Relationships between iodine and some chemical elements in normal thyroid of males investigated by neutron activation and inductively coupled plasma atomic emission spectrometry

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Abstract
Thyroid diseases rank second among endocrine disorders, and prevalence of the diseases is higher in the elderly as compared to the younger population. An excess or deficiency of chemical element contents in thyroid play important role in goitro- and carcinogenesis of gland. The correlations with age of the twenty chemical element (ChE), including I, and I/ChE content ratios, as well as inter-thyroidal relationships between ChE contents and I/ChE content ratios in normal thyroid of 73 males (mean age 37.3 years, range 2.0-80) was investigated by two methods: instrumental neutron activation analysis and inductively coupled plasma atomic emission spectrometry. Our data reveal that the I and Ca contents, as well as I/B, I/K, I/Li, I/Mg, I/Mn, and I/P content ratios increase, while Al, B, Ba, K, Li, Mn, and P decrease in the normal thyroid of male during a lifespan. Therefore, a goitrogenic and tumorogenic effect of excessive I and Ca level and inadequate Al, B, Ba, K, Li, Mn, and P level in the thyroid of old males, as well as a disturbance in intrathyroidal I/B, I/K, I/Li, I/Mg, I/Mn, and I/P relationships with increasing age may be assumed. Furthermore, it was found that the levels of Al, B, Ba, Br, Ca, Cl, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn in the thyroid gland are interconnected and depend on the content of I in it. Because I plays a decisive role in the function of the thyroid gland, the data obtained allow us to conclude that, along with I, such ChE as Al, B, Ba, Br, Ca, Cl, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn, if not directly, then indirectly, are involved in the process of thyroid hormone synthesis.

Keywords: thyroid; chemical elements; age-related changes; intrathyroidal chemical elements relationships; neutron activation analysis; inductively coupled plasma atomic emission spectrometry

Introduction

According to the World Health Organization (WHO), thyroid diseases rank second among endocrine disorders after diabetes mellitus. More than 665 million people in the world have endemic goiter or suffer from other thyroid pathologies. At the same time, according to the same statistics, the increase in the number of thyroid diseases in the world is 5% per year [1]. It has been suggested that risk factors for the development of thyroid disorders may be numerous factors, including genetics, radiation, autoimmune diseases, as well as adverse environmental factors, such as an increase in the content of various chemicals in the environment [2].

Chemical elements (ChE) are among these various chemicals, because their levels in the environment have increased significantly over the past hundred years as a result of the industrial revolution and the tremendous technological changes that have taken place in metallurgy, chemical production, electronics, agriculture, food processing and storage, cosmetics, pharmaceuticals and medicine. In connection with these changes, the levels and ratio of ChE entering the human body from the outside have been significantly disturbed, compared with the conditions in which human societies have lived for many millennia.

More than 50 years ago, we formulated the postulate about the somatic ChE homeostasis, which is now generally recognized [3]. According to this postulate, under evolutionary environmental conditions, the mechanisms of homeostasis of organisms maintain the levels and ratios of ChE in tissues and organs within certain limits. If the content of ChE in the environment changes significantly, the mechanisms of somatic homeostasis may respond inadequately. Inadequate response of homeostasis mechanisms leads to changes in ChE levels in tissues and organs, which, in turn, can affect their function and lead to the development of pathological conditions. The correctness of this conclusion was illustrated by us earlier on the example of the study of the role of ChE in the normal and pathophysiology of the prostate [4-24]. It was shown, in particular, that a special role in the development of pathological transformations of the prostate is played by disturbances in the relationship between ChE in the tissue and gland secretion. Moreover, it was found that changes in the relationship between ChE can be used as highly informative markers of various prostate diseases, including malignant tumors [25-39]. These findings stimulated our investigations of ChE relationships in thyroid tissue in normal and pathological conditions.

There are many studies regarding ChE content in human thyroid, using chemical techniques and instrumental methods [40-60]. However, among the published data, no works on the relationship of ChE in the normal human thyroid were found.

This work had three aims. The primary purpose of this study was to determine reliable values for the Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractions in the normal thyroid of subjects ranging from children to elderly males using instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) combined in consecutive order with destructive inductively coupled plasma atomic emission spectrometry (ICP-AES) and calculate individual values of I/Al, I/B, I/Ba, I/Br, I/Ca, I/Cl, I/Cu, I/Fe, I/K, I/Li, I/Mg, I/Mn, I/Na, I/P, I/S, I/Si, I/Sr, I/V, and I/Zn. The second aim was to compare the twenty ChE mass fractions in thyroid gland obtained in the study with published data. The final aim was to estimate the inter-thyroidal correlations between ChE contents and between I/ChE content ratios in normal thyroid of males and changes of these parameters with age.

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, 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.

Materials and Methods

Samples of the human thyroid were obtained from randomly selected autopsy specimens of 73 males (European-Caucasian) aged 2.0 to 80 years. All the deceased were citizens of Obninsk and had undergone routine autopsy at the Forensic Medicine Department of City Hospital, Obninsk. The available clinical data were reviewed for each subject. None of the subjects had a history of an intersex condition, endocrine disorder, or other chronic disease that could affect the normal development of the thyroid. None of the subjects were receiving medications or used any supplements known to affect thyroid ChE contents. The typical causes of sudden death of most of these subjects included trauma or suicide and also acute illness (cardiac insufficiency, stroke, embolism of pulmonary artery, alcohol poisoning). All right lobes of thyroid glands were divided into two portions using a titanium scalpel [61]. One tissue portion was reviewed by an anatomical pathologist while the other was used for the ChE content determination. A histological examination was used to control the age norm conformity as well as the unavailability of micro adenomatosis and latent cancer.

After the samples intended for ChE analysis were weighed, they were transferred to -20°C and stored until the day of transportation in the Medical Radiological Research Center, Obninsk, where all samples were freeze-dried and homogenized [62]. To determine the contents of the ChE by comparison with a known standard, aliquots of commercial, chemically pure compounds were used [63]. Ten subsamples of the Certified Reference Material (CRM) IAEA H-4 (animal muscle), as well as Polish CRM INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2 Mixed Polish Herbs were analyzed to estimate the precision and accuracy of results. The CRM subsamples were prepared in the same way as the samples of dry homogenized thyroid tissue.

A horizontal channel equipped with the pneumatic rabbit system of the WWR-C research nuclear reactor was applied to determine the mass fraction of Br, Ca, I, K, Mg, Mn, and Na by INAA-SLR. The neutron flux in the channel was 1.7×10^{13}n cm^{-2} s^{-1}. Method ICP-AES 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 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 tChE contents in the probe were carried out using software of a spectrometer (Thermo SPEC, version 4.1). Information detailing with the NAA-SLR and ICP-AES methods used, and other details of the analysis was presented in our previous studies on ChE contents in hair and prostate [64-70].

A dedicated computer program for INAA-SLR mode optimization was used [71]. All thyroid samples were prepared in duplicate, and mean values of ChE contents were used in final calculation. 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 and I/ ChE content ratios. Pearson's correlation coefficient was used in Microsoft Office Excel to calculate the relationship "age – ChE mass fraction" and" age – I/ChE mass fraction", as well as to identify inter-thyroidal relationships between different ChE contents and between different ChE content ratios.

Results

Table 1 depicts the similarity of the means of the Ca, K, Mg, Mn, and Na mass fractions in the normal thyroid of male determined by both NAA-SLR and ICP-AES methods.

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 obtained by both NAA-SLR and ICP-AES methods

Element	NAA-SLR M_1	ICP-AES M_2	Δ, %
Ca	1703±131	1647±118	3.3
K	6289±329	7071±299	-12.4
Mg	306±19	326±19	-6.5
Mn	1.31±0.07	1.23±0.07	6.1
Na	6820±214	7368±231	-8.0

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

Table 2 represents 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 fractions, as well as I/Al, I/B, I/Ba, I/Br, I/Ca, I/Cl, I/Cu, I/Fe, I/K, I/Li, I/Mg, I/Mn, I/Na, I/P, I/S, I/Si, I/Sr, I/V, and I/Zn mass fraction ratios in normal thyroid of males.

Table 2: 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) as well as of I/Al, I/B, I/Ba, I/Br, I/Ca, I/Cl, I/Cu, I/Fe, I/K, I/Li, I/Mg, I/Mn, I/Na, I/P, I/S, I/Si, I/Sr, I/V, and I/Zn mass fraction ratios in the normal thyroid of male (n=73)

Element	M	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Al	11.3	14.9	2.2	0.800	69.3	6.40	1.12	58.6
B	0.491	0.473	0.071	0.200	2.30	0.300	0.200	2.03
Ba	1.03	1.08	0.16	0.050	4.70	0.570	0.141	4.08
Br	13.7	7.8	1.0	1.90	32.3	10.2	2.50	30.7
Ca	1675	979	122	373	5582	1458	429	4163
Cl	3449	1450	219	1030	5920	3470	1262	5657
Cu	4.07	1.41	0.19	1.10	11.0	4.00	1.93	6.21
Fe	223	95	12	52.0	489	215	77.5	445
I	1786	940	118	220	4205	1742	239	3808
K	6680	2352	299	3698	15293	6060	3734	12355
Li	0.0225	0.0168	0.0028	0.0040	0.0977	0.0179	0.0046	0.0547
Mg	316	136	18	99.0	930	287	118	572
Mn	1.27	0.47	0.06	0.0470	2.30	1.16	0.534	2.21
Na	7094	1709	206	3700	13453	6882	4003	11350
P	4414	1366	204	2127	8996	4227	2305	6858
S	8745	1478	220	5066	11377	8806	5925	11326
Si	43.6	41.1	6.1	5.70	180	32.7	7.45	163
Sr	3.96	2.98	0.39	0.100	12.6	2.95	0.443	11.7
V	0.104	0.033	0.005	0.0510	0.200	0.100	0.0591	0.179
Zn	95.4	39.8	5.1	34.0	237	87.6	45.2	199
I/Al	463	552	91	5.14	2381	226	8.43	1647
I/B	5492	4696	772	367	17855	3753	585	17365
I/Ba	2843	2728	461	147	12107	1794	271	9043
I/Br	179	133	18	9.80	576	138	28.7	473
I/Ca	1.40	1.25	0.17	0.140	7.45	1.06	0.204	4.59
I/Cl	0.729	0.544	0.082	0.094	2.65	0.587	0.212	2.21
I/Cu	501	377	54	48.9	1688	429	50.0	1396
I/Fe	10.6	9.8	1.3	0.450	56.8	7.69	0.894	31.2
I/K	0.312	0.209	0.029	0.0209	0.865	0.275	0.0277	0.786
I/Li	125546	129183	23202	7881	631702	101604	8150	391353
I/Mg	7.08	5.86	0.85	0.550	29.3	5.84	0.674	19.1
I/Mn	1675	1414	206	200	7066	1334	233	5469
I/Na	0.269	0.157	0.020	0.0275	0.728	0.238	0.0357	0.558
I/P	0.431	0.322	0.053	0.0320	1.31	0.365	0.0495	1.18
I/S	0.199	0.109	0.018	0.0230	0.387	0.194	0.0285	0.383
I/Si	92.9	109	18	1.31	368	46.6	4.91	358
I/Sr	1233	3003	425	37.0	20690	567	65.1	5415
I/V	18456	13417	2206	1222	57078	16923	2282	49139
I/Zn	21.5	13.4	1.8	2.32	60.4	21.7	2.79	49.8

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 comparison of our results with published data for the Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn contents in the human thyroid is shown in Table 3.

Table 3: 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 thyroid according to data from the literature in comparison with our results (mg/kg, dry mass basis)

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)**	M±SD
Al	33.6 (12)	0.33 (-) [40]	420 (25) [41]	11.3±14.9
B	0.151 (2)	0.084 (3) [42]	0.46 (3) [42]	0.491±0.473
Ba	0.67 (7)	0.0084 (83) [43]	≤5.0 (16) [44]	1.03±1.08
Br	18.1 (11)	5.12 (44) [45]	284±44 (14) [46]	13.7±7.8
Ca	1600 (17)	840±240 (10) [47]	3800±320 (29) [47]	1675±979
Cl	6800 (5)	804±80 (4) [48]	8000 (-) [49]	3449±1450
Cu	6.1 (57)	1.42 (120) [50]	220±22 (10) [48]	4.07±1.41
Fe	252 (21)	56 (120) [50]	2444±700 (14) [46]	223±95
I	1888 (95)	159±8 (23) [51]	5772±2708 (50) [52]	1786±940
K	4400 (17)	46.4±4.8 (4) [48]	6090 (17) [44]	6680±2352
Li	6.3 (2)	0.092 (-) [53]	12.6 (180) [54]	0.0225±0.0168
Mg	390 (16)	3.5 (-) [40]	840±400 (14) [55]	316±136
Mn	1.82 (36)	0.44±11 (12) [56]	69.2±7.2 (4) [48]	1.27±0.47
Na	8000 (9)	438 (-) [57]	10000±5000 (11) [55]	7094±1709
P	3200 (10)	16 (7) [58]	7520 (60) [45]	4414±1366
S	11000 (3)	4000 (-) [49]	11800 (44) [45]	8745±1478
Si	16.0 (3)	0.97 (-) [40]	143±6 (40) [59]	43.6±41.1
Sr	0.73 (9)	0.55±0.26 (21) [42]	46.8±4.8 (4) [48]	3.96±2.98
V	0.042 (6)	0.012 (2) [60]	18±2 (4) [48]	0.104±0.033
Zn	118 (51)	32 (120) [50]	820±204 (14) [46]	95.4±39.8

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

To estimate the effect of age on the ChE contents and I/ ChE content ratios Pearson's correlation coefficient was used (Table 4).

Table 4: Correlations between age (years) and chemical element content (mg/kg, dry tissue), as well as between age and I/chemical element mass fraction ratios in the normal male thyroid (*r* – coefficient of correlation)

Element	**Al**	**B**	**Ba**	**Br**	**Ca**	**Cl**	**Cu**	**Fe**	**I**	**K**
r	-0.29[a]	-0.37[a]	-0.41[b]	0.11	0.45[c]	0.11	-0.09	-0.11	0.32[a]	-0.32[a]
Element	Li	Mg	Mn	Na	P	S	Si	Sr	V	Zn
r	-0.31[a]	0.08	-0.35[a]	-0.10	-0.30[a]	-0.14	-0.24	0.09	-0.02	-0.10
Ratio	I/Al	I/B	I/Ba	I/Br	I/Ca	I/Cl	I/Cu	I/Fe	I/K	I/Li
r	0.26	0.50[b]	0.29	0.15	0.06	0.06	0.26	0.23	0.43[c]	0.33[a]
Ratio	I/Mg	I/Mn	I/Na	I/P	I/S	I/Si	I/Sr	I/V	I/Zn	
r	0.28[a]	0.37[b]	0.25	0.33[a]	0.32	0.29	0.07	0.17	0.18	

Significant values: [a] *p*£0.05, [b] *p*£0.01.

The data of inter-thyroidal correlation (values of *r* – Pearson's coefficient of correlation) including all ChE and I/ChE content ratios identified by us are presented in Tables 5 and 6, respectively.

Table 5: Inter correlations of the chemical element mass fractions in the normal male thyroid (*r* – coefficient of correlation)

El	**Al**	**B**	**Ba**	**Br**	**Ca**	**Cl**	**Cu**	**Fe**	**I**	**K**
Al	**1.00**	-0.01	0.14	0.08	-0.10	0.18	0.58[c]	0.56[c]	-0.42[b]	0.34[a]
B	-0.01	**1.00**	0.56[c]	0.21	-0.09	-0.42[b]	-0.06	-0.10	0.13	0.15
Ba	0.14	0.56[c]	**1.00**	0.01	-0.15	0.07	0.16	-0.16	0.15	0.38[a]
Br	0.08	0.21	0.01	**1.00**	0.06	-0.26	0.16	-0.04	0.05	0.03
Ca	-0.10	-0.09	-0.15	0.06	**1.00**	-0.20	-0.33[a]	-0.19	0.23	-0.22
Cl	0.18	-0.42[b]	0.07	-0.26	-0.20	**1.00**	0.07	0.10	0.08	-0.45[b]
Cu	0.58[c]	-0.06	0.16	0.16	-0.33[a]	0.07	**1.00**	0.37[b]	-0.36[b]	0.25
Fe	0.56[c]	-0.10	-0.16	-0.04	-0.19	0.10	0.37[b]	**1.00**	-0.36[b]	0.07
I	-0.42[b]	0.13	0.15	0.05	0.23	0.08	-0.36[b]	-0.36[b]	**1.00**	-0.12
K	0.34[a]	0.15	0.38[a]	0.03	-0.22	-0.45[b]	0.25	0.07	-0.12	**1.00**
Li	0.64[c]	0.21	0.35[a]	-0.07	0.08	-0.28	0.23	0.20	-0.23	0.33[a]
Mg	0.30[a]	0.03	0.11	-0.05	0.29[a]	0.05	0.43[b]	0.27[a]	-0.20	0.25
Mn	0.29	0.13	0.01	0.07	0.04	-0.07	0.27	0.38[b]	-0.25	-0.03
Na	-0.42[b]	0.07	-0.14	-0.01	-0.20	0.51[c]	0.48[c]	0.31[a]	-0.07	-0.01
P	0.36[a]	0.10	0.34[a]	0.06	-0.30[a]	-0.34[a]	0.69[c]	0.27[a]	-0.37[a]	0.66[c]
S	-0.25	0.32[a]	-0.01	-0.08	0.13	-0.25	-0.48[c]	-0.28[a]	0.42[b]	0.03
Si	0.34[a]	0.39[b]	0.47[b]	0.08	-0.34[a]	0.28	0.33[a]	0.36[a]	-0.26	0.29
Sr	0.06	-0.06	-0.16	0.38[a]	0.32[a]	-0.23	0.02	-0.01	-0.09	-0.09
V	0.20	-0.19	0.09	-0.05	-0.17	0.02	0.50[c]	0.13	-0.09	0.07

Zn	0.26	-0.10	0.15	-0.07	-0.18	0.01	0.57[c]	0.17	-0.06	0.10
El	Li	Mg	Mn	Na	P	S	Si	Sr	V	Zn
Al	0.64[c]	0.30[a]	0.29	-0.42[b]	0.36[a]	-0.25	0.34[a]	0.06	0.20	0.26
B	0.21	0.03	0.13	0.07	0.10	0.32[a]	0.39[b]	-0.06	-0.19	-0.10
Ba	0.35[a]	0.11	0.01	-0.14	0.34[a]	-0.01	0.47[b]	-0.16	0.09	0.15
Br	-0.07	-0.05	0.07	-0.01	0.06	-0.08	0.08	0.38[a]	-0.05	-0.07
Ca	0.08	0.29[a]	0.04	-0.20	-0.30[a]	0.13	-0.34[a]	0.32[a]	-0.17	-0.18
Cl	-0.28	0.05	-0.07	0.51[c]	-0.34[a]	-0.25	0.28	-0.23	0.02	0.01
Cu	0.23	0.43[b]	0.27	0.48[c]	0.69[c]	-0.48[c]	0.33[a]	0.02	0.50[c]	0.57[c]
Fe	0.20	0.27[a]	0.38[b]	0.31[a]	0.27[a]	-0.28[a]	0.36[a]	-0.01	0.13	0.17
I	-0.23	-0.20	-0.25	-0.07	-0.37[a]	0.42[b]	-0.26	-0.09	-0.09	-0.06
K	0.33[a]	0.25	-0.03	-0.01	0.66[c]	0.03	0.29	-0.09	0.07	0.10
Li	**1.00**	0.36[a]	0.37[a]	0.21	0.24	0.07	0.17	0.05	0.28	0.24
Mg	0.36[a]	**1.00**	0.23	0.21	0.59[c]	-0.10	0.30[a]	0.14	0.23	0.26
Mn	0.37[a]	0.23	**1.00**	0.12	0.30[a]	-0.12	0.21	0.18	0.03	0.07
Na	0.21	0.21	0.12	**1.00**	0.46[b]	-0.02	0.13	-0.17	0.09	0.21
P	0.24	0.59[c]	0.30[a]	0.46[b]	**1.00**	-0.27	0.39[b]	-0.09	0.41[b]	0.47[c]
S	0.07	-0.10	-0.12	-0.02	-0.27	**1.00**	-0.34[a]	-0.06	-0.19	-0.15
Si	0.17	0.30[a]	0.21	0.13	0.39[b]	-0.34[a]	**1.00**	-0.19	-0.16	-0.05
Sr	0.05	0.14	0.18	-0.17	-0.09	-0.06	-0.19	**1.00**	0.07	0.02
V	0.28	0.23	0.03	0.09	0.41[b]	-0.19	-0.16	0.07	**1.00**	-0.15
Zn	0.24	0.26	0.07	0.21	0.47[c]	-0.15	-0.05	0.02	-0.15	**1.00**

El – element, significant values: [a] p£0.05, [b] p£0.01, [c] p£0.001.

Discussion

A good agreement of our results for the Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractions with the certified values of CRM IAEA H-4 Animal Muscle, INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2 Mixed Polish Herbs [64-70] as well as the similarity of the means of the Ca, K, Mg, Mn, and Na mass fractions in the normal thyroid of male determined by both NAA-SLR and ICP-AES methods (Table 1) demonstrates an acceptable precision and accuracy of the results obtained in the study and presented in Tables 2-6.

The content of ChE was determined in all or most of the examined samples, which made it possible to calculate the main statistical parameters: the mean value of the mass fraction (M), standard deviation (SD), standard error of the mean (SEM), minimum (Min), maximum (Max), median (Med), and percentiles with levels of 0.025 (P 0.025) and 0.975 (P 0.975), of the Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractions, as well as I/Al, I/B, I/Ba, I/Br, I/Ca, I/Cl, I/Cu, I/Fe, I/K, I/Li, I/Mg, I/Mn, I/Na, I/P, I/S, I/Si, I/Sr, I/V, and I/Zn mass fraction ratios in normal thyroid of males (Table 2). The values of M, SD, and SEM can be used to compare data for different groups of samples only under the condition of a normal distribution of the results of determining the content of ChE in the samples under study. Statistically reliable identification of the law of distribution of results requires large sample sizes, usually several hundred samples, and therefore is rarely used in biomedical research. In the conducted study, we could not prove or disprove the "normality" of the distribution of the results obtained due to the insufficient number of samples studied. Therefore, in addition to the M, SD, and SEM values, such statistical characteristics as Med, range (Min-Max) and percentiles P 0.025 and P 0.975 were calculated, which are valid for any law of distribution of the results of ChE content in thyroid tissue.

The obtained means 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 3, 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 [40-60]. The obtained mean for Li is two orders of magnitude lower the median of previously reported data. Moreover, it is outside the range of previously reported means. A number of values for chemical element mass fractions were not expressed on a dry mass basis by the authors of the cited references. Hence, we calculated these values using published data for water 75% [72] and ash 4.16% on dry mass basis [73] contents in thyroid of adults. No published data referring to I/Al, I/B, I/Ba, I/Br, I/Ca, I/Cl, I/Cu, I/Fe, I/K, I/Li, I/Mg, I/Mn, I/Na, I/P, I/S, I/Si, I/Sr, I/V, and I/Zn mass fraction ratios in human thyroid was found.

With age, the I and Ca contents, as well as I/B, I/K, I/Li, I/Mg, I/Mn, and I/P content ratios increase, while Al, B, Ba, K, Li, Mn, and P decrease (Table 4). These characteristics, particularly Ca, I/B, I/K and I/Mn, can be used to estimate the "biological age" of the male thyroid gland.

A significant direct correlation between the I and S mass fractions, as well as an inverse correlation between I and Al, I and Cu, I and Fe, and I and P mass fractions was seen in male thyroid (Table 5). Since no correlations were found between I and other ChE, it would appear that the content of B, Ba, Br, Ca, Cl, K, Li, Mg, Mn, Na, Si, Sr, V, and Zn in the thyroid gland is independent of I content. However, this is not quite so. If we bring the content of the studied ChE to the content of I (I/ ChE ratio), then there are close relationships between I/Al, I/B, I/Ba, I/Br, I/Ca, I/Cl, I/Cu, I/Fe, I/K, I/Li, I/Mg, I/Mn, I/Na, I/P, I/S, I/Si, I/Sr, I/V, and I/Zn (Table 6). From this it follows that, at least, the levels of all these ChE in the thyroid gland are interconnected and depend on the content of I in it. Because I plays a decisive role in the function of the thyroid gland, the data obtained allow us to conclude that, along with I, such ChE as Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn, if not directly, then indirectly, are involved in the process of thyroid hormone synthesis.

Table 6: Inter correlations of the I/chemical element mass fraction ratios in the normal male thyroid (*r* – coefficient of correlation)

Ratio	I/Al	I/B	I/Ba	I/Br	I/Ca	I/Cl	I/Cu	I/Fe	I/K	I/Li
I/Al	**1.00**	0.608^{c}	0.413^{a}	0.532^{c}	0.454^{b}	0.544^{c}	0.665^{c}	0.568^{c}	0.623^{c}	0.574^{c}
I/B	0.608^{c}	**1.00**	0.520^{c}	0.588^{c}	0.272	0.337^{a}	0.670^{c}	0.555^{c}	0.722^{c}	0.567^{c}
I/Ba	0.113	0.208	**1.00**	0.391^{a}	0.075	0.382^{a}	0.511^{b}	0.205	0.449^{b}	0.541^{b}
I/Br	0.532^{c}	0.588^{c}	0.391^{a}	**1.00**	0.318^{a}	0.172	0.408^{b}	0.400^{b}	0.423^{b}	0.456^{b}
I/Ca	0.454^{b}	0.272	0.075	0.318^{a}	**1.00**	0.026	0.419^{b}	0.331^{a}	0.440^{c}	0.503^{b}
I/Cl	0.544^{c}	0.337^{a}	0.382^{a}	0.172	0.026	**1.00**	0.496^{c}	0.269	0.206	0.179
I/Cu	0.665^{c}	0.670^{c}	0.511^{b}	0.408^{b}	0.419^{b}	0.496^{c}	**1.00**	0.532^{c}	0.889^{c}	0.760^{c}
I/Fe	0.568^{c}	0.555^{c}	0.205	0.400^{b}	0.331^{a}	0.269	0.532^{c}	**1.00**	0.726^{c}	0.220
I/K	0.623^{c}	0.722^{c}	0.449^{b}	0.423^{b}	0.440^{c}	0.206	0.889^{c}	0.726^{c}	**1.00**	0.646^{c}
I/Li	0.574^{c}	0.567^{c}	0.541^{b}	0.503^{b}	0.503^{b}	0.179	0.760^{c}	0.220	0.646^{c}	**1.00**
I/Mg	0.691^{c}	0.614^{c}	0.471^{b}	0.275	0.710^{c}	0.503^{c}	0.896^{c}	0.572^{c}	0.775^{c}	0.793^{c}
I/Mn	0.476^{b}	0.527^{c}	0.336^{a}	0.400^{b}	0.762^{c}	0.423^{b}	0.823^{c}	0.671^{c}	0.719^{c}	0.570^{c}
I/Na	0.643^{c}	0.642^{c}	0.367^{a}	0.452^{c}	0.445^{c}	0.792^{c}	0.829^{c}	0.639^{c}	0.723^{c}	0.565^{c}
I/P	0.641^{c}	0.679^{c}	0.461^{b}	0.538^{c}	0.519^{c}	0.520^{c}	0.966^{c}	0.657^{c}	0.916^{c}	0.820^{c}
I/S	0.675^{c}	0.610^{c}	0.383^{a}	0.631^{c}	0.631^{c}	0.633^{c}	0.825^{c}	0.692^{c}	0.871^{c}	0.620^{c}
I/Si	0.691^{c}	0.692^{c}	0.678^{c}	0.499^{b}	0.275	0.571^{c}	0.777^{c}	0.453^{b}	0.696^{c}	0.603^{c}
I/Sr	0.658^{c}	0.467^{b}	0.319	0.012	0.047	0.392^{b}	0.109	0.112	0.007	0.476^{b}
I/V	0.720^{c}	0.578^{c}	0.427^{b}	0.519^{b}	0.510^{b}	0.712^{c}	0.766^{c}	0.740^{c}	0.771^{c}	0.418^{a}
I/Zn	0.559^{c}	0.523^{c}	0.389^{a}	0.366^{b}	0.411^{b}	0.466^{b}	0.786^{c}	0.514^{c}	0.756^{c}	0.849^{c}

Ratio	I/Mg	I/Mn	I/Na	I/P	I/S	I/Si	I/Sr	I/V	I/Zn
I/Al	0.691^{c}	0.476^{b}	0.643^{c}	0.641^{c}	0.675^{c}	0.691^{c}	0.658^{c}	0.720^{c}	0.559^{c}
I/B	0.614^{c}	0.527^{c}	0.642^{c}	0.679^{c}	0.610^{c}	0.692^{c}	0.467^{b}	0.578^{c}	0.523^{c}
I/Ba	0.471^{b}	0.336^{a}	0.367^{a}	0.461^{b}	0.383^{a}	0.678^{c}	0.319	0.427^{b}	0.389^{a}
I/Br	0.275	0.400^{b}	0.452^{c}	0.538^{c}	0.631^{c}	0.499^{b}	0.012	0.519^{b}	0.366^{b}
I/Ca	0.710^{c}	0.762^{c}	0.445^{c}	0.519^{c}	0.631^{c}	0.275	0.047	0.510^{b}	0.411^{b}
I/Cl	0.503^{c}	0.423^{b}	0.792^{c}	0.520^{c}	0.633^{c}	0.571^{c}	0.392^{b}	0.712^{c}	0.466^{b}
I/Cu	0.896^{c}	0.823^{c}	0.829^{c}	0.966^{c}	0.825^{c}	0.777^{c}	0.109	0.766^{c}	0.786^{c}
I/Fe	0.572^{c}	0.671^{c}	0.639^{c}	0.657^{c}	0.692^{c}	0.453^{b}	0.112	0.740^{c}	0.514^{c}
I/K	0.775^{c}	0.719^{c}	0.723^{c}	0.916^{c}	0.871^{c}	0.696^{c}	0.007	0.771^{c}	0.756^{c}
I/Li	0.793^{c}	0.570^{c}	0.565^{c}	0.820^{c}	0.620^{c}	0.603^{c}	0.476^{b}	0.418^{a}	0.849^{c}
I/Mg	**1.00**	0.745^{c}	0.759^{c}	0.904^{c}	0.838^{c}	0.738^{c}	0.626^{c}	0.719^{c}	0.874^{c}
I/Mn	0.745^{c}	**1.00**	0.734^{c}	0.811^{c}	0.722^{c}	0.647^{c}	0.580^{c}	0.645^{c}	0.670^{c}
I/Na	0.759^{c}	0.734^{c}	**1.00**	0.815^{c}	0.903^{c}	0.602^{c}	0.321^{a}	0.843^{c}	0.734^{c}
I/P	0.904^{c}	0.811^{c}	0.815^{c}	**1.00**	0.826^{c}	0.711^{c}	0.527^{c}	0.718^{c}	0.919^{c}
I/S	0.838^{c}	0.722^{c}	0.903^{c}	0.826^{c}	**1.00**	0.562^{c}	0.673^{c}	0.860^{c}	0.804^{c}
I/Si	0.738^{c}	0.647^{c}	0.602^{c}	0.711^{c}	0.562^{c}	**1.00**	0.458^{b}	0.536^{c}	0.612^{c}
I/Sr	0.626^{c}	0.580^{c}	0.321^{a}	0.527^{c}	0.673^{c}	0.458^{b}	**1.00**	0.638^{c}	0.166
I/V	0.719^{c}	0.645^{c}	0.843^{c}	0.718^{c}	0.860^{c}	0.536^{c}	0.638^{c}	**1.00**	0.663^{c}
I/Zn	0.874^{c}	0.670^{c}	0.734^{c}	0.919^{c}	0.804^{c}	0.612^{c}	0.166	0.663^{c}	**1.00**

Significant values: ^{a} *p*£0.05, ^{b} *p*£0.01, ^{c} *p*£0.001.

Conclusion

The combination of INAA-SLR and ICP-AES is a useful analytical tool for the determination of ChE contents in the thyroid tissue samples. This method allows determine means for Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V and Zn (twenty ChE).

Our data reveal that the I and Ca contents, as well as I/B, I/K, I/Li, I/Mg, I/Mn, and I/P content ratios increase, while Al, B, Ba, K, Li, Mn, and P decrease in the normal thyroid of male during a lifespan. Therefore, a goitrogenic and tumorogenic effect of excessive I and Ca level and inadequate Al, B, Ba, K, Li, Mn, and P level in the thyroid of old males, as well as a disturbance in intrathyroidal I/B, I/K, I/Li, I/Mg, I/Mn, and I/P relationships with increasing age may be assumed. Furthermore, it was found that the levels of Al, B, Ba, Br, Ca, Cl, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn in the thyroid gland are interconnected and depend on the content of I in it. Because I plays a decisive role in the function of the thyroid gland, the data obtained allow us to conclude that, along with I, such ChE as Al, B, Ba, Br, Ca, Cl, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn, if not directly, then indirectly, are involved in the process of thyroid hormone synthesis.

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