Zaichick *et al. J Cancer Metastasis Treat* 2018;4:60
DOI: 10.20517/2394-4722.2018.52

Journal of Cancer Metastasis and Treatment

Original Article

Open Access

Levels of chemical element contents in thyroid as potential biomarkers for cancer diagnosis (a preliminary study)

Vladimir Zaichick[1], Sofia Zaichick[2]

[1]Radionuclide Diagnostics Department, Medical Radiological Research Centre, Obninsk 249036, Russia.
[2]Laboratory of Dr. Gabriela Caraveo Piso, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611-4296, USA.

Correspondence to: Dr. Vladimir Zaichick, Radionuclide Diagnostics Department, Medical Radiological Research Centre, Korolyev St. 4, Kaluga region, Obninsk 249036, Russia. E-mail: vzaichick@gmail.com

How to cite this article: Zaichick V, Zaichick S. Levels of chemical element contents in thyroid as potential biomarkers for cancer diagnosis (a preliminary study). *J Cancer Metastasis Treat* 2018;4:60. http://dx.doi.org/10.20517/2394-4722.2018.52

Received: 13 Aug 2018 **First Decision:** 2 Oct 2018 **Revised:** 28 Oct 2018 **Accepted:** 15 Nov 2018 **Published:** 28 Dec 2018

Science Editor: Bing-liang Fang **Copy Editor**: Cui Yu **Production Editor**: Huan-Liang Wu

Abstract

Aim: Thyroid cancer is an internationally important health problem. The aim of this exploratory study was to evaluate whether significantchanges in the thyroid tissue levels of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn exist in the malignantly transformed thyroid.

Methods: Thyroid tissue levels of twenty chemical elements were prospectively evaluated in 41 patients with thyroid malignant tumors and 105 healthy inhabitants. Measurements were performed using a combination of non-destructive and destructive methods: instrumental neutron activation analysis and inductively coupled plasma atomic emission spectrometry, respectively. Tissue samples were divided into two portions. One was used for morphological study while the other was intended for trace element analysis.

Results: It was found that contents of Al, B, Br, Ca, Cl, Cu, K, Mg, Mn, Na, P, S, and Si were significantly higher (approximately 3.2, 4.6, 9.3, 1.8, 2.3, 3.6, 1.6, 1.6, 1.6, 1.2, 2.5, 1.1, and 2.8 times, respectively) while content of I lower (nearly 26 times) in cancerous tissues than in normal tissues.

Conclusion: There are considerable changes in chemical element contents in the malignantly transformed tissue of thyroid.

Keywords: Thyroid malignant tumors, intact thyroid, chemical elements, biomarkers for cancer diagnosis, instrumentalneutron activation analysis, inductively coupled plasma atomic emission spectrometry

INTRODUCTION

Thyroid cancer (TC) is the most common endocrine malignancy. TC incidence has dramatically increased in the recent decades[1]. During the same period no other cancer has increased as much as TC. With the worldwide increase in the incidence of TC, it has become the fifth most common cancer in women[2-4]. In some countries, the incidence of TC has increased extremely fast, and it has been the most common cancer over the last years[5].

Although the etiology of TC is unknown, several risk factors including deficiency or excess of such micronutrient as I have been well identified[6-17]. It was also reported that the incidence of TC and mortality from this disease increases progressively with advancing age[18,19]. For example, a 37-fold increase in hazard ratio from age < 40 years to age > 70 years was shown in the study of 3,664 TC patients that received surgery and adjuvant treatment at Memorial Sloan Kettering Cancer Center from the years 1985 to 2010[19].

Besides I involved in thyroid function, other trace elements have also essential physiological functions such as maintenance and regulation of cell function, gene regulation, activation or inhibition of enzymatic reactions, and regulation of membrane function. Essential or toxic (mutagenic, carcinogenic) properties of trace elements depend on tissue-specific need or tolerance, respectively[20]. Excessive accumulation or an imbalance of the trace elements may disturb the cell functions and may result in cellular degeneration, death or malignant transformation[20-22].

In our previous study a significant positive correlation between age and some chemical element contents in the thyroid was observed[23-28]. It was concluded that an age-dependent excess of intra-thyroidal I and Zn concentration is probably one of the factors acting in both initiation and promotion stages of thyroid carcinogenesis[9,24,25], as it was earlier shown by us for I in thyroid and for Zn in prostate gland[29-34]. Moreover, it seems fair to suppose that besides I and Zn,many other chemical elementsalso play arolein the pathophysiology of the thyroid.

This work had two aims. The first was to assess the Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction contents in TC tissue using a combination of non-destructive and destructive methods: instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) and inductively coupled plasma atomic emission spectrometry, respectively. The second aim was to compare the levels of chemical elements in the malignant thyroid with those in intact (normal) gland of apparently healthy persons.

METHODS

All patients suffering from TC (n = 41, mean age Mean ± SD was 46 ± 15 years, range 16-75) were hospitalized in the Head and Neck Department of the Medical Radiological Research Centre. Thick-needle puncture biopsy of suspicious nodules of the thyroid was performed for every patient, to permit morphological study of thyroid tissue at these sites and to estimate their chemical element contents. In cases of surgical operated patients with TC the specimens of resected materials were also used for morphological and chemical investigation. In all cases the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. Histological conclusions for malignant tumors were: 25 papillary adenocarcinomas, 8 follicular adenocarcinomas, 7 solid carcinomas, and 1 reticulosarcoma.

Normal thyroids for the control group samples were removed at necropsy from 105 deceased (mean age 44 ± 21 years, range 2-87), who had died suddenly. Samples were obtained within 48 h after a sudden death. The majority of deaths were due to trauma. A histological examination in the control group was used to control the age norm conformity, as well as to confirm the absence of micro-nodules and latent cancer.

All tissue samples were divided into two portions using a titanium scalpel[35]. One was used for morphological study while the other was intended for chemical element analysis. After the samples intended for chemical element analysis were weighed, they were freeze-dried and homogenized[36].

The pounded samples weighing about 5-10 mg (for biopsy) and 100 mg (for resected materials) were used for chemical element measurement by INAA-SLR. The samples for INAA-SLR were sealed separately in thin polyethylene films washed beforehand with acetone and rectified alcohol. The sealed samples were placed in labeled polyethylene ampoules. The content of Br, Ca, Cl, I, K, Mg, Mn, and Na were determined by INAA-SLR using a horizontal channel equipped with the pneumatic rabbit system of the water-water-reactor-special research nuclear reactor (Branch of Karpov Institute, Obninsk). Thyroid samples irradiated by neutrons were measured using a gamma spectrometer. The gamma spectrometer included the 98 cm^3 Ge(Li) detector with on-line computer-based multichannel analyzer system (NUC 8100, Hungary) and provided a resolution of 1.9 keV on the ^{60}Co 1332 keV line.

After INAA-SLR investigation the thyroid samples were taken out from the polyethylene ampoules and used for inductively coupled plasma-atomic emission spectrometry (ICP-AES). The samples were decomposed in autoclaves. For this 1.5 mL of concentrated HNO_3 (nitric acid at 65%, maximum of 0.0000005% Hg; GR, ISO, Merck, Darmstadt, Germany) and 0.3 mL of H_2O_2 (pure for analysis) were added to each thyroid samples, which were placed in one-chamber autoclaves (Ancon-AT2, Ltd., Moscow, Russia) and then heated for 3 h at 160-200 °C. After autoclaving, they were cooled to room temperature and solutions from the decomposed samples were diluted with deionized water (up to 20 mL) and transferred to plastic measuring bottles. Simultaneously, the same procedure was performed in autoclaves without tissue samples (containing only HNO_3 + H_2O_2 + deionized water), and the resultant solutions were used as control samples. Sample aliquots were used to determine the Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractions by ICP-AES using the spectrometer ICAP-61 (Thermo Jarrell Ash, USA). The determination of the ChE content in aqueous solutions was made by the quantitative method using calibration solutions (High Purity Standards, USA) of 0.5 and 10 mg/L of each element. The calculations of the ChE content in the probe were carried out using software of a spectrometer (ThermoSPEC, version 4.1).

Information detailing the NAA-SLR and ICP-AES methods used and other details of the analysis were presented in our earlier publications concerning chemical element contents in human thyroid, scalp hair, and prostate[7,23,27,37-42].

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used[43]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. Ten sub-samples of certified reference material (CRM) International Atomic Energy Agency (IAEA) H-4 (animal muscle) and five sub-samples of CRM of the Institute of Nuclear Chemistry and Technology (INCT, Warszawa, Poland), INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2Mixed Polish Herbs were treated and analyzed in the same conditions as those for thyroid samples to estimate the precision and accuracy of results.

A dedicated computer program for INAA mode optimization was used[44]. All thyroid samples were prepared in duplicate, and mean values of chemical element contents were used. Mean values of chemical elements contents were used in final calculation for the Br, Fe, Rb, and Zn mass fractions measured by two methods. Using Microsoft Office Excel, a summary of the statistics, including, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for chemical element contents. The difference in the results between two age groups was evaluated by the parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test.

Table 1. Instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides data of chemical element contents in the IAEA H-4 (animal muscle) reference material compared to certified values (mg/kg on dry mass basis)

Element	Certified values			This work results
	Mean	95% confidence interval	Type	M ± SD
Br	4.1	3.5-4.7	C	5.0 ± 0.9
Ca	188	163-213	C	238 ± 59
Cl	1890	1810-1970	C	1950 ± 230
K	15800	15300-16400	C	16200 ± 3800
Mg	1050	990-1110	C	1100 ± 190
Mn	0.52	0.48-0.55	N	0.55 ± 0.11
Na	2060	1930-2180	C	2190 ± 140

M: arithmetic mean; SD: standard deviation; C: certified values; N: non-certified values

Table 2. Inductively coupled plasma-atomic emission spectrometry data of chemical element contents in certified reference materials (M ± SD, mg/kg on dry mass basis)

Element	Soya Bean Flour (INCT-SBF-4)		Tea Leaves (INCT-TL-1)		Mixed Polish Herbs (INCT-MPH-2)	
	Certificate	This work result	Certificate	This work result	Certificate	This work result
Al	45.5 ± 3.7	37.1 ± 1.4	2290 ± 280	2248 ± 61	670 ± 111	485 ± 79
B	39.9 ± 4.0	34.5 ± 1.4	26[a]	24.8 ± 1.2	-	28.8 ± 8.1
Ba	7.30 ± 0.23	7.38 ± 0.23	43.2 ± 3.9	44.7 ± 2.6	32.5 ± 2.5	32.2 ± 0.6
Ca	2467 ± 170	2737 ± 190	5820 ± 520	6296 ± 360	10800 ± 700	10250 ± 294
Cu	14.3 ± 0.5	14.2 ± 0.8	20.4 ± 1.5	19.7 ± 1.1	7.77 ± 0.53	8.28 ± 0.47
Fe	90.8 ± 4.0	80.5 ± 6.9	432[a]	493 ± 39	460[a]	459 ± 33
K	24230 ± 830	25230 ± 1090	17000 ± 1200	17810 ± 1320	19100 ± 1200	20280 ± 870
Li	-	0.0047 ± 0.0018	-	0.217 ± 0.034	-	0.574 ± 0.044
Mg	3005 ± 82	2983 ± 340	2240 ± 170	2415 ± 115	2920 ± 180	2955 ± 159
Mn	32.3 ± 1.1	30.0 ± 1.0	1570 ± 110	1628 ± 145	191 ± 12	197 ± 5
Na	-	10.2 ± 3.4	24.7 ± 3.2	24.2 ± 3.5	350[a]	338 ± 17
P	6555 ± 355	6782 ± 248	1800[a]	2457 ± 150	2500[a]	3022 ± 481
S	4245 ± 471	4468 ± 529	2470 ± 250	2500 ± 230	2410 ± 140	2409 ± 159
Si	-	26.7 ± 4.8	-	325 ± 34	-	268 ± 64
Sr	9.32 ± 0.46	8.76 ± 0.21	20.8 ± 1.7	19.8 ± 1.0	37.6 ± 2.7	37.4 ± 2.1
V	-	≤ 0.22	2.0 ± 0.4	1.8 ± 0.2	0.95 ± 0.16	0.90 ± 0.04
Zn	52.3 ± 1.3	54.8 ± 6.6	34.7 ± 2.7	36.0 ± 3.7	33.5 ± 2.1	32.0 ± 6.1

M: arithmetic mean; SD: standard deviation; a: informative values

RESULTS

Table 1 depicts our data for Br, Ca, Cl, K, Mg, Mn, and Na mass fractions in ten sub-samples of CRM IAEA H-4 (animal muscle) and the certified values of this material.

Table 2 presents our data for Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractions in five sub-samples of INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves and INCT-MPH-2Mixed Polish Herbs CRMs and the certified (or informative) values of this material.

The comparison of our results for the Ca, K, Mg, Mn, and Na mass fractions (mg/kg, dry mass basis) in the normal human thyroid obtained by both INAA-SLR and ICP-AES methods is shown in Table 3.

Table 4 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 Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction in normal and cancerous thyroid tissue.

Table 3. Comparison of the mean values (M ± SEM) of the chemical element mass fractions (mg/kg, on drymass basis)in the normal human thyroid (males and females combined) obtained by both instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides and inductively coupled plasma-atomic emission spectrometry methods

Element	INAA-SLR (M_1)	ICP-AES (M_2)	Δ, %
Ca	1692 ± 109	1633 ± 108	3.5
K	6071 ± 306	6764 ± 298	-11.4
Mg	285 ± 17	308 ± 17	-8.1
Mn	1.35 ± 0.07	1.21 ± 0.07	10.4
Na	6702 ± 178	7154 ± 201	-6.7

ICP-AES: inductively coupled plasma-atomic emission spectrometry; M: arithmetic mean; SEM: standard error of mean; $\Delta = [(M_1 - M_2)/M_1] \times 100\%$

The comparison of our results with published data for Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction in normal and cancerous thyroid[45-74] is shown in Table 5.

The ratios of means and the difference between mean values of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractions in normal and cancerous thyroid are presented in Table 6.

DISCUSSION

Precision and accuracy of results

A good agreement of our results for the Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Mg, Mn, Na, P, S, Sr, V, and Zn mass fractions with the certified values of CRM IAEA H-4, INCT-SBF-4, INCT-TL-1, and INCT-MPH-2 [Tables 1 and 2] as well as the similarity of the means of the Ca, K, Mg, Mn, and Na mass fractions in the normal human thyroid determined by both INAA-SLR and ICP-AES methods [Table 3] demonstrates an acceptable precision and accuracy of the results obtained in the study and presented in Tables 4-6.

The mean values and all selected statistical parameters were calculated for twenty chemical elements (Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn) mass fractions [Table 4]. The mass fraction of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn were measured in all, or a major portion of normal and cancerous tissue samples.

Comparison with published data

The means obtained for Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction, as shown in Table 5, agree well with the medians of mean values reported by other research for the human thyroid, including samples received from persons who died from different non-thyroid diseases[45-65]. The mean obtained for Li is two orders of magnitude lower than the median of previously reported data. Moreover, it is outside the range of previously reported means. The mean obtained for V is one order of magnitude higher than the median of previously reported data, but it is inside the previously reported range of means. A number of values for chemical element mass fractions were not expressed on a dry mass basis by the authors of the cited references. Hence we calculated these values using published data for water 75%[75] and ash 4.16% on dry mass basis[76] contents in thyroid of adults.

In cancerous tissues [Table 3] our results were within the range of means published for Br, Ca, Cu, Fe, I, Mg, Mn, and Zn contents. The obtained means for V was approximately three orders of magnitude lower median of previously reported mean [Table 5]. The obtained mean for Cl was almost one order of magnitude higher than the only reported result and the mean for K was some higher than the median of previously reported means and also higher than the upper level of the range of these means [Table 5]. No published data referring Al, B, Ba, Li, Na, P, S, Si, and Sr contents of cancerous thyroid tissue were found.

The ranges of means of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn levels reported in the literature for normal and for untreated cancerous thyroid vary widely [Table 5]. This can be

Table 4. Some statistical parameters of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction (mg/kg, dry mass basis) in normal and cancerous thyroid

Tissue	Element	M	SD	SEM	Min	Max	Median	*P* 0.025	*P* 0.975
Normal *n* = 105	Al	10.5	13.4	1.8	0.800	69.3	6.35	1.19	52.9
	B	0.476	0.434	0.058	0.200	2.30	0.300	0.200	1.73
	Ba	1.12	1.15	0.15	0.0480	5.00	0.680	0.0838	4.48
	Br	14.9	11.0	1.2	1.90	54.1	11.6	2.56	49.3
	Ca	1682	999	106	373	5582	1454	444	4183
	Cl	3400	1452	174	1030	6000	3470	1244	5869
	Cu	4.08	1.22	0.14	0.500	7.15	4.10	1.57	6.41
	Fe	223	95	10	52.0	489	210	72.8	432
	I	1841	1027	107	114	5061	1695	230	4232
	K	6418	2625	290	1914	15293	5948	2947	13285
	Li	0.0208	0.0155	0.0022	0.0015	0.0977	0.0178	0.0041	0.0487
	Mg	296	134	16	66.0	930	284	95.8	541
	Mn	1.28	0.56	0.07	0.470	4.04	1.15	0.537	2.23
	Na	6928	1730	175	3686	13453	6835	3974	10709
	P	4290	1578	207	496	8996	4221	1360	7323
	S	8259	2002	263	644	11377	8399	3662	11208
	Si	50.8	46.9	6.2	5.70	180	36.0	7.11	174
	Sr	3.81	2.93	0.34	0.100	12.6	2.90	0.365	11.3
	V	0.102	0.039	0.005	0.0200	0.250	0.100	0.0440	0.192
	Zn	94.8	39.6	4.2	7.10	215	88.5	34.9	196
Cancer *n* = 41	Al	33.0	25.5	7.1	4.50	96.5	21.3	5.70	85.6
	B	2.21	1.89	0.52	1.00	5.60	1.00	1.00	5.42
	Ba	1.42	1.30	0.35	0.220	4.09	0.945	0.259	3.93
	Br	139	203	36	6.20	802	50.2	7.75	802
	Ca	3013	2966	699	452	9768	1578	467	8938
	Cl	7699	2900	703	4214	14761	7216	4240	13619
	Cu	14.5	9.4	2.6	4.00	32.6	10.9	4.21	31.4
	Fe	255	168	27	60.6	880	217	74.6	673
	I	71.8	62.0	10.1	2.00	261	62.1	2.93	192
	K	10054	4018	877	1660	18814	9204	4073	17559
	Li	0.0314	0.0307	0.0090	0.0078	0.111	0.0182	0.0088	0.0995
	Mg	478	194	42	130	933	467	166	881
	Mn	2.01	1.34	0.29	0.100	5.95	1.61	0.250	5.23
	Na	8576	2433	531	4083	14048	8107	4901	12925
	P	10493	3238	866	5382	15403	9694	5767	15391
	S	9448	1605	429	7139	12591	9422	7211	12204
	Si	143	156	42	18.6	523	64.2	19.8	472
	Sr	6.26	7.61	1.59	0.93	30.8	3.00	0.985	25.0
	V	0.0904	0.0308	0.0100	0.0580	0.170	0.0870	0.0600	0.154
	Zn	96.9	80.0	12.6	28.7	375	69.8	36.3	374

M: arithmetic mean; SD: standard deviation; SEM: standard error of mean; Min: minimum value; Max: maximum value; *P* 0.025: percentile with 0.025 level; *P* 0.975: percentile with 0.975 level

explained by a dependence of element content on many factors, including the region of the thyroid, from which the sample was taken, age, gender, ethnicity, mass of the gland, and the cancer stage. Not all these factors were strictly controlled in cited studies. Another leading cause, in our opinion, of inter-observer variability can be attributed to the accuracy of the analytical techniques, sample preparation methods, and inability of taking uniform samples from the affected tissues. It was insufficient quality control of results in these studies. In many reported papers tissue samples were ashed or dried at high temperature for many hours. In other cases, thyroid samples were treated with solvents (distilled water, ethanol, formalin *etc*.). There is evidence that by using these methods some quantities of certain trace elements are lost as a result of this treatment, which concerns not only such volatile halogen as Br, but also other trace elements investigated in the study[36,77,78].

Table 5. Median, minimum and maximum value of means Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn contents in the normal and cancerous thyroid according to data from the literature in comparison with our results (mg/kg, dry mass basis)

Tissue	Published data [Reference]			This work
Element	**Median of means (*n*)***	**Min of means M or M ± SD, (*n*)****	**Max of means M or M ± SD, (*n*)****	**Males and females M ± SD**
Normal				
Al	33.6 (12)	0.33 (-)[45]	420 (25)[46]	10.5 ± 13.4
B	0.151 (2)	0.084 (3)[47]	0.46 (3)[47]	0.476 ± 0.434
Ba	0.67 (7)	0.0084 (83)[48]	≤ 5.0 (16)[49]	1.12 ± 1.15
Br	18.1 (11)	5.12 (44)[50]	284 ± 44 (14)[51]	16.3 ± 11.6
Ca	1600 (17)	840 ± 240 (10)[52]	3800 ± 320 (29)[52]	1663 ± 999
Cl	6800 (5)	804 ± 80 (4)[53]	8000 (-)[54]	3400 ± 1452
Cu	6.1 (57)	1.42 (120)[55]	220 ± 22 (10)[53]	3.93 ± 1.43
Fe	252 (21)	56 (120)[55]	2444 ± 700 (14)[51]	223 ± 95
I	1888 (95)	159 ± 8 (23)[56]	5772 ± 2708 (50)[57]	1841 ± 1027
K	4400 (17)	46.4 ± 4.8 (4)[53]	6090 (17)[49]	6418 ± 2625
Li	6.3 (2)	0.092 (-)[58]	12.6 (180)[59]	0.0208 ± 0.0154
Mg	390 (16)	3.5 (-)[45]	840 ± 400 (14)[60]	296 ± 134
Mn	1.82 (36)	0.44 ± 11 (12)[61]	69.2 ± 7.2 (4)[53]	1.28 ± 0.56
Na	8000 (9)	438 (-)[62]	10000 ± 5000 (11)[60]	6928 ± 1730
P	3200 (10)	16 (7)[63]	7520 (60)[50]	4290 ± 1578
S	11000 (3)	4000 (-)[54]	11800 (44)[50]	8259 ± 2002
Si	16.0 (3)	0.97 (-)[45]	143 ± 6 (40)[64]	50.8 ± 46.9
Sr	0.73 (9)	0.55 ± 0.26 (21)[47]	46.8 ± 4.8(4)[53]	3.81 ± 2.93
V	0.042 (6)	0.012 (2)[65]	18 ± 2 (4)[53]	0.102 ± 0.039
Zn	118 (51)	32 (120)[55]	820 ± 204 (14)[51]	94.8 ± 39.7
Cancerous				
Al	-	-	-	33.0 ± 25.5
B	-	-	-	2.21 ± 1.89
Ba	-	-	-	1.42 ± 1.30
Br	15.7 (4)	9.6 (1)[66]	160 ± 112 (3)[67]	139 ± 203
Ca	1572 (6)	390 (1)[68]	3544 (1)[66]	3013 ± 2966
Cl	940 (1)	940 ± 92 (4)[53]	940 ± 92 (4)[53]	7699 ± 2900
Cu	6.8 (11)	4.7 ± 1.8 (22)[69]	51.6 ± 5.2 (4)[53]	14.5 ± 9.4
Fe	316 (8)	69 ± 51 (3)[68]	5588 ± 556 (4)[53]	255 ± 168
I	78.8 (12)	< 23 ± 10 (8)[70]	800 (1)[71]	71.8 ± 62.0
K	6878 (4)	636 ± 64 (4)[54]	7900 (1)[72]	10054 ± 4018
Li	-	-	-	0.0314 ± 0.0307
Mg	320 (2)	316 ± 84 (45)[69]	544 ± 272 (6)[73]	478 ± 194
Mn	1.83 (4)	1.6 ± 0.8 (22)[69]	186 ± 18 (4)[53]	2.01 ± 1.34
Na	-	-	-	8576 ± 2433
P	-	-	-	10493 ± 3238
S	-	-	-	9448 ± 1605
Si	-	-	-	143 ± 156
Sr	-	-	-	6.26 ± 7.61
V	81.2 (1)	81.2 ± 8.4 (4)[53]	81.2 ± 8.4 (4)[53]	0.0904 ± 0.0308
Zn	112 (13)	48 ± 8 (5)[74]	494 ± 37 (2)[72]	96.9 ± 80.0

M; arithmetic mean; SD: standard deviation; (*n*)*: number of all references; (*n*)**: number of samples

Effect of malignant transformationon chemical element contents

From Table 6, it is observed that in cancerous tissue the mass fractions of Al, B, Br, Ca, Cl, Cu, P, and Si are approximately 3, 5, 9, 2, 2, 4, 2, and 3 times, respectively, higher than the mass fractions of K, Mg, Mn, Na, and S, which are almost 57%, 61%, 57%, 24%, and 14%, respectively, higher than in normal tissues of the thyroid. In contrast, the mass fraction of I is almost 26 times lower. Thus, if we accept the chemical element contents in thyroid glands in the control group as a norm, we have to conclude that with a malignant transformation the levels of Al, B, Br, Ca, Cl, Cu, K, Mg, Mn, Na, P, and S in thyroid tissue significantly increased whereas the levels of I drastically decreased.

Table 6. Differences between mean values (M ± SEM) of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction (mg/kg, dry mass basis) in normal and cancerous thyroid

Element	Thyroid tissue				Ratio
	Norm *n* = 105	Cancer *n* = 41	Student's *t*-test *P*	*U*-test *P*	Cancer to norm
Al	10.5 ± 1.8	33.0 ± 7.1	**0.0083**	**≤ 0.01**	3.14
B	0.476 ± 0.058	2.21 ± 0.52	**0.0062**	≤ 0.01	4.64
Ba	1.12 ± 0.15	1.42 ± 0.35	0.446	> 0.05	1.27
Br	14.9 ± 1.2	139 ± 36	**0.0016**	**≤ 0.01**	9.33
Ca	1682 ± 106	3013 ± 699	0.076	**≤ 0.05**	1.79
Cl	3400 ± 174	7699 ± 703	**0.000013**	**≤ 0.01**	2.26
Cu	4.08 ± 0.14	14.5 ± 2.6	**0.0017**	**≤ 0.01**	3.55
Fe	223 ± 10	255 ± 27	0.278	> 0.05	1.14
I	1841 ± 107	71.8 ± 10.1	**0.00000000001**	**≤ 0.01**	0.039
K	6418 ± 290	10054 ± 877	**0.00060**	**≤ 0.01**	1.57
Li	0.0208 ± 0.0022	0.0314 ± 0.0090	0.265	> 0.05	1.51
Mg	296 ± 16	478 ± 42	**0.00043**	**≤ 0.01**	1.61
Mn	1.28 ± 0.07	2.01 ± 0.29	**0.024**	**≤ 0.01**	1.57
Na	6928 ± 175	8576 ± 531	**0.0069**	**≤ 0.01**	1.24
P	4290 ± 207	10493 ± 866	**0.0000054**	**≤ 0.01**	2.45
S	8259 ± 263	9448 ± 429	**0.027**	**≤ 0.01**	1.14
Si	50.8 ± 6.2	143 ± 42	**0.047**	**≤ 0.01**	2.81
Sr	3.81 ± 0.34	6.26 ± 1.59	0.144	> 0.05	1.64
V	0.102 ± 0.005	0.0904 ± 0.0100	0.305	> 0.05	0.89
Zn	94.8 ± 4.2	96.9 ± 12.6	0.877	> 0.05	1.02

M: arithmetic mean; SEM: standard error of mean; statistically significant values are in bold

Role of chemical elements in malignant transformation of the thyroid

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

Al

The trace element Al is not described as essential, because no biochemical function has been directly connected to it. At this stage of our knowledge, there is no doubt that Al overload impacts negatively on human health, including the thyroid function[79].

B

Trace element B is known to influence the activity of many enzymes[80]. Numerous studies have demonstrated beneficial effects of B on human health, including anti-inflammatorystimulus - which reduces levels of inflammatory biomarkers, such as high-sensitivity C-reactive protein and tumor necrosis factor α; as well as raises levels of antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase[81]. Why B content in cancerous thyroid is higher than normal level and how an excess of B acts on thyroid are still to be cleared.

Br

This is one of the most abundant and ubiquitous of the recognized trace elements in the biosphere. Inorganic bromide is the ionic form of bromine which exerts therapeutic as well as toxic effects. An enhanced intake of bromide could interfere with the metabolism of iodine at the whole-body level. In the thyroid gland the biological behavior of bromide is more similar to the biological behavior of iodide[82]. In our previous studies,

we found a significant age-related increase of Br content in human thyroid[23,26-28]. Therefore, a goitrogenic and, probably, carcinogenic effect of excessive Br levels in the thyroid of old females was assumed. On the one hand, elevated levels of Br in TC tissues, observed in the present study, support this conclusion. But, on the other hand, bromide compounds, especially KBr, NaBr, and NH_4Br are frequently used as sedatives in Russia[83]. It may be the reason for elevated levels of Br in specimens of patients with TC. Nevertheless, the accumulation of Br in neoplastic thyroid tissues could possibly be explored for diagnosis of TC.

Ca

In addition to the elevated Br level, an excess in Ca mass fractions in thyroid tissue may contribute to harmful effects on the gland. Many reviews and numerous papers raise the concern about role of Ca in the prostate, breast, lung and other organ malignant transformation[84-94].

Cl

Cl is a ubiquitous, extracellular electrolyte essential to more than one metabolic pathway. Cl exists in the form of chloride in the human body. In the body, it is mostly present as sodium chloride. Therefore, as usual, there is a correlation between Na and Cl contents in tissues and fluids of human body. It is well known that Cl mass fractions in samples depend mainly on the extracellular water volume, including the blood volumes, in tissues[95]. Cancerous tissues are predominantly highly vascularized lesions. Thus, it is possible to speculate that thyroid malignant tumors are characterized by an increase of the mean value of the Cl mass fraction because the level of tumor vascularization is higher than that in normal thyroid tissue. Overall, the elevated levels of Cl in neoplastic thyroids could possibly be explored for diagnosis of TC.

Cu

Cu is a ubiquitous element in the human body which plays many roles at different levels. Various Cu-enzymes (such as amine oxidase, ceruloplasmin, cytochrome-c oxidase, dopamine-monooxygenase, extracellular SOD, lysyl oxidase, peptidylglycineamidating monoxygenase, Cu/Zn SOD, and tyrosinase) mediate the effects of Cu deficiency or excess. Cu excess can have severe negative impacts. Cu generates oxygen radicals and many investigators have hypothesized that excess copper might cause cellular injury via an oxidative pathway, giving rise to enhanced lipid peroxidation, thiol oxidation, and, ultimately, DNA damage[96-98]. Thus, Cu accumulation in thyroid parenchyma with age may be involved in oxidative stress, dwindling gland function, and increasing risk of goiter or cancer[26,28]. The significantly elevated level of Cu in thyroid malignant tumors, observed in the present study, supports this speculation. However, an overall comprehension of Cu homeostasis and physiology, which is not yet acquired, is mandatory to establish the exact role of Cuin the thyroid malignant tumors etiology and metabolism. Anyway, the accumulation of Cu in neoplastic thyroids could possibly be explored for diagnosis of TC.

I

Compared to other soft tissues, the human thyroid gland has higher levels of I, because this element plays an important role in its normal functions, through the production of thyroid hormones (thyroxin and triiodothyronine) which are essential for cellular oxidation, growth, reproduction, and the activity of the central and autonomic nervous system. Malignant transformation is accompanied by a loss of tissue-specific functional features, which leads to a significant reduction in I content associated with functional characteristics of the human thyroid tissue. Drastically low level of I content in neoplastic thyroids could possibly be explored for diagnosis of TC.

K

An uncontrollable cell proliferation characterizes the malignant tumors. Therefore, morphological structures of TC tissue differ from the structure of normal thyroid parenchyma. Because K is mainly an intracellular electrolyte, an elevated level of K content in the TC tissue might reflect the increase of ratio “mass of

transformed thyroid cell - mass of follicular colloid". Nevertheless, the accumulation of K in neoplastic thyroids could possibly be explored for diagnosis of TC.

Mg

Mg is abundant in the human body. This element is essential for the functions of more than 300 enzymes (e.g., alkaline phosphatases, ATP-ases, phosphokinases, the oxidative phosphorylation pathway). It plays a crucial role in many cell functions such as energy metabolism, protein and DNA syntheses, and cytoskeleton activation. Moreover, Mg plays a central role in determining the clinical picture associated with thyroid disease[99]. Experimental data have shown that high doses of magnesium increase the activity of the thyroid gland[100]. Magnesium deficiency can influence bioavailability and tissue distribution of selenium which then appears diminished[101]. From these data, one can conclude that Mg is involved in the thyroid function. If so, significant reduction in Mg content can be associate with TC, because malignant transformation is accompanied by a loss of thyroid-specific functional features. However, it is well known that malignant tumors usually have higher Mg levels than normal tissues[102-107], possibly caused by the "retention" of Mg by the tumor[108], as a result of the high Mg requirement of growing cells. In addition, cultured proliferating cells have long been known to contain more magnesium than quiescent cells, and experimental conditions that decreased magnesium availability affected cell proliferation rate[109]. Thus, the elevated levels of Mg in neoplastic thyroids could possibly be explored for the diagnosis of TC.

Mn

The trace element Mn is a cofactor for numerous enzymes, playing many functional roles in living organisms. The Mn-containing enzyme, Mn-SOD, is the principal antioxidant enzyme which neutralizes the toxic effects of reactive oxygen species (ROS). It has been speculated that Mn interferes with thyroid hormone binding, transport, and activity at the tissue level[110]. There is the opinion that Mn deficiencies in humans are rare and humans maintain stable tissue levels of this trace element[111]. It was reported that intracellular Mn content was positively correlated with Mn-SOD, suggesting that the intracellular Mn level is associated with Mn-SOD activity[112]. However, an overall comprehension of Mn homeostasis and physiology, which is not yet acquired, is mandatory to establish Mn exact role in the thyroid malignant tumors etiology and metabolism. Anyway, the accumulation of Mn in neoplastic thyroids could possibly be explored for diagnosis of TC.

Na

The knowledge concerning ion regulation in many normal and abnormal cell processes has had a rapid development. It was found, among other regulations, that sodium-calcium exchange is associated with the cytoskeleton and the cell membrane. A hypothesis was eventually established that a wide variety of pathological phenomena ranging from acute cell death to chronic processes, such as neoplasia, have a common series of cellular reactions[113]. In accordance with this hypothesis, concentrations of sodium were found to be enhanced in human and animal neoplastic tissues[114,115]. Moreover, the hypothesis that physiological and biochemical changes are associated with proliferating malignant tumors may cause an increase in total tissue sodium concentration was tested with non-invasive, quantitative ^{23}Na magnetic resonance imaging in patients with benign and malignant breast tumors. It was shown that elevated Na concentrations in breast lesions appear to be a cellular-level indicator associated with malignancy[116]. In addition, Na is mainly an extracellular electrolyte and its elevated level in malignant tumors might be linked with a high tumor vascularization (see Chlorine). Anyway, it seems that the accumulation of Na is a generic property of malignant tumors.

P

P is necessary for several, various biological roles in the signal transduction of cells and energy exchange of human body. About 80%-90% of phosphorus is founded in teeth and bones in the form of hydroxyapatite.

Calcium phosphates are one of the main constituents of mineral deposits in aortic wall and tissues[117]. Thus, the high P level in TC can be intimately linked with tumor calcification[86-96].

S

Proteins contain between 3% and 6% of sulfur amino acids. Sulfur amino acids contribute substantially to the maintenance and integrity of the cellular systems by influencing the cellular redox state and the capacity to detoxify toxic compounds, free radicals and ROS[118]. ROS are generated during normal cellular activity and may exist in excess in some pathophysiological conditions, such as inflammation. Therefore exploring fundamental aspects of sulfur metabolism such as the antioxidant effects of sulfur-containing amino acids[119] may help elucidate the mechanism by which the S content increases in TC. Thus, it might be assumed that the elevated S level in cancerous thyroid reflects an increase in concentration of ROS in malignant tissue.

Our findings show that mass fraction of Al, B, Br, Ca, Cl, Cu, I, K, Mg, Mn, Na, P, and S are significantly different in TC as compared to normal thyroid tissues [Table 6]. Thus, it is plausible to assume that levels of these chemical elements in thyroid tissue can be used as tumor markers. However, this subject needs in additional studies.

Limitations

This study has several limitations. Firstly, analytical techniques employed in this study measure only twenty element (Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn) mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of chemical elements investigated in normal and cancerous thyroid tissue. Secondly, the sample size of TC group was relatively small. It does not allow us to carry out the investigations of chemical element contents in TC group using differentials like gender, histological types of tumors, stage of disease, and dietary habits of healthy persons and patients with TC. Lastly, the generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on cancer-specific tissue Al, B, Br, Ca, Cl, Cu, I, K, Mg, Mn, Na, P, and S level alteration and shows the necessity to continue chemical element research of malignant thyroid tumors.

DECLARATIONS

Acknowledgments

The authors are extremely grateful to Profs. Vtyurin BM and Medvedev VS, Medical Radiological Research Center, Obninsk, as well as to Dr. Choporov Yu, Head of the Forensic Medicine Department of City Hospital, Obninsk, for supplying thyroid samples. We are also grateful to Dr. Karandaschev V, Dr. Nosenko S, and Moskvina I, Institute of Microelectronics Technology and High Purity Materials, Chernogolovka, Russia, for their help in ICP-MS analysis.

Authors' contributions

Collected thyroid samples, designed the INAA and ICP-AES of samples, carried out the statistical analysis of results: Zaichick V

Managed the literature searches, wrote the first draft of the manuscript, translated the manuscript into English: Zaichick S

Read and approved the final manuscript: Zaichick V, Zaichick S

Availability of data and materials

Data were obtained in Radionuclide Diagnostic Department, Medical Radiological Research Center, Obninsk 249036, Russia. The data are available in electronic format as Excel and Word files upon request.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

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

Consent for publication

Not applicable.

REFERENCES

1. Kilfoy BA, Zheng T, Holford TR, Han X, Ward MH, et al. International patterns and trends in thyroid cancer incidence, 1973-2002. Cancer Causes Control 2009;20:525-31.
2. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CACancer J Clin 2010;60:277-300.
3. Pellegriti G, Frasca F, Regalbuto C, Squatrito S, Vigneri R. Worldwide increasing incidence of thyroid cancer: update on epidemiology and risk factors. J Cancer Epidemiol 2013;2013:965212.
4. Wiltshire JJ, Drake TM, Uttley L, Balasubramanian SP.Systematic review of trends in the incidence rates of thyroid cancer.Thyroid 2016;26:1541-52.
5. Jung KW, Won YJ, Kong HJ, Oh CM, Lee DH, et al. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2011. Cancer Res Treat 2014;46:109-23.
6. Zaichick VYe, Tsyb AF, Vtyurin BM. Trace elements and thyroid cancer. Analyst 1995;120:817-21.
7. Zaichick VYe, Choporov Yu. Determination of the natural level of human intra-thyroid iodine by instrumental neutron activation analysis. J Radioanal Nucl Chem 1996;207:153-61.
8. Zaichick V, Zaichick S. Normal human intrathyroidal iodine. Sci Total Environ 1997;206:39-56.
9. Zaichick V. Iodine excess and thyroid cancer. J Trace Elem Exp Med 1998;11:508-9.
10. Zaichick V. In vivo and in vitro application of energy-dispersive XRF in clinical investigations: experience and the future. J Trace Elem Exp Med 1998;11:509-10.
11. Zaichick V, Iljina T. Dietary iodine supplementation effect on the rat thyroid 131I blastomogenic action. In: Anke M, Arnhold W, Bergmann H, Bitsch R, Dorn W, et al., editors. Die Bedentung der Mengen-und Spurenelemente. 18. Arbeitstangung. Jena: Friedrich-Schiller-Universität; 1998. pp. 294-306.
12. Zaichick VY, Zaichick SV. Energy-dispersive X-ray fluorescence of iodine in thyroid puncture biopsy specimens. J Trace Microprobe Tech 1999;17:219-32.
13. Zaichick V. Human intrathyroidal iodine in health and non-thyroidal disease. In: Abdulla M, Bost M, Gamont S, Arnaud P, Chazot G, editors. New aspects of trace element research.London and Tokyo: Smith-Gordon and Nishimura; 1999. pp.114-9.
14. Zaichick V. Relevance of, and potentiality for in vivo intrathyroidal iodine determination. Ann NY Acad Sci 2000;904:630-1.
15. Cho BY, Choi HS, Park YJ, Lim JA, Ahn HY, et al.Changes in the clinicopathological characteristics and outcomes of thyroid cancer in Korea over the past four decades.Thyroid 2013;23:797-804.
16. Shan Z, Chen L, Lian X, Liu C, Shi B, et al.Iodine status and prevalence of thyroid disorders after introduction of mandatory universal salt iodization for 16 years in China: a cross-sectional study in 10 cities. Thyroid 2016;26:1125-30.
17. Zimmermann MB, Galetti V. Iodine intake as a risk factor for thyroid cancer: a comprehensive review of animal and human studies.Thyroid Res2015;8:8.
18. McNally RJ, Blakey K, James PW, Gomez Pozo B, Basta NO, et al.Increasing incidence of thyroid cancer in Great Britain, 1976-2005: age-period-cohort analysis. Eur J Epidemiol 2012;27:615-22.
19. Ganly I, Nixon IJ, Wang LY, Palmer FL, Migliacci JC, et al. Survival from differentiated thyroid cancer: what has age got to do with it? Thyroid 2015 25:1106-14.
20. Zaichick V. Medical elementology as a new scientific discipline. J Radioanal Nucl Chem 2006;269:303-9.
21. Beyersmann D, Hartwig A. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol 2008;82:493-512.
22. Martinez-Zamudio R, Ha HC. Environmental epigenetics in metal exposure. Epigenetics2011;6:820-7.

23. Zaichick V, Zaichick S. Age-related changes of Br, Ca, Cl, I, K, Mg, Mn, and Na contents in intact thyroid of females investigated by neutron activation analysis. Curr Updates Aging 2017;1:2-9.
24. Zaichick V, Zaichick S. Age-related changes of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in intact thyroid of males investigated by neutron activation analysis. Curr Trends Biomedical Eng and Biosci 2017;4:555644.
25. Zaichick V, Zaichick S. Age-related changes of trace element contents in intact thyroid of females investigated by neutron activation analysis. J Gerontol Geriatr Med 2017;3:015.
26. Zaichick V, Zaichick S. Age-related changes of some trace element contents in intact thyroid of males investigated by energy dispersive X-ray fluorescent analysis. MOJ Gerontol Ger 2017;1:133-40.
27. Zaichick V, Zaichick S. Age-related changes of Br, Ca, Cl, I, K, Mg, Mn, and Na contents in intact thyroid of males investigated by neutron activation analysis. J Aging Age Relat Dis 2017;1:1002.
28. Zaichick V, Zaichick S. Age-related changes of some trace element contents in intact thyroid of females investigated by energy dispersive X-ray fluorescent analysis. Scientific J Geriatr Med 2017;1:31-8.
29. Zaichick V, Zaichick S. Trace element contents in adenocarcinoma of human prostate investigated by energy dispersive X-ray fluorescent analysis. J Adenocarcinoma 2016;1:1.
30. Zaichick V, Zaichick S. Trace element contents in adenocarcinoma of the human prostate gland investigated byneutron activation analysis. Cancer Res Oncol 2016;1:002.
31. Zaichick V, Zaichick S. The Comparison between the contents and interrelationships of 17 chemical elements in normal and cancerous prostate gland. JPS Open Access 2016;1:105.
32. Zaichick V, Zaichick S. Prostatic tissue levels of 43 trace elements in patients with prostate adenocarcinoma. Cancer Clin Oncol 2016;5:79-94.
33. Zaichick V, Zaichick S. Wynchank S. Intracellular zinc excess as one of the main factors in the etiology of prostate cancer. J Analytical Oncol 2016;5:124-131.
34. Zaichick V. Differences between 66 chemical element contents in normal and cancerous prostate. J Analytical Oncol 2017;6:37-56.
35. Zaichick V, Zaichick S. Instrumental effect on the contamination of biomedical samples in the course of sampling. J Analytical Chemistry 1996;51:1200-5.
36. Zaichick V, Zaichick S. A search for losses of chemical elements during freeze-drying of biological materials. J Radioanal Nucl Chem 1997;218:249-53.
37. Zaichick S, Zaichick V. The effect of age and gender on 37 chemical element contents in scalp hair of healthy humans. Biol Trace Elem Res 2010;134:41-54.
38. Zaichick V, Nosenko S, Moskvina I. The effect of age on 12 chemical element contents in intact prostate of adult men investigated byinductively coupled plasma atomic emission spectrometry. Biol Trace Elem Res 2012;147:49-58.
39. Zaichick V, Zaichick S. NAA-SLR and ICP-AES Application in the assessment of mass fraction of 19 chemical elements in pediatric and young adult prostate glands. Biol Trace Elem Res 2013;156:357-66.
40. Zaichick V, Zaichick S. Determination of trace elements in adults and geriatric prostate combining neutron activation with inductively coupled plasma atomic emission spectrometry. Open J Biochem 2014;1:16-33.
41. Zaichick S, Zaichick V. INAA application in the age dynamics assessment of Br, Ca, Cl, K, Mg, Mn, and Na content in the normal human prostate. J Radioanal Nucl Chem 2011;288:197-202.
42. Zaichick V, Zaichick S. The effect of age on Br, Ca, Cl, K, Mg, Mn, and Na mass fraction in pediatric and young adult prostate glands investigated by neutron activation analysis. Appl Radiat Isot 2013;82:145-51.
43. Zaichick VYe. Applications of synthetic reference materials in the medical radiological research centre. Fresenius J Anal Chem 1995;352:219-23.
44. Korelo AM, Zaichick V. Software to optimize the multielement INAA of medical and environmental samples. In: Nazarov VM, editor. Activation Analysis in Environment Protection. Dubna, Russia: Joint Institute for Nuclear Research; 1993. pp.326-32. (in Russian)
45. Kortev AI, Dontsov GI, Lyascheva AP. Bio-elements in human pathology. Sverdlovsk: Middle-Ural publishing-house; 1972. (in Russian)
46. Kamenev VF. Biological role of trace elements in the human and animal organisms of the East Siberia and the Far East. Ulan-Ude; 1963. pp. 12-6. (in Russian)
47. Tipton IH, Cook MJ. Trace elements in human tissue. Part II. Adult subjects from the United States. Health Phys 1963;9:103-45.
48. Reyitblat MA, Kropachyev AM. Some trace elements in the normal thyroid of Perm Prikam'e inhabitants. Proceedings Perm Medical Institute 1967;78:157-64. (in Russian)
49. Forssen A. Inorganic elements in the human body.I. Occurrence of Ba, Br, Ca, Cd, Cs, Cu, K, Mn, Ni, Sn, Sr, Y and Zn in the human body. Ann Med Exp Biol Fenn 1972;50:99-162.
50. Zhu H, Wang N, Zhang Y, Wu Q, Chen R, et al. Element contents in organs and tissues of Chinese adult men. Health Phys 2010;98:61-73.
51. Salimi J, Moosavi K, Vatankhah S, Yaghoobi A. Investigation of heavy trace elements in neoplastic and non-neoplastic human thyroid tissue: a study by proton - induced X-ray emissions. Iran J Radiat Res 2004;1:211-6.
52. Boulyga SF, Zhuk IV, Lomonosova EM, KievetzMK, DenschlagHO, et al. Determination of microelements in thyroids of the inhabitants of Belarus by neutron activation analysis using the k0-method. J Radioanal Nucl Chem 1997;222:11-4.
53. Reddy SB, Charles MJ, Kumar MR, Reddy BS, Anjaneyulu C, et al. Trace elemental analysis of adenoma and carcinoma thyroid by PIXE method. Nucl Instrum Methods Phys Res B 2002;196:333-9.
54. Woodard HQ, White DR. The composition of body tissues. Br J Radiol 1986;59:1209-18.
55. Ataullakhanov IA. Age changes in the contents of manganese, cobalt, coper, zinc, and iron in the endocrine glands of women. Problemy Endocrinologii 1969;15:98-102. (in Russian)
56. Neimark II, Timoshnikov VM. Development of carcinoma of the thyroid gland in person residing in the focus of goiter endemic. Problemy Endocrinologii 1978;24:28-32. (in Russian)
57. Zabala J, Carrión N, Murillo M, Quintana M, Chirinos J, et al. Determination of normal human intrathyroidal iodine in Caracas population.

J Trace Elem Med Bio 2009;23:9-14.
58. Zakutinskiy DI, Parfeynov UyD, Selivanova LN. Handbook on the toxicology of radioisotopes. Moscow: State Publishing House of Medical Literature; 1962. (in Russian)
59. Remis AM. Proceedings of the second all-Union conference of endocrinologists. Moscow: Nauka; 1962. pp. 330-1. (in Russian)
60. Soman SD, Joseph KT, Raut SJ, Mulay CD, Parameshwaran M, et al. Studies of major and trace element content in human tissues. Health Phys 1970;19:641-56.
61. Teraoka H. Distribution of 24 elements in the internal organs of normal males and the metallic workers in Japan. Arch Environ Health 1981;36:155-65.
62. Boulyga SF, Becker JS, Malenchenko AF, Dietze HJ. Application of ICP-MS for multielement analysis in small sample amounts of pathological thyroid tissue. Microchim Acta 2000;134:215-22.
63. Novikov GV, Vlasova ZA. Biological role of trace elements and their use in agriculture and medicine. Leningrad: Nauka; 1970. pp. 6-7. (in Russian)
64. Bredikhin LM, Soroka VP. Exchange of microelements in goiter patients in the process of surgical treatment. Vrachebnoe Delo 1969;51:81-4. (in Russian)
65. Byrne AR, Kosta L. Vanadium in foods and in human body fluids and tissues. Sci Total Environ 1978;10:17-30.
66. Jundt FC, Purser KH, Kubo H, Schenk EA. Proton-induced X-ray analysis of trace elements in tissue sections. J Histochem Cytochem 1974;22(1):1-6.
67. Vlasova ZA. Biological role of trace elements and their use in agriculture and medicine. Leningrad: Nauka; 1970. pp. 164-5. (in Russian)
68. Maeda K, Yokode Y, Sasa Y, Kusuyama H, Uda M. Multielemental analysis of human thyroid glands using particle induced X-ray emission (PIXE). Nucl Instrum Methods Phys Res B 1987;22:188-90.
69. Al-Sayer H, Mathew TC, Asfar S, Khourshed M, Al-Bader A,, et al. Serum changes in trace elements during thyroid cancers. Mol Cell Biochem 2004;260:1-5.
70. Nishida M, Sakurai H, Tezuka U, Kawada J, Koyama M, et al. Alterations in manganese and iodide contents in human thyroid tumors; a correlation between the contents of essential trace elements and the states of malignancy. Clinica Chimica Acta 1990;187:181-7.
71. Tadros TG, Maisey MN, Ng Tang Fui SC, Turner PC. The iodine concentration in binign and malignant thyroid nodules measured by X-Ray fluorescence. Brit J Radiol 1981;54:626-9.
72. Zagrodzki P, Nicol F, Arthur JR, Słowiaczek M, Walas S, et al. Selenoenzymes, laboratory parameters, and trace elements in different types of thyroid tumor. Biol Trace Elem Res 2010;134:25-40.
73. Kaya G, Avci H, Akdeniz I, Yaman M. Determination of trace and minor metals in benign and malign human thyroid tissues. Asian J Chem 2009;21:5718-26.
74. Yaman M, Akdeniz I. Sensitivity enhancement in flame atomic absorption spectrometry for determination of copper in human thyroid tissues. Anal Sci 2004;20:1363-6.
75. Katoh Y, Sato T, Yamamoto Y. Determination of multielement concentrations in normal human organs from the Japanese. Biol Trace Elem Res2002;90:57-70.
76. Schroeder HA, Tipton IH, Nason AP. Trace metals in man: strontium and barium. J Chron Dis 1972;25:491-517.
77. Zaichick V. Sampling, sample storage and preparation of biomaterials for INAA in clinical medicine, occupational and environmental health. In: IAEA, editor. Harmonization of health-related environmental measurements using nuclear and isotopic techniques. Vienna: IAEA; 1997. pp. 123-33.
78. Zaichick V. Losses of chemical elements in biological samples under the dry aching process. Trace Elements Med 2004;5:17-22. (in Russian)
79. Krewski D, Yokel RA, Nieboer E, Borchelt D, Cohen J, et al. Human health risk assessment for aluminium, aluminium oxide, and aluminium hydroxide. J Toxicol Environ Health B Crit Rev 2007;10 Suppl 1:1-269.
80. Naghii MR, Mofid M, Asgari AR, Hedayati M, Daneshpour MS. Comparative effects of daily and weekly boron supplementation on plasma steroid hormones and proinflammatory cytokines. J Trace Elem Med Biol 2011;25:54-8.
81. Pizzorno L. Nothing boring about boron. Integr Med (Encinitas) 2015;14:35-48.
82. Pavelka S. Radiometric determination of thyrotoxic effects of some xenobiotics. Rad Applic 2016;1:155-8.
83. Maschkovsky MD. The Medicaments.15th ed. Moscow: Novaya Volna; 2005. pp.72-86.
84. Legrand G, Humez S, Slomianny C, Dewailly E, Vanden Abeele F, et al. Ca2+ pools and cell growth. Evidence for sarcoendoplasmic Ca2+-ATPases 2B involvement in human prostate cancer cell growth control. J Biol Chem 2001;276:47608-14.
85. Munaron L. Calcium signalling and control of cell proliferation by tyrosine kinase receptors (review). Int J Mol Med 2002;10:671-6.
86. Capiod T, Shuba Y, Skryma R, Prevarskaya N. Calcium signalling and cancer cell growth. Subcell Biochem2007;45:405-27.
87. Roderick HL, Cook SJ. Ca2+ signalling checkpoints in cancer: remodelling Ca2+ for cancer cell proliferation and survival. Nat Rev Cancer 2008;8:361-75.
88. Flourakis M, Prevarskaya N. Insights into Ca2+ homeostasis of advanced prostate cancer cells. Biochim Biophys Acta2009;1793:1105-9.
89. Yang H, Zhang Q, He J, Lu W. Regulation of calcium signaling in lung cancer. J Thorac Dis 2010;2:52-6.
90. McAndrew D, Grice DM, Peters AA, Davis FM, Stewart T, et al. ORAI1-mediated calcium influx in lactation and in breast cancer. Mol Cancer Ther 2011;10:448-60.
91. Zaichick V, Zaichick S. INAA application in the assessment of chemical element mass fractions in adult and geriatric prostate glands. Appl Radiat Isot 2014;90:62-73.
92. Zaichick V, Zaichick S, Davydov G. Differences between chemical element contents in hyperplastic and nonhyperplastic prostate glands investigated by neutron activation analysis. Biol Trace Elem Res 2015;164:25-35.
93. Zaichick V, Zaichick S. Age-related changes in concentration and histological distribution of Br, Ca, Cl, K, Mg, Mn, and Na in nonhyperplastic prostate of adults. EJBMSR 2016;4:31-48.

94. Zaichick V, Zaichick S, Rossmann M. Intracellular calcium excess as one of the main factors in the etiology of prostate cancer. AIMS Mol Sci 2016;3:635-47.
95. Zaichick V. X-ray fluorescence analysis of bromine for the estimation of extracellular water. Appl Radiat Isot 1998;49:1165-9.
96. Li Y, Trush MA. DNA damage resulting from the oxidation of hydroquinone by copper: role for a Cu(II)/Cu(I) redox cycle and reactive oxygen generation. Carcinogenesis 1993;14:1303-11.
97. Becker TW, Krieger G, Witte I. DNA single and double strand breaks induced by aliphatic and aromatic aldehydes in combination with copper (II). Free Radic Res 1996;24:325-32.
98. Glass GA, Stark AA. Promotion of glutathione-gamma-glutamyl transpeptidase-dependent lipid peroxidation by copper and ceruloplasmin: the requirement for iron and the effects of antioxidants and antioxidant enzymes. Environ Mol Mutagen 1997;29:73-80.
99. Chandra AK, Goswami H, Sengupta P. Effects of magnesium on cytomorphology and enzyme activities in thyroid of rats. Indian J Exp Biol 2014;52:787-92.
100. Jiménez A, Planells E, Aranda P, Sánchez-Viñas M, Llopis J. Changes in bioavailability and tissue distribution of selenium caused by magnesium deficiency in rats. J Am Coll Nutr 1997;16:175-80.
101. Durlach J, Bara M, Guiet-Bara A, Collery P. Relationship between magnesium, cancer and carcinogenic or anticancer metals. Anticancer Res 1986;6:1353-61.
102. Mulay IL, Roy R, Knox BE, Suhr NH, Delaney WE. Trace-metal analysis of cancerous and non-cancerous human tissues. J Natl Cancer Inst 1971;47:1-13.
103. Anghileri LJ, Miller ES, Robinette J, Prasad KN, Lagerborg VA. Calcium metabolism in tumors. II. Calcium, magnesium and phosphorus in human and animal tumors. Oncology 1971;25:193-209.
104. Digiesi V, Bandinelli R, Bisceglie P, Santoro E. Magnesium in tumoral tissues, in the muscle and serum of subjects suffering from neoplasia. Biochem Med 1983;29:360-3.
105. Szmeja Z, Kończewska H. Red blood cell, serum and tissue magnesium levels in subjects with laryngeal carcinoma. ORL J Otorhinolaryngol Relat Spec 1983;45:102-7.
106. Ranade SS, Panday VK. Major metals in human cancer: calcium, magnesium, sodium and potassium. Sci Total Environm 1985;41:79-89.
107. Taylor JS, Vigneron DB, Murphy-Boesch J, Nelson SJ, Kessler HB, et al. Free magnesium levels in normal human brain and brain tumors: 31P chemical-shift imaging measurements at 1.5 T. Proc Natl Acad Sci USA 1991;88:6810-4.
108. Seltzer MH, Rosato FE, Fletcher MJ. Serum and tissue magnesium levels in human breast carcinoma. J Surg Res 1970;10:159-62.
109. Wolf FI, Cittadini AR, Maier JA. Magnesium and tumors: Ally or foe?Cancer Treat Rev 2009;35:378-82.
110. Soldin OP, Aschner M. Effects of manganese on thyroid hormone homeostasis: potential links. Neurotoxicology 2007;28:951-6.
111. Aschner JL, Aschner M. Nutritional aspects of manganese homeostasis.Mol Aspects Med2005;26:353-62.
112. Hasegawa S, Koshikawa M, Takahashi I, Hachiya M, Furukawa T, et al. Alterations in manganese, copper, and zinc contents, and intracellular status of the metal-containing superoxide dismutase in human mesothelioma cells. J Trace Elem Med Biol 2008;22:248-55.
113. Trump BF, Berezesky IK, Phelps PC. Sodium and calcium regulation and the role of the cytoskeleton in the pathogenesis of disease: a review and hypothesis.Scan Electron Microsc 1981;(Pt 2):434-54.
114. Ranade SS, Panday VK. Major metals in human cancer: calcium, magnesium, sodium and potassium.Sci Total Environ 1985;41:79-89.
115. Romeu A, Arola L, Alemany M. Essential metals in tissues and tumor of inbred C57BL/6 mice during the infective cycle of Lewis lung carcinoma.Cancer Biochem Biophys 1986;9:53-66.
116. Ouwerkerk R, Jacobs MA, Macura KJ, Wolff AC, Stearns V, et al. Elevated tissue sodium concentration in malignant breast lesions detected with non-invasive 23Na MRI.Breast Cancer Res Treat 2007;106:151-60.
117. Kowalska J, Gajda M, Kwiatek WM, Franczyk-Zarów M, Kostogrys RB, et al. Chemical composition of atherosclerotic plaques of apoE/LDLR-double knockout mice by synchrotron radiation FTIR microspectroscopy. Acta Physica Polonica 2012;121:555-60.
118. Townsend DM, Tew KD, Tapiero H. Sulfur containing amino acids and human disease. Biomed Pharmacother 2004;58:47-55.
119. Atmaca G. Antioxidant effects of sulfur-containing amino acids. Yonsei Med J 2004;45:776-88.