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Research Article

Investigation of Association between High Risk of Female Subclinical Hypothyroidism and Inadequate Quantities of Intra-Thyroidal Trace Elements using Neutron Activation and Inductively Coupled Plasma Mass Spectrometry

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Abstract

Introduction: The prevalence of subclinical hypothyroidism is 10 - 15 times more common in women than in men. Trace elements play important roles in thyroid function. The aim of this exploratory study was to evaluate whether significant difference of trace element contents exists between female and male thyroids and how it can be related to the etiology of subclinical hypothyroidism.

Methods: Thyroid tissue levels of 37 trace elements: Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Ga, Gd, Hg, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Ti, Tl, U, Y, and Zn were prospectively evaluated in 105 healthy persons (33 females and 72 males). Measurements were performed using a combination of non-destructive and destructive methods: instrumental neutron activation analysis and inductively coupled plasma mass spectrometry, respectively. Tissue samples were divided into two portions. One was used for morphological study while the other was intended for trace element analysis.

Results: It was found that for ages before 40 years means of Al, B, Cs, Fe, Li, Rb, Tl, and Zn content in female thyroid were lower than those in male thyroid. For ages over 40 years mean of Co content in female thyroid was higher whereas mean of Hg content was lower than those in male thyroid.

Conclusions: Inappropriate content of intra-thyroidal Al, B, Co, Cs, Fe, Hg, Li, Rb, Tl, and Zn and particularly an excess of Co and a deficiency of Fe and Zn can be associated with the etiology of female subclinical hypothyroidism.

Keywords: Subclinical Hypothyroidism; Female Thyroid; Trace Elements; Neutron Activation Analysis; Inductively Coupled Plasma Mass Spectrometry

Abbreviations

SCH: Subclinical Hypothyroidism; TE: Trace Elements; BSS: Biological Synthetic Standards; CRM/SRM: Certified/Standard Reference Materials; INAA-LLR: Instrumental Neutron Activation Analysis with High Resolution Spectrometry of Long-Lived Radionuclides; ICP-MS: Inductively Coupled Plasma Mass Spectrometry; IAEA: International Atomic Energy Agency.

Introduction

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 [1-11]. 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 trace elements (TE), 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 TE depend on tissue-specific need or tolerance, respectively [25]. Both TE deficiencies as well as overexposures may disturb the thyroidal cell functions [25].

The reliable data on TE mass fractions in normal human thyroid separately for female and male gland is apparently extremely limited. There are a few studies regarding TE content in human thyroid, using chemical techniques and instrumental methods [26-45]. 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 TE are lost as a result of such treatment [46-48]. Moreover, only a few of these studies employed quality control using certified/standard reference materials (CRM/SRM) for determination of the TE contents.

Therefore, nondestructive techniques such as instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR) combined with subsequent, destructive inductively coupled plasma atomic emission spectrometry (ICP-MS) 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 TE as silver (Ag), aluminum (Al), boron (B), beryllium (Be), bismuth (Bi), cadmium (Cd), cerium (Ce), cobalt (Co), chromium (Cr), cesium (Cs), dysprosium (Dy), erbium (Er), iron (Fe), gallium (Ga), gadolinium (Gd), mercury (Hg), lanthanum (La), lithium (Li), manganese (Mn), molybdenum (Mo), niobium (Nb), neodymium (Nd), nickel (Ni), lead (Pb), praseodymium (Pr), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), samarium (Sm), tin (Sn), terbium (Tb), titanium (Ti), thallium (Tl), uranium (U), yttrium (Y), and zinc (Zn) contents in intact (normal) thyroid gland of apparently healthy persons using the combination of INAA-LLR and ICP-MS analysis. The second aim was to compare the levels of TE in the thyroid tissue of all females and males investigated in the study. The final aim was to compare the levels of TE 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.

Materials 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, SD) was 30.9 ± 10.1 years and in group 2 (n = 22) mean age was 66.3 ± 12.8 years. For males in group 1 (n = 44) mean age was 25.3 ± 10.0 years and in group 2 (n = 28) mean age was 57.4 ± 14.5 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 [49]. One tissue portion was reviewed by an anatomical pathologist while the other was used for the TE 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 TE analysis were weighed, they were freeze-dried and homogenized [50-52]. The pounded sample weighing about 50 mg was used for trace element measurement by INAA-LLR. The samples for INAA-LLR were wrapped separately in a high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule. Biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used as standards [53]. In addition to BSS, aliquots of commercially available pure compounds were also used.

After NAA-LLR investigation the thyroid samples were taken out from the aluminum foils and used for ICP-MS. The samples were decomposed in autoclaves; 1.5 mL of concentrated HNO_3 (nitric acid at 65%, maximum (max) of 0.0000005% Hg; GR, ISO, Merck, Darmstadt, Germany) and 0.3 mL of H_2O_2 (pure for analysis) were added to thyroid samples, placed in one-chamber autoclaves (Ancon-AT2, Ltd., Moscow, Russia) and then heated for 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 (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 [54-61].

Instrumentation and methods

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

The samples were measured for period from 10 to 30 days after irradiation. The duration of measurements was from 20 min to 10 hours subject to pulse counting rate. Spectrometric measurements were performed using a coaxial 98-cm^3 Ge (Li) detector and a spectrometric unit (NUC 8100), including a PC-coupled multichannel analyzer. Resolution of the spectrometric unit was 2.9-keV at the ^{60}Co 1,332-keV line.

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

Information detailing with the NAA-LLR and ICP-MS methods used, and other details of the analysis was presented in our previous publication [54-61].

Computer programs and statistic

A dedicated computer program for INAA mode optimization was used [62]. All thyroid samples were prepared in duplicate, and mean values of TE contents were used in final calculation. Mean values was also used for TE contents that were measured by two different methods. Using Microsoft Office Excel, arithmetic mean, standard deviation, and standard error of mean was calculated for TE 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 Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions (mg/kg, dry mass basis) in the normal thyroids of females and males obtained by both NAA-LLR and ICP-MS methods is shown in table 1.

Gender	Element	NAA-LLR M_1	ICP-MS M_2	Δ, %
Males	Ag	0.0156 ± 0.0021	0.0121 ± 0.0018	22.4
	Co	0.0352 ± 0.0031	0.0334 ± 0.0032	5.1
	Cr	0.520 ± 0.041	0.427 ± 0.039	17.9
	Fe	222 ± 12	223 ± 15	-0.5
	Hg	0.0437 ± 0.0048	0.0970 ± 0.0102	-122
	Rb	7.89 ± 0.58	8.38 ± 0.61	-6.2
	Sb	0.108 ± 0.010	0.0705 ± 0.0097	34.7
	Se	2.36 ± 0.17	2.10 ± 0.17	11.0
	Zn	103 ± 5.5	95.4 ± 5.1	7.4
Females	Ag	0.0140 ± 0.0020	0.0129 ± 0.0041	7.9
	Co	0.0505 ± 0.0064	0.0479 ± 0.0069	5.1
	Cr	0.573 ± 0.049	0.496 ± 0.057	13.4
	Fe	232 ± 22	217 ± 19	6.5
	Hg	0.0389 ± 0055	0.0471 ± 0.0087	-21.1
	Rb	6.16 ± 0.48	6.38 ± 0.53	-3.6
	Sb	0.116 ± 0.012	0.098 ± 0.014	15.5
	Se	2.22 ± 0.23	2.16 ± 0.23	0.5
	Zn	85.7 ± 7.4	83.2 ± 8.1	2.9

Table 1: Comparison of the mean values (M±SEM) of the chemical element mass fractions (mg/kg, dry mass basis) in the normal thyroid of males and females obtained by both NAA-LLR and ICP-MS methods.
M: Arithmetic Mean; SEM: Standard Error of Mean, $\Delta=[(M_1 - M_2)/M_1] \cdot 100\%$.

The comparison of our results (Mean ± Standard Deviation) for group of females and males combined with published data for Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Ga, Gd, Hg, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Ti, Tl, U, Y, and Zn mass fraction in normal human thyroid is shown in table 2.

The ratios of means and the difference between mean values Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Ga, Gd, Hg, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Ti, Tl, U, Y, and Zn mass fractions in normal thyroid of females and males are presented in table 3.

Because, in our previous studies age-dependents of many TE in thyroid gland was found [63-71], the comparison between TE contents in thyroid of females and males separately in age group 1 and 2 was also performed (Tables 4 and 5).

Discussion

Precision and accuracy of results

A good agreement of our results for TE 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 [54-61] as well as the similarity of the means of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions in the normal human thyroids determined by both NAA-LLR and ICP-MS methods (Table 1) demonstrates an acceptable precision and accuracy of the results obtained in the study and presented in tables 2-5.

Element	This work	Published data [Reference]		
	Males and females (combined) M ± SD	Median of means (n)*	Minimum of means M or M ± SD, (n)**	Maximum of means M or M ± SD, (n)**
Ag	0.0133 ± 0.0114	0.25 (12)	0.000784 (16) [1]	1.20 ± 1.24 (105) [2]
Al	10.5 ± 13.4	33.6 (12)	0.33 (-) [3]	420 (25) [4]
B	0.476 ± 0.434	0.151 (2)	0.084 (3) [5]	0.46 (3) [5]
Be	0.00052 ± 0.00060	0.042 (3)	0.000924(16) [1]	< 0.12 (-) [6]
Bi	0.0072 ± 0.0161	0.126 (4)	0.0339 (16) [1]	< 0.4 (-) [6]
Cd	2.08 ± 2.05	3.01 (15)	0.84 (180) [7]	47.6 ± 8.0 (16) [8]
Ce	0.0080 ± 0.0080	0.22 (1)	0.22 (59) [1]	0.22 (59) [1]
Co	0.039 ± 0.028	0.336 (17)	0.026 ± 0.031 (46) [9]	70.4 ± 40.8 (14) [10]
Cr	0.49 ± 0.25	0.69 (17)	0.105 (18) [5]	24.8 ± 2.4 (4) [11]
Cs	0.025 ± 0.017	0.069 (6)	0.011 ± 0.011 (14) [12]	0.109 ± 0.370 (48) [9]
Dy	0.0012 ± 0.0018	0.0011 (1)	0.0011 (60) [1]	0.0011 (60) [1]
Er	0.00038 ± 0.00038	0.0007 (1)	0.0007 (60) [1]	0.0007 (60) [1]
Fe	223 ± 90	252 (21)	56 (120) [13]	2444 ± 700 (14) [10]
Ga	0.032 ± 0.016	0.273 (3)	< 0.04 (-) [6]	1.7 ± 0.8 (-) [14]
Gd	0.00105 ± 0.00015	0.0026 (1)	0.0026 (59) [1]	0.0026 (59) [1]
Hg	0.054 ± 0.037	0.08 (13)	0.0008 ± 0.0002 (10) [14]	396 ± 40 (4) [11]
La	0.0047 ± 0.0046	0.068 (3)	0.052 (59) [1]	< 4.0 (-) [6]
Li	0.021 ± 0.015	6.3 (2)	0.092 (-) [6]	12.6 (180) [7]
Mn	1.28 ± 0.56	1.82 (36)	0.44 ± 11 (12) [17]	69.2 ± 7.2 (4) [11]
Mo	0.0836 ± 0.047	0.42 (11)	0.0288 ± 0.0096 (39) [15]	516 ± 292 (14) [10]
Nb	0.60 ± 0.90	< 4.0 (1)	<4.0 (-) [6]	< 4.0 (-) [6]
Nd	0.0040 ± 0.0034	0.0108 (1)	0.0108 (60) [2]	0.0108 (60) [1]
Ni	0.45 ± 0.34	0.96 (16)	0.00084 (83) [16]	33.6 ± 3.6 (4) [11]
Pb	0.23 ± 0.25	0.63 (22)	0.021 (83) [16]	68.8 ± 6.8 (4) [11]
Pr	0.00107 ± 0.00086	0.0034 (1)	0.0034 (59) [1]	0.0034 (59) [1]
Rb	7.5 ± 3.7	12.3 (9)	≤ 0.85 (29) [15]	294 ± 191 (14) [10]
Sb	0.094 ± 0.069	0.105 (10)	0.040 ± 0.003 (-) [17]	4.0 (-) [18]
Sc	0.0268 ± 0.0329	0.009 (4)	0.0018 ± 0.0003 (17) [19]	0.014 ± 0.005 (10) [15]
Se	2.2 ± 1.2	2.61 (17)	0.95 ± 0.08 (29) [15]	756 ± 680 (14) [10]
Sm	0.00051 ± 0.00047	0.0022 (1)	0.0022 (60) [1]	0.0022 (60) [1]
Sn	0.078 ± 0.068	0.40 (7)	0.0235 (16) [1]	-≤ 3.8 (17) [20]
Tb	0.00020 ± 0.00012	0.0002 (1)	0.00022 (60) [1]	0.00022 (60) [1]
Ti*	3.5 ± 3.5	1.42 (8)	0.084 (83) [16]	73.6 ± 7.2 (4) [11]
Tl	0.00099 ± 0.00053	< 0.2 (2)	0.00138 (16) [1]	< 0.4 (-) [6]
U	0.00044 ± 0.00043	0.05 (7)	0.00424 (16) [1]	0.428 ± 0.143 (10) [15]
Y	0.0026 ± 0.0023	< 2.9 (2)	0.00225 (16) [1]	≤ 5.9 (17) [20]
Zn	95 ± 40	118 (51)	32 (120) [13]	820 ± 204 (14) [10]

Table 2: Median, minimum and maximum value of means of trace element contents in the normal thyroid according to data from the literature in comparison with our results (mg/kg, dry mass basis).

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

Element	Thyroid tissue				Ratio
	Females 3.5 - 87 years n = 33	Males 2.0 - 80 years n = 72	t-test *p*£	U-test p	Females to Males
Ag	0.0132 ± 0.0020	0.0133 ± 0.0017	0.992	> 0.05	0.99
Al	7.43 ± 1.24	11.3 ± 2.3	0.132	> 0.05	0.66
B	0.418 ± 0.074	0.491 ± 0.071	0.483	> 0.05	0.85
Be	0.00067 ± 0.00028	0.00048 ± 0.00008	0.521	> 0.05	1.40
Bi	0.0180 ± 0.0084	0.00386 ± 0.00079	0.119	> 0.05	4.66
Cd	1.63 ± 0.48	2.22 ± 0.33	0.322	> 0.05	0.73
Ce	0.00897 ± 0.00227	0.00773 ± 0.00123	0.636	> 0.05	1.16
Co	0.0493 ± 0.0066	0.0345 ± 0.0031	0.052	**≤ 0.05**	1.43
Cr	0.535 ± 0.051	0.474 ± 0.038	0.341	> 0.05	1.13
Cs	0.0185 ± 0.0029	0.0263 ± 0.0026	0.055	**≤ 0.05**	0.70
Dy	0.00173 ± 0.00095	0.00108 ± 0.00018	0.511	> 0.05	1.60
Er	0.00050 ± 0.00017	0.00034 ± 0.00004	0.384	> 0.05	1.47
Fe	225 ± 20	222 ± 11	0.907	> 0.05	1.01
Ga	0.0309 ± 0.0060	0.0318 ± 0.0022	0.897	> 0.05	0.97
Gd	0.00147 ± 0.00048	0.00092 ± 0.00012	0.288	> 0.05	1.60
Hg	0.0400 ± 0.0057	0.0610 ± 0.0055	**0.0092**	**≤ 0.01**	0.66
La	0.00550 ± 0.00106	0.00454 ± 0.00074	0.464	> 0.05	1.21
Li	0.0153 ± 0.0024	0.0225 ± 0.0028	0.055	**≤ 0.05**	0.68
Mn	1.32 ± 0.22	1.27 ± 0.06	0.834	> 0.05	1.04
Mo	0.0755 ± 0.0169	0.0856 ± 0.0064	0.584	> 0.05	0.88
Nb	0.641 ± 0.200	0.584 ± 0.145	0.819	> 0.05	1.10
Nd	0.0046 ± 0.0012	0.00388 ± 0.00047	0.557	> 0.05	1.19
Ni	0.385 ± 0.056	0.467 ± 0.056	0.308	> 0.05	0.82
Pb	0.200 ± ± 0.032	0.242 ± 0.040	0.426	> 0.05	0.83
Pr	0.00123 ± 0.00028	0.00102 ± 0.00012	0.501	> 0.05	1.21
Rb	6.27 ± 0.48	8.07 ± 0.50	**0.011**	**≤ 0.01**	0.78
Sb	0.107 ± 0.013	0.0895 ± 0.0091	0.271	> 0.05	1.20
Sc	0.0086 ± 0.0045	0.0390 ± 0.0084	**0.0040**	**≤ 0.01**	0.22
Se	2.19 ± 0.23	2.23 ± 0.17	0.879	> 0.05	0.98
Sm	0.00057 ± 0.00013	0.00049 ± 0.00007	0.575	> 0.05	1.16
Sn	0.112 ± 0.026	0.0674 ± 0.0085	0.122	> 0.05	1.66
Tb	0.00020 ± 0.00004	0.00020 ± 0.00002	0.905	> 0.05	1.00
Ti*	3.74 ± 1.16	3.43 ± 0.51	0.807	> 0.05	1.09
Tl	0.00070 ± 0.00010	0.00099 ± 0.00008	0.031	> 0.05	0.71
U	0.00060 ± 0.00020	0.00040 ± 0.00005	0.357	> 0.05	1.50
Y	0.00304 ± 0.00082	0.00247 ± 0.00034	0.526	> 0.05	1.23
Zn	84.5 ± 7.6	99.1 ± 5.0	0.114	> 0.05	0.85

Table 3: Differences between mean values (M±SEM) of trace element mass fractions (mg/kg, dry mass basis) in normal thyroid tissue of males and females.

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**.

Element	Thyroid tissue				Ratio
	Males (MG1) 2.0 - 40 years n = 44	Females (FG1) 3.5 - 40 years n = 11	t-test $p \leq$	U-test p	FG1/MG1
Ag	0.0134 ± 0.0025	0.0125 ± 0.0032	0.817	> 0.05	0.93
Al	13.2 ± 3.4	5.95 ± 0.97	**0.049**	≤ **0.05**	0.45
B	0.607 ± 0.105	0.361 ± 0.070	0.061	≤ **0.05**	0.59
Be	0.00055 ± 0.00011	0.00088 ± 0.00046	0.522	> 0.05	1.60
Bi	0.00416 ± 0.00101	0.018 ± 0.012	0.272	> 0.05	4.33
Cd	1.45 ± 0.27	1.34 ± 0.53	0.858	> 0.05	0.92
Ce	0.0072 ± 0.0016	0.0070 ± 0.0030	0.942	> 0.05	0.97
Co	0.0367 ± 0.00471	0.0311 ± 0.0046	0.400	> 0.05	0.85
Cr	0.476 ± 0.049	0.495 ± 0.066	0.818	> 0.05	1.04
Cs	0.0259 ± 0.0037	0.0160 ± 0.0032	0.056	≤ **0.05**	0.62
Dy	0.00125 ± 0.00026	0.0021 ± 0.0014	0.567	> 0.05	1.68
Er	0.00036 ± 0.00005	0.00059 ± 0.00025	0.395	> 0.05	1.64
Fe	229 ± 14	174 ± 25	0.077	≤ **0.05**	0.76
Ga	0.0340 ± 0.0032	0.0285 ± 0.0086	0.562	> 0.05	0.84
Gd	0.00108 ± 0.00018	0.00141 ± 0.00074	0.673	> 0.05	1.31
Hg	0.0589 ± 0.0079	0.0402 ± 0.0078	0.102	> 0.05	0.68
La	0.00389 ± 0.00077	0.00423 ± 0.00120	0.813	> 0.05	1.09
Li	0.0273 ± 0.0040	0.0131 ± 0.0033	**0.012**	≤ **0.01**	0.48
Mn	1.43 ± 0.09	1.21 ± 0.14	0.209	> 0.05	0.85
Mo	0.0845 ± 0.0090	0.072 ± 0.027	0.669	> 0.05	0.85
Nb	0.597 ± 0.223	0.349 ± 0.155	0.368	> 0.05	0.58
Nd	0.00379 ± 0.00065	0.0046 ± 0.0019	0.683	> 0.05	1.21
Ni	0.527 ± 0.084	0.357 ± 0.051	0.094	> 0.05	0.68
Pb	0.249 ± 0.058	0.140 ± 0.026	0.096	> 0.05	0.56
Pr	0.00095 ± 0.00016	0.00118 ± 0.00044	0.638	> 0.05	1.24
Rb	8.50 ± 0.67	4.93 ± 0.64	**0.00050**	≤ **0.01**	0.58
Sb	0.0900 ± 0.0105	0.0762 ± 0.00084	0.312	> 0.05	0.85
Sc	0.0458 ± 0.0107	0.0290 ± 0.0281	0.656	> 0.05	0.63
Se	1.95 ± 0.18	1.80 ± 0.24	0.637	> 0.05	0.92
Sm	0.00054 ± 0.00011	0.00039 ± 0.00017	0.497	> 0.05	0.72
Sn	0.0575 ± 0.0108	0.076 ± 0.023	0.476	> 0.05	1.32
Tb	0.00022 ± 0.00002	0.000213 ± 0.000060	0.949	> 0.05	0.97
Ti*	3.15 ± 0.55	3.39 ± 1.30	0.869	> 0.05	1.08
Tl	0.00103 ± 0.00010	0.00060 ± 0.00016	**0.043**	≤ **0.01**	0.58
U	0.00049 ± 0.00007	0.00063 ± 0.00029	0.648	> 0.05	1.29
Y	0.00282 ± 0.00047	0.0032 ± 0.0012	0.767	> 0.05	1.13
Zn	100.7 ± 6.6	58.3 ± 9.3	**0.0013**	≤ **0.01**	0.58

Table 4: Differences between mean values (M ± SEM) of trace element mass fractions (mg/kg, dry mass basis) in normal thyroid tissue of males and females aged 2 - 40 years.

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**.

Element	Thyroid tissue				Ratio
	Males (MG2) 41 - 80 years n = 28	Females (FG2) 41 - 87 years n = 22	t-test $p \leq$	U-test p	FG2/MG2
Ag	0.0130 ± 0.0018	0.0138 ± 0.0027	0.806	> 0.05	1.06
Al	8.3 ± 1.7	9.8 ± 2.7	0.643	> 0.05	1.18
B	0.289 ± 0.029	0.533 ± 0.177	0.263	> 0.05	1.84
Be	0.00036 ± 0.00008	0.00037 ± 0.00013	0.933	> 0.05	1.03
Bi	0.00338 ± 0.00130	0.017 ± 0.012	0.324	> 0.05	5.03
Cd	3.65 ± 0.64	2.10 ± 0.95	0.213	> 0.05	0.58
Ce	0.00852 ± 0.00194	0.0118 ± 0.0034	0.444	> 0.05	1.38
Co	0.0310 ± 0.0031	0.0635 ± 0.0099	**0.0063**	≤ **0.01**	2.05
Cr	0.471 ± 0.063	0.566 ± 0.076	0.339	> 0.05	1.20
Cs	0.0271 ± 0.0033	0.0225 ± 0.0056	0.503	> 0.05	0.83
Dy	0.00081 ± 0.00020	0.00095 ± 0.00014	0.578	> 0.05	1.17
Er	0.00031 ± 0.00008	0.00032 ± 0.00005	0.978	> 0.05	1.03
Fe	209 ± 17	264 ± 25	0.079	> 0.05	1.26
Ga	0.0281 ± 0.0021	0.0358 ± 0.0069	0.354	> 0.05	1.27
Gd	0.00067 ± 0.00011	0.00156 ± 0.00055	0.182	> 0.05	2.33
Hg	0.0639 ± 0.0074	0.0391 ± 0.0084	**0.035**	≤ **0.01**	0.61
La	0.00553 ± 0.00146	0.00728 ± 0.00176	0.460	> 0.05	1.32
Li	0.0145 ± 0.0022	0.0178 ± 0.0032	0.430	> 0.05	1.23
Mn	1.06 ± 0.07	1.54 ± 0.45	0.423	> 0.05	1.45
Mo	0.0874 ± 0.0083	0.081 ± 0.014	0.712	> 0.05	0.93
Nb	0.565 ± 0.145	1.11 ± 0.40	0.252	> 0.05	1.96
Nd	0.00401 ± 0.00067	0.0046 ± 0.0008	0.581	> 0.05	1.15
Ni	0.372 ± 0.057	0.424 ± 0.120	0.707	> 0.05	1.14
Pb	0.229 ± 0.052	0.284 ± 0.047	0.445	> 0.05	1.24
Pr	0.00113 ± 0.00020	0.00132 ± 0.00028	0.588	> 0.05	1.17
Rb	7.30 ± 0.71	7.26 ± 0.57	0.965	> 0.05	0.99
Sb	0.0886 ± 0.0174	0.1296 ± 0.0198	0.129	> 0.05	1.46
Sc	0.0255 ± 0.0132	0.0045 ± 0.0043	0.173	> 0.05	0.18
Se	2.72 ± 0.31	2.47 ± 0.35	0.589	> 0.05	0.91
Sm	0.00041 ± 0.00006	0.00083 ± 0.00017	0.066	> 0.05	2.02
Sn	0.0824 ± 0.0133	0.168 ± 0.048	0.152	> 0.05	2.04
Tb	0.00017 ± 0.00002	0.000183 ± 0.000048	0.766	> 0.05	1.08
Ti*	3.91 ± 1.02	4.30 ± 2.39	0.884	> 0.05	1.10
Tl	0.00093 ± 0.00012	0.00083 ± 0.00010	0.531	> 0.05	0.89
U	0.00025 ± 0.00003	0.00052 ± 0.00020	0.280	> 0.05	2.08
Y	0.00191 ± 0.00044	0.0027 ± 0.0006	0.317	> 0.05	1.41
Zn	96.4 ± 7.6	103.7 ± 8.5	0.527	> 0.05	1.08

Table 5: Differences between mean values (M ± SEM) of trace element mass fractions (mg/kg, dry mass basis) in normal thyroid tissue of males and females aged 41 - 87 years.

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**.

Comparison with published data

The obtained means for Al, B, Cd, Cr, Cs, Dy, Er, Fe, Hg, Mn, Ni, Pb, Pr, Rb, Sb, Sc, Se, Tb, Ti, and Zn mass fraction in thyroids of females and males, as shown in table 2, 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 [26-45]. The obtained means for Ag, Co, Mo, Sn, and Y are lower medians but inside the range of previously reported means, while means for Be, Bi, Ce, Ga, Gd, La, Li, Nb, Nd, Sm, Tl, and U are outside. Moreover, means of Bi, Ce, La, Nb, Nd, and U content obtained in the study are almost one order of magnitude lower the minimal value of published results. 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% [34] and ash 4.16% on dry mass basis [72] contents in thyroid of adults.

The range of means of Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Ga, Gd, Hg, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Ti, Tl, U, Y, and Zn level reported in the literature for normal human thyroid vary widely (Table 2). 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

Pronounced differences in Co, Cs, Hg, Li, Rb, and Sc mass fraction were observed between female and male thyroid (Table 3). The mean of Co mass fraction in female thyroids was 1.4 time higher while the means of Cs, Hg, Li, Rb, and Sc mass fractions were respectively 30%, 34%, 32%, 22%, and 78% lower than in male thyroids. During the first 40 years of life (Age group 1) the situation with TE contents in female thyroids was some different than that for older females ((Age group 2). In Age group 1 differences between Al, B, Cs, Fe, Li, Rb, Tl, and Zn contents in female and male thyroids were detected (Table 4). In Age group 1 of females with mean age 30.9 years the Al, B, Cs, Fe, Li, Rb, Tl, and Zn contents in thyroid were respectively 55%, 41%, 38%, 24%, 52%, 42%, 42% and 42% 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 Co and Hg content in female and male thyroids was observed. The mean of Co content in female thyroids was 2.05 times higher, whereas the mean of Hg 39% lower than that in male thyroids. In Age group 2 differences between the Al, B, Cs, Fe, Li, Rb, Tl, 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 greater in women than in men [4,10], we can accept that the levels of TE 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, Cs, Fe, Li, Rb, Tl, and Zn contents in female thyroid parenchyma. In age over 40 deficiencies of Al, B, Cs, Fe, Li, Rb, Tl, and Zn contents in female thyroid disappear and an excess of Co as well as deficiency Hg 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 [73]. 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 [74]. 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 [75]. Moreover, B helps the conversion of the storage form of thyroid hormone, T_4, to T_3, the active form.

Cobalt

Co is widely used in a bijouterie production. It may be one of the reasons of the higher level of this TE content in female thyroids than in that in male thyroids. Health effects of high Co occupational, environmental, dietary and medical exposure are characterized by a complex clinical syndrome, mainly including neurological, cardiovascular and endocrine deficits, including hypothyroidism

[76,77]. Moreover, Co is genotoxic and carcinogenic, mainly caused by oxidative DNA damage by reactive oxygen species, perhaps combined with inhibition of DNA repair [78]. Therefore, an excessive Co level in the thyroid of elderly females might inhibit thyroid hormonal synthesis.

Cesium

In humans blood Cs concentrations were inversely associated with thyroid hormones fT_3 and T_3 [79]. It means that impaired thyroid function is a common effect of Cs exposure, for example, through drinking water. Why Cs 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.

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 [54,55]. The Fe deficiency in young females needs in correction [80].

Mercury

Hg is one of the most dangerous environmental pollutants [81]. The International Agency for Research on Cancer classified Hg as certain or probable carcinogen [82]. This metal involved in many processes that lead to genotoxicity, including generation of free radicals and oxidative stress, action on microtubules, influence on DNA repair mechanisms and direct interaction with DNA molecules [83]. Hg may decrease fertility in women and men and also harm a developing fetus [81]. However, long-term effects of chronic exposure to relatively low levels of Hg compounds are still not fully understood.

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 [84].

Rubidium

As for Rb, there is very little information about its effects in organisms. No negative environmental effects have been reported. Rb is only slightly toxic on an acute toxicological basis and would pose an acute health hazard only when ingested in large quantities [85]. Rb has some function in immune responce [86], probably by supporting cell differentiation [87]. Both potassium (K) and Rb are in the first group of the periodic table. Rb, like K, seems to be concentrated in the intracellular space and transfered through membrane by the Na+K+-ATPase pump. Thus, the low Rb level in the thyroid of women compared with men might reflect the reduced ratio "Volume of thyroid cells / Volume of follicular colloid" in the female thyroid. Thyroid function depends in part on the total volume of active thyroid cells. From this it might be concluded that the reduced level of active cells in the thyroids of women compared to men increases risk of hypothyroidism.

Thallium

Tl considered as the most toxic among TE [88]. Tl is a suspected human carcinogen [89].

Zinc

Zn is a most essential TE 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 TE can result in hypothyroidism [90,91]. 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 37 TE (Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Ga, Gd, Hg, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Ti, Tl, U, Y, and Zn) mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of TE 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, Cs, Fe, Li, Rb, Tl, and Zn contents in thyroid parenchyma of females aged before 40 years and on an excess of Co accompanied a deficiency of Hg for ages over 40. Present study shows also the necessity the need to continue research on the role of inadequate contents of intra-thyroidal TE in the etiology of female SCH.

Conclusion

Our data indicate that there is a statistically significant gender-related difference between TE 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 TE 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, Co, Cs, Fe, Hg, Li, Rb, Tl, and Zn and particularly an excess of Co and a deficiency of Fe and Zn, in the etiology of female SCH may be assumed.

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Conflict of Interest

The authors have not declared any conflict of interests.

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Volume 2 Issue 9 December 2018