

Selenium Content in Lesions of Bone as Osteosarcoma Marker

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Abstract

Objectives: Childhood bone cancer often is difficult to detect in its early stages. One of the most important differential diagnostics is between osteomyelitis (OM) and osteosarcoma (OS). To clarify the possible role of selenium (Se) as osteosarcoma marker, a non-destructive neutron activation analysis were performed.

Methods: The Se content was measured in three groups of samples: normal bone samples from 10 persons with intact bone, and also in samples, obtained from open biopsies or after operation of 10 patients with OM and 27 patients with OS. The difference in the results between Se contents in the three groups was evaluated by the parametric Student's t-test and non-parametric Wilcoxon-Mann-Whitney U-test.

Results: In the OS tissue the mean mass fraction of Se was 6.2 and 11.0 times higher than in inflamed and normal bone, respectively. The level of Se mass fraction was checked as tumor marker.

Conclusions: It was shown that the level of Se mass fraction can be recommended as an additional high informative test for differential diagnosis between OS and normal or inflamed bone.

Keywords: Selenium; Human Bone; Osteomyelitis; Osteosarcoma; Differential Diagnostics; Neutron Activation Analysis

Abbreviations

OS: Osteosarcoma; OM: Osteomyelitis; TE: Trace Elements; INAA-LLR: Instrumental Neutron Activation Analysis with High Resolution Spectrometry of Long-Lived Radionuclides; SRM: Standard Reference Material; CRM: Certified Reference Material; BSS: Biological Synthetic Standards

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 give be subject to inflammation, benign or malignant tumors. Childhood bone cancer often is difficult to detect in its 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 diagnostics is between an inflammation and a malignant process such as osteogenic sarcoma.

Osteomyelitis (OM) is an inflammation of the bone and generally refers to a bacterial infection of bone [2]. OM occurs most commonly in children, and the overall prevalence is 1 case per 5000 children [3]. OM typically affects the most rapidly growing ends of long bones and is more common in the lower extremity, the metaphysis of the distal femur and of the proximal tibia being the most common sites of infection [4,5].

Osteogenic sarcoma or osteosarcoma (OS) is the most common primary bone malignancy in children and young adults. Overall, this malignancy is rare, with an annual incidence of approximately one per 100000 [6,7]. OS can occur in any bone but most commonly affect the metaphysis of long bones in the appendicular skeleton (80%) [8].

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, and infiltration of the adjacent tissue. However, clinical imaging is not useful as a routine examination in the diagnosis of OS for it may be confused with OM [3-10]. Definitive diagnosis must be achieved using biopsy and histopathologic evaluation. 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, inflamed 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, inflamed and malignant lesions of bone and has a potential to become a valuable diagnostic tool [14,15,27,39].

A high selenium (Se) level was reported in malignant tumors of the ovary [40], lung [41], prostate [42-50], breast [51,52], gastro intestinal tract [53], and also in cancers of the stomach [54] and thyroid [55]. Moreover, in our previous study elevated levels of Se were found in such malignant tumors of bone as Ewing's sarcoma [56], chondrosarcoma [57], and malignant giant cell tumor of bone [58]. To our knowledge, no data are available for the Se content of OM and OS, to permit distinction between inflamed bone and malignant tumor.

Aim of the Study

This work had three aims. The first was to obtain reliable data for Se content in three groups of bone tissue samples – intact bone, OM and OS using non-destructive instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR). The second aim was to compare the Se content in the different groups of samples. The final aim was to evaluate the diagnostic significance of Se content as tumor marker.

Materials and Methods

Samples

Forty-seven children, adolescents and adults were included in this study. The subjects were divided into three groups: control (1), OM (2) and OS (3). The reference/control group consisted of 10 persons with intact bone (2 females and 8 males, aged from 11 to 44 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 10 patients with OM (3 females and 7 males aged between 9 and 21 years) and 27 patients with OS (9 females and 18 males, from 6 to 71 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 scrape samples [59,60]. All bone and tumor tissue samples were freeze dried, until constant mass was obtained, and homogenized. Then samples weighing about 50 mg were wrapped separately in high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

Method and reference materials

To determine contents of the element 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 [61,62]. 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 50 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 Se 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 $10^{13} \text{ n}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$. 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 ($T_{1/2}=14.3 \text{ d}$) background, a beryllium filter was used. A coaxial 98 cm^3 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 [31,33,34,62].

A dedicated computer program of INAA mode optimization was used [63]. 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 Se mass fraction. The differences in the results between intact bone, OM and OS were evaluated using the parametric Student's t-test and non-parametric Wilcoxon-Mann-Whitney U-test.

Results

Figure 1 shows individual data sets for Se mass fraction (mg/kg dry tissue) in intact bone (1) and also in bone affected by osteomyelitis (2) and osteosarcoma (3).

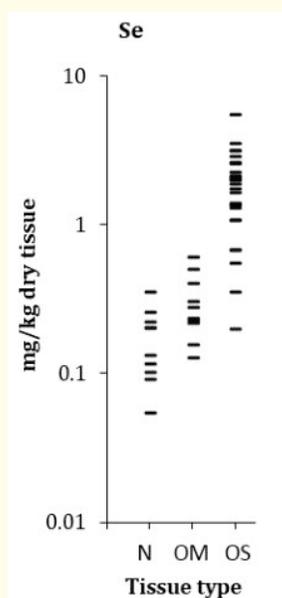


Figure 1: Individual data sets for Se mass fraction (mg/kg dry tissue) in intact bone (N) and also in bone affected by osteomyelitis (OM) and osteosarcoma (OS).

Table 1 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 Se mass fraction in the samples of intact bone, osteomyelitis and osteogenic sarcoma.

Tissue	M	SD	SEM	Min	Max	Med	P0.025	P0.975
Intact bone	0.176	0.092	0.029	0.0550	0.358	0.169	0.0633	0.336
Osteomyelitis	0.310	0.155	0.049	0.130	0.610	0.260	0.137	0.588
Osteogenic sarcoma	1.93	1.15	0.22	0.200	5.59	1.92	0.305	4.27

Table 1: Basic statistical parameters for Se mass fraction (mg/kg; dry mass basis) in tissue of intact bone; osteomyelitis and osteogenic sarcoma 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 inflammation or malignant transformation on the Se mass fraction in bone is shown in table 2.

Groups of samples	Normal (N)	Osteomyelitis (OM)	t-test p≤	U-test p	Ratio OM/N
Osteomyelitis and Normal	0.176 ± 0.029	0.310 ± 0.049	0.032	≤0.01	1.76
Groups of samples	Norm (N)	Osteogenic sarcoma (OS)	t-test p≤	U-test p	Ratio OS/N
Osteogenic sarcoma and Normal	0.176 ± 0.029	1.93 ± 0.22	0.000001	≤0.01	11.0
Groups of samples	Osteomyelitis (OM)	Osteogenic sarcoma (OS)	t-test p≤	U-test p	Ratio OS/OM
Osteogenic sarcoma and Osteomyelitis	0.310 ± 0.049	1.93 ± 0.22	0.000001	≤0.01	6.23

Table 2: Differences between mean values ($M \pm SEM$) of Se mass fraction (mg/kg; dry mass basis) in tissue of normal bone; osteomyelitis and osteogenic sarcoma. 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**.

Table 3 depicts parameters ($M \pm SD$) of the importance (sensitivity, specificity and accuracy) of Se mass fraction for the diagnosis of OS (estimation is made for "OS or normal and inflamed bone").

Element	Limit for OS	Sensitivity	Specificity	Accuracy
Se	≥ 0.65 mg/kg dry tissue	89 ± 6	100 - 5	94 ± 4

Table 3: Parameters ($M \pm SD$) of the importance (sensitivity, specificity and accuracy) of Se mass fraction for the diagnosis of OS (estimation is made for "OS or normal and inflamed bone"). M: Arithmetic Mean; SD: Standard Deviation.

Results for two age groups of patients with osteogenic sarcoma were compared to check a possible age-dependence of Se mass fraction in tumor (Table 4).

Age group 1 6 - 20 years (n=20)	Age group 2 21 - 71 years (n=7)	t-test p≤	U-test p
2.07 ± 0.28	1.51 ± 0.27	0.159	> 0.05

Table 4: Comparison of mean values ($M \pm SEM$) of Se mass fraction (mg/kg, dry mass basis) in tumor between two age groups of patients with osteogenic sarcoma.

Gender-dependence of Se mass fraction in tumor was investigated by a comparison between the group of males and females with osteogenic sarcoma (Table 5).

Males (n = 18)	Females (n = 9)	t-test p≤	U-test p
1.92 ± 0.31	1.94 ± 0.26	0.952	>0.05

Table 5: Comparison of mean values ($M \pm SEM$) of Se mass fraction (mg/kg, dry mass basis) in tumor between the group of males and females with osteogenic sarcoma.

Good agreement with the certified data of CRM for Se mass fraction determined by INAA-LLR indicate an acceptable accuracy for Se mass fraction obtained in the study of intact bone, inflamed bone and tumor tissue samples presented in figure 1 and tables 1-3.

From table 2 it is observed that in the OM tissue the mean mass fraction of Se is 1.7 times higher than that in normal bone tissues. In the OS tissue the mean mass fractions of Se is 11.0 times higher in comparison with normal bone tissues. In the OS tissue the mean mass fraction of Se is 6.2 times higher than that in inflamed bone.

Significant differences between levels of Se mass fraction in OM and OS tissues suggest potential use of this element mass fraction as OS marker. Figure 1 shows individual data set for Se mass fraction (mg/kg dry tissue) in all samples of intact bone (1), OM (2) and OS tissue (3). Thus, quantities of the Se mass fraction in tissue of bone lesions could become a powerful diagnostic tool. As is evident from tables 2 and, particularly, from individual data set (Figure 1), the Se mass fraction is potentially the informative test for a differential diagnosis. For example, if 0.65 mg/kg dry tissue is the value of Se mass fraction assumed to be the lower limit for OS (Figure 1) and the estimation is made for "OS or intact and inflamed bone", the following values are obtained:

Sensitivity = $\{ \text{True Positives (TP)} / [\text{TP} + \text{False Negatives (FN)}] \} \cdot 100\% = 89 \pm 6\%$;

Specificity = $\{ \text{True Negatives (TN)} / [\text{TN} + \text{False Positives (FP)}] \} \cdot 100\% = 100-5\%$;

Accuracy = $[(\text{TP} + \text{TN}) / (\text{TP} + \text{FP} + \text{TN} + \text{FN})] \cdot 100\% = 94 \pm 4\%$.

The number of people (samples) examined was taken into account for calculation of confidence intervals [64]. In other words, if Se mass fraction in a biopsy sample of bone lesion is higher than 0.65 mg/kg, one could diagnose OS with an accuracy $94 \pm 4\%$. Results of the test does not depend on age and gender of patients (Table 4 and 5).

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 [11,60,65]. 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 biggest disadvantage of INAA is a requirement to use nuclear reactor for samples irradiation by neutrons. Among up-to-date analytical technologies a non-destructive energy dispersive X-ray fluorescence analysis (EDXRF) is one of the simplest, fast, reliable, efficient, and available techniques. There are many different kinds of EDXRF devices on the market and technical possibilities of this method improve rapidly. By now, as was shown in our recent study [66], this method allows accurate measurement at least 11 chemical element contents in microprobes of biological materials, including such important tumor marker as Se. Thus, EDXRF methods may be recommended for using in clinical practice for measurement of OS markers in biopsy samples of bone lesions.

Limitations

The role played by Se in malignant 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 [67]. However the cause of increased Se in OM and particularly in OS of bone is not completely understood and requires further studies. There are many other TE, besides Se, associated with different levels of oxidative stress and carcinogenesis. Thus, future studies should be directed toward using different analytical methods which will extend the list of TE investigated in normal bone as well as in the OM and OS tissue. In addition, the sample size of control and OM group was relatively small. Despite these limitations, for the first time this study provides evidence that the level of Se mass fractions have altered in OM and OS tissue and shows the necessity to continue TE research of these diseases of bone.

Conclusion

In the OS tissue the mean mass fractions of Se is significantly higher than in normal and inflamed bone. It was shown that the level of Se mass fraction is the informative parameter. Thus, the level of Se mass fraction can be used as an additional high informative test for differential diagnosis between OS and normal or inflamed bone.

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Conflict of Interest

There is no any financial interest or any conflict of interest.

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