Evaluation of Fifty Trace Element Contents in Thyroid Adenomas using a Combination of Instrumental Neutron Activation Analysis and Inductively Coupled Plasma Mass Spectrometry

Journal of Cancer and Oncology Research

Research Article

Vladimir Zaichick

Radionuclide Diagnostics Department, Medical Radiological Research Centre, Obninsk, 249036, Russia.

***Correspondence author**

Vladimir Zaichick
Radionuclide Diagnostics Department
Medical Radiological Research Centre
Obninsk
Russia

Submitted : 12 Aug 2021 ; Published : 13 Nov 2021

Abstract

Introduction: *Thyroid adenomas (TA) are benign tumors, but there is a 20% possibility of malignant transformation. The distinguishing between the TA and thyroid cancer is tricky, therefore new TA biomarkers are needed. Furthermore, the role of trace elements (TE) in etiology and pathogenesis of TA is unclear.*

Aim: *The aim of this exploratory study was to evaluate whether significant changes in the thyroid tissue levels of TE exist in the adenomatous transformed thyroid.*

Methods: *Thyroid tissue levels of fifty TE were prospectively evaluated in 19 patients with TA and 105 healthy inhabitants. Measurements were performed using a combination of non-destructive and destructive methods: instrumental neutron activation analysis and inductively coupled plasma mass spectrometry, respectively. Tissue samples were divided into two portions. One was used for morphological study while the other was intended for TE analysis.*

Results: *It was found that contents of Ag, Al, B, Cr, Fe, Hg, Mo, and Zn are significantly higher in TA than in normal thyroid tissues.*

Conclusion: *There are considerable changes in some TE contents in adenomatous tissue of thyroid. Thus, it is reasonable to assume that the levels of these TE in thyroid tissue can be used as TA markers. However, this topic needs additional studies.*

Keywords: Thyroid adenomas; Intact thyroid; Trace elements; Biomarkers for thyroid adenoma diagnosis; Instrumental neutron activation analysis; Inductively coupled plasma mass spectrometry.

Introduction

Thyroid adenomas (TA) are homogenous, solitary, encapsulated benign tumors, more common in females, and have a good prognosis. However, because there is a 20% possibility of malignant transformation, TA should be differentiated from other thyroid nodular diseases such as nodular goiter (NG) and thyroid cancer (TC). The distinguishing between the TA and TC is tricky, therefore new differential diagnostics and TA biomarkers are needed [1].

For more than twenty centuries, there has been a prevailing view that NG, including TA, is a minor consequence of iodine (I) deficiency. However, NG has been found to be a frequent disease even in those countries and regions where the population is never exposed to I deficiency [2]. Moreover, it was shown that I excess has severe consequences on human health and associated with the presence of thyroidal disfunctions and autoimmunity, nodular and diffuse goiters, benign and malignant tumors of gland [3-5]. It was also demonstrated that besides the I deficiency and excess many other dietary, environmental, and occupational factors are associated with the NG incidence [6, 7]. Among them a disturbance of evolutionary stable input of many trace elements (TE) in human body after industrial revolution plays a significant role in etiology of thyroidal disorders [8].

Besides iodine involved in thyroid function, TE have basic physiological functions such as maintaining and regulating cell function, regulating genes, activating or inhibiting enzymatic reactions, and regulating membrane function [9]. The essential or toxic (goitogenic, mutagenic, and carcinogenic) properties of ChE depend on the tissue-specific need or tolerance, respectively [9]. Excessive accumulation or an imbalance of the ChE may disturb the cell functions and may result in cellular degeneration, death, benign or malignant transformation [9, 10].

In our previous studies the complex of in vivo and in vitro nuclear analytical and related methods was developed and used for the investigation of I and other ChE contents in the normal and pathological thyroid [11-17]. Level of I in the normal thyroid was investigated in relation to age, gender and some non-thyroidal diseases [18, 19]. After that, variations of ChE content with age in the thyroid of males and females were studied and age- and gender-dependence of some ChE was observed [20-36]. Furthermore, a significant difference between some ChE contents in normal and cancerous thyroid was demonstrated [37-42].

So far, the etiology and pathogenesis of TA has to be considered as multifactorial. The present study was performed to clarify the role of some TE in the TA etiology. With this in mind, our aim was to assess the silver (Ag), aluminum (Al), arsenic (As), gold (Au), boron (B),, beryllium (Be), bismuth (Bi), cadmium (Cd), cerium (Ce), cobalt (Co), chromium (Cr), cesium (Cs), dysprosium (Dy), iron (Fe), erbium (Er), europium (Eu), gallium (Ga), gadolinium (Gd), mercury (Hg), holmium (Ho), iridium (Ir), lanthanum (La), lithium (Li), lutecium (Lu), manganese (Mn), molybdenum (Mo), niobium (Nb), neodymium (Nd), nickel (Ni), lead (Pb), palladium (Pd), praseodymium (Pr), platinum (Pt), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), samarium (Sm), tin (Sn), terbium (Tb), tellurium (Te), thorium (Th), titanium (Ti), thallium (Tl), thulium (Tm), uranium (U), yttrium (Y), ytterbium (Yb), zinc (Zn), and zirconium (Zr) mass fraction in TA tissue using a combination of non-destructive and destructive methods: instrumental neutron activation analysis with high resolution spectrometry of Long-lived radionuclides (INAA-LLR) and inductively coupled plasma mass spectrometry (ICPMS), respectively. A further aim was to compare the levels of these fifty TE in the adenomatous thyroid with those in normal gland of apparently healthy persons.

Material and Methods

Samples

All patients suffered from TA (n=19, 16 females and 3 males, mean age M±SD was 41±11 years, range 22-55) were hospitalized in the Head and Neck Department of the Medical Radiological Research Centre and an informed consent was taken from the subjects. 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 TE contents. For all patients the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. Histological conclusion for all thyroidal lesions was the TA.

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. Histological examination was used in the control group to match the age criteria, as well as to confirm the absence of micro-nodules and underlying cancer.

Sample preparation

All tissue samples were divided into two portions using a titanium scalpel [43]. One was used for morphological study while the other was intended for TE analysis. After the samples intended for TE analysis were weighed, they were freeze-dried and homogenized [44].

The pounded sample weighing about about 5-10 mg (for biopsy) and 50-100 mg (for resected materials) was used for TE measurement by non-destructive INAA-LLR.

After INAA-LLR investigation the thyroid samples were used for ICP-MS. The samples were decomposed in autoclaves. Simultaneously, the same procedure was performed in autoclaves without tissue samples (only HNO3+H2O2+ deionized water), and the resultant solutions were used as control samples.

Certified Reference Materials

To determine contents of the TE by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [45]. 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 and IAEA HH-1 Human Hair from the International Atomic Energy Agency (IAEA), and also five sub-samples 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 our earlier publications [30, 36, 42].

Instrumentation and methods

A vertical channel of 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. The mass fractions 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 were determined by ICP-MS method using an ICP-MS Thermo-Fisher "X-7" Spectrometer (Thermo Electron, USA). The TE 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 TE contents in human thyroid [30, 36, 42].

Computer programs and statistic

A dedicated computer program for INAA=LLR mode optimization was used [46]. All thyroid samples were prepared in duplicate, and mean values of TE contents were used. Mean values of TE contents were used in final calculation for the Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions measured by two methods INAA-LLR and ICPMS. 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 TE mass fractions. The difference in the results between two groups (normal and adenomatous thyroid) 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

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	≤0.0049	-	-	<0.003	0.0200	-	-	-
Au	≤0.0050	-	-	<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	0.000300	0.100	0.00270	0.000500	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	0.00122	0.00183	0.00025	0.000300	0.0121	0.000630	0.000300	0.00519
Er	0.000377	0.000367	0.000050	0.000100	0.00220	0.000275	0.000100	0.00110
Eu	≤0.00039	-	-	<0.0002	0.00190	-	-	-
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	0.000400	0.00650	0.000600	0.000400	0.00425
Hg	0.0543	0.0373	0.0043	0.00700	0.151	0.0460	0.00983	0.150
Ho	≤0.00040	-	-	<0.0001	0.00420	-	-	-
Ir	≤00.00028	-	-	<0.0002	0.0010	-	-	-
La	0.00475	0.00461	0.00062	0.000400	0.0219	0.00270	0.000400	0.0171
Li	0.0208	0.0155	0.0022	0.00150	0.0977	0.0178	0.00412	0.0487
Lu	≤0.00020	-	-	<0.0001	0.00100	-	-	-
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	0.00390	0.00073	0.00020	0.00350
Pt	≤0.00057	-	-	<0.00020	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	0.000200	0.0860	0.00640	0.000418	0.0860
Se	2.22	1.24	0.14	0.320	5.80	1.84	0.776	5.58
Sm	0.000507	0.000469	0.000064	0.000100	0.00210	0.000350	0.000100	0.00150
Sn	0.0777	0.0677	0.0091	0.00900	0.263	0.0550	0.00900	0.242
Tb	0.000198	0.000116	0.000016	0.0000800	0.000600	0.000150	0.000100	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	0.000932	0.000511	0.000068	0.000100	0.00290	0.000900	0.000294	0.00216
Tm	≤0.00014	-	-	<0.0001	0.00040	-	-	-
U	0.000443	0.000434	0.000059	0.000100	0.00260	0.00030	0.000100	0.00131
Y	0.00260	0.00234	0.00032	0.00100	0.0110	0.00170	0.00100	0.00942
Yb	≤0.00059	-	-	<0.0003	0.00570	-	-	-
Zn	94.8	39.6	4.2	7.10	215	88.9	34.9	196
Zr	≤0.081	-	-	<0.03	0.480	-	-	-

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 2: Some statistical parameters of 50 trace element mass fraction (mg/kg, dry mass basis) in the normal thyroid (n=105)

Element	M	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Ag	0.181	0.180	0.050	0.00120	0.679	0.198	0.0129	0.552
Al	34.2	24.1	9.1	8.70	78.4	30.6	9.53	74.1
As	<0.004	-	-	-	-	-	-	-
Au	0.0287	0.0293	0.0110	0.00300	0.0709	0.0240	0.00323	0.0705
B	3.38	2.74	1.12	1.00	7.30	3.00	1.00	7.01
Be	0.00181	0.00222	0.00090	0.000200	0.00600	0.00125	0.000200	0.00550
Bi	0.112	0.157	0.064	0.0113	0.422	0.0591	0.0119	0.382
Cd	2.78	2.51	0.95	0.310	6.39	3.25	0.311	6.21
Ce	0.0246	0.0174	0.0090	0.00730	0.0459	0.0225	0.00780	0.0448
Co	0.0660	0.0469	0.0135	0.0159	0.159	0.0439	0.0190	0.149
Cr	1.18	1.38	0.24	0.144	7.30	0.659	0.200	4.47
Cs	0.0216	0.0232	0.0050	0.00760	0.114	0.0147	0.00793	0.0760
Dy	<0.005	-	-	-	-	-	-	-
Er	0.00400	0.00390	0.00200	0.00100	0.00900	0.00200	0.00100	0.00900
Eu	<0.001	-	-	-	-	-	-	-
Fe	571	675	174	52.3	2563	368	53.4	2142
Ga	0.0223	0.0097	0.0050	0.0100	0.0300	0.0245	0.0107	0.0300
Gd	<0.001	-	-	-	-	-	-	-
Hg	1.16	1.26	0.34	0.193	5.20	0.885	0.254	4.07

Ho	<0.0002	-	-	-	-	-	-	-
Ir	<0.0003	-	-	-	-	-	-	-
La	0.0116	0.0105	0.0060	0.00540	0.0237	0.00560	0.00541	0.0228
Li	0.0401	0.0236	0.0100	0.0185	0.0680	0.0341	0.0186	0.0678
Lu	<0.0002	-	-	-	-	-	-	-
Mn	1.67	1.88	0.54	0.100	6.12	0.805	0.210	5.50
Mo	0.233	0.145	0.055	0.0460	0.448	0.199	0.0586	0.441
Nb	<0.013	-	-	-	-	-	-	-
Nd	0.0141	0.0047	0.0030	0.00960	0.0190	0.0137	0.00981	0.0187
Ni	3.95	3.39	1.39	0.480	9.00	3.35	0.508	8.73
Pb	1.86	3.29	1.24	0.260	9.30	0.660	0.289	8.06
Pd	<0.012	-	-	-	-	-	-	-
Pr	0.00475	0.00345	0.00200	0.00120	0.00930	0.00425	0.00136	0.00899
Pt	<0.0002	-	-	-	-	-	-	-
Rb	8.96	3.19	0.82	3.60	16.4	9.00	4.13	15.0
Sb	0.140	0.034	0.0449	0.466	0.105	0.105	0.0449	0.394
Sc	0.0286	0.0451	0.0140	0.000300	0.140	0.00710	0.000300	0.128
Se	3.01	2.43	0.65	0.720	10.6	2.25	0.941	8,68
Sm	0.00252	0.00263	0.00099	0.000400	0.00800	0.00140	0.000470	0.00725
Sn	0.0756	0.0443	0.0170	0.0331	0.157	0.0548	0.0360	0.151
Tb	<0.0001	-	-	-	-	-	-	-
Te	<0.007	-	-	-	-	-	-	-
Th	0.0229	0.0293	0.0011	0.00200	0.0783	0.00500	0.00200	0.0736
Ti	<0.4	-	-	-	-	-	-	-
Tl	0.00238	0.00164	0.00067	0.00110	0.00540	0.00190	0.00111	0.00508
Tm	<0.0003	-	-	-	-	-	-	-
U	0.00083	0.00035	0.00020	0.000440	0.00110	0.00095	0.000466	0.00109
Y	0.0115	0.0140	0.0060	0.00310	0.0361	0.00520	0.00312	0.0335
Yb	0.000375	0.000236	0.000118	0.000200	0.000700	0.000300	0.000200	0.000678
Zn	129	58	13	57.7	251	137	61.3	225
Zr	0.080	0.059	0.029	0.0310	0.165	0.0620	0.0333	0.157

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

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 adenomatous thyroid tissue, respectively. The As, Au, Eu, Ho, Ir, Lu, Pd, Pt, Te, Th, Tm, Yb, and Zr mass fractions in normal thyroid samples were determined in a few samples. The possible upper limit of the mean (≤M) for these TE 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:

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

where Ci is the individual value of the TE mass fraction in sample -i, ni is number of samples with mass fraction higher than the DL, nj is number of samples with mass fraction lower than 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, Ti, and Tm contents in all samples of adenomatous thyroid were under DL.

Element	Published data [Reference]			This work
	Median of means (n)*	Min of means M or M±SD, (n)**	Max of means M or M±SD, (n)**	M±SD
Ag	0.110 (1)	0.110±0.045 (16) [47]	0.110±0.045 (16) [47]	0.181±0.180
Al	-	-	-	34.2±24.1
As	35 (2)	0.00612 (46) [48]	70.8±6.8 (4) [49]	<0.004
Au	-	-	-	0.0287±0.0293
B	-	-	-	3.38±2.74
Be	-	-	-	0.00181±0.00222
Bi	-	-	-	0.112±0.157
Cd	0.522 (2)	0.172 (46) [48]	0.872±0.704 (13) [50]	2.78±2.51
Ce	-	-	-	0.0246±0.0174
Co	46.4 (1)	46.4±4.8 (4) [49]	46.4±4.8 (4) [49]	0.0660±0.0469
Cr	76 (2)	6,00±5.32 (9) [51]	146±14 (4) [49]	1.18±1.38
Cs	-	-	-	0.0216±0.0232
Dy	-	-	-	<0.005
Er	-	-	-	0.00400±0.00390
Eu	-	-	-	<0.001
Fe	566 (3)	54.6±36.1 (5) [52]	2100±208 (4) [49]	571±675
Ga	-	-	-	0.0223±0.0097
Gd	-	-	-	<0.001
Hg	79 (1)	79.2±8.0 (4) [49]	79.2±8.0 (4) [49]	1.16±1.26
Ho	-	-	-	<0.0002
Ir	-	-	-	<0.0003
La	-	-	-	0.0116±0.0105
Li	-	-	-	0.0401±0.0236
Lu	-	-	-	<0.0002
Mn	1.28 (4)	0.40 (46) [48]	57.6±6.0 (4) [49	1.67±1.88
Mo	0.128 (1)	0.128±0.064 (16) [47]	0.128±0.064 (16) [47]	0.233±0.145
Nb	-	-	-	<0.013
Nd	-	-	-	0.0141±0.0047
Ni	6.5 (2)	0.580±0.384 (16) [47]	12.4±4.4 (4) [49]	3.95±3.39
Pb	2.04 (2)	0.22 (46) [48]	46.4±4.8 (4) [49]	1.86±3.29
Pd	-	-	-	<0.012
Pr	-	-	-	0.00475±0.00345
Pt	-	-	-	<0.0002
Rb	7.0 (1)	7.0 (10) [53]	7.0 (10) [53]	8.96±3.19
Sb	-	-	-	0.140±0.117
Sc	-	-	-	0.0286±0.0451
Se	1.88 (4)	0.316 (46) [48]	3.16±2.88 (9) [51]	3.01±2.43
Sm	-	-	-	0.00252±0.00263
Sn	-	-	-	0.0756±0.0443
Tb	-	-	-	<0.0001
Te	-	-	-	<0.007
Th	-	-	-	0.0229±0.0293
Ti*	63.6 (1)	63.6±6.4 (4) [49]	63.6±6.4 (4) [49]	<0.4

Tl	-	-	-	0.00238±0.00164
Tm	-	-	-	<0.0003
U	0.00052 (1)	0.00052 (46) [48]	0.00052 (46) [48]	0.00083±0.00035
Y	-	-	-	0.0115±0.0140
Yb	-	-	-	0.000375±0.000236
Zn	68.5 (8)	23.1 (2) [54]	1236±560 (2) [51]	129±58
Zr	-	-	-	0.080±0.059

M –arithmetic mean, SD – standard deviation, Min – minimum, Max – maximum, (n)* – number of all references, (n)** – number of samples, “-“ – no information.

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

The comparison of our results with published data for TE mass fraction in adenomatous thyroid [47-54] is shown in Table 4.

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 adenomatous thyroid are presented in Table 5.

Element	Thyroid tissue				Ratio
	Norm n=105	Adenoma n=19	Student’s t-test $p \leq$	U-test p	Adenoma to Norm
Ag	0.0133±0.0013	0.181±0.050	**0.0058**	**≤0.01**	13.6
Al	10.5±1.8	34.2±9.1	**0.040**	**≤0.01**	3.26
B	0.476±0.058	3.38±1.12	**0.048**	**≤0.01**	7.10
Be	0.00052±0.00008	0.00181±0.00090	0.210	>0.05	3.48
Bi	0.0072±0.0022	0.112±0.064	0.164	>0.05	15.6
Cd	2.08±0.27	2.78±0.95	0.501	>0.05	1.34
Ce	0.0080±0.0011	0.0246±0.0090	0.152	>0.05	3.08
Co	0.0390±0.0031	0.0660±0.0135	0.075	>0.05	1.69
Cr	0.495±0.031	1.18±0.24	**0.0037**	**≤0.01**	2.38
Cs	0.0245±0.0022	0.0216±0.0050	0.507	>0.05	0.88
Er	0.000377±0.000050	0.00400±0.00200	0.072	>0.05	10.6
Fe	222.8±9.6	571±174	0.066	**≤0.05**	2.57
Ga	0.0316±0.0021	0.0223±0.0050	0.148	>0.05	0.71
Hg	0.0543±0.0043	1.16±0.34	**0.0060**	**≤0.01**	21.4
La	0.00475±0.00062	0.0116±0.0060	0.378	>0.05	2.44
Li	0.0208±0.0022	0.0401±0.0100	0.103	>0.05	1.93
Mn	1.28±0.07	1.67±0.54	0.488	>0.05	1.30
Mo	0.0836±0.0062	0.233±0.055	**0.034**	**≤0.01**	2.79
Nd	0.0041±0.0004	0.0141±0.0030	0.062	>0.05	3.44
Ni	0.449±0.046	3.95±1.39	0.053	>0.05	8.80
Pb	0.233±0.033	1.86±1.24	0.239	>0.05	7.98
Pr	0.00107±0.00011	0.00475±0.00200	0.122	>0.05	4.44
Rb	7.54±0.39	8.96±0.82	0.134	>0.05	1.19
Sb	0.0947±0.0075	0.140±0.034	0.219	>0.05	1.48
Sc	0.0268±0.0060	0.0286±0.0140	0.909	>0.05	1.07
Se	2.22±0.14	3.01±0.65	0.249	>0.05	1.36
Sm	0.000507±0.000064	0.00252±0.00099	0.089	>0.05	4.97
Sn	0.0777±0.0091	0.0756±0.0170	0.917	>0.05	0.97

Tl	0.000932±.000068	0.00238±0.00067	0.083	>0.05	2.55
U	0.000443±0.000059	0.00083±0.00020	0.184	>0.05	1.87
Y	0.00260±0.00032	0.0115±0.0060	0.229	>0.05	4.42
Zn	94.8±4.2	129±13	**0.023**	**≤0.01**	1.36

M – arithmetic mean, SEM – standard error of mean, Statistically significant values are in bold

Table 5: Differences between mean values (M±SEM) of trace element mass fractions (mg/kg, dry mass basis) in normal and adenomatous thyroid

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 adenomatous thyroid are presented in Table 5.

Discussion

Precision and accuracy of results

A good agreement of our results for the TE mass fractions with the certified values of CRM IAEA H-4, IAEA HH-1, INCT-SBF-4, INCT-TL-1, and INCT-MPH-2, as was shown in previous studies [30,36,42], 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 acceptable precision and accuracy of the results obtained in the study and presented in Tables 2-5.

Comparison with published data

Published data on TE of TA are very limited (Table 4). A number of values for TE mass fractions presented in Table 4 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%) [55] and ash (4.16% on dry mass basis) [56] contents in thyroid of adults.

In adenomatous tissues (Table 4) our results were comparable with published data for Ag, Cd, Fe, Mn, Mo, Ni, Pb, Rb, Se, U, and Zn contents. The obtained means for As were approximately four order of magnitude, for Co – three order of magnitude, for Cr, Hg, and Ti – two order of magnitude lower median of previously reported means and were outside the range of cited means (Table 4). No published data referring Al, Au, B, Be, Bi, Ce, Cs, Dy, Er, Eu, Ga, Gd, Ho, Ir, La, Li, Lu, Nb, Nd, Pd, Pr, Pt, Sb, Sc, Sm, Sn, Tb, Te, Th, Tl, Tm, Y, Yb, and Zr contents of adenomatous thyroid were found.

The ranges of means of TE content reported in the literature for normal [30, 36, 42] and for adenomatous thyroid (Tables 4) vary widely. This can be explained by a dependence of TE 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 adenoma stage. Not all these factors were strictly controlled in cited studies. Another and, in our opinion, the main reason for the inter-observer discrepancy can be attributed to the accuracy of the analytical techniques, sample preparation methods, and the inability to take standardized samples from 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 TE are lost as a result of this treatment That concern not only such volatile halogen as Br, but also other TE investigated in the study [57,58].

Effect of adenomatous transformation on trace element contents

From Table 5, it is observed that in adenomatous tissue the mass fraction of Ag, Al, B, Cr, Fe, Hg, Mo, and Zn are approximately 13.6, 3.3, 7.1, 2.4, 2.6, 21.4, 2.8, and 1.4 times, respectively, higher than in normal tissues of the thyroid. In contrast, the mass fraction of Cd, Ga, and Sn are 39%, 34%, and 41%, respectively, lower. Thus, if we accept the TE contents in thyroid glands in the control group as a norm, we have to conclude that with a adenomatous transformation the levels of Ag, Al, B, Cr, Fe, Hg, Mo, and Zn in affected thyroid tissue significantly increased.

Role of trace elements in adenomatous transformation of the thyroid

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

Our findings show that mass fraction of Ag, Al, B, Cr, Fe, Hg, Mo, and Zn are significantly different in TA as compared to normal thyroid tissues (Tables 5). Thus, it is plausible to assume that levels of these TE in affected thyroid tissue can be used as TA markers. However, this subjects needs in additional studies.

Limitations

This study has several limitations. Firstly, analytical techniques employed in this study measure only fifty TE mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of TE investigated in normal thyroid and adenomatous thyroid tissue. Secondly, the sample size of TA group was relatively small. It was not allow us to carry out the investigations of TE contents in TA group using differentials like gender, histological types of adenoma, stage of disease, and dietary habits of healthy persons and patients with TA. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on adenoma-specific tissue Ag, Al, B, Cr, Fe, Hg, Mo, and Zn level alteration and shows the necessity the need to continue TE research of adenomatous thyroid.

Conclusion

In this work, TE measurements in tissue samples from normal thyroid and TA were performed using two useful analytical methods: non-destructive neutron activation analysis with high-resolution long-lived radionuclide spectrometry and inductively coupled plasma mass spectrometry. The combination of these methods has been shown to be a suitable analytical tool for the determination of fifty TE (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) in tissue samples from healthy and affected human thyroid, including needle biopsy samples. It was observed that the content of Ag, Al, B, Cr, Fe, Hg, Mo, and Zn in adenomatous thyroid tissues increased significantly. In our opinion, the presented study data strongly suggest that TE plays an important role in thyroid health and the etiology of TA. It was assumed that the differences in TE levels in affected thyroid tissue could be used as TA markers.

Acknowledgements

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

Conflict of Interest

No conflict of interest applies to the work described in this manuscript.

Ethics 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.

Funding

There were no any sources of funding that have supported this work.

References

1. Kant R, Davis A, Verma V (2020). Thyroid nodules: Advances in evaluation and management. *Am Fam Physician, 102*(5), 298-304.
2. Derwahl M, Studer H (2000). Multinodular goitre: 'much more to it than simply iodine deficiency'. *Baillieres Best Pract Res Clin Endocrinol Metab , 14*(4), 577-600.
3. Zaichick V (1998). Iodine excess and thyroid cancer. *J Trace Elements in Experimental Medidicne, 11*(4), 508-509.
4. Zaichick V, Iljina T (1998). Dietary iodine supplementation effect on the rat thyroid 131I blastomogenic action. In: Die Bedentung der Mengen- und Spurenelemente. 18. Arbeitstangung. *Friedrich-Schiller-Universität, Jena*, 294-306.
5. Kim S, Kwon YS, Kim JY, Hong KH, Park YK (2019). Association between Iodine Nutrition Status and Thyroid Disease-Related Hormone in Korean Adults: Korean National Health and Nutrition Examination Survey VI (2013-2015). *Nutrients, 11*(11), 2757.
6. Stojsavljević A, Rovčanin B, Krstić D, Jagodić J, Borković-Mitić S, Paunović I, et al (2019). Cadmium as main endocrine disruptor in papillary thyroid carcinoma and the significance of Cd/Se ratio for thyroid tissue pathophysiology. *J Trace Elem Med Biol,* 55, 190-195.
7. Liu M, Song J, Jiang Y, Lin Y, Peng J, Liang H, et al (2021). A case-control study on the association of mineral elements exposure and thyroid tumor and goiter. *Ecotoxicol Environ Saf*, 208, 111615.
8. Zaichick V (2006). Medical elementology as a new scientific discipline. *J Radioanal Nucl Chem*, 269, 303-309.
9. Moncayo R, Moncayo H (2017). A post-publication analysis of the idealized upper reference value of 2.5 mIU/L for TSH: Time to support the thyroid axis with magnesium and iron especially in the setting of reproduction medicine. *BBA Clin, 7*, 115–119.
10. Martinez-Zamudio R, Ha HC (2011). Environmental epigenetics in metal exposure. *Epigenetics, 6*(7), 820-827.
11. Zaĭchik VE, Raibukhin YuS, Melnik AD, Cherkashin VI (1970). Neutron-activation analysis in the study of the behavior of iodine in the organism. *Med Radiol (Mosk), 15*(1), 33-36.
12. Zaĭchik VE, Matveenko EG, Vtiurin BM, Medvedev VS (1982). Intrathyroid iodine in the diagnosis of thyroid cancer. *Vopr Onkol, 28*(3), 18-24.
13. Zaichick V, Tsyb AF, Vtyurin BM (1995). Trace elements and thyroid cancer. *Analyst, 120*(3), 817-821.
14. Zaichick VYe, Choporov YuYa (1996). Determination of the natural level of human intra-thyroid iodine by instrumental neutron activation analysis. *J Radioanal Nucl Chem, 207*(1), 153-161.
15. Zaichick V (1998). In vivo and in vitro application of energy-dispersive XRF in clinical investigations: experience and the future. *J Trace Elements in Experimental Medidicne , 11*(4), 509-510.

16. Zaichick V, Zaichick S (1999). Energy-dispersive X-ray fluorescence of iodine in thyroid puncture biopsy specimens. *J Trace Microprobe Tech, 17*(2), 219-232.

17. Zaichick V (2000). Relevance of, and potentiality for in vivo intrathyroidal iodine determination. *Ann N Y Acad Sci*, 904, 630-632.

18. Zaichick V, Zaichick S (1997). Normal human intrathyroidal iodine. *Sci Total Environ, 206*(1), 39-56.

19. Zaichick V (1999). Human intrathyroidal iodine in health and non-thyroidal disease. In: New aspects of trace element research (Eds: M.Abdulla, M.Bost, S.Gamon, P.Arnaud, G.Chazot). Smith-Gordon, London, and Nishimura,Tokyo, p.114-119.

20. Zaichick V, Zaichick S (2017). Age-related changes of some trace element contents in intact thyroid of females investigated by energy dispersive X-ray fluorescent analysis. *Trends Geriatr Healthc, 1*(1), 31-38.

21. Zaichick V, Zaichick S (2017). Age-related changes of some trace element contents in intact thyroid of males investigated by energy dispersive X-ray fluorescent analysis. *MOJ Gerontol Ger, 1*(5), 00028.

22. Zaichick V, Zaichick S (2017). 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* 1, 5.1.

23. Zaichick V, Zaichick S (2017). 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, 1*(1):1002.

24. Zaichick V, Zaichick S (2017). 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*, 3, 015.

25. Zaichick V, Zaichick S (2017). 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 Biosci, 4*(4), 555644.

26. Zaichick V. and Zaichick S (2018). Effect of age on chemical element contents in female thyroid investigated by some nuclear analytical methods. *MicroMedicine, 6*(1), 47-61.

27. Zaichick V, Zaichick S (2018). Neutron activation and X-ray fluorescent analysis in study of association between age and chemical element contents in thyroid of males. *Op Acc J Bio Eng Bio Sc, 2*(4), 202-212.

28. Zaichick V, Zaichick S (2018). Variation with age of chemical element contents in females' thyroids investigated by neutron activation analysis and inductively coupled plasma atomic emission spectrometry. *J Biochem Analyt Stud, 3*(1), 1-10.

29. Zaichick V and Zaichick S (2018). Association between Age and Twenty Chemical Element Contents in Intact Thyroid of Males. *SM Gerontol Geriatr Res, 2*(1), 1014.

30. Zaichick V, Zaichick S (2018). Associations between age and 50 trace element contents and relationships in intact thyroid of males. *Aging Clin Exp Res, 30*(9), 1059–1070.

31. Zaichick V, Zaichick S (2018). Possible role of inadequate quantities of intra-thyroidal bromine, rubidium and zinc in the etiology of female subclinical hypothyroidism. *EC Gynaecology, 7*(3), 107-115.

32. Zaichick V, Zaichick S (2018). Possible role of inadequate quantities of intra-thyroidal bromine, calcium and magnesium in the etiology of female subclinical hypothyroidism. *Int Gyn and Women's Health, 1*(3), IGWHC.MS.ID.000113.

33. Zaichick V, Zaichick S (2018). Possible role of inadequate quantities of intra-thyroidal cobalt, rubidium and zinc in the etiology of female subclinical hypothyroidism. *Womens Health Sci J, 2*(1), 000108.

34. Zaichick V, Zaichick S (2018). Association between female subclinical hypothyroidism and inadequate quantities of some intra-thyroidal chemical elements investigated by X-ray fluorescence and neutron activation analysis. *Gynaecology and Perinatolog, 2*(4), 340-355.

35. Zaichick V, Zaichick S (2018). Investigation of association between the high risk of female subclinical hypothyroidism and inadequate quantities of twenty intra-thyroidal chemical elements. *Clin Res: Gynecol Obstet, 1*(1), 1-18.

36. Zaichick V, Zaichick S (2018). Investigation of association between the high risk of female subclinical hypothyroidism and inadequate quantities of intra-thyroidal trace elements using neutron activation and inductively coupled plasma mass spectrometry. *Acta Scientific Medical Sciences, 2*(9), 23-37.

37. Zaichick V. Zaichick S (2018). Trace element contents in thyroid cancer investigated by energy dispersive X-ray fluorescent analysis. *American Journal of Cancer Research and Reviews*, 2, 5.

38. Zaichick V., Zaichick S (2018). Trace element contents in thyroid cancer investigated by instrumental neutron activation analysis. *J Oncol Res, 2*(1), 1-13.

39. Zaichick V, Zaichick S (2018). Variation in selected chemical element contents associated with malignant tumors of human thyroid gland. *Cancer Studies, 2*(1), 2.

40. Zaichick V, Zaichick S (2018). Twenty chemical element contents in normal and cancerous thyroid. *Int J Hematol Blo Dis, 3*(2), 1-13.

41. Zaichick V, Zaichick S (2018). Levels of chemical element contents in thyroid as potential biomarkers for cancer diagnosis (a preliminary study). *J Cancer Metastasis Treat, 4*, 60.

42. Zaichick V, Zaichick S (2018). Fifty trace element contents in normal and cancerous thyroid. *Acta Scientific Cancer Biology , 2*(8), 21-38.

43. Zaichick V, Zaichick S (1996). Instrumental effect on the contamination of biomedical samples in the course of sampling. *The Journal of Analytical Chemistry, 51*(12), 1200-1205.

44. Zaichick V, Zaichick S (1997). A search for losses of chemical elements during freeze-drying of biological materials. *J Radioanal Nucl Chem, 218*(2), 249-253.

45. Zaichick V (1995). Applications of synthetic reference materials in the Medical Radiological Research Centre. *Fresenius J Anal Chem, 35*2, 219-223.

46. Korelo AM, Zaichick V (1993). Software to optimize the multielement INAA of medical and environmental samples. In: Activation Analysis in Environment Protection. *Joint Institute for Nuclear Research, Dubna, Russia*, p.326-332.

47. Predtechenskaya VC (1975). Nucleic acids and trace elements in thyroid pathology. *Proceedings of the Voronezh Medical Faculty*, 94, 85-87.

48. Stojsavljević A, Rovčanin B, Krstić D, Borković-Mitić S, Paunović I, Kodranov I, et al (2019). Evaluation of trace metals in thyroid tissues: Comparative analysis with benign and malignant thyroid diseases. *Ecotoxicol Environ Saf*, 183, 109479.

49. Reddy SB, Charles MJ, Kumar MR, Reddy BS, Anjaneyulu Ch, Raju GJN, Sundareswar B, Vijayan V (2002). Trace elemental analysis of adenoma and carcinoma thyroid by PIXE method. Nucl Instrum Methods Phys Res B: Beam *Interactions with Materials and Atoms, 196*(3-4), 333-339.

50. Yaman M (2005). The improvement of sensitivity in lead and cadmium determinations using flame atomic absorption spectrometry. *Anal Biochem, 339*(1), 1-8.

51. Zagrodzki P, Nicol F, Arthur JR, Słowiaczek M, Walas S, Mrowiec H, Wietecha-Posłuszny R (2010). Selenoenzymes, laboratory parameters, and trace elements in different types of thyroid tumor. *Biol Trace Elem Res, 134*(1), 25-40.

52. Maeda K, Yokode Y, Sasa Y, Kusuyama H, Uda M. Multielemental analysis of human thyroid glands using particle induced X-ray emission (PIXE) (1987). *Nucl Instrum Methods Phys Res B, 22*(1-3), 188-190.

53. Kvicala, Havelka J, Zeman J, Nemec J (1991). Determination of some trace elements in the thyroid gland by INAA. *J. Radioanal. Nucl. Chem, 149*(2), 267-274.

54. Koch HJ, Smith ER (1956). The determination of copper and zinc in normal and pathologic thyroid tissue. *J Clin Endocrinol, 16*(1), 23-129.

55. Katoh Y, Sato T, Yamamoto Y (2002). Determination of multielement concentrations in normal human organs from the Japanese. *Biol Trace Elem Res, 90*(1-3), 57-70.

56. Schroeder HA, Tipton IH, Nason AP (1972). Trace metals in man: strontium and barium. *J Chron Dis, 25*(9), 491-517.

57. Zaichick V (1997). 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. *IAEA*, Vienna, p. 123-133.

58. Zaichick V (2004). Losses of chemical elements in biological samples under the dry aching process. *Trace Elements in Medicine, 5*(3), 17–22.