Journal of Cancer Therapy, 2015, 6, 493-503
Published Online June 2015 in SciRes. http://www.scirp.org/journal/jct
http://dx.doi.org/10.4236/jct.2015.66053

The Content of Silver, Cobalt, Chromium, Iron, Mercury, Rubidium, Antimony, Selenium, and Zinc in Osteogenic Sarcoma

Sofia Zaichick[1], Vladimir Zaichick[2*]

[1]Department of Medicine, University of Illinois, College of Medicine at Chicago, Chicago, USA
[2]Radionuclide Diagnostics Department, Medical Radiological Research Centre, Obninsk, Russia
Email: [*]vezai@obninsk.com

Received 30 April 2015; accepted 31 May 2015; published 3 June 2015

Abstract

Objectives: To clarify the role of trace elements in the etiology and the pathogenesis of the osteogenic sarcoma (osteosarcoma), a non-destructive neutron activation analysis with high resolution spectrometry of long-lived radionuclides was performed. Methods: The silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), rubidium (Rb), antimony (Sb), selenium (Se), and zinc (Zn) mass fraction, Rb/Co, Rb/Fe, Rb/Se, and Rb/Zn mass fraction ratios as well as Co × Zn, Fe × Zn, Sb × Zn, Se × Zn, Co × Se, and Fe × Se mass fraction multiplications 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 tumor samples, obtained from open biopsies or after operation of 27 patients with osteosarcoma (9 females and 18 males, 6 to 71 years old). The reliability of difference in the results between intact bone and osteosarcoma tissues was evaluated by Student's t-test. Results: In the osteosarcoma tissue the mass fractions of Co, Cr, Fe, Sb, Se, and Zn are significantly higher while the mass fraction of Rb is lower than in normal bone tissues. Moreover, we found significantly lower values of Rb/Co, Rb/Fe, Rb/Se, and Rb/Zn mass fraction ratios as well as significant higher mean values of Co × Zn, Fe × Zn, Sb × Zn, Se × Zn, Co × Se, and Fe × Se mass fractions multiplications in the osteosarcoma tissue compared to intact bone. In the osteosarcoma tissue many correlations between trace elements found in the control group were no longer evident. Conclusion: In osteosarcoma transformed bone tissues the trace element homeostasis is significantly disturbed.

Keywords

Trace Elements, Human Bone, Osteogenic Sarcoma, Neutron Activation Analysis

[*]Corresponding author.

How to cite this paper: Zaichick, S. and Zaichick, V. (2015) The Content of Silver, Cobalt, Chromium, Iron, Mercury, Rubidium, Antimony, Selenium, and Zinc in Osteogenic Sarcoma. *Journal of Cancer Therapy*, **6**, 493-503.
http://dx.doi.org/10.4236/jct.2015.66053

1. Introduction

The roles of trace elements in the development and inhibition of cancer 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, including cancer [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 tumors form a heterogeneous group of benign or malignant neoplastic diseases since they 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.

Osteogenic sarcoma (osteosarcoma), a highly malignant primary bone tumor that is derived from primitive bone-forming mesenchymal cells, is the most common primary bone malignancy [30] [31]. Overall, this malignancy is rare, with an annual incidence of approximately one per 100,000 [32] [33]. Osteosarcoma has a bimodal age distribution, having the first peak during adolescence and the second peak in older adulthood. The first peak is in the 10 - 14-year-old age group, coinciding with the pubertal growth spurt. The second osteosarcoma peak is in adults older than 65 years of age [30] [31]. There is a male predilection of 1.35/1 as well as a difference in incidence according to racial origin, in the sense that Blacks are affected more frequently [30]. Osteosarcomas can occur in any bone but most commonly affect the metaphysis of long bones in the appendicular skeleton (80%) [34]. The most common sites are the femur (42%, with 75% of tumors in the distal femur), the tibia (19%, with 80% of tumors in the proximal tibia), and the humerus (10%, with 90% of tumors in the proximal humerus). Other likely locations are the skull or jaw (8%) and the pelvis (8%) [30]. No single morphologic or functional imaging method provides findings for a specific diagnosis of osteosarcoma. Therefore, obtaining a histologic specimen of the lesion in all patients is essential in recognizing this tumor and planning therapy. The etiology of osteosarcoma is not well understood, however significant interest and effort in this cancer led to the identification of numerous etiologic agents. Therapeutic radiation, Paget disease of bone, electrical burn, or trauma all are thought to be other factors that may contribute to the pathogenesis of osteosarcoma. Moreover, several chemical agents such as metals, including beryllium, uranium, plutonium, and amercium were shown to be potent inducers of osteosarcoma [35] [36].

The data available on chemical elements in osteosarcoma tissue is extremely limited to permit conclusion about their role in malignant transformation or pathogenesis [37]-[39].

The aim of the study was to compare and to correlate the contents and selected combinations of trace element in two groups of samples (normal bone and osteosarcoma tissue). 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).

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

2. Materials and Methods

2.1. Materials

Fifty-four children, adolescents and adults were included in this study. The subjects were divided into two groups: reference and osteosarcoma. The reference group consisted of 27 patients with intact bone (12 females and 15 males, aged from 16 to 49 years, M ± SD 34 ± 11 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 27 patients with osteosarcoma (9 females and 18 males, 6 to 71 years old, M ± SD 23 ± 20 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 [40] [41]. All bone and tumor tissue samples were freeze

dried, until constant mass was obtained, and homogenized. Then samples weighing about 100 mg were wrapped separately in high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

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 100 mg were analyzed in the same conditions as bone and tumor samples to estimate the precision and accuracy of the results.

2.2. Methods

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 cm^3 Ge (Li) detector and a spectrometric unit (NUC 8100), including a PC-coupled multichannel analyzer, were used for measurements. The spectrometric unit provided 2.9 keV resolution at the ^{60}Co 1332 keV line. The information of used nuclear reactions, radionuclides, gamma-energies, and other details of the analysis including the quality control of results were reported by us before [22] [24] [25] [43].

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 and their selected combinations. The reliability of difference in the results between intact bone and osteosarcoma tissue was evaluated by Student's t-test. For the estimation of the Pearson correlation coefficient between different pairs of the trace element mass fractions in intact bone and osteosarcoma tissue the Microsoft Office Excel program was also used.

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 osteosarcoma tissue (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 osteosarcoma tissue.

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

Table 3 represents the basic statistical parameters for Rb/Co, Rb/Fe, Rb/Se, and Rb/Zn mass fractions ratios as well as Co × Zn, Fe × Zn, Sb × Zn, Se × Zn, Co × Se, and Fe × Se mass fractions multiplications in tissue of intact bone and osteogenic sarcoma.

The ratio of means and the reliability of difference between mean values Rb/Co, Rb/Fe, Rb/Se, and Rb/Zn mass fractions ratios as well as Co × Zn, Fe × Zn, Sb × Zn, Se × Zn, Co × Se, and Fe × Se mass fractions multiplications in tissue of intact bone and osteogenic sarcoma are presented in **Table 4**.

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

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 non-destructive 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 spectrome-

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 osteogenic sarcoma.

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
Osteogenic sarcoma, n = 27								
Ag	0.0074	0.0188	0.0036	0.00064	0.0967	0.0018	0.00067	0.0530
Co	0.0490	0.0370	0.0071	0.00790	0.145	0.0456	0.0110	0.145
Cr	0.555	0.385	0.074	0.142	1.51	0.385	0.153	1.39
Fe	247	141	27	36.0	601	247	38.6	524
Hg	0.0077	0.0068	0.0013	0.00023	0.0214	0.0045	0.00041	0.0212
Rb	2.17	1.38	0.26	0.275	6.29	1.92	0.539	5.47
Sb	0.0365	0.0465	0.0089	0.00330	0.197	0.0190	0.00712	0.176
Se	1.93	1.15	0.22	0.200	5.59	1.92	0.305	4.27
Zn	192	79	15	72.7	418	181	89.1	340

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 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 osteogenic sarcoma.

Element	Intact bone M_1	Osteogenic sarcoma M_2	Ratio M_2/M_1	Student's *t*-test
Ag	0.0027 ± 0.0005	0.0074 ± 0.0036	2.72	$p \leq 0.210$
Co	0.0107 ± 0.0014	0.0490 ± 0.0071	4.58	**$p \leq 0.000013$**
Cr	0.274 ± 0.057	0.555 ± 0.074	2.03	**$p \leq 0.00514$**
Fe	51.2 ± 9.3	247 ± 27	4.82	**$p \leq 0.000000097$**
Hg	0.0057 ± 0.0014	0.0077 ± 0.0013	1.35	$p \leq 0.327$
Rb	3.68 ± 0.48	2.17 ± 0.26	0.59	**$p \leq 0.0134$**
Sb	0.0151 ± 0.0032	0.0365 ± 0.0089	2.42	**$p \leq 0.0317$**
Se	0.176 ± 0.029	1.93 ± 0.22	11.0	**$p \leq 0.000000022$**
Zn	80.6 ± 3.0	192 ± 15	2.38	**$p \leq 0.000000079$**

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

Table 3. Basic statistical parameters for Rb/Co, Rb/Fe, Rb/Se, and Rb/Zn mass fraction ratios as well as Co × Zn, Fe × Zn, Sb × Zn, Se × Zn, Co × Se, and Fe × Se mass fraction multiplications in tissue of intact bone and osteogenic sarcoma.

Mass fractions combinations	M	SD	SEM	Min	Max	Med	P0.025	P0.975
Intact bone, n = 27								
Rb/Co	368	201	61	28.1	784	397	51.8	716
$(Rb/Fe) \times 10^4$	1442	1308	394	56.1	4022	922	119	3726
Rb/Se	266	124	39	27.1	491	288	49.6	453
$(Rb/Zn) \times 10^4$	425	178	54	114	795	414	161	753
$(Co \times Zn) \times 10^2$	88	66	13	25.9	294	66.3	32.2	276
Fe × Zn	4013	3546	709	554	14,757	2436	627	12,021
$(Sb \times Zn) \times 10^2$	140	99	31	42.0	393	119	43.8	344
(Se × Zn) × 10	163	90	28	46.8	305	139	50.7	295
$(Co \times Se) \times 10^5$	301	356	112	34.0	1235	198	44.6	1073
Fe × Se	13.7	18.5	5.9	1.22	61.9	7.38	1.31	54.0
Osteogenic sarcoma, n = 27								
Rb/Co	74	85	16	13.2	344	45.7	13.4	300
$(Rb/Fe) \times 10^4$	118	107	21	26.6	461	77.1	27.0	407
Rb/Se	23.9	35.5	6.8	2.27	136	8.51	2.42	107
$(Rb/Zn) \times 10^4$	124	79	15	20.5	328	114	22.7	324
$(Co \times Zn) \times 10^2$	870	729	140	202	2784	700	215	2784
Fe × Zn	45,782	33,527	6452	4824	161,669	33,642	6576	116,862
$(Sb \times Zn) \times 10^2$	740	1103	212	44.2	4887	333	81.6	3721
(Se × Zn) × 10	3393	2246	432	560	9950	2921	857	9043
$(Co \times Se) \times 10^5$	10,256	9562	1840	158	40,080	7615	354	33,375
Fe × Se	530	505	97	11.8	2029	416	29.9	1952

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 ± SEM), ratio of means and the reliability of difference between mean values of Rb/Co, Rb/Fe, Rb/Se, and Rb/Zn mass fraction ratios as well as Co × Zn, Fe × Zn, Sb × Zn, Se × Zn, Co × Se, and Fe × Se mass fraction multiplications in tissue of intact bone and osteogenic sarcoma.

Mass fractions combinations	Intact bone M_1	Osteogenic sarcoma M_2	Ratio M_2/M_1	Student's *t*-test
Rb/Co	368 ± 61	74 ± 16	0.20	**$p \le 0.000597$**
$(Rb/Fe) \times 10^4$	1442 ± 394	118 ± 21	0.082	**$p \le 0.00730$**
Rb/Se	266 ± 39	23.9 ± 6.8	0.090	**$p \le 0.000139$**
$(Rb/Zn) \times 10^4$	425 ± 54	124 ± 15	0.29	**$p \le 0.000181$**
$(Co \times Zn) \times 10^2$	88 ± 13	870 ± 140	9.89	**$p \le 0.00000755$**
Fe × Zn	4013 ± 709	45782 ± 6452	11.4	**$p \le 0.00000072$**
$(Sb \times Zn) \times 10^2$	140 ± 31	740 ± 212	5.29	**$p \le 0.00939$**
(Se × Zn) × 10	163 ± 28	3393 ± 432	20.8	**$p \le 0.000000061$**
$(Co \times Se) \times 10^5$	301 ± 112	10256 ± 1840	34.1	**$p \le 0.0000115$**
Fe × Se	13.7 ± 5.9	530 ± 97	38.7	**$p \le 0.0000149$**

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

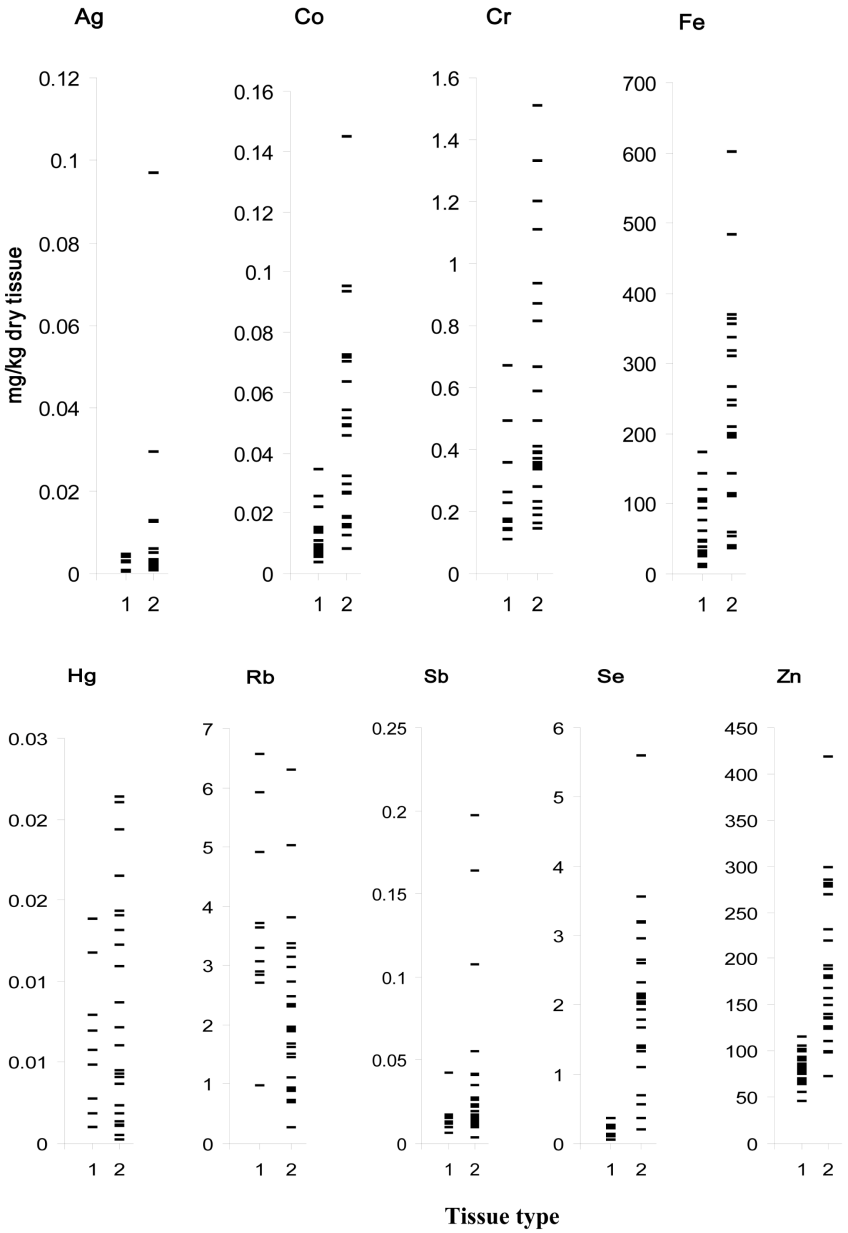

Figure 1. Individual data sets for Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions (mg/kg, dry mass basis) in intact bone (1) and osteogenic sarcoma (2).

try, 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/tumor 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.

Table 5. Intercorrelations of pairs of the trace element mass fraction in tissue of intact bone and osteogenic sarcoma.

Tissue	E	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	-
Osteogenic	Ag	0.56[b]	0.33	0.35	0.24	0.67[c]	0.02	−0.21	−0.02
sarcoma	Co	-	0.66[b]	0.80[c]	0.26	0.44[a]	0.09	0.21	−0.39[a]
N = 27	Cr	0.66[b]	-	0.73[c]	0.21	0.29	0.08	0.38	−0.18
	Fe	0.80[c]	0.73[c]	-	0.34	0.44[a]	0.15	0.36	−0.17
	Hg	0.26	0.21	0.34	-	0.34	0.35	0.09	−0.13
	Rb	0.44[a]	0.29	0.44[a]	0.34	-	0.13	−0.30	0.40[a]
	Sb	0.09	0.08	0.15	0.35	0.13	-	0.04	0.11
	Se	0.21	0.38	0.36	0.09	−0.30	0.04	-	−0.37
	Zn	−0.39[a]	−0.18	−0.17	−0.13	0.40[a]	0.11	−0.37	-

E element, statistically significant difference: [a]$p \le 0.05$, [b]$p \le 0.01$, [c]$p \le 0.001$.

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]-[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 osteosarcoma tissue presented in **Tables 1-5**.

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 (**Figure 1**). In the osteosarcoma group the mass fraction of all nine trace elements were determined in all samples (**Figure 1**).

Table 2 shows that in the osteosarcoma tissue the mean mass fraction of Ag, Co, Cr, Fe, Hg, Sb, Se, and Zn is higher while the mean mass fraction of Rb is lower than in the normal bone tissues. However, in osteosarcoma only the mean mass fractions of Co ($p \le 0.000013$), Cr ($p \le 0.0051$), Fe ($p \le 0.000000097$), Sb ($p \le 0.0317$), Se ($p \le 0.000000022$), and Zn ($p \le 0.000000079$) are significantly increased and the mean mass fraction of Rb ($p \le 0.0134$) is significantly decreased when compared with those in normal bone. Our findings that the Fe, Se and Zn mass fractions are significantly higher in the tumor than in the normal bone tissue agree well with published data [37]-[39].

Different directions of mass fraction changes suggest potential use of mass fraction ratios of these trace elements as osteosarcoma markers. A simple multiplication of two or more trace element mass fractions, which change in one direction, can improve the difference between such characteristics of intact bone and osteosarcoma tissues. These conclusions were the main reason for calculating Rb/Co, Rb/Fe, Rb/Se, and Rb/Zn mass fraction ratios, as well as Co × Zn, Fe × Zn, Sb × Zn, Se × Zn, Co × Se, and Fe × Se mass fractions multiplications (**Table 3**). It was found that significant lower mean values of all selected mass fraction ratios as well as significant higher mean values of multiplications were typical of osteosarcoma tissue compared with intact bone (**Table 4**). No published data referring to ratios or multiplications of trace element mass fractions in the osteo-

sarcoma tissue were found.

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 5**). 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 osteosarcoma tissue many significant correlations between trace elements found in the control group are no longer evident, for example, direct correlation between Fe and Se, etc. (**Table 5**). However, direct correlations between Co and Ag ($r = 0.56$, $p \leq 0.01$), Co and Cr ($r = 0.66$, $p \leq 0.01$), Co and Rb ($r = 0.44$, $p \leq 0.05$), Cr and Fe ($r = 0.73$, $p \leq 0.001$), Fe and Rb ($r = 0.44$, $p \leq 0.05$), and also Rb and Zn ($r = 0.40$, $p \leq 0.05$), as well as inverse correlation between Co and Zn ($r = -0.39$, $p \leq 0.05$) were observed (**Table 5**). Thus, if we accept the levels and relationships of trace element mass fraction in the intact bone samples of control group as a norm, we have to conclude that with a malignant transformation the levels and relationships of trace elements in bone significantly change. No published data referring to correlations between trace element mass fractions in the osteosarcoma tissue were found.

The changes in trace element contents of cancerous tissues in comparison with non-cancerous tissues may be attributed to a cause or effect of malignant transformation. Bone is a mineralized connective tissue. 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]. Osteosarcoma is classified as a bone tumor. Our previous findings showed that the means of the Ca and P mass fraction in the osteosarcoma tissue are lower than in normal bone, but the mean of Ca/P ratio is similar [46]. It suggested that osteosarcoma 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 osteosarcoma tissue samples was 4.8 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. Osteosarcoma is considered a highly vascularized bone tumor [47]. Thus, it is possible to speculate that osteosarcoma is characterized by an increase of the mean value of the Fe mass fraction because the level of tumor vascularization is higher than that in normal bone. As we found, there is a direct correlation between Fe and Co as well as between Fe and Cr mass fractions in osteosarcoma tissue (**Table 5**). Therefore an increased level of Co and Cr in the osteosarcoma may be closely connected with a high Fe content in tumor tissue (**Table 2**).

In the osteosarcoma tissue the mean Se mass fractions is 11.0 times higher ($p \leq 0.000000022$) than in normal bone (**Table 2**). The high Se level was reported in malignant tumors of ovary [48], lung [49], prostate [50], breast [51] [52], intestine [53], and in gastric cancer tissue [54]. 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 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 [55]. However the cause of increased Se in cancerous tissue and particularly in the osteosarcoma is not completely understood and requires further studies.

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 bone samples and samples of intraosseous lesions weighing about 100 mg. In the osteosarcoma tissue the mass fractions of Co, Cr, Fe, Sb, Se, and Zn are significantly higher while the mass fraction of Rb is lower than in normal bone tissues. Moreover, significantly lower of Rb/Co, Rb/Fe, Rb/Se, and Rb/Zn mass fraction ratios as well as significant higher mean values of Co × Zn, Fe × Zn, Sb × Zn, Se × Zn, Co × Se, and Fe × Se mass fractions multiplications are typical of the osteosarcoma tissue compared to intact bone. In the osteosarcoma 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 osteosarcoma transformed bone tissues the trace element homeostasis is significantly disturbed.

Acknowledgements

The authors are grateful to the late Prof. V.A. Bizer, Medical Radiological Research Center, Obninsk, for supplying the osteosarcoma samples.

References

[1] Ulmer, D.D. (1973) Metals—From Privation to Pollution. *Federation Proceedings*, **32**, 1758-1762.

[2] Zaichick, V. (2006) Medical Elementology as a New Scientific Discipline. *Journal of Radioanalytical and Nuclear Chemistry*, **269**, 303-309. http://dx.doi.org/10.1007/s10967-006-0383-3

[3] Zherbin, E.A. and Zaichick, V. (1976) Several Aspects of Applied Neutron Activation in Medicine: The Present State and Development of Activation Analysis in the Institute of Medical Radiology. *Proceedings of the* 2*nd Meeting on New Nuclear-Physical Methods Used in Solving Scientific-Technical and National Economic Problems*, Join Institute of Nuclear Research, Dubna, 104-126. (Article in Russian)

[4] Kalashnikov, V.M. and Zaichick, V. (1977) Bone Analysis of N, F and P by Photonuclear Activation. *Voprosy Meditsinskoĭ Khimii*, **23**, 122-127. (Article in Russian)

[5] Zaichick, V. (1993) The *in Vivo* Neutron Activation Analysis of Calcium in the Skeleton of Normal Subjects, with Hypokinesia and Bone Diseases. *Journal of Radioanalytical and Nuclear Chemistry*, **169**, 307-316. http://dx.doi.org/10.1007/BF02042988

[6] Zaichick, V. (1994) Instrumental Activation and *X*-Ray Fluorescent Analysis of Human Bones in Health and Disease. *Journal of Radioanalytical and Nuclear Chemistry*, **179**, 295-303. http://dx.doi.org/10.1007/BF02040164

[7] Zaichick, V. and Ovchjarenko, N.N. (1996) *In Vivo* X-Ray Fluorescent Analysis of Ca, Zn, Sr, and Pb in Frontal Tooth Enamel. *Journal of Trace and Microprobe Techniques*, **14**, 143-152.

[8] Zaichick, V. and Morukov, B. (1998) *In Vivo* Bone Mineral Studies on Volunteers during a 370-Day Antiorthostatic Hypokinesia Test. *Applied Radiation and Isotopes*, **49**, 691-694. http://dx.doi.org/10.1016/S0969-8043(97)00205-4

[9] Zaichick, V. (1998) *In Vivo* and *in Vitro* Application of Energy-Dispersive XRF in Clinical Investigations: Experience and the Future. *Journal of Trace Elements in Experimental Medicine*, **11**, 509-510.

[10] Zaichick, V., Ovchjarenko, N. and Zaichick, S. (1999) *In Vivo* Energy Dispersive X-Ray Fluorescence for Measuring the Content of Essential and Toxic Trace Elements in Teeth. *Applied Radiation and Isotopes*, **50**, 283-293. http://dx.doi.org/10.1016/S0969-8043(97)10150-6

[11] Zaichick, V. and Snetkov, A. (2000) Bone Composition in Children with Rickets-Like Diseases before and during Treatment. In: Anke, M., *et al*., Eds., *Mengen- und Spurenelemente*: 20. *Arbeitstagung*. Friedrich-Schiller-Universitat, Jena, 1109-1117.

[12] Zaichick, V., Dyatlov, A. and Zaihick, S. (2000) INAA Application in the Age Dynamics Assessment of Major, Minor, and Trace Elements in the Human Rib. *Journal of Radioanalytical and Nuclear Chemistry*, **244**, 189-193. http://dx.doi.org/10.1023/A:1006797006026

[13] Sastri, C.S., Iyengar, V., Blondiaux, G., Tessier, Y., Petri, H., Hoffmann, P., Aras, N.K., Zaichick, V. and Ortner, H.M. (2001) Fluorine Determination in Human and Animal Bones by Particle-Induced Gamma-Ray Emission. *Fresenius*' *Journal of Analytical Chemistry*, **370**, 924-929. http://dx.doi.org/10.1007/s002160100900

[14] Zaichick, V. and Tzaphlidou, M. (2002) Determination of Calcium, Phosphorus, and the Calcium/Phosphorus Ratio in Cortical Bone from the Human Femoral Neck by Neutron Activation Analysis. *Applied Radiation and Isotopes*, **56**, 781-786. http://dx.doi.org/10.1016/S0969-8043(02)00066-0

[15] Tzaphlidou, M. and Zaichick, V. (2002) Neutron Activation Analysis of Calcium/Phosphorus Ratio in Rib Bone of Healthy Humans. *Applied Radiation and Isotopes*, **57**, 779-783. http://dx.doi.org/10.1016/S0969-8043(02)00171-9

[16] Tzaphlidou, M. and Zaichick, V. (2003) Calcium, Phosphorus, Calcium-Phosphorus Ratio in Rib Bone of Healthy Humans. *Biological Trace Element Research*, **93**, 63-74. http://dx.doi.org/10.1385/BTER:93:1-3:63

[17] Zaichick, V. and Tzaphlidou, M. (2003) Calcium and Phosphorus Concentrations and Calcium/Phosphorus Ratio in Trabecular Bone from Femoral Neck of Healthy Humans as Determined by Neutron Activation Analysis. *Applied Radiation and Isotopes*, **58**, 623-627. http://dx.doi.org/10.1016/S0969-8043(03)00092-7

[18] Zaichick, V. (2004) INAA Application in the Age Dynamics Assessment of Ca, Cl, K, Mg, Mn, Na, P, and Sr Contents in the Cortical Bone of Human Femoral Neck. *Journal of Radioanalytical and Nuclear Chemistry*, **259**, 351-354. http://dx.doi.org/10.1023/B:JRNC.0000017317.64992.04

[19] Zaichick, V. (2004) Sex and Age Related Ca/P Ratio in Trabecular Bone of Iliac Crest of Healthy Humans. In: Anke, M., *et al*., Eds., *Macro and Trace Elements*: 22 *Workshop*, Vol. 1, Friedrich-Schiller-Universitat, Jena, 248-255.

[20] Tzaphlidou, M. and Zaichick, V. (2004) Sex and Age Related Ca/P Ratio in Cortical Bone of Iliac Crest of Healthy Humans. *Journal of Radioanalytical and Nuclear Chemistry*, **259**, 347-349. http://dx.doi.org/10.1023/B:JRNC.0000017316.20693.45

[21] Zaichick, V. (2007) INAA Application in the Assessment of Selected Elements in Cancellous Bone of Human Iliac Crest. *Journal of Radioanalytical and Nuclear Chemistry*, **271**, 573-576. http://dx.doi.org/10.1007/s10967-007-0308-9

[22] Zaichick, V. and Zaichick, S. (2009) Instrumental Neutron Activation Analysis of Trace Element Contents in the Rib Bone of Healthy Men. *Journal of Radioanalytical and Nuclear Chemistry*, **281**, 47-52. http://dx.doi.org/10.1007/s10967-009-0084-9

[23] Zaichick, V. (2009) Neutron Activation Analysis of Ca, Cl, K, Mg, Mn, Na, P, and Sr Contents in the Crowns of Human Permanent Teeth. *Journal of Radioanalytical and Nuclear Chemistry*, **281**, 41-45. http://dx.doi.org/10.1007/s10967-009-0083-x

[24] Zaichick, S. and Zaichick, V. (2010) The Effect of Age and Gender on 38 Chemical Element Contents in Human Iliac Crest Investigated by Instrumental Neutron Activation Analysis. *Journal of Trace Elements in Medicine and Biology*, **24**, 1-6. http://dx.doi.org/10.1016/j.jtemb.2009.07.002

[25] Zaichick, S. and Zaichick, V. (2010) The Effect of Age and Gender on 38 Chemical Element Contents in Human Femoral Neck Investigated by Instrumental Neutron Activation Analysis. *Biological Trace Element Research*, **137**, 1-12. http://dx.doi.org/10.1007/s12011-009-8554-z

[26] Zaichick, S. and Zaichick, V. (2012) Neutron Activation Analysis of Ca, Cl, Mg, Na, and P Content in Human Bone Affected by Osteomyelitis or Osteogenic Sarcoma. *Journal of Radioanalytical and Nuclear Chemistry*, **293**, 241-246. http://dx.doi.org/10.1007/s10967-012-1645-x

[27] Zaichick, V. (2013) Chemical Elements of Human Bone Tissue Investigated by Nuclear Analytical and Related Methods. *Biological Trace Element Research*, **153**, 84-99. http://dx.doi.org/10.1007/s12011-013-9661-4

[28] Zaichick, V. (2013) Data for the Reference Man: Skeleton Content of Chemical Elements. *Radiation Environmental Biophysics*, **52**, 65-85. http://dx.doi.org/10.1007/s00411-012-0448-3

[29] Zaichick, V. and Zaichick, S. (2014) The Ca, Cl, Mg, Na, and P Mass Fractions in Human Bone Affected by Ewing's Sarcoma. *Biological Trace Element Research*, **159**, 32-38. http://dx.doi.org/10.1007/s12011-014-9966-y

[30] Ottaviani, G. and Jaffe, N. (2009) The Epidemiology of Osteosarcoma. *Cancer Treatment Research*, **152**, 3-13. http://dx.doi.org/10.1007/978-1-4419-0284-9_1

[31] Savage, S.A. and Mirabello, L. (2011) Using Epidemiology and Genomics to Understand Osteosarcoma Etiology. *Sarcoma*, **2011**, Article ID: 548151. http://dx.doi.org/10.1155/2011/548151

[32] Wittig, J.C., Bickels, J., Priebat, D., Jelinek, J., Kellar-Graney, K., Shmookler, B. and Malawer, M.M. (2002) Osteosarcoma: A Multidisciplinary Approach to Diagnosis and Treatment. *American Family Physician*, **65**, 1123-1132.

[33] Marina, N., Gebhardt, M., Teot, L. and Gorlick, R. (2004) Biology and Therapeutic Advances for Pediatric Osteosarcoma. *Oncologist*, **9**, 422-441. http://dx.doi.org/10.1634/theoncologist.9-4-422

[34] Di Caprio, M.R., Lindskog, D.M., Husted, D., Reith, J. and Scarborough, M.T. (2005) Knee Pain in a 14-Year-Old Girl. *Clinical Orthopaedics and Related Research*, **435**, 267-275. http://dx.doi.org/10.1097/01.blo.0000150577.14047.01

[35] Fuchs, B. and Pritchard, D.J. (2002) Etiology of Osteosarcoma. *Clinical Orthopaedics and Related Research*, **397**, 40-52. http://dx.doi.org/10.1097/00003086-200204000-00007

[36] Ellender, M., Harrison, J.D., Pottinger, H. and Thomas, J.M. (2001) Induction of Osteosarcoma and Acute Myeloid Leukaemia in CBA/H Mice by the Alpha-Emitting Nuclides, Uranium-233, Plutonium-239 and Amercium-241. *International Journal Radiation Biology*, **77**, 41-52. http://dx.doi.org/10.1080/095530001453104

[37] Janes, J.M., McCall, J.T. and Elveback, L.R. (1972) Trace Metals in Human Osteogenic Sarcoma. *Mayo Clinic Proceedings*, **47**, 476-478.

[38] Milachowski, K.A. and Keyl, W. (1984) Mineral and Trace Element Determination in Human Osteosarcoma. Case Report. *Zeitschrift für Orthopädie und ihre Grenzgebiete*, **122**, 848-850. (In German) http://dx.doi.org/10.1055/s-2008-1045079

[39] Golubkina, N.A., Aliev, M.D. and Kushlinskiĭ, N.E. (1995) Selenium in Blood Serum and in Tumors of Patients with Benign and Malignant Bone Neoplasms. *Voprosy Meditsinskoĭ Khimii*, **41**, 50-54. (In Russian)

[40] Zaichick, V. and Zaichick, S. (1996) Instrumental Effect on the Contamination of Biomedical Samples in the Course of Sampling. *The Journal of Analytical Chemistry*, **51**, 1200-1205. (In Russian)

[41] Zaichick, V. (1997) Sampling, Sample Storage and Preparation of Biomaterials for INAA in Clinical Medicine, Occu-

pational and Environmental Health. In: Parr, R., Ed., *Harmonization of Health-Related Environmental Measurements Using Nuclear and Isotopic Techniques*, International Atomic Energy Agency, Vienna, 123-133.

[42] Zaichick, V. (1995) Application of Synthetic Reference Materials in the Medical Radiological Research Centre. *Fresenius' Journal of Analytical Chemistry*, **352**, 219-223. http://dx.doi.org/10.1007/BF00322330

[43] Zaichick, V., Kolotov, A.V. and Dogadkin, N. (2002) Comparative Analysis of Major and Trace Elements in Bone Reference Materials, IAEA H-5 (Animal Bone) and NIST SRM 1486 (Bone Meal). In: Anke, M., *et al*., Eds., *Macro and Trace Elements*: 21 *Workshop*, Friedrich Schiller University, Jena, 39-47.

[44] Korelo, A. and Zaichick, V. (1993) Software to Optimize the Multielement INAA of Medical and Environmental Samples. In: *Activation Analysis in Environment Protection*, Join Institute of Nuclear Research, Dubna, 326-332. (In Russian)

[45] Zaichick, V. (2004) Losses of Chemical Elements in Biological Samples under the Dry Ashing Process. *Trace Elements in Medicine*, **5**, 17-22. (In Russian)

[46] Zaichick, S. and Zaichick, V. (2012) Neutron Activation Analysis of Ca, Cl, Mg, Na, and P Content in Human Bone Affected by Osteomyelitis or Osteogenic Sarcoma. *Journal of Radioanalytical and Nuclear Chemistry*, **293**, 241-246. http://dx.doi.org/10.1007/s10967-012-1645-x

[47] Kunz, P., Fellenberg, J., Moskovszky, L., Sápi, Z., Krenacs, T., Machado, I., Poeschl, J., Lehner, B., Szendrõi, M., Ruef, P., Bohlmann, M., Bosch, A.L., Ewerbeck, V., Kinscherf, R. and Fritzsching, B. (2015) Improved Survival in Osteosarcoma Patients with Atypical Low Vascularization. *The Annals of Surgical Oncology*, **22**, 489-496. http://dx.doi.org/10.1245/s10434-014-4001-2

[48] Xue, F.X. and Zhang, S.W. (1991) Selenium Concentrations in Serum, Hair and Tumor Tissue from Patients with Ovarian Tumors. *Zhonghua Fu Chan Ke Za Zhi*, **26**, 290-292.

[49] Zachara, B.A., Marchaluk-Wiśniewska, E., Maciag, A., Pepliński, J., Skokowski, J. and Lambrecht, W. (1997) Decreased Selenium Concentration and Glutathione Peroxidase Activity in Blood and Increase of These Parameters in Malignant Tissue of Lung Cancer Patients. *Lung*, **175**, 321-332. http://dx.doi.org/10.1007/PL00007578

[50] Zachara, B.A., Szewczyk-Golec, K., Tyloch, J., Wolski, Z., Szylberg, T., Stepien, S., Kwiatkowski, S., Bloch-Boguslawska, E. and Wasowicz, W. (2005) Blood and Tissue Selenium Concentrations and Glutathione Peroxidase Activities in Patients with Prostate Cancer and Benign Prostate Hyperplasia. *Neoplasma*, **52**, 248-254.

[51] Raju, G.J.N., Sarita, P., Kumar, M.R., Murty, G., Reddy, B.S., Lakshminarayana, S., Vijayan, V., Lakshmi, P., Gavarasana, S. and Reddy, S.B. (2006) Trace Elemental Correlation Study in Malignant and Normal Breast Tissue by PIXE Technique. *Nuclear Instruments and Methods in Physics Research B*, **247**, 361-367. http://dx.doi.org/10.1016/j.nimb.2006.02.007

[52] Charalabopoulos, K., Kotsalos, A., Batistatou, A., Charalabopoulos, A., Vezyraki, P., Peschos, D., Kalfakakou, V. and Evangelou, A. (2006) Selenium in Serum and Neoplastic Tissue in Breast Cancer: Correlation with CEA. *The British Journal of Cancer*, **95**, 674-676. http://dx.doi.org/10.1038/sj.bjc.6603292

[53] Kucharzewski, M., Braziewicz, J., Majewska, U. and Gózdz, S. (2003) Selenium, Copper, and Zinc Concentrations in Intestinal Cancer Tissue and in Colon and Rectum Polyps. *Biological Trace Element Research*, **92**, 1-10. http://dx.doi.org/10.1385/BTER:92:1:1

[54] Charalabopoulos, K., Kotsalos, A., Batistatou, A., Charalabopoulos, A., Peschos, D., Vezyraki, P., Kalfakakou, V., Metsios, A., Charalampopoulos, A., Macheras, A., Agnantis, N. and Evangelou, A. (2009) Serum and Tissue Selenium Levels in Gastric Cancer Patients and Correlation with CEA. *Anticancer Research*, **29**, 3465-3467.

[55] Gupta-Elera, G., Garrett, A.R., Robison, R.A. and O'Neill, K.L. (2012) The Role of Oxidative Stress in Prostate Cancer. *European Journal of Cancer Prevention*, **21**, 155-162. http://dx.doi.org/10.1097/CEJ.0b013e32834a8002