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Research Article

Investigation of Association between the High Risk of Female Subclinical Hypothyroidism and Inadequate Quantities of Twenty Intra-Thyroidal Chemical Elements

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Abstract

Subclinical hypothyroidism does affect fertility. The prevalence of subclinical hypothyroidism is 10-15 times more common in women than in men. Chemical elements, including trace elements, play important roles in thyroid function and fertility. The aim of this study was to evaluate whether significant difference of chemical element contents exists between female and male thyroids and how they can be related to the etiology of subclinical hypothyroidism. Thyroid tissue levels of twenty chemical elements: Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn were prospectively evaluated in 105 healthy persons (33 females and 72 males). It was found that for ages before 40 years means of Al, B, Ca, Cu, Fe, Li, Mg, S, and Zn content in female thyroid were lower than those in male thyroid. For ages over 40 years means of Ba, Br and Si content in female thyroid was higher whereas mean of Mg content was lower than those in male thyroid. Thus, inappropriate content of intra-thyroidal Al, B, Ba, Br, Ca, Cu, Fe, Li, Mg, S, Si, and Zn can be associated with the etiology of female subclinical hypothyroidism.

Keywords: Subclinical Hypothyroidism; Female Thyroid; Chemical Elements; Neutron Activation Analysis; Inductively Coupled Plasma Atomic Emission Spectrometry

Abbreviations

SCH - Subclinical Hypothyroidism;
ROS - Reactive Oxygen Species;
ChE - Chemical Elements;
BSS - Biological Synthetic Standards;

CRM/SRM - Certified/Standard Reference Materials;
INAA-SLR - Instrumental Neutron Activation Analysis with High Resolution Spectrometry of Short-Lived Radionuclides;
ICP-AES - Inductively Coupled Plasma Atomic Emission Spectrometry;
IAEA - International Atomic Energy Agency.

Introduction

Adequate thyroid function is important to maintain normal reproduction, because thyroid dysfunction affects fertility in various ways resulting in abnormal ovulatory cycles, luteal phase defects, high prolactin levels, and sex hormone imbalances [1,2]. Therefore, normal thyroid function is necessary for fertility, and to sustain a healthy pregnancy [2]. From large population studies, which measured thyroid function, and systematic reviews of this subject carried out in the 1990s to 2010s, it is known that untreated hypothyroidism is a common condition all over the world [2-11]. The prevalence of subclinical hypothyroidism (SCH) is between 1% and 10% in different countries [2-11] and almost everywhere it is 10-15 times more common in women than in men [4,10]. Form such a great gender-related difference in the prevalence of SCH arises a question about a specific sensitivity of female thyroid tissue to some external and internal factors.

Although the etiology of SCH and other thyroidal disorders is unknown in detail, several risk factors including deficiency or excess of such micronutrients as iodine (I) has been well identified [12-23]. Besides I involved in thyroid function, other chemical elements (ChE), including trace elements, also play important roles such as stabilizers, structural elements, maintenance and regulation of cell function, gene regulation, enzyme cofactors, activation or inhibition of enzymatic reactions, normal peripheral utilization of thyroid hormones and regulation of cell membrane function [24]. Essential or toxic properties of ChE depend on tissue-specific need or tolerance, respectively [25]. Both ChE deficiencies as well as overexposures may disturb the thyroidal cell functions [25].

The reliable data on ChE mass fractions in normal human thyroid separately for female and male gland is apparently extremely limited. There are a few studies regarding ChE content in human thyroid, using chemical techniques and instrumental methods [26-46]. However, the majority of these data are based on measurements of processed tissue and in many studies tissue samples are ashed before analysis. In other cases, thyroid samples are treated with solvents (distilled water, ethanol etc) and then are dried at a high temperature for many hours. There is evidence that certain quantities of ChE are lost as a result of such treatment [47-49]. Moreover, only a few of these studies employed quality control using certified/standard reference materials (CRM/SRM) for determination of the ChE contents.

Therefore, nondestructive techniques such as instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) combined with subsequent, destructive inductively coupled plasma atomic emission spectrometry (ICP-AES) provides a good alternative for multi-element determination in samples of thyroid parenchyma. This combination of methods provides a possibility to ensure data quality assurance using a comparison of results obtained for some elements by both methods. This work had three aims.

The primary purpose of this study was to determine reliable values for such ChE as aluminum (Al), boron (B), barium (Ba), bromine (Br), calcium (Ca), chlorine (Cl), coper (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) contents in intact (normal) thyroid gland of apparently healthy persons using the combination of INAA-SLR and ICP-AES analysis. The second aim was to compare the levels of ChE in the thyroid tissue of all females and males investigated in the study. The final aim was to compare the levels of ChE in the thyroid tissue of females and males in age group 1 (≤ 40 years) and in age group 2 (>40 years).All studies were approved by the Ethical Committee of the Medical Radiological Research Centre, Obninsk, Russia. 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.

Material and Methods

Samples

Samples of the human thyroid were obtained from randomly selected autopsy specimens of 33 females (European-Caucasian, aged 3.5 to 87 years) and 72 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. Age ranges for subjects were divided into two age groups, with group 1 (≤ 40 years), and group 2 (>40 years). For females in group 1 (n=11) mean age (±standard error of mean, SEM) was 30.9 ± 3.1 years and in group 2 (n=22) mean age was 66.3 ± 2.7 years. For males in group 1 (n=36) mean age was 22. 5 ± 1.4 years and in group 2 (n=36) mean age was 52.4 ± 2.4 years.

These groups were selected to reflect the condition of thyroid tissue in the children, teenagers, young adults and first period of adult life (group 1) and in the second period of adult life as well as in old age (group 2).

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 trace element contents. The typical causes of sudden death of most of these subjects included trauma or suicide and also acute untreated illness (cardiac insufficiency, stroke, embolism of pulmonary artery, alcohol poisoning).

Sample preparation

All right lobes of thyroid glands were divided into two portions using a titanium scalpel [50]. 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 microadenomatosis and latent cancer. After the samples intended for ChE analysis were weighed, they were freeze-dried and homogenized [51-53]. The sample weighing about 100 mg was used for chemical element measurement by INAA-SLR. The samples for INAA-SLR were sealed separately in thin polyethylene films washed beforehand with acetone and rectified alcohol. The sealed samples were placed in labeled polyethylene ampoules. Biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used as standards [54].

In addition to BSS, aliquots of commercially available pure compounds were also used. After NAA-SLR investigation the thyroid samples were taken out from the polyethylene ampoules and used for ICP-AES. The samples were decomposed in autoclaves. For this 1.5 mL of concentrated HNO3 (nitric acid at 65 %, maximum (max) of 0.0000005 % Hg; GR, ISO, Merck, Darmstadt, Germany) and 0.3 mL of H_2O_2 (pure for analysis) were added to each thyroid samples, which were placed in one-chamber autoclaves (Ancon-AT2, Ltd., Moscow, Russia) and then heated

for 3 h at 160–200 °C. After autoclaving, they were cooled to room temperature and solutions from the decomposed samples were diluted with deionized water (up to 20 mL) and transferred to plastic measuring bottles. Simultaneously, the same procedure was performed in autoclaves without tissue samples (containing only HNO_3+H_2O_2+ deionized water), and the resultant solutions were used as control samples.

Certified Reference Materials

For quality control, ten subsamples of the certified reference materials (CRM) IAEA H-4 Animal Muscle from the International Atomic Energy Agency (IAEA), and also five 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 earlier publications [55-62].

Instrumentation and methods

A horizontal channel equipped with the pneumatic rabbit system of the WWR-C research nuclear reactor was applied to determine the mass fractions of Br, Ca, K, Mg, Mn, and Na by INAA-SLR. The neutron flux in the channel was 1.7×10^{13}n cm^{-2} s^{-1}. Ampoules with thyroid samples, biological synthetic standards [53], intralaboratory-made standards, and CRM were put into polyethylene rabbits and then irradiated separately for 180 s. Copper foils were used to assess neutron flux. The measurement for each sample was made twice, 1 and 120 min after irradiation. The durations of the first and second measurements were 10 and 20 min, respectively. The gamma spectrometer included the 100 cm^3 Ge(Li) detector and on-line computer-based multichannel analyzer system. The spectrometer provided a resolution of 1.9 keV on the ^{60}Co 1332 keV line. 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 (Thermo SPEC, version 4.1). Information detailing with the NAA-SLR and ICP-AES methods used and other details of the analysis were presented in our previous publication [55-62].

Computer programs and statistic

A dedicated computer program for INAA mode optimization was used [63]. All thyroid samples were prepared in duplicate, and mean values of ChE contents were used in final calculation. Mean values was also used for ChE contents that were measured by two different 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 females and males (age group 1 and 2 combined), as well as between females and males separately in age group 1 and group 2 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 Ca, K, Mg, Mn, and Na mass fractions (mg/kg, dry mass basis)in the normal human thyroids (females and males combined) obtained by both NAA-SLR and ICP-AES methods is shown in (Table 1).

(Table 2) 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 thyroid tissue of female and male.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 human thyroid is shown in (Table 3).

(Table 1): Comparison of the mean values (M ± 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 M1	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 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) in normal human thyroid

Gender	Element	M	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Males n=72	Al	11.3	14.9	2.2	0.8	69.3	6.4	1.12	58.6
	B	0.491	0.473	0.071	0.2	2.3	0.3	0.2	2.03
	Ba	1.03	1.08	0.16	0.05	4.7	0.57	0.141	4.08
	Br	13.7	7.8	1	1.9	32.3	10.2	2.5	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	1.1	11	4	1.93	6.21
	Fe	223	95	12	52	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	0.003	0.004	0.0977	0.0179	0.0046	0.0547
	Mg	316	136	18	99	930	287	118	572
	Mn	1.27	0.47	0.06	0.047	2.3	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	5.7	180	32.7	7.45	163
	Sr	3.96	2.98	0.39	0.1	12.6	2.95	0.443	11.7
	V	0.104	0.033	0.005	0.051	0.2	0.1	0.0591	0.179
	Zn	95.4	39.8	5.1	34	237	87.6	45.2	199

Females n=33	Al	7.43	4.49	1.24	2.5	17.2	5.5	2.77	16.6
	B	0.418	0.257	0.074	0.2	1	0.315	0.2	0.89
	Ba	1.42	1.38	0.38	0.048	5	0.77	0.121	4.34
	Br	22.4	16.2	3.23	5	66.9	16.3	5	59.2
	Ca	1630	1071	219	461	4256	1132	539	3902
	Cl	3317	1480	290	1200	6000	3375	1388	5906
	Cu	3.4	1.41	0.35	0.5	5.9	3.35	1.1	5.79
	Fe	225	98	20	52	435	199	64	391
	I	1956	1199	219	114	5061	1562	309	4662
	K	5605	3272	732	1914	13700	5058	2360	13230
	Li	0.0153	0.0078	0.002	0.0015	0.0251	0.0152	0.003	0.025
	Mg	230	105	26	66	426	216	73.5	403
	Mn	1.32	0.84	0.22	0.55	4.04	1.1	0.603	3.28
	Na	6533	1744	324	3686	10450	6739	4088	9924
	P	3860	2175	603	496	7368	3422	585	7337
	S	6579	2662	738	644	9921	7097	1238	9868
	Si	75.7	58.1	16.1	6.9	176	47.6	9.9	175
	Sr	3.3	2.77	0.69	0.2	10.9	2.85	0.369	9.18
	V	0.097	0.056	0.016	0.02	0.25	0.1	0.026	0.211
	Zn	83.2	41.2	8.1	6.1	166	84.9	6.16	156

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): 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)**	Males and females M±SD
Al	33.6 (12)	0.33 (-) [26]	420 (25) [27]	10.5 ± 13.4
B	0.151 (2)	0.084 (3) [28]	0.46 (3) [28]	0.476 ± 0.434
Ba	0.67 (7)	0.0084 (83) [29]	≤ 5.0 (16) [30]	1.12 ± 1.15
Br	18.1 (11)	5.12 (44) [31]	284 ± 44 (14) [32]	16.3 ± 11.6
Ca	1600 (17)	840 ± 240 (10) [33]	3800 ± 320 (29) [33]	1663 ± 999
Cl	6800 (5)	804 ± 80 (4) [34]	8000 (-) [35]	3400 ± 1452

Cu	6.1 (57)	1.42 (120) [36]	220 ± 22 (10) [34]	3.93 ± 1.43
Fe	252 (21)	56 (120) [36]	2444 ± 700 (14) [32]	223 ± 95
I	1888 (95)	159 ± 8 (23) [37]	5772 ± 2708 (50) [38]	1841 ± 1027
K	4400 (17)	46.4 ± 4.8 (4) [34]	6090 (17) [30]	6418 ± 2625
Li	6.3 (2)	0.092 (-) [39]	12.6 (180) [40]	0.0208 ± 0.0154
Mg	390 (16)	3.5 (-) [26]	840 ± 400 (14) [41]	296 ± 134
Mn	1.82 (36)	0.44 ± 11 (12) [42]	69.2 ± 7.2 (4) [34]	1.28 ± 0.56
Na	8000 (9)	438 (-) [43]	10000 ± 5000 (11) [41]	6928 ± 1730
P	3200 (10)	16 (7) [44]	7520 (60) [31]	4290 ± 1578
S	11000 (3)	4000 (-) [35]	11800 (44) [31]	8259 ± 2002
Si	16.0 (3)	0.97 (-) [26]	143 ± 6 (40) [45]	50.8 ± 46.9
Sr	0.73 (9)	0.55 ± 0.26 (21) [28]	46.8 ± 4.8 (4) [34]	3.81 ± 2.93
V	0.042 (6)	0.012 (2) [46]	18 ± 2 (4) [34]	0.102 ± 0.039
Zn	118 (51)	32 (120) [36]	820 ± 204 (14) [32]	94.8 ± 39.7
M –arithmetic mean, SD – standard deviation, (n)* – number of all references, (n)** – number of samples				

The ratios of means and the difference between mean values 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 thyroid of females and males are presented in (Table 4). Because, in our previous studies age-dependents of many ChE in thyroid gland was found [64-72], the comparison between ChE contents in thyroid of females and males separately in age group 1 and also in age group 2 was performed (Tables 5-6).

(Table 4): 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 thyroid tissue of males and females

Element	Thyroid tissue				Ratio
	Males 2.0-80 years n=72	Females 3.5-87 years n=33	Student's t-test p≤	U-test p	Females to Males
Al	11.3 ± 2.2	7.43 ± 1.24	0.132	> 0.05	0.66
B	0.491 ± 0.071	0.418 ± 0.074	0.483	> 0.05	0.85
Ba	1.03 ± 0.16	1.42 ± 0.38	0.372	> 0.05	1.38
Br	13.7 ± 1.0	22.4 ± 3.23	**0.016**	**≤ 0.01**	1.64
Ca	1675 ± 122	1630 ± 219	0.858	> 0.05	0.97

Cl	3449 ± 219	3317 ± 290	0.718	> 0.05	0.96
Cu	4.07 ± 0.19	3.40 ± 0.35	0.104	> 0.05	0.84
Fe	223 ± 12	225 ± 20	0.925	> 0.05	1.01
I	1786 ± 118	1956 ± 219	0.497	> 0.05	1.1
K	6680 ± 299	5605 ± 732	0.186	> 0.05	0.84
Li	0.0225 ± 0.0028	0.0153 ± 0.0024	0.054	**≤ 0.05**	0.68
Mg	316 ± 18	23 ± 26	**0.012**	**≤ 0.01**	0.73
Mn	1.27 ± 0.06	1.32 ± 0.22	0.834	> 0.05	1.04
Na	7094 ± 206	6533 ± 324	0.15	> 0.05	0.92
P	4414 ± 204	3860 ± 603	0.398	> 0.05	0.87
S	8745 ± 220	6579 ± 738	**0.014**	**≤ 0.01**	0.75
Si	43.6 ± 6.1	75.7 ± 16.1	0.082	**≤ 0.05**	1.74
Sr	3.96 ± 0.39	3.30 ± 0.69	0.414	> 0.05	0.83
V	0.104 ± 0.005	0.097 ± 0.016	0.709	> 0.05	0.93
Zn	95.4 ± 5.1	83.2 ± 8.1	0.114	> 0.05	0.87
M – arithmetic mean, SEM – standard error of mean, *t*-test - Student's *t*-test, U-test - Wilcoxon-Mann-Whitney *U*-test, Sstatistically significant values are in **bold**.					

(Table 5): 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 thyroid tissue of males and females aged 2-40 years

Element	Thyroid tissue				Ratio
	Males (MG1) 2.0-40 years n = 44	Females (FG1) 3.5-40 years n=11	Student's t-test p≤	U-test p	FG1/MG1
Al	13.2 ± 3.4	5.9 5± 0.97	**0.049**	**≤ 0.01**	0.45
B	0.607 ± 0.105	0.361 ± 0.070	0.061	**≤ 0.05**	0.59
Ba	1.31 ± 0.25	0.819 ± 0.278	0.206	> 0.05	0.63
Br	12.5 ± 1.3	13.3 ± 2.5	0.793	> 0.05	1.06
Ca	1453 ± 87	945 ± 91	**0.00047**	**≤ 0.01**	0.65
Cl	3236 ± 314	4109 ± 544	0.19	> 0.05	1.27
Cu	4.03 ± 0.27	2.94 ± 0.45	0.053	**≤ 0.05**	0.73
Fe	228 ± 16	174 ± 25	0.089	**≤ 0.05**	0.77
I	1601 ± 146	1876 ± 346	0.476	> 0.05	1.17
K	6875 ± 370	5545 ± 1145	0.296	> 0.05	0.81
Li	0.0273 ± 0.0040	0.0131 ± 0.0033	**0.0125**	**≤ 0.01**	0.48
Mg	318 ± 18	234 ± 41	0.093	**≤ 0.05**	0.74
Mn	1.43 ± 0.09	1.21 ± 0.14	0.209	> 0.05	0.85
Na	7134 ± 271	6217 ± 549	0.16	> 0.05	0.87

P	4593 ± 298	3427 ± 842	0.225	> 0.05	0.75
S	8986 ± 275	6049 ± 1114	**0.034**	**≤ 0.01**	0.67
Si	47.1 ± 7.3	57.3 ± 20.5	0.649	> 0.05	1.22
Sr	3.97 ± 0.44	3.62 ± 1.04	0.76	> 0.05	0.91
V	0.105 ± 0.006	0.104 ± 0.023	0.988	> 0.05	0.99
Zn	99.3 ± 7.0	56.8 ± 10.6	**0.0032**	**≤ 0.01**	0.57
M – arithmetic mean, SEM – standard error of mean, *t*-test - Student's *t*-test, U-test - Wilcoxon-Mann-Whitney *U*-test, statistically significant values are in **bold**.					

(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 thyroid tissue of males and females aged 41-87 years

Element	Thyroid tissue				Ratio
	Males (MG2) 41-80 years n=28	Females (FG2) 41-87 years n=22	Student's t-test p≤	U-test p	FG2/MG2
Al	8.27 ± 1.7	9.80 ± 2.67	0.643	> 0.05	1.19
B	0.289 ± 0.029	0.533 ± 0.177	0.263	> 0.05	1.84
Ba	0.613 ± 0.085	2.37 ± 0.75	0.077	**≤ 0.05**	3.87
Br	15.4 ± 1.7	26.8 ± 4.3	**0.021**	**≤ 0.01**	1.74
Ca	1980 ± 256	2042 ± 301	0.877	> 0.05	1.03
Cl	3662 ± 305	2965 ± 318	0.122	> 0.05	0.81
Cu	4.15 ± 0.24	4.17 ± 0.47	0.975	> 0.05	1
Fe	213 ± 17	264 ± 25	0.099	> 0.05	1.24
I	2048 ± 190	2002 ± 288	0.895	> 0.05	0.98
K	6410 ± 498	5654 ± 995	0.507	> 0.05	0.88
Li	0.0145 ± 0.0022	0.0178 ± 0.0033	0.43	> 0.05	1.23
Mg	313 ± 36	225 ± 35	0.099	**≤ 0.05**	0.72
Mn	1.06 ± 0.07	1.45 ± 0.45	0.423	> 0.05	1.37
Na	7037 ± 321	6675 ± 405	0.488	> 0.05	0.95
P	4120 ± 215	4552 ± 812	0.631	> 0.05	1.1
S	8348 ± 357	7426 ± 697	0.282	> 0.05	0.89
Si	37.9 ± 11.1	105 ± 22	**0.036**	**≤ 0.01**	2.77
Sr	3.93 ± 0.75	2.77 ± 0.68	0.263	> 0.05	0.7
V	0.102 ± 0.008	0.086 ± 0.017	0.446	> 0.05	0.84
Zn	88.3±6.3	102.6±9.0	0.204	> 0.05	1.16

M – arithmetic mean, SEM – standard error of mean, *t*-test - Student's *t*-test, U-test - Wilcoxon-Mann-Whitney *U*-test, Statistically significant values are in **bold**

Discussion

Precision and accuracy of results

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 [55-62] as well as the similarity of the means of the Ca, K, Mg, Mn, and Na mass fractions in the normal human thyroids 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 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 in thyroid of female and male (Table 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 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 [26-46]. 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. The mean obtained for V is one order of magnitude higher the median of previously reported data, but it is inside the previously reported range of 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% [73] and ash 4.16% on dry mass basis [74] contents in thyroid of adults.

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 human thyroid vary widely (Table 3). 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, and mass of the gland. 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 insufficient quality control of results in these studies.

Gender-related differences

Strongly pronounced differences in Br, Mg, and S mass fraction were observed between female and male thyroid (Table 4). The means of Br and Si mass fraction in female thyroids were respectively 1.6 and 1.7 time higher while the means of Li, Mg and S mass fractions were respectively 32%, 27% and 25% lower than in male thyroids. During the first 40 years of life (Age group 1) the situation with ChE contents in female thyroids was some different than that for older females ((Age group 2). In Age group 1 differences between Al, B, Ca, Cu, Fe, Li, Mg, S, and Zn contents in female and male thyroids were detected (Table 5). In Age group 1 of females with mean age 30.9 years the Al, B, Ca, Cu, Fe, Li, Mg, S, and Zn contents in thyroid were respectively 55%, 41%, 35%, 27%, 23%, 52%, 26%, 33% and 43% lower than in thyroid of males from the same age group. For ages over 40 years (Age group 2) a statistically significant difference between the Ba, Br Mg, and Si content in female and male thyroids was observed. The means of Ba, Br, and Si content in female thyroids were respectively 3.9, 1.7, and 2.8 times higher, whereas the mean of Mg

28% lower than those in male thyroids. In Age group 2 differences between the Al, B, Ca, Cu, Fe, Li, S and Zn contents in thyroids of females and males, previously found in the Age group 1, was no longer evident.

Because the prevalence of SCH is 10-15 times more greater in women than in men [4,10], we can accept that the levels of ChE mass fractions in male thyroids as more suitable (perhaps optimal) for normal function of the gland. If so, we have to conclude that for ages before 40 years there is a significant deficiency of Al, B, Ca, Cu, Fe, Li, Mg, S, and Zn contents in female thyroid parenchyma. In age over 40 deficiencies of Al, B, Ca, Cu, Fe, Li, S, and Zn contents in female thyroid disappear and an excess of Ba, Br and Si is now seen.

Role of intra-thyroidal chemical elements in the gland function

Aluminum

Trace element Al is not described as essential, because no biochemical function has been directly connected to it. At this stage of our knowledge, no doubt that the Al overload negatively impacts human health, including the thyroid function [75]. Why Al content in normal thyroid of females aged before 40 lower than that in male thyroid and how this deficiency acts on the female thyroid are still to be cleared.

Boron

Trace element B is known to influence the activity of many enzymes [76]. 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 [77]. Moreover, B helps the conversion of the storage form of thyroid hormone, T_4, to T_3, the active form.

Barium

Trace element Ba has been shown to affect thyroid function in occupationally exposed persons. Urinary Ba content was associated with decreased T_4 and T_3 level in blood serum [78]. Why in age over 40 the Ba content in normal female thyroid higher than that in male thyroid and how this excess acts on the female thyroid are still to be cleared.

Bromine

The Br is one of the most abundant and ubiquitous of ChE in the biosphere. Inorganic bromide compounds, especially potassium bromide (KBr), sodium bromide (NaBr), and ammonium bromide (NH4Br), are frequently used as sedatives in Russia [79]. This may be the reason for elevated levels of Br in female thyroid, because females particularly if aged over 40 years use sedatives more intensively than males. Inorganic bromide exerts therapeutic as well as toxic effects. An enhanced intake of bromide could interfere with the metabolism of iodine at the whole-body level, for both elements have similar chemical properties, and are adjacent halogens. So in the thyroid gland the biological behavior of bromide is similar to that of iodide [80]. Therefore, an excessive Br level in the thyroid of elderly females might inhibit thyroid hormonal synthesis.

Calcium

Despite the fact that Ca is the most abundant ChE in a human body its role in thyroid health is poorly understood. However, a significant direct correlation between serum Ca and thyroid stimulating hormone (TSH) level was confirmed by the results of many studies [81-83]. The reduced Ca content in female thyroid parenchyma in comparison with the optimal level characteristic of male thyroid can reflect some deficiency of this element in female body. Thus, a deficiency of Ca inhibits TSH secretion and, as consequence, thyroid

function.

Copper

Cu, like Fe and Zn, is also a ubiquitous element in the human body which plays many roles at different levels. Various cuproenzymes (such as amine oxidase, ceruloplasmin, cytochrome-c oxidase, dopamine-monooxygenase, extracellular superoxide dismutase, lysyl oxidase, peptidylglycineamidating monoxygenase, Cu/Z superoxide dismutase, and tyrosinase) mediate the effects of Cu deficiency or excess. For females, serum levels of Cu are associated with increased levels of thyroid hormones T_3 and T_4 [84]. Thus, Cu deficiency can have severe negative impacts on thyroid function.

Iron

The low Fe level in the thyroid of young women compared with men can be attributed to physiological characteristics of the female body related to reproduction and pre-menopausal physiology [55,56]. The Fe deficiency in young females needs in correction [85].

Lithium

The results of lifelong lithium-poor nutrition of animals show that lithium is essential to the fauna, and thus, to humans as well. It was shown that lithium-poor nutrition has a negative influence on feed intake, organism growth, skin properties, reproduction performance, milk production, mortality, and on some enzyme activity, mainly the enzymes of the citrate cycle, glycolysis, and of nitrogen metabolism [86].

Magnesium

Current biochemical evidence about the elements required to maintain thyroid function shows that these not only include dietary iodine and selenium (Se) but also Mg, because magnesium-ATP contributes to the active process of iodine uptake [85]. Moreover, Mg deficiency can influence bioavailability and tissue distribution of Se which then appears diminished [87]. Similar Ca, there is a significant direct correlation between serum Mg and TSH level [81]. From these data, one can conclude that Mg is involved in the thyroid function. The reduced Ca content in female thyroid parenchyma in comparison with the optimal level characteristic of male thyroid may reflect some deficiency of this element in female body, while a deficiency of Mg has to associate with hypothyroidism.

Sulfur

S is available to humans in their diets, derived mainly from proteins, and yet only 2 of the 20 amino acids normally present in proteins contains sulfur – methionine and cysteine. One of these amino acids, methionine, cannot be synthesized by our bodies and therefore has to be supplied by the diet. Proteins contain between 3 and 6% of sulfur amino acids. Sulfur amino acids contribute substantially to the maintenance and integrity of the cellular systems by influencing the cellular redox state and the capacity to detoxify toxic compounds, free radicals and reactive oxygen species [88]. ROS are generated during normal cellular activity and may exist in excess in some pathophysiological conditions, such as inflammation. Thus, S deficiency can be harmful for thyroid.

Silicon

There is evidence to suggest that excessive Si intake disturbers the endocrine balance between thyrotropin and thyroid hormones [89]. Moreover, the influence of Si on Ca and Mg metabolism was demonstrated [90]. The Si involvement in mineral metabolism may help to clarify the relationships between the elevated Si level and reduced Ca and Mg levels in female thyroid.

Zinc

Zn is a most essential ChE for humans. Today more than 300 proteins and over 100 DNA-binding proteins that require Zn have been classified. Zn is required for the

synthesis of thyroid hormones, and deficiency of this ChE can result in hypothyroidism [91,92]. Thus, a Zn deficiency in female thyroid parenchyma observed in the present study may be one of the reasons for the higher incidence of SCH in females in comparison with males.

Study limitations

This study has two 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 thyroid tissue. Secondly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on a significant deficiency of Al, B, Ca, Cu, Fe, Li, Mg, S, and Zn contents in thyroid parenchyma of females aged before 40 years and on an excess of Ba, Br and Si accompanied a deficiency of Mg for ages over 40. Present study shows also the necessity the need to continue research on the role of inadequate contents of intra-thyroidal ChE in the etiology of female SCH.

Conclusion

Our data indicate that there is a statistically significant gender-related difference between ChE levels in thyroid tissue of females and males that depends on age. Subclinical hypothyroidism is a multietiological and multifactorial complex condition. The complete understanding of the role of inadequate levels of some ChE in thyroid parenchyma in the etiology of SCH requires a global vision of their different mechanisms of action, which is not yet possible with the present state of knowledge. However, from the results of our study it follows that an involvement of inadequate contents of intra-thyroidal Al, B, Ba, Br, Ca, Cu, Fe, Li, Mg, S, Si, and Zn in the etiology of female SCH may be assumed.

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Conflict of interest

There is no any financial interest or any conflict of interest.

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