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Research Article

Fifty Trace Element Contents in Normal and Cancerous Thyroid

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Abstract

Introduction: 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 trace elements exist in the malignantly transformed thyroid.

Methods: Thyroid tissue levels of fifty trace elements were prospectively evaluated in 41 patients with thyroid malignant tumors and 105 healthy inhabitants. Measurements were performed using non-destructive instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides combined with inductively coupled plasma mass spectrometry. 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 Ag, Al, B, Bi, Ce, Cr, Hg, Mn, Mo, Ni, Pb, Rb, Sm, and Tl were approximately 10.5, 3.1, 4.6, 9.3, 3.5, 3.7, 16.9, 1.6, 3.5, 9.8, 4.9, 1.7, 3.8, and 3.3 times higher, respectively, while content of Sc was lower (nearly 3.4 times) in cancerous tissues than in normal tissues.

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

Keywords: Thyroid Malignant Tumors; Intact Thyroid; Trace Elements; Instrumental Neutron Activation Analysis; Inductively Coupled Plasma Mass Spectrometry

Abbreviations

TC: Thyroid Cancer; INAA-LLR: Instrumental Neutron Activation Analysis with High Resolution Spectrometry of Long-Lived Radionuclides; ICP-MS: Inductively Coupled Plasma Mass Spectrometry; BSS: Biological Synthetic Standards; CRM: Certified Reference Materials; IAEA: International Atomic Energy Agency

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 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 trace element contents in the thyroid was observed [23-28]. It was concluded that an age-dependent excess of intrathyroidal 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 trace elements also play a role in the pathophysiology of the thyroid.

This work had two aims. The first was to determine reliable values for trace element mass fractions in TC tissue using two instrumental analytical methods: neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA - LLR) and inductively coupled plasma mass spectrometry (ICP-MS). The second aim was to compare the levels of trace elements in the malignant thyroid with those in intact (normal) gland of apparently healthy persons.

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.

Materials and Method

Samples

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.

Sample preparation

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 [36-38].

The pounded sample weighing about 5 - 10 mg (for biopsy) and 50 - 100 mg (for resected materials) was used for trace element measurement by INAA-LLR. The samples for INAA-LLR were wrapped separately in a high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

After INAA-LLR investigation the thyroid samples were taken out from the aluminum foils and used for ICP-MS. The samples were decomposed in autoclaves; 1.5 mL of concentrated HNO_3 (nitric acid at 65%, maximum (max) of 0.0000005% Hg; GR, ISO, Merck, Darmstadt, Germany) and 0.3 mL of H_2O_2 (pure for analysis) were added to thyroid samples, placed in one-chamber autoclaves (Ancon-AT2, Ltd., Moscow, Russia) and then heated for 3h 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 (only HNO_3+H_2O_2+ deionized water), and the resultant solutions were used as control samples.

Certified Reference Materials

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [39]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. For quality control, ten subsamples of the certified reference materials (CRM) IAEA H-4 Animal Muscle from the International Atomic Energy Agency (IAEA), and also five subsamples INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves and INCT-MPH-2 Mixed Polish Herbs from the Institute of Nuclear Chemistry and Technology (INCT, Warszawa, Poland) were analyzed simultaneously with the investigated thyroid tissue samples. All samples of CRM were treated in the same way as the thyroid tissue samples. Detailed results of this quality assurance program were presented in earlier publications [40-46].

Instrumentation and methods

A vertical channel of nuclear reactor was applied to determine the content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn by INAA-LLR. The quartz ampoule with thyroid samples, standards, and certified reference material was soldered, positioned in a transport aluminum container and exposed to a 24-hour neutron irradiation in a vertical channel with a neutron flux of $1.3 \cdot 10^{13}$ n · cm^{-2}. s^{-1}. Ten days after irradiation samples were reweighed and repacked.

The samples were measured for period from 10 to 30 days after irradiation. The duration of measurements was from 20 minutes to 10 hours subject to pulse counting rate. Spectrometric measurements were performed using a coaxial 98-cm^3 Ge (Li) detector and a spectrometric unit (NUC 8100, Hungary), including a PC-coupled multichannel analyzer. Resolution of the spectrometric unit was 2.9-keV at the 60Co 1.332-keV line.

Sample aliquots were used to determine the content of Ag, Al, As, Au, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Sb, Se, Sm, Sn, Tb, Te, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr by ICP-MS using an ICP-MS ThermoFisher "X-7" Spectrometer (Thermo Electron, USA). The trace element concentrations in aqueous solutions were determined by the quantitative method using multi elemental calibration solutions ICP-MS-68A and ICP-AM-6-A produced by High-Purity Standards (Charleston, SC 29423, USA). Indium was used as an internal standard in all measurements.

Information detailing with the INAA-LLR and ICP-MS methods used, and other details of the analysis was presented in our previous publication concerning trace element contents in human prostate and scalp hair [40-46].

Computer programs and statistic

A dedicated computer program for INAA mode optimization was used [47]. All thyroid samples were prepared in duplicate, and mean values of trace element contents were used. Mean values of trace elements contents were used in final calculation for the Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, 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 trace element mass fractions. 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

The comparison of our results for the Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions (mg/kg, dry mass basis) in the normal human thyroid obtained by both INAA-LLR and ICP-MS methods is shown in table 1.

Element	NAA-LLR M_1	ICP-MS M_2	Δ, %
Ag	0.0151 ± 0.0016	0.0122 ± 0.0014	19.2
Co	0.0399 ± 0.0030	0.0378 ± 0.0031	5.3
Cr	0.539 ± 0.032	0.451 ± 0.033	16.3
Fe	225 ± 11	221 ± 12	1.8
Hg	0.0421 ± 0.0041	0.0794 ± 0.0114	-88.5
Rb	7.37 ± 0.44	7.79 ± 0.46	-5.7
Sb	0.111 ± 0.008	0.079 ± 0.008	28.8
Se	2.32 ± 0.14	2.12 ± 0.14	8.6
Zn	97.8 ± 4.5	91.8 ± 4.3	6.1

Table 1: Comparison of the mean values (M±SEM) of the chemical element mass fractions (mg/kg, on dry-mass basis) in the normal thyroid of males and females obtained by both NAA-LLR and ICP-MS methods.

M: Arithmetic Mean; SEM: Standard Error of Mean,
$\Delta = [(M_1 - M_2)/M_1] \cdot 100\%$.

Tables 2 and 3 present 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, Al, As, Au, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Fe, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Sb, Sc, Se, Sm, Sn, Tb, Te, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr mass fractions in normal and cancerous thyroid tissue, respectively. The As, Au, Eu, Ho, Ir, Lu, Pd, Pt, Te, Th, Tm, Yb, and Zr mass fractions in normal thyroid samples and Au, Eu, Th, Ti, and Zr in cancerous samples were determined in a few samples. The possible upper limit of the mean (≤ M) for these trace elements was calculated as the average mass fraction, using the value of the detection limit (DL) instead of the individual value when the latter was found to be below the DL:

$$\leq M = \left(\sum_{i}^{n_j} C_i + DL \cdot n_j \right) / n$$

where C_i is the individual value of the trace-element mass fraction in sample -i, n_i is number of samples with mass fraction higher than the DL, n_j is number of samples with mass fraction lower than

Element	M	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Ag	0.0133	0.0114	0.0013	0.00160	0.0789	0.0102	0.00187	0.0333
Al	10.5	13.4	1.8	0.80	69.3	6.35	1.19	52.9
As	≤ 0.005	-	-	< 0.003	0.0200	-	-	-
Au	≤ 0.005	-	-	< 0.002	0.0203	-	-	-
B	0.476	0.434	0.058	0.200	2.30	0.300	0.200	1.73
Be	0.00052	0.00060	0.00008	0.0001	0.0031	0.00030	0.0001	0.0022
Bi	0.0072	0.0161	0.0022	0.00030	0.100	0.00270	0.00050	0.0523
Cd	2.08	2.05	0.27	0.0110	8.26	1.37	0.113	7.76
Ce	0.0080	0.0080	0.0011	0.00100	0.0348	0.00475	0.00134	0.0293
Co	0.0390	0.0276	0.0031	0.0100	0.140	0.0285	0.0130	0.124
Cr	0.495	0.261	0.031	0.130	1.30	0.430	0.158	1.08
Cs	0.0245	0.0166	0.0022	0.00220	0.0924	0.0198	0.00667	0.0723
Dy	0.0012	0.0018	0.0003	0.00030	0.0121	0.000630	0.00030	0.00519
Er	0.00038	0.00037	0.00005	0.00010	0.0022	0.000275	0.00010	0.00110
Eu	≤ 0.00039	-	-	< 0.0002	0.0019	-	-	-
Fe	222.8	89.5	9.6	52.0	474	222	67.8	425
Ga	0.0316	0.0156	0.0021	0.0100	0.0810	0.0295	0.0100	0.0700
Gd	0.00105	0.00109	0.00015	0.00040	0.0065	0.000600	0.00040	0.00425
Hg	0.0543	0.0373	0.0043	0.00700	0.151	0.0460	0.00983	0.150
Ho	≤ 0.0004	-	-	< 0.0001	0.0042	-	-	-
Ir	≤ 00.0003	-	-	< 0.0002	0.0010	-	-	-
La	0.00475	0.00461	0.00062	0.00040	0.0219	0.00270	0.00040	0.0171
Li	0.0208	0.0155	0.0022	0.00150	0.0977	0.0178	0.00412	0.0487
Lu	≤ 0.0002	-	-	< 0.0001	0.0010	-	-	-
Mn	1.28	0.56	0.07	0.470	4.04	1.15	0.537	2.23
Mo	0.0836	0.0470	0.0062	0.0104	0.299	0.0776	0.0278	0.211
Nb	0.597	0.898	0.120	0.0130	3.77	0.188	0.0130	3.26
Nd	0.0041	0.0034	0.0004	0.00020	0.0165	0.0030	0.00064	0.0137
Ni	0.449	0.344	0.046	0.0740	1.80	0.330	0.120	1.39
Pb	0.233	0.246	0.033	0.0230	1.60	0.180	0.0328	0.776
Pd	≤ 0.022	-	-	< 0.014	0.0700	-	-	-
Pr	0.00107	0.00086	0.00011	0.00010	0.0039	0.00073	0.00020	0.00350
Pt	≤ 0.0006	-	-	< 0.0002	0.0138	-	-	-
Rb	7.54	3.65	0.39	1.21	22.6	6.84	3.54	17.4
Sb	0.0947	0.0692	0.0075	0.00470	0.308	0.0808	0.0117	0.279
Sc	0.0268	0.0329	0.0060	0.00020	0.0860	0.00640	0.00042	0.0860
Se	2.22	1.24	0.14	0.320	5.80	1.84	0.776	5.58
Sm	0.00051	0.00047	0.00006	0.00010	0.0021	0.000350	0.00010	0.00150
Sn	0.0777	0.0677	0.0091	0.00900	0.263	0.0550	0.00900	0.242
Tb	0.00020	0.00012	0.00002	0.00008	0.00060	0.000150	0.00010	0.000470
Te	≤ 0.0057	-	-	< 0.003	0.0185	-	-	-
Th	≤ 0.0032	-	-	< 0.002	0.0100	-	-	-

Ti*	3.50	3.53	0.47	0.440	14.5	2.30	0.602	13.0
Tl	0.00093	0.00051	0.00007	0.00010	0.0029	0.000900	0.00029	0.00216
Tm	≤ 0.00014	-	-	< 0.0001	0.0004			
U	0.00044	0.00043	0.00006	0.00010	0.0026	0.00030	0.00010	0.00131
Y	0.00260	0.00234	0.00032	0.00100	0.0110	0.00170	0.00100	0.00942
Yb	≤ 0.0005	-	-	< 0.0003	0.0057	-	-	-
Zn	94.8	39.6	4.2	7.10	215	88.9	34.9	196
Zr	≤ 0.081	-	-	< 0.03	0.480	-	-	-

Table 2: Some statistical parameters of 50 trace element mass fraction (mg/kg, dry mass basis) in the normal thyroid.

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.

Element	M	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Ag	0.139	0.141	0.028	0.00750	0.536	0.0841	0.00800	0.501
Al	33.0	25.5	7.1	4.50	96.5	21.3	5.7	85.6
As	< 0.004	-	-	-	-	-	-	-
Au	≤ 0.014	-	-	< 0.003	0.073	-	-	-
B	2.21	1.89	0.52	1.00	5.6	1.00	1.00	5.42
Be	0.00047	0.00013	0.00004	0.00020	0.00072	0.00050	0.00023	0.00067
Bi	0.067	0.083	0.023	0.00480	0.335	0.0471	0.00879	0.258
Cd	1.13	1.82	0.49	0.0290	6.83	0.460	0.0322	5.55
Ce	0.0277	0.0275	0.0080	0.00470	0.0874	0.0161	0.00476	0.0836
Co	0.0499	0.0292	0.0050	0.00420	0.143	0.0456	0.0159	0.129
Cr	1.85	1.81	0.15	0.0390	3.50	0.515	0.0941	3.05
Cs	0.0298	0.0287	0.0090	0.00660	0.112	0.0223	0.00800	0.0926
Dy	< 0.005	-	-	-	-	-	-	-
Er	< 0.001	-	-	-	-	-	-	-
Eu	≤ 0.0016	-	-	< 0.001	0.0022	-	-	-
Fe	255	168	27	60.6	880	217	74.6	673
Ga	0.0342	0.0111	0.0030	0.0200	0.0640	0.0300	0.0225	0.0578
Gd	< 0.001	-	-	-	-	-	-	-
Hg	0.915	0.826	0.146	0.0685	3.75	0.771	0.0689	2.85
Ho	< 0.0002	-	-	-	-	-	-	-
Ir	< 0.0003	-	-	-	-	-	-	-
La	0.0134	0.0124	0.0040	0.00430	0.0443	0.00930	0.00438	0.0393
Li	0.0315	0.0307	0.0090	0.00780	0.111	0.0182	0.00885	0.0995
Lu	< 0.0002	-	-	-	-	-	-	-
Mn	2.01	1.34	0.29	0.100	5.95	1.61	0.250	5.23
Mo	0.292	0.112	0.031	0.0936	0.534	0.309	0.107	0.488
Nb	< 0.013	-	-	-	-	-	-	-
Nd	0.0156	0.0143	0.0050	0.00330	0.0412	0.00940	0.00377	0.0401
Ni	4.38	2.24	0.65	0.270	7.30	4.35	0.691	7.27

Pb	1.14	1.16	0.33	0.240	4.44	0.850	0.262	3.77
Pd	< 0.012	-	-	-	-	-	-	-
Pr	0.0078	0.0130	0.0040	0.000920	0.0463	0.00420	0.00102	0.0372
Pt	< 0.0002	-	-	-	-	-	-	-
Rb	12.65	4.87	0.76	5.10	27.4	12.3	5.50	21.7
Sb	0.107	0.075	0.014	0.0160	0.334	0.0870	0.0174	0.302
Sc	0.0077	0.0129	0.0020	0.00020	0.0565	0.00230	0.000200	0.0447
Se	2.04	1.06	0.19	0.143	4.80	1.76	0.627	4.37
Sm	0.00194	0.00174	0.00048	0.000500	0.0067	0.00100	0.000536	0.00574
Sn	0.0697	0.0487	0.0140	0.0138	0.182	0.0636	0.0173	0.173
Tb	< 0.0001	-	-	-	-	-	-	-
Te	< 0.007	-	-	-	-	-	-	-
Th	≤ 0.0256	-	-	< 0.0089	0.0973	-	-	-
Ti*	≤ 0.98	-	-	< 0.4	3.0	-	-	-
Tl	0.00307	0.00197	0.00100	0.00060	0.00700	0.00250	0.000840	0.00685
Tm	< 0.0003	-	-	-	-	-	-	-
U	0.00514	0.01109	0.00400	0.000550	0.0326	0.00108	0.000611	0.0273
Y	0.0123	0.0117	0.0040	0.00230	0.0343	0.00840	0.00245	0.0324
Yb	< 0.0002	-	-	-	-	-	-	-
Zn	96.9	80.0	12.6	28.7	375	69.8	36.3	374
Zr	≤ 0.149	-	-	< 0.03	0.81	-	-	-

Table 3: Some statistical parameters of 50 trace element mass fraction (mg/kg, dry mass basis) in the cancerous thyroid.

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.

the DL, and $n = n_i + n_j$ is number of samples that were investigated. The As, Dy, Er,Gd, Ho, Ir, Lu, Nb, Pd, Pt, Tb, Te, and Yb contents in all samples of cancerous thyroid were under detection limit.

The comparison of our results with published data for trace element mass fraction in normal and cancerous thyroid [48-78] is shown in table 4 and 5, respectively.

Element	Published data [Reference]			This work
	Median of means (n)*	Minimum of means or M ± SD, (n)**	Maximum of means M or M ± SD, (n)**	Males and females (combined) M ± SD
Ag	0.25 (12)	0.000784 (16) [48]	1.20 ± 1.24 (105) [49]	0.0133 ± 0.0114
Al	33.6 (12)	0.33 (-) [50]	420 (25) [51]	10.5 ± 13.4
As	0.079 (11)	0.0256 ± 0.0420 (8) [52]	500 ± 48 (4) [53]	≤0.0049
Au	0.084 (3)	0.0014 ± 0.0002 (10) [54]	< 0.4 (-) [55]	≤0.0050
B	0.151 (2)	0.084 (3) [56]	0.46 (3) [56]	0.476 ± 0.434
Be	0.042 (3)	0.000924(16) [48]	< 0.12 (-) [55]	0.00052 ± 0.00060
Bi	0.126 (4)	0.0339 (16) [48]	< 0.4 (-) [55]	0.0072 ± 0.0161
Cd	3.01 (15)	0.84 (180) [57]	47.6 ± 8.0 (16) [58]	2.08 ± 2.05
Ce	0.22 (1)	0.22 (59) [48]	0.22 (59) [48]	0.0080 ± 0.0080

Co	0.336 (17)	0.026 ± 0.031 (46) [59]	70.4 ± 40.8 (14) [60]	0.039 ± 0.028
Cr	0.69 (17)	0.105 (18) [56]	24.8 ± 2.4 (4) [53]	0.49 ± 0.25
Cs	0.069 (6)	0.0112 ± 0.0109 (14) [61]	0.109 ± 0.370 (48) [59]	0.025 ± 0.017
Dy	0.00106 (1)	0.00106 (60) [48]	0.00106 (60) [48]	0.0012 ± 0.0018
Er	0.00068 (1)	0.00068 (60) [48]	0.00068 (60) [48]	0.00038 ± 0.00038
Eu	0.0036 (1)	0.0036 (60) [48]	0.0036 (60) [48]	≤ 0.00039
Fe	252 (21)	56 (120) [62]	2444 ± 700 (14) [60]	223 ± 90
Ga	0.273 (3)	< 0.04 (-) [55]	1.7 ± 0.8 (-) [63]	0.032 ± 0.016
Gd	0.00256 (1)	0.00256 (59) [48]	0.00256 (59) [48]	0.00105 ± 0.00015
Hg	0.08 (13)	0.0008 ± 0.0002 (10) [54]	396 ± 40 (4) [53]	0.054 ± 0.037
Ho	0.00016 (1)	0.00016 (60) [48]	0.00016 (60) [48]	≤ 0.00040
Ir	-	-	-	≤ 0.00028
La	0.068 (3)	0.052 (59) [48]	<4.0 (-) [55]	0.0047 ± 0.0046
Li	6.3 (2)	0.092 (-) [55]	12.6 (180) [57]	0.021 ± 0.015
Lu	0.00022 (1)	0. 00022 (60) [48]	0. 00022 (60) [48]	≤ 0.00020
Mn	1.82 (36)	0.44 ± 11 (12) [64]	69.2 ± 7.2 (4) [53]	1.28 ± 0.56
Mo	0.42 (11)	0.0288 ± 0.0096 (39) [54]	516 ± 292 (14) [60]	0.0836 ± 0.047
Nb	< 4.0 (1)	< 4.0 (-) [55]	<4.0 (-) [55]	0.60 ± 0.90
Nd	0.0108 (1)	0.0108 (60) [48]	0.0108 (60) [48]	0.0041 ± 0.0034
Ni	0.96 (16)	0.00084 (83) [65]	33.6 ± 3.6 (4) [53]	0.45 ± 0.34
Pb	0.63 (22)	0.021 (83) [65]	68.8 ± 6.8 (4) [53]	0.23 ± 0.25
Pd	-	-	-	≤ 0.022
Pr	0.0034 (1)	0.0034 (59) [48]	0.0034 (59) [48]	0.00107 ± 0.00086
Pt	0.00017 (1)	0.00017 (59) [48]	0.00017 (59) [48]	≤ 0.00057
Rb	12.3 (9)	≤ 0.85 (29) [54]	294 ± 191 (14) [60]	7.5 ± 3.7
Sb	0.105 (10)	0.040 ± 0.003 (-) [66]	4.0 (-) [67]	0.095 ± 0.069
Sc	0.009 (4)	0.0018 ± 0.0003 (17) [68]	0.014 ± 0.005 (10) [54]	0.0268 ± 0.0329
Se	2.61 (17)	0.95 ± 0.08 (29) [54]	756 ± 680 (14) [60]	2.2 ± 1.2
Sm	0.00216 (1)	0.00216 (60) [48]	0.00216 (60) [48]	0.00051 ± 0.00047
Sn	0.40 (7)	0.0235 (16) [48]	-≤ 3.8 (17) [69]	0.078 ± 0.068
Tb	0.00022(1)	0.000224 (60) [48]	0.000224 (60) [48]	0.00020 ± 0.00012
Te	109 (1)	109 ± 82 (7) [70]	109 ± 82 (7) [70]	≤ 0.0057
Th	0.00456 (2)	0.00383 (15) [71]	0.00528 (60) [48]	≤ 0.0032
Ti*	1.42 (8)	0.084 (83) [66]	73.6 ± 7.2 (4) [53]	3.5 ± 3.5
Tl	< 0.2 (2)	0.00138 (16) [48]	< 0.4 (-) [55]	0.00093 ± 0.00051
Tm	0.00012 (1)	0.000124 (60) [48]	0.000124 (60) [48]	≤ 0.00014
U	0.05 (7)	0.00424 (16) [48]	0.428 ± 0.143 (10) [54]	0.00044 ± 0.00043
Y	< 2.9 (2)	0.00225 (16) [48]	≤ 5.9 (17) [69]	0.0026 ± 0.0023
Yb	0.00056 (1)	0.00056 (60) [48]	0.00056 (60) [48]	≤ 0.00059
Zn	118 (51)	32 (120) [62]	820 ± 204 (14) [60]	95 ± 40
Zr	< 0.4 (3)	0.188 (60) [48]	< 4.0 (-) [55]	≤ 0.082

Table 4: Median, minimum and maximum value of means of trace element contents in the normal thyroid according to data from the literature in comparison with our results (mg/kg, dry mass basis).

M: Arithmetic Mean; SD: Standard Deviation, (n)* - Number of All References, (n)** - Number of Samples.

Element	Published data [Reference]			This work
	Median of means (n)*	Minimum of means M or M ± SD, (n)**	Maximum of means M or M ± SD, (n)**	Males and females (combined) M ± SD
Ag	-	-	-	0.139 ± 0.141
Al	-	-	-	33.0 ± 25.5
As	10.2 (2)	9.2 ± 11.6 (3) [60]	11.2 ± 1.2 (4) [53]	< 0.004
Au	-	-	-	≤ 0.014
B	-	-	-	2.21 ± 1.89
Be	-	-	-	0.00047 ± 0.00013
Bi	-	-	-	0.067 ± 0.083
Cd	0.764 (1)	0.764 ± 0.140 (5) [72]	0.764 ± 0.140 (5) [72]	1.13 ± 1.82
Ce	-	-	-	0.0277 ± 0.0275
Co	71.6 (3)	2.48 ± 0.85 (18) [73]	94.4 ± 69.6 (3) [60]	0.0499 ± 0.0292
Cr	2.74 (2)	1.04 ± 0.52 (4) [74]	119 ± 12 (4) [53]	1.85 ± 1.81
Cs	-	-	-	0.0298 ± 0.0287
Dy	-	-	-	< 0.005
Er	-	-	-	< 0.001
Eu	-	-	-	≤ 0.0016
Fe	316 (8)	69 ± 51 (3) [75]	5588 ± 556 (4) [53]	255 ± 168
Ga	-	-	-	0.0342 ± 0.0111
Gd	-	-	-	< 0.001
Hg	30.8 (1)	30.8 ± 3.2 (4) [53]	30.8 ± 3.2 (4) [53]	0.915 ± 0.826
Ho	-	-	-	< 0.0002
Ir	-	-	-	< 0.0003
La	-	-	-	0.0134 ± 0.0124
Li	-	-	-	0.0315 ± 0.0307
Lu	-	-	-	< 0.0002
Mn	1.83 (4)	1.6 ± 0.8 (22) [76]	186 ± 18 (4) [53]	2.01 ± 1.34
Mo	-	-	-	0.292 ± 0.112
Nb	-	-	-	< 0.013
Nd	-	-	-	0.0156 ± 0.0143
Ni	18.6 (3)	1.62 ± 0.78 (6) [77]	30.8 ± 2.8 (4) [53]	4.38 ± 2.24
Pb	3.24 (4)	0.764 ± 0.140 (5) [72]	72 (1) [78]	1.14 ± 1.16
Pd	-	-	-	< 0.012
Pr	-	-	-	0.0078 ± 0.0130
Pt	-	-	-	< 0.0002
Rb	14.7 (2)	11,5 (10) [68]	17.8 ± 9.7 (5) [68]	12.65 ± 4.87
Sb	-	-	-	0.107 ± 0.075
Sc	-	-	-	0.0077 ± 0.0129
Se	2.16 (7)	1.00 ± 0.24 (3) [74]	241 ± 296 (3) [60]	2.04 ± 1.06
Sm	-	-	-	0.00194 ± 0.00174
Sn	-	-	-	0.0697 ± 0.0487
Tb	-	-	-	< 0.0001
Te	-	-	-	< 0.007

Th	-	-	-	≤ 0.0256
Ti*	112 (1)	112 ± 44 (4) [53]	112 ± 44 (4) [53]	≤ 0.98
Tl	-	-	-	0.00307 ± 0.00197
Tm	-	-	-	< 0.0003
U	-	-	-	0.00514 ± 0.01109
Y	-	-	-	0.0123 ± 0.0117
Yb	-	-	-	< 0.0002
Zn	112 (13)	48 ± 8 (5) [72]	494 ± 37 (2) [74]	96.9 ± 80.0
Zr	-	-	-	≤ 0.149

Table 5: Median, minimum and maximum value of means of trace element contents in the cancerous thyroid according to data from the literature in comparison with our results (mg/kg, dry mass basis).

M: Arithmetic Mean, SD *- Standard Deviation, (n)* - Number of All References, (n)** - Number of Samples.

The ratios of means and the difference between mean values of Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Fe, Ga, Hg, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tl, U, Y, and Zn mass fractions in normal and cancerous thyroid are presented in table 6.

Element	Thyroid tissue				Ratio
	Norm n = 105	Cancer n = 41	Student's t-test p ≤	U - test p	Cancer to Norm
Ag	0.0133 ± 0.0013	0.139 ± 0.028	**0.00013**	**≤ 0.01**	10.5
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
Be	0.00052 ± 0.00008	0.00047 ± 0.00004	0.589	> 0.05	0.90
Bi	0.0072 ± 0.0022	0.067 ± 0.023	**0.024**	**≤ 0.01**	9.31
Cd	2.08 ± 0.27	1.13 ± 0.49	0.103	> 0.05	0.54
Ce	0.0080 ± 0.0011	0.0277 ± 0.0080	**0.025**	**≤ 0.01**	3.46
Co	0.0390 ± 0.0031	0.0499 ± 0.0050	0.082	> 0.05	1.28
Cr	0.495 ± 0.031	1.85 ± 0.15	**0.026**	**≤ 0.01**	3.74
Cs	0.0245 ± 0.0022	0.0298 ± 0.0090	0.573	> 0.05	1.22
Fe	222.8 ± 9.6	255 ± 27	0.270	> 0.05	1.14
Ga	0.0316 ± 0.0021	0.0342 ± 0.0030	0.519	> 0.05	1.08
Hg	0.0543 ± 0.0043	0.915 ± 0.146	**< 0.000001**	**≤ 0.01**	16.9
La	0.00475 ± 0.00062	0.0134 ± 0.0040	0.070	> 0.05	2.82
Li	0.0208 ± 0.0022	0.0315 ± 0.0090	0.265	> 0.05	1.51
Mn	1.28 ± 0.07	2.01 ± 0.29	**0.025**	**≤ 0.01**	1.57
Mo	0.0836 ± 0.0062	0.292 ± 0.031	**0.000017**	**≤ 0.01**	3.49
Nd	0.0041 ± 0.0004	0.0156 ± 0.0050	0.056	> 0.05	3.80
Ni	0.449 ± 0.046	4.38 ± 0.65	**0.000079**	**≤ 0.01**	9.76
Pb	0.233 ± 0.033	1.14 ± 0.33	**0.020**	**≤ 0.01**	4.89
Pr	0.00107 ± 0.00011	0.0078 ± 0.0040	0.115	> 0.05	7.29
Rb	7.54 ± 0.39	12.65 ± 0.76	**< 0.000001**	**≤ 0.01**	1.68
Sb	0.0947 ± 0.0075	0.107 ± 0.014	0.388	> 0.05	1.13
Sc	0.0268 ± 0.0060	0.0077 ± 0.0020	**0.0053**	**≤ 0.01**	0.29

Se	2.22 ± 0.14	2.04 ± 0.19	0.457	> 0.05	0.92
Sm	0.000507 ± 0.000064	0.00194 ± 0.00048	**0.012**	**≤ 0.01**	3,83
Sn	0.0777 ± 0.0091	0.0697 ± 0.0140	0.627	> 0.05	0.90
Tl	0.000932 ± .000068	0.00307 ± 0.00100	**0.0020**	**≤ 0.01**	3.29
U	0.000443 ± 0.000059	0.00514 ± 0.00400	0.270	> 0.05	11.6
Y	0.00260 ± 0.00032	0.0123 ± 0.0040	0.071	> 0.05	4.73
Zn	94.8 ± 4.2	96.9 ± 12.6	0.877	> 0.05	1.02

Table 6: Differences between mean values (M ± SEM) of trace element mass fractions (mg/kg, dry mass basis) in normal and cancerous thyroid.

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 trace element mass fractions with the certified values of CRM IAEA H-4 and CRM IAEA HH-1 [40-46] as well as the similarity of the means of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions in the normal human thyroid determined by both INAA-LLR and ICP-MS methods (Table 1) demonstrates an acceptable precision and accuracy of the results obtained in the study and presented in tables 2-6.

Comparison with published data

Values obtained for Al, B, Cd, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, Lu, Mn, Nb, Nd, Ni, Pb, Pr, Pt, Rb, Sb, Sc, Se, Sm, Tb, Th, Ti, Tm, Yb, Zn, and Zr contents in the normal human thyroid (Table 4) agree well with median of mean values reported by other researches [48-71]. The obtained means for Ag, Au, Co, Ga, Mo, Sn, and Y were almost one-three orders of magnitude lower median of previously reported means but inside the range of means (Table 4). The mean obtained for As, Be, Bi, Ce, Eu, La, Li, Tl, and U were also one-three orders of magnitude lower than the median of previously reported data and outside the range of previously reported means (under a minimal value of published means). The mean obtained for Te was five orders of magnitude lower than the only reported result [70]. Data cited in Table 4 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%) [59] and ash (4.16% on dry mass basis) [79] contents in thyroid of adults. No published data referring Ir and Pd contents of normal thyroid tissue were found.

In cancerous tissues (Table 5) our results were comparable with published data for Cd, Cr, Fe, Mn, Ni, Pb, Rb, Se, and Zn contents. The obtained means for As, Co, Hg, and Ti were approximately four, three, two, and two, respectively, orders of magnitude lower median of previously reported means and outside the range of these means (Table 5). No published data referring Ag, Al, Au, B, Be, Bi, Ce, Cs, Dy, Er, Eu, Ga, Gd, Ho, Ir, La, Li, Lu, Mo, Nb, Nd, Pd, Pr, Sb, Sc, Sm, Sn, Tb, Te, Th, Tl, Tm, U, Y, Yb, and Zr contents of cancerous thyroid tissue were found.

The ranges of means of trace element content reported in the literature for normal and for untreated cancerous thyroid vary widely (Tables 4 and 5 respectively). 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 [80-82].Insufficient quality control in previous studies, when checking the accuracy of obtained results with the help of international CRM's was not used, can also explain why our data for such elements as Ag, As, Au, Be, Bi, Ce, Co, Eu, Ga, Hg, La, Li, Mo, Sn, Tl, U, and Y are very different from those that was published in the literature.

Effect of malignant transformation on trace element contents

From table 6, it is observed that in cancerous tissue the mass fraction of Ag, Al, B, Bi, Ce, Cr, Hg, Mn, Mo, Ni, Pb, Rb, Sm, and Tl are approximately 10.5, 3.1, 4.6, 9.3, 3.5, 3.7, 16.9, 1.6, 3.5, 9.8, 4.9, 1.7, 3.8, and 3.3 times, respectively, higher than in normal tissues of the thyroid. In contrast, the mass fraction of Sc is almost 3.4 times lower. Thus, if we accept the trace 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, Al, B, Bi, Ce, Cr, Hg, Mn, Mo, Ni, Pb, Rb, Sm, and Tl in thyroid tissue significantly increased whereas the levels of Sc 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 [83]. 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 [84]. 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 [85]. More detailed knowledge of the Ag toxicity can lead to a better understanding of the impact on human health, including thyroid function.

Aluminum

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 [86]. Why Al content in cancerous thyroid is higher than normal level and how an excess of Al acts on thyroid are still to be cleared.

Boron

Trace element B is known to influence the activity of many enzymes [87]. Numerous studies have demonstrated beneficial effects of B on human health, including anti-inflammatory stimulus - reduces levels of inflammatory biomarkers, such as high-sensitivity C-reactive protein (hs-CRP) and tumor necrosis factor α (TNF-α); as well as raises levels of antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase [88]. However, there is evidence that slightly increased environmental concentrations of B can accelerate the appearance of transformation marks in the thyroid gland of hypothyroid rats [89]. 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.

Bismuth

Trace metal B is the heaviest stable element. There is only limited information on Bi compounds effects and fate in the human body, but Bi is seen as the least toxic heavy metal for humans. It is widely used in medical applications for its good antibacterial properties [90]. Until now Bi is not considered a human carcinogen. However, in recent publication Bi effects on thyroid function was shown [91]. Moreover, it was found that Bi replaces catalytic or structural metals such as iron, nickel and zinc in metalloproteins and the inorganic Bi derivatives can cause DNA single-strand breaks [92]. Why Bi content in cancerous thyroid is higher than normal level and how an excess of Bi acts on thyroid are still to be cleared.

Cerium

Ce is one among rare earth elements (REEs). REEs are a series of 17 chemical elements. They include scandium (Sc), yttrium (Y), lanthanum (La) and the lanthanide series from Ce to lutetium (Lu), in the periodic table. Their adverse health effects, including toxicity affected embryogensis, fertilization, cytogenetic and redox endpoints, are well known [93,94]. However, the available information is insufficient to ascertain the mutagenicity and carcinogenicity of Ce or Ce compounds. Why Ce content in cancerous thyroid is higher than normal level and how an excess of Ce acts on thyroid are still to be cleared.

Chromium

Cr-compounds are cytotoxic, genotoxic, and carcinogenic in nature. Some Cr forms, including hexavalent chromium (Cr^{6+}), 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 [95]. The lung cancer risk is prevalent in pigment chromate handlers, ferrochromium production workers, stainless steel welders, and chrome-platers [96]. 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 Cr^{6+} in drinking water induced tumors in the mouse small intestine [97]. Many other animal experiments and in vitro studies demonstrate also that Cr can induce oxidative stress and exert cytotoxic effects [98]. 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 [96].

Mercury

Hg is one of the most dangerous environmental pollutants [99]. 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 [100]. Hg may also harm a developing fetus and decrease fertility in men and women [99]. Besides these effects, Hg has been classified as certain or probable carcinogen by the International Agency for Research on Cancer [94]. For example, in Hg polluted area thyroid cancer incidence was almost 2 times higher than in in adjacent control areas [101].

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 [102]. 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 [103].

Manganese

Trace element Mn is a cofactor for numerous enzymes, playing many functional roles in living organisms. The Mn-containing enzyme, manganese superoxide dismutase (Mn-SOD), is the principal antioxidant enzyme which neutralizes the toxic effects of reactive oxygen species. It has been speculated that Mn interferes with thyroid hormone binding, transport, and activity at the tissue level [104]. There is opinion that Mn deficiencies in humans are rare and humans maintain stable tissue levels of this trace element [105]. It was reported that intracellular Mn content was positively correlated with manganese-containing superoxide dismutase (Mn - SOD), suggesting that the intracellular Mn level is associated with Mn-SOD activity [106]. 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.

Molybdenum

Mo is an essential trace element and part of a complex called molybdenum co-factor, which is required for three mammalian enzymes-xanthine oxidase, aldehyde oxidase and sulphite oxidase [107]. Mo-dependent enzymes operate in the oxidative system of thyroid epithelial cells and also play role in the release of T3 from the thyroid gland. However, there is data that even a slight increase Mo in the diet may accelerate and/or promote the process of thyroid cell transformation, thus acting as a tumor-promoting agent rather than a carcinogen [89]. Why Mo content in cancerous thyroid is higher than normal level and how an excess of Mo acts on thyroid are still to be cleared.

Nickel

The peripheral connection between inorganic Ni and autoimmune thyroid diseases was mentioned in the literature [108]. Moreover, well known that human exposure to highly nickel-polluted environments, such as those associated with nickel refining, electroplating, and welding, has the potential to produce not only thyroid diseases but a variety of pathologic effects. Among them are skin allergies, lung fibrosis, and cancer of the respiratory tract [109]. The exact mechanisms of nickel-induced carcinogenesis are not known. However, there is data that Ni-induced oxidative stress triggers cell proliferation, a process of great significance for cancer [110].

Lead

Pb is highly cytotoxic. It affects hormonal secretion and hormonal-induced cell responses. The epidemiological evidence for an association between Pb exposures and human cancer risk has been strengthened by many studies [111]. Why Pb content in cancerous thyroid is higher than normal level and how an excess of Pb acts on thyroid are still to be cleared.

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 [112]. Rb has some function in immune responce [113], probably by supporting cell differentiation [114]. 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 [114]. 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.

Scandium and Samarium

Sc and Sm are REEs (see, Cerium). REEs are not described as essential for humans, because no biochemical function has been directly connected to it. At this stage of our knowledge, no doubt that REEs overload negatively impact human health [93,94]. Why Sc content in cancerous thyroid is lower while Sm content is higher than normal level and how a deficiency of Sc and an excess of Sm acts on thyroid are still to be cleared.

Thallium

Tl is a ubiquitous natural metal considered as the most toxic among trace elements. Moreover, Tl is a suspected human carcinogen [115]. Why Tl content in cancerous thyroid is higher than normal level and how an excess of Tl acts on thyroid are still to be cleared.

Our findings show that mass fraction of Ag, Al, B, Bi, Ce, Cr, Hg, Mn, Mo, Ni, Pb, Rb, Sc, Sm, and Tl are significantly different in TC as compared to normal thyroid tissues (Tables 6). Thus, it is plausible to assume that levels of these trace 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 fifty trace element 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 allowed us to carry out the investigations of trace 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, Al, B, Bi, Ce, Cr, Hg, Mn, Mo, Ni, Pb, Rb, Sc, Sm, and Tl level alteration and shows the necessity the need to continue trace element research of malignant thyroid tumors.

Conclusion

In this work, trace elemental measurements were carried out in the tissue samples of normal thyroid and malignant tumors of thyroid using two instrumental analytical methods: non-destructive neutron activation analysis with high resolution spectrometry of long-lived radionuclides and inductively coupled plasma mass spectrometry. It was shown that the combination of these methods is an adequate analytical tool for the estimation of fifty trace element contents in the tissue samples of human thyroid, including needle-biopsy cores. It was observed that in cancerous tissues content of Ag, Al, B, Bi, Ce, Cr, Hg, Mn, Mo, Ni, Pb, Rb, Sm, and Tl significantly increased whereas the levels of Sc decrease in a comparison with the normal thyroid tissues. In our opinion, the increase in levels of Ag, Al, B, Bi, Ce, Cr, Hg, Mn, Mo, Ni, Pb, Rb, Sm, and Tl, as well as the decrease in levels of Sc 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 of these trace elements in thyroid tissue can be used as tumor markers.

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Conflict of Interest

The authors have not declared any conflict of interests.

Bibliography

1. Kilfoy BA., *et al*. "International patterns and trends in thyroid cancer incidence, 1973-2002". *Cancer Causes Control* 20 (2009): 525-531.

2. Jemal RSA., *et al*. "Cancer statistics, 2010". *Cancer Journal Clinicians* 60.5 (2010): 277-300.

3. Pellegriti G., *et al*. "Worldwide increasing incidence of thyroid cancer: update on epidemiology and risk factors". *Journal of Cancer Epidemiology* 2013 (2013): 10.

4. Wiltshire JJ., *et al*. "Systematic review of trends in the incidence rates of thyroid cancer". *Thyroid* 26.11 (2016): 1541-1552.

5. Jung K., *et al*. "Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2011". *Cancer Research and Treatment* 46.2 (2014): 109-123.

6. Zaichick V., *et al*. "Trace elements and thyroid cancer". *Analyst* 120 (1995): 817-821.

7. Zaichick V and Choporov Yu. "Determination of the natural level of human intra-thyroid iodine by instrumental neutron activation analysis". *Journal of Radioanalytical and Nuclear Chemistry* 207.1 (1996): 153-161.

8. Zaichick V and Zaichick S. "Normal human intrathyroidal iodine". *Science of the Total Environment* 206.1 (1997): 39-56.

9. Zaichick V. "Iodine excess and thyroid cancer". *Journal of Trace Elements in Experimental Medicine* 11.4 (1998): 508-509.

10. Zaichick V. "In vivo and in vitro application of energy-dispersive XRF in clinical investigations: experience and the future". *Journal of Trace Elements in Experimental Medicine* 11.4 (1998): 509-510.

11. Zaichick V and Iljina T. "Dietary iodine supplementation effect on the rat thyroid 131 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 and Zaichick S. "Energy-dispersive X-ray fluorescence of iodine in thyroid puncture biopsy specimens". *Journal of Trace and Microprobe Techniques* 17.2 (1999): 219-232.

13. Zaichick V. "Human intrathyroidal iodine in health and non-thyroidal disease". In: New aspects of trace element research. Abdulla M., *et al*. editors. 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". *Annals of the New York Academy of Sciences* 904 (2000): 630-632.

15. Cho BY., *et al*. "Changes in the clinicopathological characteristics and outcomes of thyroid cancer in Korea over the past four decades". *Thyroid* 23.7 (2013): 797-804.

16. Shan Z., *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* 26.8 (2016): 1125-1130.

17. Zimmermann MB and Galetti V. "Iodine intake as a risk factor for thyroid cancer: a comprehensive review of animal and human studies". *Thyroid Research* 8 (2015): 8.

18. McNally RJ., *et al*. "Increasing incidence of thyroid cancer in Great Britain, 1976-2005: age-period-cohort analysis". *European Journal of Epidemiology* 27.8 (2012): 615-622.

19. Ganly I., *et al*. "Survival from differentiated thyroid cancer: What has age got to do with it?" *Thyroid* 25.10 (2015): 1106-1114.

20. Zaichick V. "Medical elementology as a new scientific discipline". *Journal of Radioanalytical and Nuclear Chemistry* 269 (2006): 303-309.

21. Beyersmann D and Hartwig A. "Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms". *Archives of Toxicology* 82.8 (2008): 493-512.

22. Martinez-Zamudio R and Ha HC. "Environmental epigenetics in metal exposure". *Epigenetics* 6.7 (2011): 820-827.

23. Zaichick V and 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". *Current Updates in Aging* 1.1 (2017).

24. Zaichick V and 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*". Current Trends in Biomedical Engineering and Biosciences* 4.4 (2017): 555-644.

25. Zaichick V and 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". *Journal of Gerontology and Geriatric Medicine* 3 (2017): 015.

26. Zaichick V and Zaichick S. "Age-related changes of some trace element contents in intact thyroid of males investigated by energy dispersive X-ray fluorescent analysis*". MOJ Gerontology and Geriatrics* 1.5 (2017): 00028.

27. Zaichick V and 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". *Journal of Aging and Age Related Diseases* 1.1 (2017): 1002.

28. Zaichick V and Zaichick S. "Age-related changes of some trace element contents in intact thyroid of females investigated by energy dispersive X-ray fluorescent analysis". *Trends in Geriatric Healthcare* 1.1 (2017): 31-38.

29. Zaichick V and Zaichick S. "Trace element contents in adenocarcinoma of human prostate investigated by energy dispersive X-ray fluorescent analysis". *Journal of Adenocarcinoma* 1.1 (2016): 1-7.

30. Zaichick V and Zaichick S. "Trace element contents in adenocarcinoma of the human prostate gland investigated by neutron activation analysis". *Cancer Research and Oncology* 1.1 (2016): 1-10.

31. Zaichick V and Zaichick S. "The Comparison between the contents and interrelationships of 17 chemical elements in normal and cancerous prostate gland". *Journal of Prostate Cancer* 1.1 (2016): 105.

32. Zaichick V and Zaichick S. "Prostatic tissue levels of 43 trace elements in patients with prostate adenocarcinoma". *Cancer and Clinical Oncology* 5.1 (2016): 79-94.

33. Zaichick V., *et al.* "Intracellular zinc excess as one of the main factors in the etiology of prostate cancer". *Journal of Analytical Oncology* 5.3 (2016): 124-131.

34. Zaichick V. "Differences between 66 chemical element contents in normal and cancerous prostate". *Journal of Analytical Oncology* 6.1 (2017): 37-56.

35. Zaichick V and Zaichick S. "Instrumental effect on the contamination of biomedical samples in the course of sampling". *Journal of Analytical Chemistry* 51.12 (1996): 1200-1205.

36. Zaichick V and Zaichick S. "A search for losses of chemical elements during freeze-drying of biological materials". *Journal of Radioanalytical and Nuclear Chemistry* 218.2 (1997): 249-253.

37. Zaichick V and Tsislyak YuV. "A simple device for bio-sample lyophilic drying". *Laboratornoe Delo* 2 (1978): 109-110.

38. Zaichick V and Tsislyak YuV. "A modified adsorptive and cryogenic lyophilizer for biosample concentrations". *Laboratornoe Delo* 2 (1981):100-101.

39. Zaichick V. "Applications of synthetic reference materials in the medical Radiological Research Centre". *Fresenius Journal of Analytical Chemistry* 352 (1995): 219-223.

40. Zaichick S and 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". *International Journal of Applied Radiation and Isotopes* 69 (2011): 827-833.

41. Zaichick V and Zaichick S. "INAA application in the assessment of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction in pediatric and young adult prostate glands". *Journal of Radioanalytical and Nuclear Chemistry* 298.3 (2013): 1559-1566.

42. Zaichick V and Zaichick S. "Relations of the Al, B, Ba, Br, Ca, Cl, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, and Zn mass fractions to morphometric parameters in pediatric and nonhyperplastic young adult prostate glands". *Bio Metals* 27.2 (2014): 333-348.

43. Zaichick S., *et al*. "Mass fractions of 52 trace elements and zinc trace element content ratios in intact human prostates investigated by inductively coupled plasma mass spectrometry". *Biological Trace Element Research* 149.2 (2012): 171-183.

44. Zaichick V and Zaichick S. "Use of neutron activation analysis and inductively coupled plasma mass spectrometry for the determination of trace elements in pediatric and young adult prostate". *American Journal of Analytical Chemistry* 4 (2013): 696-706.

45. Zaichick V and Zaichick S. "Use of INAA and ICP-MS for the assessment of trace element mass fractions in adult and geriatric prostate". *Journal of Radioanalytical and Nuclear Chemistry* 301.2 (2014): 383-397.

46. Zaichick S and Zaichick V. "The effect of age and gender on 37 chemical element contents in scalp hair of healthy humans". *Biological Trace Element Research* 134.1 (2010): 41-54.

47. Korelo AM and 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.

48. Zhu H., *et al*. "Element contents in organs and tissues of Chinese adult men". *Health Physics* 98.1 (2010): 61-73

49. 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* 80 (1969): 135-144.

50. Kortev AI., *et al*. "Bio-elements in human pathology". Sverdlovsk (Russia): Middle-Ural publishing-house (1972).

51. Kamenev VF. "Trace element contents in the thyroid gland of adult person". In: *Trace elements in agriculture and medicine* (1963): 12-16.

52. Kosta L., *et al*. "Trace elements in human thyroid with special reference to the observed accumulation of mercury following long-term exposure". In: *Comparative Studies of Food and Environmental Contamination. Proceedings of Symposium* (1974): 541-550.

53. Reddy SB., *et al*. "Trace elemental analysis of adenoma and carcinoma thyroid by PIXE method". *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 196.3-4 (2002): 333-339.

54. Boulyga SF., *et al*. "Determination of microelements in thyroids of the inhabitants of Belarus by neutron activation analysis using the k0-method". *Journal of Radioanalytical and Nuclear Chemistry* 222.1-2 (1997): 11-14.

55. Zakutinskiy DI., *et al*. "Handbook on the toxicology of radioisotopes". Moscow: *Meditcinskaya Literatura* (1962).

56. Tipton IH and Cook MJ. "Trace elements in human tissue. Part II. Adult subjects from the United States". *Health Physics* 9.2 (1963): 103-145.

57. Remiz AM. "Endemic goiter and trace elements in Kabardino-Balkaria ASSR". In: *All-Union conference on endocrinology* (1962): 330-331.

58. Uetani M., *et al*. "Tissue cadmium (Cd) concentrations of people living in a Cd polluted area, Japan". *Biometals* 19.5 (2006): 521-525.

59. Katoh Y., *et al*. "Determination of multielement concentrations in normal human organs from the Japanese". *Biological Trace Element Research* 90.1-3 (2002): 57-70.

60. Salimi J., *et al*. "Investigation of heavy trace elements in neoplastic and non-neoplastic human thyroid tissue: A study by proton - induced X-ray emissions". *Iran Journal of Radiation Research* 1.4 (2004): 211-216.

61. Yamagata N. "The concentration of common cesium and rubidium in human body". *Journal of Radiation Research* 3.1 (1962): 9-30.

62. Ataulchanov IA. "Age-related changes of manganese, cobalt, coper, zinc, and iron contents in the endocrine glands of females". *Problemy Endocrinologii* 15.2 (1969): 98-102.

63. Belozerov ES. "Content of Ga trace element in some human tissues and organs, including thyroid gland". *Zdravoohranenie Turkmenii* 4 (1965): 15-16.

64. Teraoka H. "Distribution of 24 elements in the internal organs of normal males and the metallic workers in Japan". *Archives of Environmental Health* 36.4 (1981): 155-165.

65. Reytblat MA and Kropacheyv AM. "Some trace elements in the normal thyroid of Perm Prikam'e inhabitants". *Proceedings of Perm Medical Institute* 78 (1967): 157-164.

66. Boulyga SF., *et al*. "Application of ICP-MS for multielement analysis in small sample amounts of pathological thyroid tissue". *Microchemical Acta*134.3-4 (2000): 215-222.

67. 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* (1981): 58-62.

68. Kvicala J., *et al*. "Determination of some trace elements in the thyroid gland by INAA". *Journal of Radioanalytical and Nuclear Chemistry* 149.2 (1991): 267-274.

69. Forssen A. "Inorganic elements in the human body". *Annales Medicinae Experimentalis et Biologiae Fenniae* 50 (1972): 99-162.

70. Soman SD., *et al*. "Studies of major and trace element content in human tissues". *Health Physics* 19.5 (1970): 641-656.

71. Wang J., *et al*. "Determination of trace Cs, Th and U in ten kinds of human autopsy tissues by ICP-MS". *Spectroscopy and Spectral Analysis* 24.9 (2004): 1117-1120.

72. Yaman M and Akdeniz I. "Sensitivity enhancement in flame atomic absorption spectrometry for determination of copper in human thyroid tissues". *Analytical Sciences* 20.9 (2004): 1363-1366.

73. Neimark II and Timoschnikov VM. "Development of carcinoma of the thyroid gland in person residing in the focus of goiter endemic". *Problemy Endocrinilogii* 24.3 (1978): 28-32.

74. Zagrodzki P., *et al*. "Selenoenzymes, laboratory parameters, and trace elements in different types of thyroid tumor". *Biological Trace Element Research* 134.1 (2010): 25-40.

75. Maeda K., *et al*. "Multielemental analysis of human thyroid glands using particle induced X-ray emission (PIXE)". *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 22.1-3 (1987): 188-190.

76. Al-Sayer H., *et al*. "Serum changes in trace elements during thyroid cancers". *Molecular and Cellular Biochemistry* 260.1 (2004): 1-5.

77. Kaya G., *et al*. "Determination of Trace and Minor Metals in Benign and Malign Human Thyroid Tissues". *Asian Journal of Chemistry* 21.7 (2009): 5718-5726.

78. Jundt FC., *et al*. "Proton-induced X-ray analysis of trace elements in tissue sections". *Journal of Histochemistry and Cytochemistry* 22.1 (1974): 1-6.

79. Schroeder HA., *et al*. "Trace metals in man: strontium and barium". *Journal of Chronic Diseases* 25.9 (1972): 491-517.

80. 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* (1997): 123-133.

81. Zaichick V and Zaichick S. "A search for losses of chemical elements during freeze-drying of biological materials". *Journal of Radioanalytical and Nuclear Chemistry* 218.2 (1997): 249-253.

82. Zaichick V. "Losses of chemical elements in biological samples under the dry aching process". *Trace Elements in Medicine* 5.3 (2004): 17-22.

83. Lansdown AB. "Critical observations on the neurotoxicity of silver". *Critical Reviews in Toxicology* 37.3 (2007): 237-250.

84. Lansdown AB. "Silver in health care: antimicrobial effects and safety in use". *Current Problems in Dermatology* 33 (2006): 17-34.

85. Drake PL and Hazelwood KJ. "Exposure-related health effects of silver and silver compounds: a review". *Annals of Occupational Hygiene* 49.7 (2005): 575-585.

86. Krewski D., *et al*. "Human health risk assessment for aluminium, aluminium oxide, and aluminium hydroxide". *Journal of Toxicology and Environmental Health, Part B* 10 (2007): 1-269.

87. Naghii MR., *et al*. "Comparative effects of daily and weekly boron supplementation on plasma steroid hormones and proinflammatory cytokines". *Journal of Trace Elements in Medicine and Biology* 25 (2011): 54-58.

88. Pizzorno L. "Nothing boring about boron". *Integrative Medicine (Encinitas)* 14 (2015): 35-48.

89. Luca E., *et al*. "Intake of Boron, Cadmium, and Molybdenum enhances rat thyroid cell transformation". *Journal of Experimental and Clinical Cancer Research* 36 (2017): 73.

90. Mohan R. "Green bismuth". *Nature Chemistry* 2 (2010): 336-336.

91. Noori Jassim AM., *et al*. "Biochemical study for bismuth oxide and tellurium nanoparticles on thyroid hormone levels in serum and saliva of patients with chronic renal failure". *International Journal of Chemical Sciences* 11.3 (2013): 1299-1313.

92. Thomas F., *et al*. "Medical Use of Bismuth: The Two Sides of the Coin". *Journal of Clinical Toxicology* S3 (2012): 004.

93. Zaichick S., *et al*. "Accumulation of rare earth elements in human bone within the lifespan". *Metallomics* 3 (2011): 186-194.

94. Pagano G., *et al*. "Heavy rare earth elements affect early life stages in *Paracentrotus lividus* and *Arbacia lixula* sea urchins". *Environmental Research* 154 (2017): 240-246.

95. Järup L. "Hazards of heavy metal contamination". *British Medical Bulletin* 68 (2003): 167-182.

96. Nigam A., *et al*. "Cytogenomics of hexavalent chromium (Cr 6+) exposed cells: a comprehensive review". *Indian Journal of Medical Research* 139.3 (2014): 349-370.

97. Zhitkovich A. "Chromium in drinking water: sources, metabolism, and cancer risks". *Chemical Research in Toxicology* 24.10 (2011): 1617-1629.

98. Ding SZ., *et al*. "Epithelial-mesenchymal transition during oncogenic transformation induced by hexavalent chromium involves reactive oxygen species-dependent mechanism in lung epithelial cells". *Toxicology and Applied Pharmacology* 269.1 (2013): 61-71.

99. Clarkson TW and Magos L. "The toxicology of mercury and its chemical compounds". *Critical Reviews in Toxicology* 36 (2006): 609-662.

100. Hazelhoff MH., *et al*. "Gender related differences in kidney injury induced by mercury". *International Journal of Molecular Sciences* 13 (2012): 10523-10536.

101. Malandrino P., *et al*. "Increased thyroid cancer incidence in a basaltic volcanic area is associated with non-anthropogenic pollution and biocontamination". *Endocrine* 53.2 (2016): 471-479.

102. Abnoos H., *et al*. "Developmental study of mercury effects on the fruit fly (Drosophila melanogaster)". *Interdisciplinary Toxicology* 6.1 (2013): 34-40.

103. Crespo-López ME., *et al*. "Mercury and human genotoxicity: critical considerations and possible molecular mechanisms". *Pharmacological Research* 60.4 (2009): 212-220.

104. Soldin OP and Aschner M. "Effects of manganese on thyroid hormone homeostasis". *Neurotoxicology* 28 (2007): 951-956.

105. Aschner JL and Aschner M. "Nutritional aspects of manganese homeostasis". *Molecular Aspects of Medicine* 26 (2005): 353-362.

106. Hasegawa S., *et al*. "Alterations in manganese, copper, and zinc contents, and intracellular status of the metal-containing superoxide dismutase in human mesothelioma cells". *Journal of Trace Elements in Medicine and Biology* 22.3 (2008): 248-255.

107. Sardesai VM. "Molybdenum: an essential trace element". *Nutrition in Clinical Practice* 8 (1993): 277-281.

108. Takir M., *et al*. "Thyroid Hormone and Ultrasonographical Analyses in Patients with Nickel Allergy". *European Journal of Medicine and Oncology* 1.3 (2017): 145-148.

109. Kasprzak KS., *et al*. "Nickel carcinogenesis". *Mutation Research/ Fundamental and Molecular Mechanisms of Mutagenesis* 533.1-2 (2003): 67-97.

110. Mohammadi-Bardbori A and Rannug A. "Arsenic, cadmium, mercury and nickel stimulate cell growth via NADPH oxidase activation". *Chemico-Biological Interactions* 224 (2014): 183-188.

111. Silbergeld EK. "Facilitative mechanisms of lead as a carcinogen". *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 533 (2003): 121-133.

112. Johnson GT., *et al*. "Acute toxicity of cesium and rubidium compounds". *Toxicology and Applied Pharmacology* 32.2 (1975): 239-245.

113. Jones JM., *et al*. "Effects of lithium and rubidium on immune responses of rats". *Toxicology Letters* 52 (1990): 163-168.

114. Petrini M., *et al*. "Rubidium is a possible supporting element for bone marrow leukocyte differentiation". *Haematologica* 75 (1990): 27-31.

115. Emsley J. "Thallium. The element of murder. A history of poison". Oxford University Press Inc (2005).

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