Coefficient) of Pairs of the Trace Element Mass Fractions in Tissue of Intact Bone and Bone Affected by Osteomyelitis

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
n = 27	Co	-	0.16	0.55[b]	0.79[b]	-0.10	0.08	0.52	0.17
	Cr	0.16	-	-0.48	0.51	0.56[a]	-0.31	-0.08	0.46
	Fe	0.55[b]	-0.48	-	0.09	-0.54	-0.25	0.60[a]	-0.17
	Hg	0.79[b]	0.51	0.09	-	0.18	-0.13	0.35	-0.14
	Rb	-0.10	0.56[a]	-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[a]	0.35	-0.06	0.04	-	0.24
	Zn	0.46	0.46	-0.17	-0.14	0.34	0.22	0.24	-
Osteomyelitis	Ag	0.45	0.16	0.33	0.71[a]	0.37	0.16	0.07	-0.78[b]
n = 10	Co	-	0.28	-0.74[a]	0.49	-0.17	0.22	0.43	-0.43
	Cr	0.28	-	-0.22	-0.25	-0.43	0.08	-0.53	-0.28
	Fe	-0.74[a]	-0.22	-	0.48	0.56[a]	-0.11	-0.70[a]	-0.24
	Hg	0.49	-0.25	0.48	-	0.63[a]	0.30	0.45	-0.57[a]
	Rb	-0.17	-0.43	0.56[a]	0.63[a]	-	-0.12	0.21	-0.12
	Sb	0.22	0.08	-0.11	0.30	-0.12	-	0.01	0.16
	Se	0.43	-0.53	-0.70[a]	0.45	0.21	0.01	-	-0.12
	Zn	-0.43	-0.28	-0.24	-0.57[a]	-0.12	0.16	-0.12	-

*S*tatistically significant difference: [a] - $P \leq 0.05$, [b] - $P \leq 0.01$, [c] - $P \leq 0.001$.

tive 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, 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 trace element contents in the fresh bone samples and combining trace element 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 [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, 41, 45]. 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 [22, 24, 25, 43]. 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 bone affected by osteomyelitis presented in Figure **1** and Tables **1-3**.

In the 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 the osteomyelitis group the mass fraction of all nine trace elements were determined in all samples.

In the osteomyelitis group the mean mass fraction of Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn is higher than in the normal bone tissues (Table **2**). However, in the osteomyelitis group only the mean mass fractions of Co (P=0.023), Cr (P=0.025), Fe (P=0.0092), Se (P=0.032), and Zn (P=0.0000017) are significantly increased when compared with those in normal bone.

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 between Rb and Cr (r = 0.56, $P \leq 0.05$) mass fractions (Table **3**). In the same group a pronounced 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 the bone affected by osteomyelitis many significant correlations between trace elements found in the control group are no longer evident, for example, direct correlations between Fe and Se, as well as between Fe and Co are transformed into inverse correlations, etc. (Table **3**). However, direct correlations between Fe and Rb (r = 0.56, $P \leq 0.05$), Hg and Ag (r = 0.71, $P \leq 0.05$), and also Hg and Rb (r = 0.63, $P \leq 0.05$), as well as inverse correlation between Hg and Zn (r = -0.57, $P \leq 0.05$), and also between Zn and Ag r = -0.78, $P \leq 0.01$) were observed (Table **3**). 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 an inflammation the levels and relationships of trace elements in bone significantly change. No published data referring to correlations between trace element mass fractions in the inflamed bone tissue were found.

The changes in trace element contents of inflamed bone in comparison with intact bone tissues may be attributed to a cause or effect of inflammation. 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 trace elements are bone-seeking elements and they are closely associated with hydroxyapatite [24-28]. Osteomyelitis is classified as a bone inflammation. Our previous findings showed that the means of the Ca and P mass fraction as well as Ca/P ratio in the bone affected by osteomyelitis are similar those in normal bone [46]. It suggested that inflammation do not effect on bone hydroxyapatite content.

Our findings show that the mean of the Fe mass fraction in the inflamed bone samples was almost two 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. The blood supply to the affected area is increased substantially during the inflammatory response [47]. Thus, it is possible to speculate that bone affected by osteomyelitis is characterized by an increase of the mean value of the Fe mass fraction because the level of blood is higher than that in normal bone.

Inflammatory processes are initiated and regulated by a great variety of inflammatory mediators including metalloproteases [48]. Most metalloproteases require Zn, but some use Co. Inflammation may induce a high perturbation in the intracellular and intercellular homeostasis as well as an imbalance between pro-oxidant and antioxidant enzyme activities in bone tissue [49]. Recent studies indicate that transition metals contents, including Cr, associated with the levels of oxidative stress in tissue [50]. Because of that elevated levels of Co, Cr, and Zn in inflamed bone tissue may result from oxidative stress in osteomyelitis.

In the inflamed bone tissue the mean Se mass fractions is 1.8 times higher ($p \leq 0.032$) than in normal bone (Table **2**). The cause of increased Se in inflamed tissues remains unknown, but in general it is accepted that certain proteins containing Se can mediate the protective effects against oxidative stress. However the role of Se in inflamed bone tissue is not completely understood and requires further studies.

Limitations: The low number of bone samples (10 patients with osteomyelitis, 3 females and 7 males, 9 to 21 years old) examined in this study does not allow statistical comparisons of trace element accumulation or dynamics between different age (before and after puberty) and gender (females and males) groups. Therefore it cannot be fully excluded that the presented results for inflamed bone 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 osteomyelitis and to extend the list of trace elements investigated.

5. CONCLUSION

INAA-LLR 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 intact bone samples and samples of intraosseous lesions weighing about 100mg. In the bone affected by osteomyelitis the mass fractions of Co, Cr, Fe, Se, and Zn are significantly higher than in normal bone tissues. In the inflamed bone 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 inflamed bone the trace element homeostasis is significantly disturbed. The studies on the role of trace elements in the etiology of osteomyelitis have to be continued.

ACKNOWLEDGEMENTS

The authors are grateful to the late Prof. V.A. Bizer, Medical Radiological Research Center, Obninsk, for supplying the samples of bone affected by osteomyelitis.

AUTHORS' CONTRIBUTION

Vladimir Zaichick and Sofia Zaichick contributed equally to this work.

CONFLICT OF INTERESTS

Authors have declared that no competing interests exist.

ABBREVIATIONS

HIV= human immunodeficiency virus

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

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Received on 09-11-2015 Accepted on 23-11-2015 Published on 11-05-2016

DOI: http://dx.doi.org/10.12974/2313-0954.2016.03.01.1