

Evaluation of twenty chemical element contents in thyroid adenomas using neutron activation analysis and inductively coupled plasma atomic emission spectrometry

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Abstract

Thyroid adenomas (TA) are benign tumors, but there is a 20% possibility of malignant transformation. The distinguishing between the TA and thyroid cancer (TC) is tricky, therefore new TA biomarkers are needed. Furthermore, the role of chemical elements (ChE) in etiology and pathogenesis of TA is unclear. The aim of this exploratory study was to evaluate whether significant changes in the thyroid tissue levels of twenty chemical elements (ChE) Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn exist in the adenomatous transformed thyroid. Thyroid tissue levels of twenty ChE 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 atomic emission spectrometry, respectively. Tissue samples were divided into two portions. One was used for morphological study while the other was intended for ChE analysis. It was found that contents of Al, B, Br, Cl, Cu, Na, and Zn are significantly higher whereas the levels of I some lower. in TA than in normal tissues. It was supposed that the changes in levels Al, B, Br, Cl, Cu, I, Na, and Zn in thyroid tissue can be used as TA markers.

Keywords: Thyroid adenomas; Intact thyroid; Chemical elements; Biomarkers for adenoma diagnosis; Instrumental neutron activation analysis; Inductively coupled plasma atomic emission spectrometry

1. Introduction

Thyroid adenomas (TA) are homogenous, solitary, encapsulated benign tumors, more common in females, and have a good prognosis [1]. 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 [2,3].

For over 20th century, there was the dominant opinion that NG, including TA, is the simple consequence of iodine (I) deficiency. However, it was found that NG is a frequent disease even in those countries and regions where the population is never exposed to I shortage [4]. Moreover, it was shown that I excess has severe consequences on human health and associated with the presence of thyroidal dysfunctions and autoimmunity, nodular and diffuse goiters, adenomas and malignant tumors of gland [5-8]. It was also demonstrated that besides the I deficiency and excess many other dietary, environmental, and occupational factors are associated with the NG incidence [9-11]. Among them a disturbance of evolutionary stable input of many (ChE) in human body after industrial revolution plays a significant role in etiology of thyroidal disorders [12].

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Besides I involved in thyroid function, other ChE 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 [13]. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of ChE depend on tissue-specific need or tolerance, respectively [13]. Excessive accumulation or an imbalance of the ChE may disturb the cell functions and may result in cellular degeneration, death, benign or malignant transformation [13-15].

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 [16-22]. Level of I in the normal thyroid was investigated in relation to age, gender and some non-thyroidal diseases [23,24]. 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 [25-41]. Furthermore, a significant difference between some ChE contents in normal and cancerous thyroid was demonstrated [42-47].

To date, 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. Having this in mind, our aim was to assess the aluminum (Al), boron (B), barium (Ba), bromine (Br), calcium (Ca), chlorine (Cl), copper (Cu), iron (Fe), I, potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), sulfur (S), silicon (Si), strontium (Sr), vanadium (V), and zinc (Zn) mass fraction contents in TA tissue using a combination of non-destructive and destructive methods: instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) and inductively coupled plasma atomic emission spectrometry (ICPAES), respectively. A further aim was to compare the levels of these twenty ChE in the adenomatous thyroid with those in normal gland of apparently healthy persons.

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre (MRRC), Obninsk. All the 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 with comparable ethical standards.

2. Material and methods

All patients suffered from TA (n=19, 16 females and 3 males, mean age $M \pm SD$ was 41 ± 11 years, range 22-55) 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 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. Samples were obtained within 48 hours after a sudden death. The majority of deaths were due to trauma. A histological examination in the control group was used to control the age norm conformity, as well as to confirm the absence of micro-nodules and latent cancer.

All tissue samples were divided into two portions using a titanium scalpel [48]. One was used for morphological study while the other was intended for ChE analysis. After the samples intended for ChE analysis were weighed, they were freeze-dried and homogenized [49].

The pounded samples weighing about 10 mg (for biopsy) and 100 mg (for resected materials) were used for ChE measurement by INAA-SLR. The content of Br, Ca, Cl, I, K, Mg, Mn, and Na were determined by INAA-SLR using a horizontal channel equipped with the pneumatic rabbit system of the WWR-c research nuclear reactor (Branch of Karpov Institute, Obninsk). After non-destructive INAA-SLR investigation the thyroid samples were used for ICP-AES. The samples were decomposed in autoclaves. Simultaneously, the same procedure was performed in autoclaves without tissue samples (containing only $HNO_3 + H_2O_2 +$ deionized water), and the resultant solutions were used as control samples. Sample aliquots were used to determine the Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractions by ICP-AES using the Spectrometer ICAP-61 (Thermo Jarrell Ash, USA). The determination of the ChE content in aqueous solutions was made by the quantitative method using calibration solutions (High Purity Standards, USA) of 0.5 and 10 mg/L of each element. The calculations of the ChE content in the probe were carried out using software of a spectrometer (ThermoSPEC, version 4.1).

Information detailing with the NAA-SLR and ICP-AES methods used and other details of the analysis were presented in our earlier publications concerning ChE contents in human thyroid, scalp hair, and prostate[33,34,50-55].

To determine contents of the ChE by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [56]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. Ten sub-samples of certified reference material (CRM) IAEA H-4 (animal muscle) and five sub-samples of CRM of the Institute of Nuclear Chemistry and Technology (INCT, Warszawa, Poland) INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2 Mixed Polish Herbs were treated and analyzed in the same conditions that thyroid samples to estimate the precision and accuracy of results.

A dedicated computer program for INAA mode optimization was used [57]. All thyroid samples were prepared in duplicate, and mean values of ChE contents were used. Mean values of ChE contents were used in final calculation for the Br, Fe, Rb, and Zn mass fractions measured by two methods. Using Microsoft Office Excel, a summary of the statistics, including, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for ChE contents. The difference in the results between two groups (normal thyroid and TA) was evaluated by the parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test.

3. Results

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

Table 1 INAA-SLR data of chemical element contents in the IAEA H-4 (animal muscle) reference material compared to certified values (mg/kg on dry mass basis)

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

Mean - arithmetical mean, SD - standard deviation, C - certified values, N - non-certified values

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

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

Table 2 ICP-AES data of chemical element contents in Certified Reference Materials ($M \pm SD$, mg/kg on dry mass basis)

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

M - arithmetic mean, SD - standard deviation, ^a Informative values**Table 3** Comparison of the mean values ($M \pm SEM$) of the chemical element mass fractions (mg/kg, on dry-mass basis) in the normal human thyroid (males and females combined) obtained by both NAA-SLR and ICP-AES methods

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

M - arithmetic mean, SEM - standard error of mean, $\Delta = [(M_1 - M_2)/M_1] \cdot 100\%$.

Table 4 presents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction in normal and adenomatous thyroid.

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

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Normal n=105	Al	10.5	13.4	1.8	0.800	69.3	6.35	1.19	52.9
	B	0.476	0.434	0.058	0.200	2.30	0.300	0.200	1.73
	Ba	1.12	1.15	0.15	0.0480	5.00	0.680	0.0838	4.48
	Br	14.9	11.0	1.2	1.90	54.1	11.6	2.56	49.3
	Ca	1682	999	106	373	5582	1454	444	4183
	Cl	3400	1452	174	1030	6000	3470	1244	5869
	Cu	4.08	1.22	0.14	0.500	7.15	4.10	1.57	6.41
	Fe	223	95	10	52.0	489	210	72.8	432
	I	1841	1027	107	114	5061	1695	230	4232
	K	6418	2625	290	1914	15293	5948	2947	13285
	Li	0.0208	0.0155	0.0022	0.0015	0.0977	0.0178	0.0041	0.0487
	Mg	296	134	16	66.0	930	284	95.8	541
	Mn	1.28	0.56	0.07	0.470	4.04	1.15	0.537	2.23
	Na	6928	1730	175	3686	13453	6835	3974	10709
	P	4290	1578	207	496	8996	4221	1360	7323
	S	8259	2002	263	644	11377	8399	3662	11208
	Si	50.8	46.9	6.2	5.70	180	36.0	7.11	174
	Sr	3.81	2.93	0.34	0.100	12.6	2.90	0.365	11.3
	V	0.102	0.039	0.005	0.0200	0.250	0.100	0.0440	0.192
Zn	94.8	39.6	4.2	7.10	215	88.5	34.9	196	
Adenoma n=19	Al	34.3	24.1	9.1	8.70	78.4	30.6	9.53	74.1
	B	3.38	2.74	1.12	1.00	7.30	3.00	1.00	7.01
	Ba	3.06	4.07	1.54	0.410	11.7	1.40	0.454	10.7
	Br	394	397	125	11.6	1080	203	22.5	1033
	Ca	1370	1030	311	52.0	3582	1252	136	3353
	Cl	7722	3785	1262	1757	13824	9085	2043	113179
	Cu	17.6	14.0	5.7	4.10	35.2	13.8	4.28	35.0
	Fe	429	405	108	52.3	1360	335	53.3	1326
	I	962	1013	232	131	3906	476	170	3591
	K	5603	2727	756	797	10099	5741	937	9600
	Li	0.0401	0.0236	0.0100	0.0185	0.0680	0.0341	0.0186	0.0678
	Mg	236	108	30	15.0	397	269	36.9	376
	Mn	1.67	1.88	0.54	0.100	6.12	0.805	0.210	5.50
	Na	9747	4746	1316	2319	18734	9100	2728	18038
	P	4930	1945	735	2982	8932	4300	3072	8395
	S	10536	2968	1122	7865	16706	9486	8017	15970
	Si	114	106	40	15.2	346	83.7	24.6	310
	Sr	3.30	2.18	0.63	0.420	6.70	2.85	0.582	6.69
	V	0.140	0.041	0.020	0.110	0.200	0.125	0.111	0.195
Zn	129	50	13	57.7	251	137	61.3	225	

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

Tissue	Published data [Reference]			This work
Element	Median of means (n)*	Minimum of means M or M±SD, (n)**	Maximum of means M or M±SD, (n)**	Males and females M±SD
Norm				
Al	33.6 (12)	0.33 (-) [58]	420 (25) [59]	10.5±13.4
B	0.151 (2)	0.084 (3) [60]	0.46 (3) [60]	0.476±0.434
Ba	0.67 (7)	0.0084 (83) [61]	≤5.0 (16) [62]	1.12±1.15
Br	18.1 (11)	5.12 (44) [63]	284±44 (14) [64]	16.3±11.6
Ca	1600 (17)	840±240 (10) [65]	3800±320 (29) [65]	1663±999
Cl	6800 (5)	804±80 (4) [66]	8000 (-) [67]	3400±1452
Cu	6.0 (61)	0.16 (83) [61]	220±22 (10) [66]	3.93±1.43
Fe	252 (21)	56 (120) [68]	3360 (25) [59]	223±95
I	1888 (95)	159±8 (23) [69]	5772±2708 (50) [70]	1841±1027
K	4300 (17)	46.4±4.8 (4) [66]	6090 (17) [62]	6418±2625
Li	6.3 (2)	0.092 (-) [71]	12.6 (180) [72]	0.0208±0.0154
Mg	390 (16)	3.5 (-) [58]	1520 (20) [73]	296±134
Mn	1.62 (40)	0.076 (83) [61]	69.2±7.2 (4) [66]	1.28±0.56
Na	8000 (9)	438 (-) [74]	10000±5000 (11) [75]	6928±1730
P	2860 (10)	16 (7) [76]	7520 (60) [63]	4290±1578
S	11000 (3)	4000 (-) [67]	11800 (44) [63]	8259±2002
Si	16.0 (3)	0.97 (-) [58]	143±6 (40) [77]	50.8±46.9
Sr	0.61 (9)	0.055 (83) [61]	46.8±4.8 (4) [66]	3.81±2.93
V	0.065 (6)	0.0124 (2) [78]	18±2 (4) [66]	0.102±0.039
Zn	110 (56)	2.1 (-) [58]	820±204 (14) [64]	94.8±39.7
Adenoma				
Al	-	-	-	34.3±24.1
B	-	-	-	3.38±2.74
Ba	-	-	-	3.06±4.07
Br	38 (4)	11 (5) [79]	777 (1) [80]	394±397
Ca	2298(4)	900 (1) [65]	3500 (1) [65]	1370±1030
Cl	864 (1)	864±84 (4) [66]	864±84 (4) [66]	7722±3785
Cu	11.0 (7)	1.24 (46) [81]	29 (5) [79]	17.6±14.0
Fe	92.5 (4)	15 (5) [79]	2100±208 (4) [66]	429±405
I	640(13)	80 (1) [79]	2800 (1) [82]	962±1013

K	3650 (3)	72,8±7,2 (4) [66]	5600 (1) [79]	5603±2727
Li	-	-	-	0.0401±0.0236
Mg	-	-	-	236±108
Mn	1.28 (4)	0.40 (46) [81]	57,6±6,0 (4) [66]	1.67±1.88
Na	-	-	-	9747±4746
P	-	-	-	4930±1945
S	-	-	-	10536±2968
Si	-	-	-	114±106
Sr	27.2 (1)	27.2±2.4 (4) [66]	27.2±2.4 (4) [66]	3.30±2.18
V	16.8 (1)	16.8±1.6 (4) [66]	16.8±1.6 (4) [66]	0.140±0.041
Zn	68.5 (8)	21.0 (130) [83]	330±282 (9) [84]	129±50

M – arithmetic mean, SD – standard deviation, (n)* – number of all references, (n)** – number of samples.

The comparison of our results with published data for Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction in normal and adenomatous thyroid [58-84] is shown in Table 5.

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

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

Element	Thyroid tissue				Ratio Adenoma to Norm
	Norm n=105	Adenoma n=19	Student's t-test <i>p</i> ≤	U-test <i>p</i>	
Al	10.5±1.8	34.3±9.1	0.040	≤ 0.01	3.27
B	0.476±0.058	3.38±1.12	0.048	≤ 0.01	7.10
Ba	1.12±0.15	3.06±1.54	0.256	>0.05	2.73
Br	14.9±1.2	394±125	0.014	≤ 0.01	26.4
Ca	1682±106	1370±311	0.360	>0.05	0.81
Cl	3400±174	7722±1262	0.0089	≤ 0.01	2.27
Cu	4.08±0.14	17.6±5.7	0.064	≤ 0.05	4.31
Fe	223±10	429±108	0.081	>0.05	1.92
I	1841±107	962±232	0.0020	≤ 0.01	0.52
K	6418±290	5603±756	0.330	>0.05	0.87
Li	0.0208±0.0022	0.0401±0.0100	0.103	>0.05	1.93
Mg	296±16	236±30	0.088	>0.05	0.80
Mn	1.28±0.07	1.67±0.54	0.488	>0.05	1.30
Na	6928±175	9747±1316	0.054	≤ 0.05	1.41
P	4290±207	4930±735	0.430	>0.05	1.15
S	8259±263	10536±1122	0.091	>0.05	1.28
Si	50.8±6.2	114±40	0.165	>0.05	2.24
Sr	3.81±0.34	3.30±0.63	0.482	>0.05	0.87
V	0.102±0.005	0.140±0.020	0.159	>0.05	1.37
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.

4. Discussion

4.1. Precision and accuracy of results

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

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

4.2. Comparison with published data

The means obtained for Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction, as shown in Table 5, agree well with the medians of mean values reported by other researches for the human thyroid, including samples received from persons who died from different non-thyroid diseases. The mean obtained for Li is two orders of magnitude lower than the median of previously reported data. Moreover, it is outside the range of previously reported means. A number of values for ChE mass fractions were not expressed on a dry mass basis by the authors of the cited references. Hence we calculated these values using published data for water 75% [85] and ash 4.16% on dry mass basis [86] contents in thyroid of adults.

In adenomatous tissues our results for Br, Ca, Cu, Fe, I, Mn, and Zn contents were within the range of published means, while mean for K was some higher the upper level of the previously reported range of means (Table 5). Only one published article on Cl, Sr, and V contents in the adenomatous thyroid samples was found in the literature [66]. The mean obtained in the present study for Sr and V content in the adenomatous thyroid were approximately one and two order of magnitude, respectively, lower, whereas for Cl was one order of magnitude higher than published results. No published data referring Al, B, Ba, Li, Mg, Na, P, S, and Si contents of adenomatous thyroid were found.

The range of means of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn level reported in the literature for normal and for adenomatous thyroid vary widely (Table 5). This can be explained by a dependence of ChE content on many factors, including “normality” of thyroid samples (see above), the region of the thyroid, from which the sample was taken, age, gender, ethnicity, mass of the gland, and the adenoma stage, histology and functional activity. 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 sample preparation methods some quantities of certain ChE are lost as a result of this treatment That concern not only such volatile halogen as Br, but also other ChE investigated in the study [87,88].

4.3. Effect of adenomatous transformation on ChE contents

From Table 6, it is observed that in TA the mass fraction of Al, B, Br, Cl, Cu, Na and Zn are approximately 3.3, 7.1, 26.4, 2.3, 4.3, 1.41, and 1.4 times, respectively, higher than in normal tissues of the thyroid. In contrast, the mass fraction of I is 48% lower. Thus, if we accept the ChE contents in thyroid glands in the control group as a norm, we have to conclude that with a adenomatous transformation the levels of Al, B, Br, Cl, Cu, Na, and Zn in thyroid tissue significantly increased, whereas the level of I decreased.

4.4. Role of ChE in adenomatous transformation of the thyroid

Characteristically, elevated or reduced levels of ChE 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 ChE in adenomatous thyroid researchers try to determine the role of the deficiency or excess of each ChE in the TA etiology. In our opinion, abnormal levels of many ChE in TA could be and cause, and also effect of adenomatous transformation. From the results of such kind studies, it is not always possible to decide whether the measured decrease or increase in ChE level in pathologically altered tissue is the reason for alterations or vice versa.

4.4.1. 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 [89].

4.4.2. Boron

Trace element B is known to influence the activity of many enzymes [90]. 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 [91]. Why B content in adenomatous thyroid is higher than normal level and how an excess of B acts on thyroid are still to be cleared.

4.4.3. Bromine

This is one of the most abundant and ubiquitous of the recognized trace elements in the biosphere. Inorganic bromide is the ionic form of Br which exerts therapeutic as well as toxic effects. An enhanced intake of bromide could interfere with the metabolism of I at the whole-body level. In the thyroid gland the biological behavior of bromide is more similar to the biological behavior of iodide [92].

In our previous studies, we found a significant age-related increase of Br content in human thyroid [27,28,31-34]. Therefore, a goitrogenic and tumorigenic effect of excessive Br levels in the thyroid of old females was assumed. On the one hand, elevated levels of Br in TA, observed in the present study, supports this conclusion. But, on the other hand, bromide compounds, especially potassium bromide (KBr), sodium bromide (NaBr), and ammonium bromide (NH₄Br), are frequently used as sedatives in Russia [93]. It may be the reason for elevated levels of Br in specimens of patients with TA.

4.4.4. Chlorine

Cl is a ubiquitous, extracellular electrolyte essential to more than one metabolic pathway. Cl exists in the ionic form (chloride) in the human body. In the body, it is mostly present as sodium chloride. Therefore, as usual, there is a correlation between Na and Cl contents in tissues and fluids of human body. It is well known that Cl mass fractions in samples depend mainly on the extracellular water volume, including the blood volumes, in tissues [94]. TA tissues are predominantly highly vascularized lesions [95]. Thus, it is possible to speculate that TA are characterized by an increase of the mean value of the Cl mass fraction because the level of adenoma vascularization is higher than that in normal thyroid tissue.

4.4.5. Copper

Cu is a ubiquitous element in the human body which plays many roles at different levels. Various Cu-enzymes (such as amine oxidase, ceruloplasmin, cytochrome-c oxidase, dopamine-monooxygenase, extracellular superoxide dismutase, lysyl oxidase, peptidylglycineamidating monooxygenase, Cu/Zn superoxide dismutase, and tyrosinase) mediate the effects of Cu deficiency or excess. Cu excess can have severe negative impacts. Cu generates oxygen radicals and many investigators have hypothesized that excess copper might cause cellular injury via an oxidative pathway, giving rise to enhanced lipid peroxidation, thiol oxidation, and, ultimately, DNA damage [96]. Thus, Cu accumulation in thyroid parenchyma with age may be involved in oxidative stress, dwindling gland function, and increasing risk of goiter, adenoma, or cancer [25,26,31,33,34]. The significantly elevated level of Cu in adenomatous thyroid, observed in the present study, supports this speculation. However, an overall comprehension of Cu homeostasis and physiology, which is not yet acquired, is mandatory to establish Cu exact role in the TA etiology and metabolism.

4.4.6. Iodine

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

4.4.7. Sodium

Knowledge concerning ion regulation in many normal and abnormal cell processes has had a rapid development. It was found, among other regulations, that sodium-calcium exchange is associated with the cytoskeleton and the cell membrane. A hypothesis was eventually established that a wide variety of pathological phenomena ranging from acute cell death to chronic processes, such as neoplasia, all have a common series of cellular reactions [97]. Furthermore, iodide (I^-), an essential constituent of the thyroid hormones, is actively transported into the thyroid via the Na^+/I^- symporter (NIS), a key plasma membrane glycoprotein [98]. In addition, Na is mainly an extracellular electrolyte and its elevated level in TA might link with a higher adenoma vascularization in comparison with the normal thyroid (see *Chlorine*).

4.4.8. Zinc

Zn is active in more than 300 proteins and over 100 DNA-binding proteins, including the tumor suppressor protein p53, a Zn-binding transcription factor acting as a key regulator of cell growth and survival upon various forms of cellular stress. p53 is mutated in half of human tumors and its activity is tightly regulated by metals and redox mechanisms. On the other hand, excessive intracellular Zn concentrations may be harmful to normal metabolism of cells [99]. By now much data has been obtained related both to the direct and indirect action of intracellular Zn on the DNA polymeric organization, replication and lesions, and to its vital role for cell division [100,101]. Other actions of Zn have been also described. They include its action as a potent anti-apoptotic agent [102-106]. All these facts allowed us to speculate that age-related overload Zn content in female thyroid, as was found in our previous study [25,29,31,33], is probably one of the factors in etiology of thyroid goiter, TA, and malignant tumors. Therefore, the elevated Zn level in TA in comparison with normal level, detected in this study, supports our hypothesis.

Our findings show that mass fraction of Al, B, Br, Cl, Cu, I, Na, and Zn are significantly different in TA as compared to normal thyroid tissues (Tables 6). Thus, it is plausible to assume that levels of these ChE in 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 twenty ChE (Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn) mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of ChE investigated in normal and adenomatous thyroid. Secondly, the sample size of TA group was relatively small. It was not allow us to carry out the investigations of ChE contents in TA group using differentials like gender, histological and functional 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 Al, B, Br, Cl, Cu, I, Na, and Zn level alteration and shows the necessity to continue ChE research of adenomatous thyroid.

5. Conclusion

In this work, ChE measurements were carried out in the tissue samples of normal thyroid and TA using the combination of non-destructive INAA-SLR and destructive ICP-AES methods. It was shown that the combination of these methods is an adequate analytical tool for the determination of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn content in the tissue samples of human thyroid, including needle-biopsy cores. It was observed that in adenomatous tissues content of Al, B, Br, Cl, Cu, Na, and Zn significantly increased whereas the level of I decreased in a comparison with the normal thyroid tissues. In our opinion, the increase in levels of Al, B, Br, Cl, Cu, Na, and Zn, as well as the decrease in level of I in adenomatous tissue might demonstrate an involvement of these ChE in etiology and pathogenesis of TA. It was supposed that the changes in levels Al, B, Br, Cl, Cu, I, Na, and Zn in thyroid tissue can be used as TA markers.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares that he has no competing interests.

Statement of ethical approval

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre (MRRC), Obninsk. All the 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 with comparable ethical standards.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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