Symbiosis

www.symbiosisonline.org
www.symbiosisonlinepublishing.com

Research Article International Journal of Hematology and Blood Disorders Open Access

Twenty Chemical Element Contents in Normal and Cancerous Thyroid

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

[1]*Radionuclide Diagnostics Department, Medical Radiological Research Centre, Russia*

[2]*Laboratory of Dr. Gabriela Caraveo Piso, Feinberg School of Medicine, Northwestern University, USA*

Received: August 8, 2018; Accepted: August 17, 2018; Published: August 22, 2018

***Corresponding author:** V Zaichick, Professor of Radionuclide Diagnostics Department, Medical Radiological Research Centre, Russia, Tel: +7 (48439) 60289; Fax: +7 (495) 956 1440; E-mail: vezai@obninsk.com*

Abstract

Thyroid cancer is an internationally important health problem. The aim of this exploratory study was to evaluate whether significant changes in the thyroid tissue levels of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn exist in the malignantly transformed thyroid. 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 non-destructive energy-dispersive X-Ray fluorescent analysis combined with instrumental neutron activation analysis with high resolution spectrometry of short– and long-lived radionuclides. Tissue samples were divided into two portions. One was used for morphological study while the other was intended for chemical element analysis. It was found that contents of Ag, Br, Cl, Co, Cr, Cu, Hg, K, Mg, Na, and Rb were significantly higher (approximately 12.8, 9.3, 2.3, 1.4, 1.6, 3.4, 19.6, 1.6, 1.6, 1.3, and 1.5 times, respectively) while content of I lower (nearly 26 times) in cancerous tissues than in normal tissues. In our opinion, the increase in levels of Ag, Br, Cl, Co, Cr, Cu, Hg, K, Mg, Na, and Rb, as well as the decrease in levels of I in cancerous tissue might demonstrate an involvement of these elements in etiology and pathogenesis of malignant thyroid tumors. It was supposed that the changes in levels Ag, Br, Cl, Co, Cr, Cu, Hg, I, K, Mg, Na, and Rb in thyroid tissue can be used as tumor markers.

Keywords: Thyroid malignant tumors; Intact thyroid; Chemical elements; Energy-dispersive X-Ray fluorescent analysis; Instrumental neutron activation analysis

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 for the last years [5].

Although the etiology of TC is unknown, several risk factors including deficiency or excess of such micronutrient as iodine (I) have been well identified [6-17]. It was also reported that 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 showed in the study of 3664 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 chemical 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 chemical elements depend on tissue-specific need or tolerance, respectively [20]. Excessive accumulation or an imbalance of the chemical 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 zinc (Zn) concentration are 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 elements also play a role in the pathophysiology of the thyroid.

This work had two aims. The first was to assess the silver (Ag), bromine (Br), calcium (Ca), chlorine (Cl), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), I, potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), strontium (Sr), and Zn mass fraction contents in TC tissue using three non-destructive instrumental analytical methods: energy dispersive X-ray fluorescent (EDXRF), neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR), and neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR). 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.

Material and Methods

All patients suffered from TC (n=41, mean age M±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 trace element contents. 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. 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].

For EDXRF the pounded sample weighing about 8 mg was applied to the piece of Scotch tape serving as an adhesive fixing backing. The content of Br, Cu, Fe, Rb, Sr, and Zn were determined by EDXRF. Details of the relevant facility for this method, source with 109Cd radionuclide, methods of analysis and the results of quality control were presented in our earlier publications concerning the EDXRF analysis of human thyroid and prostate tissue [26, 28, 37-39].

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 WWR-c research nuclear reactor (Branch of Karpov Institute, Obninsk). Details of used neutron flux, nuclear reactions, radionuclides, gamma-energies, and spectrometric unit were presented in our earlier publications concerning the INAA-SLR chemical element contents in human thyroid, scalp hair, and prostate [7, 23, 27, 40]

In a few days after INAA-SLR all thyroid samples were repacked separately in a high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule and used for INAA-LLR. A vertical channel of the WWR-c research nuclear reactor (Branch of Karpov Institute, Obninsk).was applied to determine the content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn by INAA-LLR. Details of used neutron flux, nuclear reactions, radionuclides, gamma-energies, and spectrometric unit were presented in our earlier publications concerning the INAA-LLR chemical element contents in human thyroid, scalp hair, and prostate [24, 25, 40, 41]

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [42]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. For each method ten certified reference material IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) sub-samples were treated and analyzed in the same conditions that thyroid samples to estimate the precision and accuracy of results.

A dedicated computer program for INAA mode optimization was used [43]. 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, and 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.

Results

Table 1 depicts our data for Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction mass fractions in ten sub-samples of IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) certified reference material and the certified values of this material.

The comparison of our results for the Br, Fe, Rb, and Zn mass fractions (mg/kg, dry mass basis) in the normal human thyroid obtained by both EDXRF and INAA methods is shown in Table 2.

Table 3 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 Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction mass fraction in normal and cancerous thyroid tissue.

The comparison of our results with published data for Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction in normal and cancerous thyroid [44-69] is shown in Table 4.

The ratios of means and the difference between mean values of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fractions in normal and cancerous thyroid are presented in Table 5.

Table1: EDXRF, INAA-SLR and INAA-LLR data of chemical element contents in certified reference material IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) compared to certified values (mg/kg, dry mass basis)

Element	IAEA H-4 animal muscle	This work results	IAEA HH-1 human hair	This work results
Ag	-	0.033±0.008	0.19±0.06[b]	0.18±0.05
Br	4.1±1.1[a]	5.0±09	4.2±2.1[b]	3.9±1.6
Ca	188±58[b]	238±59	522±160[a]	525±42
Cl	1890±130[b]	1950±230	2265±478[a]	2210±340
Co	0.0027±0.0010[b]	0.0034±0.0008	5.97±0.42[a]	5.4±1.1
Cr	0.06±0.04[b]	0.071±0.010	0.27±0.16[b]	≤0.3
Cu	4.0±1.0[a]	3.9±1.1	10.2±3.2[a]	-
Fe	49.1±6.5[a]	47.0±1.0	23.7±3.1[a]	25.1±4.3
Hg	0.014±0.005[b]	0.015±0.004	1.70±0.09[a]	1.54±0.14
I	0.08±0.10[b]	<1.0	20.3±8.9[b]	19.1±6.2
K	15840±1440[a]	16200±3800	9.2±5.2[b]	10.7±4.0
Mg	1050±140[a]	1100±190	62.0±9.6[b]	64.7±18.6
Mn	0.52±0.08[a]	0.55±0.11	0.85±0.25[a]	0.93±0.16
Na	2060±330[a]	2190±140	12.6±4.8[b]	14.0±2.7
Rb	18.7±3.5[a]	22±4	0.94±0.09[b]	0.89±0.17
Sb	0.0056±0.0031[b]	0.0061±0.0021	0.031±0.010[b]	0.033±0.009
Sc	0.0059±0.0034[b]	0.0015±0.0009	-	-
Se	0.28±0.08[a]	0.281±0.014	0.35±0.02[a]	0.37±0.08
Sr	-	<1	0.82±0.16[b]	1.24±0.57
Zn	86.3±11.5[a]	91±2	174±9[a]	173±17

M – arithmetical mean, SD – standard deviation, a – certified values, b – information values.

Table 2: Comparison of the mean values (M±SEM) of the chemical element mass fractions (mg/kg, dry mass basis) in the normal human thyroid obtained by both EDXRF and INAA methods

Element	EDXRF (1)	INAA (2)	Δ=[(M1 – M2)/M1] ·100%
Br	13.8±1.3	16.3±1.3 (INAA-SLR)	-18
Fe	222±11	225±11 (INAA-LLR)	-1.4
Rb	9.0±0.7	7.4±0.4 (INAA-LLR)	18
Zn	112±5	98±5 (INAA-LLR)	12.5

M – Arithmetic mean, SEM – standard error of mean.

Table 3: Some statistical parameters of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction (mg/kg, dry mass basis) in normal and cancerous thyroid

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Normal n=105	Ag	0.0151	0.0140	0.0016	0.0012	0.0800	0.0121	0.0017	0.0454
	Br	14.9	11	1.2	1.9	54.1	11.6	2.56	49.3
	Ca	1711	1022	109	414	6230	1458	460	3805
	Cl	3400	1452	174	1030	6000	3470	1244	5869
	Co	0.0399	0.0271	0.003	0.0046	0.14	0.0327	0.0134	0.124
	Cr	0.539	0.272	0.032	0.13	1.3	0.477	0.158	1.08
	Cu	4.23	1.52	0.18	0.5	7.5	4.15	1.57	7.27
	Fe	223	93	10	51	512	221	74.2	433
	Hg	0.0421	0.0358	0.0041	0.0065	0.18	0.0304	0.0091	0.15
	I	1841	1027	107	114	5061	1695	230	4232
	K	6071	2773	306	1740	14300	5477	2541	13285
	Mg	285	139	17	66	930	271	81.6	541
	Mn	1.35	0.54	0.07	0.51	4.18	1.32	0.537	2.23
	Na	6702	1764	178	3050	13453	6690	3855	10709
	Rb	8.16	4.55	0.49	1.66	29.4	7.37	3.08	19.3
	Sb	0.111	0.072	0.008	0.0047	0.308	0.103	0.0117	0.28
	Sc	0.0046	0.0038	0.0008	0.0002	0.0143	0.0042	0.00035	0.0131
	Se	2.32	1.29	0.14	0.439	5.8	2.01	0.775	5.65
	Sr	4.55	3.22	0.37	0.1	13.7	3.7	0.483	12.3
	Zn	105.1	40.1	4.3	7.1	221	104.9	39.2	186
Cancer n=41	Ag	0.193	0.215	0.041	0.0075	1.02	0.147	0.008	0.705
	Br	139	203	36	6.2	802	50.2	7.75	802
	Ca	2397	2368	558	452	8309	1302	467	7428
	Cl	7699	2900	703	4214	14761	7216	4240	13619
	Co	0.055	0.0309	0.006	0.0042	0.143	0.0497	0.0159	0.129
	Cr	0.835	0.859	0.157	0.039	3.5	0.46	0.0941	3.05
	Cu	14.5	9.4	2.6	4	32.6	10.9	4.21	31.4
	Fe	243	177	29	55.1	887	200	58.2	679
	Hg	0.824	0.844	0.149	0.0685	3.75	0.475	0.0689	2.85
	I	71.8	62	10	2	261	62.1	2.93	192
	K	9655	4444	970	1660	19225	8746	3381	19035
	Mg	450	232	51	122	1033	408	126	931
	Mn	1.9	1.41	0.32	0.1	5.79	1.59	0.1	5.37
	Na	8556	2959	646	4083	17284	7264	4704	14543
	Rb	12.6	4.6	0.7	5.5	27.4	11.2	5.84	19.8
	Sb	0.124	0.081	0.015	0.016	0.381	0.108	0.0174	0.315
	Sc	0.0077	0.0129	0.002	0.0002	0.0565	0.0023	0.0002	0.0447
	Se	2.04	1.02	0.18	0.143	4.7	1.8	0.663	4.33
	Sr	6.25	7.83	1.63	0.93	30.8	3	0.985	25
	Zn	89.7	57.6	10.8	36.7	326	67.7	37.7	324

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.

Table 4: Median, minimum and maximum value of means Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, 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)***	**Minimum of means M or M±SD,(n)****	**Maximum of Means M or M±SD,(n)****	**Males and females M±SD**
Normal				
Ag	0.25 (12)	0.000784 (16) [44]	1.20±1.24 (105) [45]	0.0151±0.0140
Br	18.1 (11)	5.12 (44) [44]	284±44 (14) [46]	14.9±10.9
Ca	1600 (17)	840±240 (10) [47]	3800±320 (29) [47]	1692±1022
Cl	6800 (5)	804±80 (4) [48]	8000 (-) [49]	3400±1452
Co	0.336 (17)	0.026±0.031 (46) [50]	70.4±40.8 (14) [46]	0.0399±0.0271
Cr	0.69 (17)	0.105 (18) [51]	24.8±2.4 (4) [48]	0.539±0.272
Cu	6.1 (57)	1.42 (120) [52]	220±22 (10) [48]	4.23±1.52
Fe	252 (21)	56 (120) [52]	2444±700 (14) [46]	223±93
Hg	0.08 (13)	0.0008±0.0002 (10) [47]	396±40 (4) [48]	0.0421±0.0358
I	1888 (95)	159±8 (23) [53]	5772±2708 (50) [54]	1841±1027
K	4400 (17)	46.4±4.8 (4) [48]	6090 (17) [55]	6071±2773
Mg	390 (16)	3.5 (-) [56]	840±400 (14) [57]	285±139
Mn	1.82 (36)	0.44±11 (12) [58]	69.2±7.2 (4) [48]	1.35±0.58
Na	8000 (9)	438 (-) [59]	10000±5000 (11) [57]	6702±1764
Rb	12.3 (9)	≤0.85 (29) [47]	294±191 (14) [46]	8.20±4.54
Sb	0.105 (10)	0.040±0.003 (-) [59]	4.0 (-) [60]	0.111±0.072
Sc	0.009 (4)	0.0018±0.0003 (17) [61]	0.014±0.005 (10) [47]	0.0046±0.0038
Se	2.61 (17)	0.95±0.08 (29) [47]	756±680 (14) [47]	2.32±1.29
Sr	0.73 (9)	0.55±0.26 (21) [51]	46.8±4.8 (4) [48]	4.55±3.22
Zn	118 (51)	32 (120) [52]	820±204 (14) [46]	105±40
Cancerous				
Ag	-	-	-	0.193±0.215
Br	15.7 (4)	9.6 (1) [62]	160±112 (3) [46]	139±203
Ca	1572 (6)	390 (1) [63]	3544 (1) [62]	2397±2368
Cl	940 (1)	940±92 (4) [48]	940±92 (4) [48]	7699±2900
Co	71.6 (3)	2.48±0.85 (18) [53]	94.4±69.6 (3) [46]	0.0550±0.0309
Cr	2.74 (2)	1.04±0.52 (4) [64]	119±12 (4) [48]	0.835±0.839
Cu	6.8 (11)	4.7±1.8 (22) [65]	51.6±5.2 (4) [48]	14.5±9.4
Fe	316 (8)	69±51 (3) [63]	5588±556 (4) [48]	243±177
Hg	30.8 (1)	30.8±3.2 (4) [48]	30.8±3.2 (4) [48]	0.824±0.844
I	78.8 (12)	<23±10 (8) [66]	800 (1) [67]	71.8±62.0
K	6878 (4)	636±64 (4) [49]	7900 (1) [64]	9655±4444
Mg	320 (2)	316±84 (45) [65]	544±272 (6) [68]	450±232
Mn	1.83 (4)	1.6±0.8 (22) [65]	186±18 (4) [48]	1.90±1.41
Na	-	-	-	8556±2959
Rb	14.7 (2)	11,5 (10) [61]	17.8±9.7 (5) [61]	12.6±4.6
Sb	-	-	-	0.124±0.081

Sc	-	-	-	0.0077±0.0129
Se	2.16 (7)	1.00±0.24 (3) [64]	241±296 (3) [46]	2.04±1.02
Sr	-	-	-	6.25±7.83
Zn	112 (13)	48±8 (5) [69]	494±37 (2) [64]	89.7±57.6
M –arithmetic mean, SD – standard deviation, (n)* – number of all references, (n)** – number of samples.				

Table 5: Differences between mean values (M±SEM) of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, 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
Ag	0.0151±0.0016	0.193±0.041	**0.00022**	**≤0.01**	12.8
Br	14.9±1.2	139±36	**0.0017**	**≤0.01**	9.33
Ca	1711±109	2397±558	0.243	>0.05	1.4
Cl	3400±174	7699±703	**0.000013**	**≤0.01**	2.26
Co	0.0399±0.0030	0.0550±0.0060	**0.022**	**≤0.01**	1.38
Cr	0.539±0.032	0.835±0.157	0.073	**≤0.05**	1.55
Cu	4.23±0.18	14.5±2.6	**0.0019**	**≤0.01**	3.43
Fe	223±10	243±29	0.519	>0.05	1.09
Hg	0.0421±0.0041	0.824±0.149	**0.000011**	**≤0.01**	19.6
I	1841±107	71.8±10.0	**0.00000000001**	**≤0.01**	0.039
K	6071±306	9655±970	**0.0017**	**≤0.01**	1.59
Mg	285±17	450±51	**0.0047**	**≤0.01**	1.58
Mn	1.35±0.07	1.90±0.32	0.107	>0.05	1.41
Na	6702±178	8556±646	**0.011**	**≤0.01**	1.28
Rb	8.16±0.49	12.6±0.7	**0.0000029**	**≤0.01**	1.54
Sb	0.111±0.008	0.124±0.015	0.423	>0.05	1.12
Sc	0.0046±0.0008	0.0077±0.0020	0.223	>0.05	1.67
Se	2.32±0.14	2.04±0.18	0.235	>0.05	0.88
Sr	4.55±0.37	6.25±1.63	0.319	>0.05	1.37
Zn	105.1±4.3	89.7±10.8	0.191	>0.05	0.85
M – arithmetic mean, SEM – standard error of mean, Statistically significant values are in **bold.**					

Discussion

Precision and accuracy of results

A good agreement of our results for the Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fractions with the certified values of CRM IAEA H-4 and CRM IAEA HH-1 (Table 1) as well as the similarity of the means of the Br, Fe, Rb, and Zn mass fractions in the normal human thyroid determined by both EDXRF and INAA methods (Table 2) demonstrates an acceptable precision and accuracy of the results obtained in the study and presented in Tables 3-5.

The mean values and all selected statistical parameters were calculated for twenty chemical elements (Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn) mass fractions (Table 3). The mass fraction of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn were measured in all, or a major portion of normal and cancerous tissue samples.

Comparison with published data

Values obtained for Br, Ca, Cl, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, and Zn contents in the normal human thyroid (Table 4) agree well with median of mean values reported by other researches [41-55]. The obtained means for Ag and Co were almost one order of magnitude lower whereas mean for Sr was almost one order of magnitude higher median of previously reported means but inside the range of means (Table 3). Data cited in Table 3 also includes samples obtained from patients

who died from different non-endocrine diseases. A number of values for trace element mass fractions were not expressed on a dry mass basis by the authors of the cited references. However, we calculated these values using published data for water (75%) [50] And ash (4.16% on dry mass basis) [70] contents in thyroid of adults.

In cancerous tissues (Table 4) our results were comparable with published data for Ca, Cu, Fe, I, Mg, Mn, Rb, Se, and Zn contents. The obtained means for Co, Hg, and Cr were approximately three, two, and one, respectively, order of magnitude lower median of previously reported means and inside the range of these means (Table 4). The obtained mean for Cl was almost one order of magnitude higher the only reported result and mean for K was some higher median of previously reported means and also higher the upper level of the range of these means (Table 4). No published data referring Ag, Na, Sb, Sc, and Sr contents of cancerous thyroid tissue were found.

The range of means of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn level reported in the literature for normal and for untreated cancerous thyroid vary widely (Table 4). This can be explained by a dependence of trace 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 and, in our opinion, leading cause 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 use of these methods some quantities of certain trace elements are lost as a result of this treatment That concern not only such volatile halogen as Br, but also other trace elements investigated in the study [71-73].

Effect of malignant transformation on chemical element contents

From Table 5, it is observed that in cancerous tissue the mass fraction of Ag, Br, Cl, Cu, and Hg are approximately 13, 9, 2, 3, and 20 times, respectively, higher and also mass fractions of Co, Cr, K, Mg, Na, and Rb are almost in 38%, 55%, 59%, 58%, 28%, and 54%, 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 Ag, Br, Cl, Co, Cr, Cu, Hg, K, Mg, Na, and Rb in thyroid tissue significantly increased whereas the levels of I drastically decrease.

Role of trace 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 thyroid cancer. 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 and cause, and also effect of malignant transformation. From the results of such kind 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.

Silver

Ag is a chemical element with no recognized trace metal value in the human body [74]. Ag in metal form and inorganic Ag compounds ionize in the presence of water, body fluids or tissue exudates. The silver ion Ag+ is biologically active and readily interacts with proteins, amino acid residues, free anions and receptors on mammalian and eukaryotic cell membranes [75]. Besides such the adverse effects of chronic exposure to Ag as a permanent bluish-gray discoloration of the skin (argyria) or eyes (argyrosis), exposure to soluble Ag compounds may produce other toxic effects, including liver and kidney damage, irritation of the eyes, skin, respiratory, and intestinal tract, and changes in blood cells [76]. More detailed knowledge of the Ag toxicity can lead to a better understanding of the impact on human health, including thyroid function. Anyway, a drastically elevated level of Ag in malignant thyroid tumors could possibly be explored for diagnosis of TC.

Bromine

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 [77].

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, supports this conclusion. But, on the other hand, bromide compounds, especially potassium bromide (KBr), sodium bromide (NaBr), and ammonium bromide (NH4Br), are frequently used as sedatives in Russia [78]. 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.

Chlorine

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 [79]. Cancerous tissues are predominantly highly vascularized lesions [80-85]. 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.

Cobalt

Health effects of high Co occupational, environmental, dietary and medical exposure are characterized by a complex clinical syndrome, mainly including neurological, cardiovascular and endocrine deficits, including hypothyroidism [86,87]. Co is genotoxic and carcinogenic, mainly caused by oxidative DNA damage by reactive oxygen species, perhaps combined with inhibition of DNA repair [88]. In our previous studies it was found a significant age-related increase of Co content in female thyroid [25]. Therefore, a goitrogenic and, probably, carcinogenic effect of excessive Co level in the thyroid of old females was assumed. Elevated level of Coin TC tissues, observed in the present study, supports this conclusion. Anyway, the accumulation of Co in malignant thyroid tumors could possibly be explored for diagnosis of TC.

Chromium

Cr-compounds are cytotoxic, genotoxic, and carcinogenic in nature. Some Cr forms, including hexavalent chromium (Cr6+), are toxicants known for their carcinogenic effect in humans. They have been classified as certain or probable carcinogens by the International Agency for Research on Cancer [89]. The lung cancer risk is prevalent in pigment chromate handlers, ferrochromium production workers, stainless steel welders, and chrome-platers [90]. Except in Cr-related industries and associated environments, Cr intoxication from environmental exposure is not common. However, it was found, that drinking water supplies in many geographic areas contain chromium in the +3 and +6 oxidation states. Exposure of animals to Cr6+ in drinking water induced tumors in the mouse small intestine [91]. Many other animal experiments and in vitro studies demonstrate also that Cr can induce oxidative stress and exert cytotoxic effects [92]. Besides reactive oxygen species (ROS) generation, oxidative stress, and cytotoxic effects of Cr exposure, a variety of other changes like DNA damage, increased formation of DNA adducts and DNA-protein cross-links, DNA strand breaks, chromosomal aberrations and instability, disruption of mitotic cell division, chromosomal aberration, premature cell division, S or G2/M cell cycle phase arrest, and carcinogenesis also occur in humans or experimental test systems [90]. Anyway, the accumulation of Cr in malignant thyroid tumors could possibly be explored for diagnosis of TC.

Copper

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 superoxide dismutase, lysyl oxidase, peptidylglycineamidating monoxygenase, Cu/Zn superoxide dismutase, 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 per oxidation, thiol oxidation, and, ultimately, DNA damage [93-95]. 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 Cu exact role in the thyroid malignant tumors etiology and metabolism. Anyway, the accumulation of Cu in neoplastic thyroids could possibly be explored for diagnosis of TC.

Mercury

Hg is one of the most dangerous environmental pollutants [96]. The growing use of this metal in diverse areas of industry has resulted in a significant increase of environment contamination and episodes of human intoxication. Hg damages the central nervous system and has irreparable effects on the kidneys [97]. Hg may also harm a developing fetus and decrease fertility in men and women [98]. Besides these effects, Hg has been classified as certain or probable carcinogen by the International Agency for Research on Cancer [89]. For example, in Hg polluted area thyroid cancer incidence was almost 2 times higher than in adjacent control areas [99].

Negative effects of Hg are due to the interference of this metal in cellular signaling pathways and protein synthesis during the period of development. Since it bonds chemically with the sulfur hydride groups of proteins, it causes damage to the cell membrane and decreases the amount of RNA [100]. Moreover, it was shown that Hg may be involved in four main processes that lead to genotoxicity: generation of free radicals and oxidative stress, action on microtubules, influence on DNA repair mechanisms and direct interaction with DNA molecules [101]. Anyway, a drastically elevated level of Hg in malignant thyroid tumors could possibly be explored for diagnosis of TC.

Iodine

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.

Potassium

An uncontrollable cell proliferation characterize 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 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.

Magnesium

Mg is abundant in the human body. This element is essential for the functions of more than 300 enzymes (e.g. alkaline phosphatase, 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 [102]. Experimental data have shown that high doses of magnesium increase the activity of the thyroid gland [103]. Magnesium deficiency can influence bioavailability and tissue distribution of selenium which then appears diminished [104]. From these data, one can conclude that Mg is involved in the thyroid function. If so, significant reduction in Mg content cam 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 have an usually higher Mg levels than do normal tissues [105-110], possibly caused by the "retention" of Mg by the tumor [111], 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 [112]. Thus, the elevated levels of Mg in neoplastic thyroids could possibly be explored for diagnosis of TC.

Sodium

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, all 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 associated with proliferating malignant tumors may cause an increase in total tissue sodium concentration was tested with non-invasive, quantitative 23Na magnetic resonance imaging in patients with benign and malignant breast tumors. It was shown that elevated Na concentrations in breast lesions appears 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 link with a high tumor vascularization (see Chlorine). Anyway, it seems that the accumulation of Na is a generic property of malignant tumors.

Rubidium

As for Rb, there is very little information about its effects in organisms. No negative environmental effects have been reported. Rb is only slightly toxic on an acute toxicological basis and would pose an acute health hazard only when ingested in large quantities [117]. Rb has some function in immune responce [118], probably by supporting cell differentiation [119]. Potassium (K) and Rb are in the first group of the periodic table. Rb, like K, seems to be concentrated in the intracellular space and transfered through membrane by the Na+K+-ATPase pump. An overload of Rb could modulate proliferative responses of the cell, as was shown for bone marrow leukocytes [78]. In our previous studies it was found a significant age-related increase of Rb content in female thyroid [25, 28]. Therefore, a goitrogenic and, probably, carcinogenic effect of excessive Rb level in the thyroid of old females was assumed. Elevated level of Rb in TC tissues, observed in the present study, supports this conclusion. Anyway, the accumulation of Rb in malignant thyroid tumors could possibly be explored for diagnosis of TC.

Our findings show that mass fraction of Ag, Br, Cl, Co, Cr, Cu, Hg, I, K, Mg, Na, and Rb are significantly different in TC as compared to normal thyroid tissues (Tables 5). Thus, it is plausible to assume that levels of these chemical elements in thyroid tissue can be used as tumor markers. However, this subjects needs in additional studies.

Limitations

This study has several limitations. Firstly, analytical techniques employed in this study measure only twenty element (Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, 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 was 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, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on cancer-specific tissue Ag, Br, Cl, Co, Cr, Cu, Hg, I, K, Mg, Na, and Rb level alteration and shows the necessity the need to continue chemical element research of malignant thyroid tumors.

Conclusion

In this work, chemical elemental measurements were carried out in the tissue samples of normal thyroid and malignant tumors of thyroid using three non-destructive instrumental analytical methods: EDXRF, INAA-SLR, and INAA-LLR. It was shown that the combination of these methods is an adequate analytical tool for the non-destructive determination of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn content in the tissue samples of human thyroid, including needle-biopsy cores.

It was observed that in cancerous tissues content of Ag, Br, Cl, Co, Cr, Cu, Hg, K, Mg, Na, and Rb significantly increased whereas the levels of I drastically decrease in a comparison with the normal thyroid tissues. In our opinion, the increase in levels of Ag, Br, Cl, Co, Cr, Cu, Hg, K, Mg, Na, and Rb, as well as the decrease in levels of I in cancerous tissue might demonstrate an involvement of these elements in etiology and pathogenesis of malignant thyroid tumors. It was supposed that the changes in levels Ag, Br, Cl, Co, Cr, Cu, Hg, I, K, Mg, Na, and Rb in thyroid tissue can be used as tumor markers.

Acknowledgements

The authors are extremely grateful to Profs. B.M. Vtyurin and V.S. Medvedev, Medical Radiological Research Center, Obninsk, as well as to Dr. Yu. Choporov, Head of the Forensic Medicine Department of City Hospital, Obninsk, for supplying thyroid samples.

Ethical Approval

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.

Contributions

This work was carried out in collaboration between two authors. Author VZ collected thyroid samples, designed the EDXRF and INAA of samples, and carried out the statistical analysis of results. Author SZ managed the literature searches, wrote the first draft of the manuscript, and translated the manuscript into English. All authors read and approved the final manuscript.

References

1. Kilfoy BA, Zheng T, Holford TR, Han X, Ward MH, Sjodin A, Zhang Y, et al. International patterns and trends in thyroid cancer incidence, 1973-2002. CCC. 2009;20(5):525-531. doi: 10.1007/s10552-008-9260-4
2. Jemal RSA, Xu J, Ward E . Cancer statistics, 2010. Cancer J Clin. 2010;60(5):277-300. doi: 10.3322/caac.20073
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. 2013:10.
4. Wiltshire JJ, Drake TM, Uttley L, Balasubramanian SP. Systematic review of trends in the incidence rates of thyroid cancer. Thyroid. 2016;26(11):1541-1552.
5. Jung K, Won Y, Kong H, Oh C, Lee DH, Lee JS. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2011. Cancer Res Treat. 2014;46(2):109-123. doi: 10.4143/crt.2014.46.2.109
6. Zaichick V, Tsyb A, Vtyurin BM. Trace elements and thyroid cancer. Analyst. 1995;120(3):817-821.
7. Zaichick V, Choporov Yu. Determination of the natural level of human intra-thyroid iodine by instrumental neutron activation analysis. J Radioanal Nucl Chem. 1996;207(1):153-161.
8. Zaichick V, Zaichick S. Normal human intrathyroidal iodine. Sci Total Environ. 1997;206(1):39-56.
9. Zaichick V. Iodine excess and thyroid cancer. J Trace Elem Exp Med. 1998;11(4):508-509.
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(4):509-510.
11. Zaichick V, Iljina T. Dietary iodine supplementation effect on the rat thyroid I blastomogenic action. In: Anke M, editors. Die Bedentung der Mengen- und Spurenelemente 18 Arbeitstangung Jena: Friedrich-Schiller-Universität. 1998:294-306.
12. Zaichick V, Zaichick S. Energy-dispersive X-ray fluorescence of iodine in thyroid puncture biopsy specimens. J Trace Microprobe Tech. 1999;17(2):219-232.
13. Zaichick V. Human intrathyroidal iodine in health and non-thyroidal disease. In: New aspects of trace element research. London and Tokyo: Smith-Gordon and Nishimura. 1999:114-119.
14. Zaichick V. Relevance of, and potentiality for in vivo intrathyroidal iodine determination. In Vivo Body Composition Studies. Ann N-Y Acad Sci. 2000;904(1):630-631.
15. Cho BY, Choi HS, Park YJ, Lim JA, Ahn HY, Lee EK, Kim KW,et al. Changes in the clinicopathological characteristics and outcomes of thyroid cancer in Korea over the past four decades. Thyroid. 2013;23(7):797-804. doi: 10.1089/thy.2012.0329
16. Shan Z, Chen L, Lian X, Liu C, Shi B, Shi L, Tong N, 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(8):1125-1130. doi: 10.1089/thy.2015.0613
17. Zimmermann MB, Galetti V. Iodine intake as a risk factor for thyroid cancer: a comprehensive review of animal and human studies. Thyroid Res. 2015;8:8. doi: 10.1186/s13044-015-0020-8
18. McNally RJ, Blakey K, James PW, Gomez Pozo B, Basta NO, Hale J. Increasing incidence of thyroid cancer in Great Britain, 1976-2005: age-period-cohort analysis. Eur J Epidemiol. 2012;27(8):615-622. doi: 10.1007/s10654-012-9710-x
19. Ganly I, Nixon IJ, Wang LY, Palmer FL, Migliacci JC, Aniss A, Sywak M, et al. Survival from differentiated thyroid cancer: What has age got to do with it?. Thyroid. 2015;25(10):1106-1114.
20. Zaichick V. Medical elementology as a new scientific discipline. J Radioanal Nucl Chem. 2006;269(2):303-309.
21. Beyersmann D, Hartwig A. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol. 2008;82(8):493-512. doi: 10.1007/s00204-008-0313-y
22. Martinez-Zamudio R, Ha HC. Environmental epigenetics in metal exposure. Epigenetics. 2011;6(7):820-827. doi: 10.4161/epi.6.7.16250
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(1).
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(4):555-644.

25. Zaichick V, Zaichick S. Age-related changes of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn 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(5):00028.
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(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. Trends Geriatr Healthc. 2017;1(1):31-38.
29. Zaichick V, Zaichick S. Trace element contents in adenocarcinoma of human prostate investigated by energy dispersive X-ray fluorescent analysis. Journal of Adenocarcinoma. 2016;1(1):1-7. doi:10.21767/2572-309X.10001
30. Zaichick V, Zaichick S. Trace element contents in adenocarcinoma of the human prostate gland investigated by neutron activation analysis. Cancer Research and Oncology. 2016;1(1):1-10.
31. Zaichick V, Zaichick S. The Comparison between the contents and interrelationships of 17 chemical elements in normal and cancerous prostate gland. Journal of Prostate Cancer. 2016;1(1):105.
32. Zaichick V, Zaichick S. Prostatic tissue levels of 43 trace elements in patients with prostate adenocarcinoma. Cancer and Clinical Oncology. 2016;5(1):79-94.
33. Zaichick V, Zaichick S. Wynchank S. Intracellular zinc excess as one of the main factors in the etiology of prostate cancer. Journal of Analytical Oncology. 2016;5(3):124-131. doi: 10.6000 / 1927-7229.2016.05.03.5
34. Zaichick V. Differences between 66 chemical element contents in normal and cancerous prostate. Journal of Analytical Oncology. 2017;6(2):37-56.
35. Zaichick V, Zaichick S. Instrumental effect on the contamination of biomedical samples in the course of sampling. The Journal of Analytical Chemistry. 1996;51(12):1200-1205.
36. Zaichick V, Zaichick S. A search for losses of chemical elements during freeze-drying of biological materials. J Radioanal Nucl Chem. 1997;218(2):249-253.
37. Zaichick S, Zaichick V. The Br, Fe, Rb, Sr, and Zn contents and interrelation in intact and morphologic normal prostate tissue of adult men investigated by energy-dispersive X-ray fluorescent analysis. X-Ray Spectr. 2011; 40(6):464-469.
38. Zaichick S, Zaichick V. Method and portable facility for energy-dispersive X-ray fluorescent analysis of zinc content in needle-biopsy specimens of prostate. X-Ray Spectr. 2010;39(2):83-89.
39. Zaichick V, Zaichick S, Davydov G. Method and portable facility for measurement of trace element concentration in prostate fluid samples using radionuclide-induced energy-dispersive X-ray fluorescent analysis. Nuclear Science and Techniques. 2016;27(6):136.
40. 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(1):41-54. doi: 10.1007/s12011-009-8456-0
41. Zaichick S., Zaichick V. The effect of age on Ag, Co, Cr, Fe, Hg, Sb, Sc, Se, and Zn contents in intact human prostate investigated by neutron activation analysis. J Appl Radiat Isot. 2011;69(6):827-833.
42. Zaichick V. Applications of synthetic reference materials in the medical Radiological Research Centre. Fresenius J Anal Chem. 1995;352(1-2):219-223.
43. Korelo AM, Zaichick V. Software to optimize the multielement INAA of medical and environmental samples. In: Activation Analysis in Environment Protection. Dubna Russia: Joint Institute for Nuclear Research. 1993:326-332.
44. Zhu H, Wang N, Zhang Y, Wu, Quan, Chen, Rusong, Gao, et al. Element contents in organs and tissues of Chinese adult men. Health Phys. 2010; 98(1): 61-73. doi: 10.1097/HP.0b013e3181bad921
45. Vlasova ZA. Dynamics of trace element contents in thyroid gland in connection with age and atherosclerosis. Proceedings of the Leningrad Institute of Doctor Advanced Training. 1969;80:135-144.
46. 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(4): 211-216.
47. Boulyga SF, Zhuk IV, Lomonosova EM, Kanash NV, Bazhanova NN. Determination of microelements in thyroids of the inhabitants of Belarus by neutron activation analysis using the k0-method. J Radioanal Nucl Chem. 1997; 222(1-2):11-14.
48. Reddy SB, Charles MJ, Kumar MR, SeetharamiReddy, ChAnjaneyulu G, JNaga Raju, BSundareswar, et al. Trace elemental analysis of adenoma and carcinoma thyroid by PIXE method. Nucl Instrum Methods Phys Res B. 2002; 196(3-4):333-339.
49. Woodard HQ, White DR. The composition of body tissues. Brit J Radiol. 1986;59(708):1209-1218.
50. Katoh Y, Sato T, Yamamoto Y. Determination of multielement concentrations in normal human organs from the Japanese. Biol Trace Elem Res. 2002;90(1-3):57-70.
51. Tipton IH, Cook MJ. Trace elements in human tissue. Part II. Adult subjects from the United States. Health Phys. 1963;9(2):103-145.
52. Ataulchanov IA. Age-related changes of manganese, cobalt, copper, zinc, and iron contents in the endocrine glands of females. Problemy Endocrinologii. 1969;15(2):98-102.
53. Neimark II, Timoschnikov VM. Development of carcinoma of the thyroid gland in person residing in the focus of goiter endemic. Problemy Endocrinilogii. 1978;24(3):28-32.
54. Zabala J, Carrion N, Murillo M, Quintana M, Chirinos J, Seijas N, Duarte L, et al. Determination of normal human intrathyroidal iodine in Caracas population. J Trace Elem Med Biol. 2009;23(1):9-14.
55. Forssen A. Inorganic elements in the human body. Ann Med Exp Biol Fenn. 1972;50(3):99-162.
56. Kortev AI, Donthov GI, Lyascheva AP. Bioelements and a human pathology. Sverdlovsk, Russia: Middle-Ural publishing-house. 1972.
57. Soman SD Joseph KT, Raut SJ, Mulay CD, Parameshwaran M,

Panday VK. Studies of major and trace element content in human tissues. Health Phys. 1970;19(5):641-656.

58. Teraoka H. Distribution of 24 elements in the internal organs of normal males and the metallic workers in Japan. Arch Environ Health. 1981;36(4):155-165.
59. Boulyga SF, Becker JS, Malenchenko AF, Dietze H-J. Application of ICP-MS for multielement analysis in small sample amounts of pathological thyroid tissue. Microchimica Acta. 2000;134(3-4):215-222.
60. Fuzailov YuM. Reaction of human and animal thyroids in the conditions of antimony sub-region of the Fergana valley. In: IX All-Union Conference on Trace Elements in Biology. Kishinev: State University. 1981:58-62.
61. Kvicala J, Havelka J, Zeman J, Nemec J. Determination of some trace elements in the thyroid gland by INAA. J Radioanal Nucl Chem. 1991;149(2):267-274.
62. 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.
63. 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(1-3):188-190.
64. Zagrodzki P, Nicol F, Arthur JR, Słowiaczek M, Walas S, Mrowiec H, Wietecha-Posłuszny R, et al. Selenoenzymes, laboratory parameters, and trace elements in different types of thyroid tumor. Biol Trace Elem Res. 2010; 134(1):25-40. doi: 10.1007/s12011-009-8454-2
65. Al-Sayer H, Mathew TC, Asfar S, Khourshed M, Al-Bader A, Behbehani A, Dashti H, et al. Serum changes in trace elements during thyroid cancers. Mol Cell Biochem. 2004;260(1-2): 1-5.
66. Nishida M, Sakurai H, Tezuka U, Kawada J, Koyama M, Takada J. 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(2):181-187.
67. Tardos 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(643):626-629.
68. 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(7):5718-5726.
69. Yaman M, Akdeniz I. Sensitivity enhancement in flame atomic absorption spectrometry for determination of copper in human thyroid tissues. Anal Sci. 2004;20(9):1363-1366.
70. Schroeder HA, Tipton IH, Nason AP. Trace metals in man: strontium and barium. J Chron Dis. 1972;25(9):491-517.
71. Zaichick V. Sampling, sample storage and preparation of biomaterials for INAA in clinical medicine, occupational and environmental health. In: Harmonization of Health-Related Environmental Measurements Using Nuclear and Isotopic Techniques. Vienna: IAEA. 1997:123-133.
72. Zaichick V, Zaichick S. A search for losses of chemical elements during freeze-drying of biological materials. J Radioanal Nucl Chem. 1997;218(2):249-253.
73. Zaichick V. Losses of chemical elements in biological samples under the dry aching process. Trace Elements in Medicine. 2004;5(3):17-22.
74. Lansdown AB. Critical observations on the neurotoxicity of silver. Crit Rev Toxicol. 2007;37(3):237-250.
75. Lansdown AB. Silver in health care: antimicrobial effects and safety in use. Curr Probl Dermatol. 2006;33:17-34.
76. Drake PL, Hazelwood KJ. Exposure-related health effects of silver and silver compounds: a review. Ann Occup Hyg. 2005;49(7):575-585.
77. Pavelka S. Radiometric determination of thyrotoxic effects of some xenobiotics. Rad Applic. 2016;1(2):155-158.
78. Maschkovsky MD. The sedatives. In: The Medicaments. 15th ed Moscow: Novaya Volna. 2005:72-86.
79. Zaichick V. X-ray fluorescence analysis of bromine for the estimation of extracellular water. J Appl Radiat Isot. 1998;49(12):1665-1669.
80. Zaichick V, Zaichick S. The silver, cobalt, chromium, iron, mercury, rubidium, antimony, selenium and zinc contents in human bone affected by Ewing's sarcoma. Journal of Cancer and Tumor International. 2015;2(1): 21-31.
81. Zaichick S, Zaichick V. The content of silver, cobalt, chromium, iron, mercury, rubidium, antimony, selenium, and zinc in osteogenic sarcoma. Journal of Cancer Therapy. 2015; 6(6): 493-503. DOI: 10.4236/jct.2015.66053
82. Zaichick V, Zaichick S. The silver, cobalt, chromium, iron, mercury, rubidium, antimony, selenium, and zinc contents in human bone affected by chondrosarcoma. Journal of Hematology and Oncology Research. 2015; 1(4): 25-36. DOI: 10.14302/issn.2372-6601.jhor-15-666
83. Zaichick V, Zaichick S. Distinguishing malignant from benign prostate using content of 17 chemical elements in prostatic tissue. Integr Cancer Sci Therap. 2016; 3(5): 579-587. DOI: 10.15761/ICST.1000208
84. Zaichick V, Zaichick S. Trace element contents in adenocarcinoma of the human prostate gland investigated by neutron activation analysis. Cancer Research & Oncology. 2016; 1(1): 1-10.
85. Zaichick V, Zaichick S. Prostatic tissue levels of 43 trace elements in patients with prostate adenocarcinoma. Cancer and Clinical Oncology. 2016; 5(1): 79-94. DOI: 10.5539/cco.v5n1p79
86. Leyssens L, Vinck B, Van Der Straeten C, Wuyts F, Maes L. Cobalt toxicity in humans-A review of the potential sources and systemic health effects. Toxicology. 2017; 387: 43-56. DOI:10.1016/j.tox.2017.05.015
87. Yu R. Cobalt Toxicity, An overlooked Cause of Hypothyroidism. J Endocrinol Thyroid Res. 2017; 1(3): 1-4. DOI: 10.19080/JETR.2017.01.555563
88. Simonsen LO, Harbak H, Bennekou P. Cobalt metabolism and toxicology--a brief update. Sci Total Environ. 2012; 432: 210-215. DOI: 10.1016/j.scitotenv.2012.06.009
89. Järup L. Hazards of heavy metal contamination. Br Med Bull. 2003; 68: 167-182.

90. Nigam A, Priya S, Bajpai P, Kumar S. Cytogenomics of hexavalent chromium (Cr 6+) exposed cells: a comprehensive review. Indian J Med Res. 2014; 139(3): 349-370.
91. Zhitkovich A. Chromium in drinking water: sources, metabolism, and cancer risks. Chem Res Toxicol. 2011; 24(10): 1617-1629. DOI: 10.1021/tx200251t
92. Ding SZ, Yang YX, Li XL, Michelli-Rivera A, Han SY, Wang L, et al., Epithelial-mesenchymal transition during oncogenic transformation induced by hexavalent chromium involves reactive oxygen species-dependent mechanism in lung epithelial cells. Toxicol Appl Pharmacol. 2013; 269(1): 61-71. DOI: 10.1016/j.taap.2013.03.006
93. 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(7): 1303-1311. DOI: 10.1093/carcin/14.7.1303
94. 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(5): 325-332. DOI:10.3109/10715769609088030
95. 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(1): 73-80.
96. Clarkson TW, Magos L. The toxicology of mercury and its chemical compounds. Crit Rev Toxicol. 2006; 36(8): 609-662. DOI:10.1080/10408440600845619
97. Hazelhoff MH, Bulacio RP, Torres AM. Gender related differences in kidney injury induced by mercury. Int J Mol Sci. 2012; 13(8): 10523-10536. DOI: 10.3390/ijms130810523
98. Clarkson TW, Magos L. The toxicology of mercury and its chemical compounds. Crit Rev Toxicol. 2006; 36(8): 609-662. DOI: 10.1080/10408440600845619
99. Malandrino P, Russo M, Ronchi A, Minoia C, Cataldo D, Regalbuto C, et al., Increased thyroid cancer incidence in a basaltic volcanic area is associated with non-anthropogenic pollution and biocontamination. Endocrine. 2016; 53(2): 471-479. DOI: 10.1007/s12020-015-0761-0
100. Abnoos H, Fereidoni M, Mahdavi-Shahri N, Haddad F, Jalal R. Developmental study of mercury effects on the fruit fly (Drosophila melanogaster). Interdiscip Toxicol. 2013; 6(1): 34-40. DOI: 10.2478/intox-2013-0007
101. Crespo-López ME, Macêdo GL, Pereira SI, Arrifano GP, Picanço-Diniz DL , do Nascimento JL , et al., Mercury and human genotoxicity: critical considerations and possible molecular mechanisms. Pharmacol Res. 2009; 60(4): 212-220. DOI: 10.1016/j.phrs.2009.02.011
102. Chandra A.K. Goswami H, Sengupta P. Effects of magnesium on cytomorphology and enzyme activities in thyroid of rats. Indian J Exp Biol. 2014; 52(8): 787-792.
103. Jiménez A. Changes in bioavailability and tissue distribution of selenium caused by magnesium deficiency in rats. J Am Coll Nutr. 1997; 16(2): 175-180. DOI: 10.1080/07315724.1997.10718669
104. Durlach J, Bara M, Guiet-Bara A, Collery P. Relationship between magnesium, cancer and carcinogenic or anticancer metals. Anticancer Res. 1986; 6(6): 1353-1361.
105. 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): 1-13.
106. 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(3): 193-209. DOI: 10.1159/000224570
107. 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(3): 360-363. DOI:10.1016/0006-2944(83)90071-6
108. Szmeja Z, Koenczewska H. Red blood cell, serum and tissue magnesium levels in subjects with laryngeal carcinoma. J Otorhinolaryngol Relat Spec. 1983; 45(2): 102-107. DOI: 10.1159/000275631
109. Ranade SS, Panday VK. Major metals in human cancer: calcium, magnesium, sodium and potassium. Sci Total Environm. 1985; 41(1): 79-89.
110. Taylor JS, Vigneron DB, Murphy-Boesch J, S J Nelson, H B Kessler, L Coia, 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(15): 6810-6814.
111. Collery P, Anghileri LJ, Coudoux P, Durlach J. Magnesium and cancer: Clinical data. Magnesium Bull. 1981; 3: 11-20.
112. Wolf FI, Cittadini ARM, Maier AM. Magnesium and tumors: Ally or foe? Cancer Treatment Reviews. 2009; 35(4): 378-382. DOI: 10.1016/j.ctrv.2009.01.003
113. Sodium and calcium regulation and the role of the cytoskeleton in the pathogenesis of disease: a review and hypothesis. 1981; (Pt 2): 434-454.
114. Ranade SS, Panday VK. Major metals in human cancer: calcium, magnesium, sodium and potassium. Sci Total Environ. 1985; 41(1): 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(1): 53-66.
116. Ouwerkerk R, Jacobs MA, Macura KJ, Wolff AC, Stearns V, Mezban SD, et al., Elevated tissue sodium concentration in malignant breast lesions detected with non-invasive 23Na MRI. Breast Cancer Res Treat. 2007; 106(2): 151-160. DOI:10.1007/s10549-006-9485-4
117. Johnson GT, Lewis TR, Wagner WD. Acute toxicity of cesium and rubidium compounds. Toxicol Appl Pharmacol. 1975; 32(2): 239-245.
118. Jones JM, Yeralan O, Hines G, Maher M, Roberts DW, Benson RW. Effects of lithium and rubidium on immune responses of rats. Toxicol Lett. 1990;52(2):163-168. DOI: 10.1016/0378-4274(90)90150-K
119. Petrini M, Vaglini F, Carulli G, Azzara A, Ambrogi F, Grassi B. Rubidium is a possible supporting element for bone marrow leukocyte differentiation. Haematologica. 1990; 75(1): 27-31.