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Association Between Female Subclinical Hypothyroidism and Inadequate Quantities of Some Intra-Thyroidal Chemical Elements Investigated by Combination of X-ray Fluorescent and Neutron Activation Analysis

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Abstract

Background: 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.

Aim: The aim of this exploratory 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.

Methods: Thyroid tissue levels of twenty chemical elements: Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn were prospectively evaluated in 105 healthy persons (33 females and 72 males). Measurements were performed using energy dispersive X-ray fluorescent analysis and instrumental neutron activation analysis with high resolution spectrometry of short- and long-lived radionuclides. Tissue samples were divided into two portions. One was used for morphological study while the other was intended for chemical element analysis.

Results: It was found that for ages before 40 years means of Ca, Fe, Mg, Rb and Zn content in female thyroids were lower than in male thyroids. For ages over 40 years means of Br and Co content in female thyroid was higher than those in male thyroid.

Conclusions: Inappropriate content of intra-thyroidal Br, Ca, Co, Fe, Mg, Rb and Zn can be associated with the etiology of female subclinical hypothyroidism.

Keywords: *Subclinical hypothyroidism; Female Thyroid; Chemical Elements; X-ray fluorescent analysis; Neutron activation analysis*

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Abbreviations: SCH, subclinical hypothyroidism; ChE: chemical elements; EDXRF: energy dispersive X-ray fluorescent analysis; INAA-SLR: neutron activation analysis with high resolution spectrometry of short-lived radionuclides; INAA-LLR: neutron activation analysis with high resolution spectrometry of long-lived radionuclides; Ag: silver; Br: bromine; Ca: calcium; Cl: chlorine; Co: cobalt; Cr: chromium; Cu: Cooper; Fe: iron; Hg: mercury; I: iodine; K: potassium; Mg: magnesium; Mn: manganese; Na: sodium: Rb: rubidium; Sb: antimony; Sc: scandium; Se: selenium; Sr: strontium; Zn: zinc; SRM: standard reference material; CRM: certified reference material; BSS: biological synthetic standards.

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 [26-43]. 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 [44-46]. Moreover, only a few of these studies employed quality control using certified/standard reference materials (CRM/SRM) for determination of the ChE contents. Sample-nondestructive techniques such as instrumental energy dispersive X-ray fluorescent analysis (EDXRF) and instrumental neutron activation analysis with high resolution spectrometry of short- and long-lived radionuclides (INAA-SLR and INAA-LLR, respectively) are good alternatives for multi-element determination in the samples of thyroid parenchyma.

This work had three aims. The primary purpose of this study was to determine reliable values for such ChE as silver (Ag), bromine (Br), calcium (Ca), chlorine (Cl), cobalt (Co), chromium (Cr), coper (Cu), iron (Fe), mercury (Hg), I, potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), strontium (Sr), and zinc (Zn) contents in intact (normal) thyroid gland of apparently healthy persons using EDXRF, INAA-SLR, and INAA-LLR 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 ($\leq$ 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, 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 [47]. 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 [48-50].

For EDXRF the pounded sample weighing about 8 mg was applied to the piece of Scotch tape serving as an adhesive fixing backing [51,52]. To determine the contents of the elements by comparison with a known standard, aliquots of commercial, chemically pure compounds were used [52]. The microliter standards prepared from aliquots of commercially available pure compounds were placed on disks made of thin, ash-free filter papers fixed on the Scotch tape pieces and dried in a vacuum.

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 [53]. In addition to BSS, aliquots of commercially available pure compounds were also used.

The 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. BSS were used as standards [53].

Certified Reference Materials

Ten subsamples of the Certified Reference Materials (CRM) IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) were analyzed to estimate the precision and accuracy of results obtained by EDXRF, INAA-SLR, and INAA-LLR. In each method the CRMs subsamples were prepared and analyzed in the same way as the samples of thyroid tissue.

Instrumentation and methods

The facility for EDXRF included an annular ^{109}Cd source with an activity of 2.56 GBq, Si (Li) detector and portable multichannel analyzer combined with a PC (NUC 8100, Hungary). Its resolution was 270 eV at the 5.9 keV line of ^{55}Fe-source. The duration of the Br, Cu, Fe, Rb, Sr, and Zn measurements was 60 min. The intensity of K_{α}-line of Br, Cu, Fe, Rb, Sr, and Zn for samples and standards was estimated on calculation basis of the total area of the corresponding photo peak in the spectra. The trace element content was calculated by the relative way of comparing between intensities of Kα-lines for samples and standards. More details of the facility and method of analysis were presented in our previous publication [51,52].

A horizontal channel equipped with the pneumatic rabbit system of the WWR-c research nuclear reactor (Karpov Institute of Physical Chemistry, Obninsk Branch) was used for INAA-SLR. The neutron flux in the channel was 1.7×10^{13}n cm^{-2} s^{-1}. Ampoules with thyroid tissue samples, SSB, intra laboratory-made standards, and certified reference material were put into polyethylene rabbits and then irradiated separately for 180 s. Copper foils were used to assess neutron flux. The measurement of each sample was made twice, 1 and 120 min after irradiation.

The duration of the first and second measurements was 10 and 20 min, respectively. Spectrometric measurements were performed using a coaxial 98-cm^3 Ge (Li) detector and a spectrometric unit (NUC 8100, Hungary), including a PC-coupled multichannel analyzer. Resolution of the spectrometric unit was 2.9-keV at the ^{60}Co 1,332-keV line. Details of used nuclear reactions, radionuclides, and gamma-energies were reported in our earlier publications concerning the INAA-SLR of ChE contents in human prostate and scalp hair [54-56].

A vertical channel of the WWR-c research nuclear reactor (Karpov Institute of Physical Chemistry, Obninsk Branch) was applied to determine the content of trace elements 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 $1.3 \cdot 10^{13}$ 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. The gamma spectrometer included the 100 cm^3 Ge (Li) detector and a spectrometric unit (NUC 8100, Hungary), including a PC-coupled multichannel analyzer. The spectrometer provided a resolution of 1.9 keV on the ^{60}Co 1332 keV line. Details of used nuclear reactions, radionuclides, and gamma-energies were presented in our earlier publications concerning the INAA-LLR of ChE contents in human prostate and scalp hair [54,55,57-59].

Computer programs and statistic

A dedicated computer program for INAA mode optimization was used [60]. 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 and 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 and Discussion

Precision and accuracy of results

Table 1 depicts our data for Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fractions in ten sub-samples of IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) certified reference material and the certified values of this material.

Element	IAEA H-4 animal muscle	This work results	IAEA HH-1 human hair	This work results
Ag	-	0.033 ± 0.008	0.19 ± 0.06[b]	0.18 ± 0.05
Br	4.1 ± 1.1[a]	5.0 ± 09	4.2 ± 2.1[b]	3.9 ± 1.6
Ca	188 ± 58[b]	238 ± 59	522 ± 160[a]	525 ± 42
Cl	1890 ± 130[b]	1950 ± 230	2265 ± 478[a]	2210 ± 340
Co	0.0027 ± 0.0010[b]	0.0034 ± 0.0008	5.97 ± 0.42[a]	5.4 ± 1.1
Cr	0.06 ± 0.04[b]	0.071 ± 0.010	0.27± 0.16[b]	≤ 0.3
Cu	4.0 ± 1.0[a]	3.9 ± 1.1	10.2 ± 3.2[a]	-
Fe	49.1 ± 6.5[a]	47.0 ± 1.0	23.7 ± 3.1[a]	25.1 ± 4.3
Hg	0.014 ± 0.005[b]	0.015 ± 0.004	1.70 ± 0.09[a]	1.54 ± 0.14
I	0.08 ± 0.10[b]	<1.0	20.3 ± 8.9[b]	19.1 ± 6.2
K	15840 ± 1440[a]	16200 ± 3800	9.2 ± 5.2[b]	10.7 ± 4.0
Mg	1050 ± 140[a]	1100 ± 190	62.0 ± 9.6[b]	64.7 ± 18.6
Mn	0.52 ± 0.08[a]	0.55 ± 0.11	0.85 ± 0.25[a]	0.93 ± 0.16
Na	2060 ± 330[a]	2190 ± 140	12.6 ± 4.8[b]	14.0 ± 2.7
Rb	18.7 ± 3.5[a]	22 ± 4	0.94 ± 0.09[b]	0.89 ± 0.17
Sb	0.0056 ± 0.0031[b]	0.0061 ± 0.0021	0.031 ± 0.010[b]	0.033 ± 0.009
Sc	0.0059 ± 0.0034[b]	0.0015 ± 0.0009	-	-
Se	0.28 ± 0.08[a]	0.281 ± 0.014	0.35 ± 0.02[a]	0.37 ± 0.08
Sr	-	<1	0.82 ± 0.16[b]	1.24 ± 0.57
Zn	86.3 ± 11.5[a]	91 ± 2	174 ± 9[a]	173 ± 17

Table 1: *EDXRF, INAA-SLR and INAA-LLR data of chemical element contents in certified reference material IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) compared to certified values ((mg/kg, dry mass basis).*

M – Arithmetical mean, SD – standard deviation, a – Certified values, b – Information values.

Good agreement of the Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn contents analyzed by INAA-LLR with the certified data of CRM IAEA H-4 and IAEA HH-1 (Table 1) indicates an acceptable accuracy of the results obtained in the study of ChE of the thyroid presented in Tables 2–6.

Gender	Element	M	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Males n=72	Ag	0.016	0.016	0.002	0.0017	0.080	0.010	0.0018	0.0661
	Br	12.3	7.9	1.0	1.90	40.7	10.0	2.50	34.8
	Ca	1703	1048	131	414	6230	1470	452	4163
	Cl	3449	1450	219	1030	5920	3470	1262	5657
	Co	0.035	0.023	0.003	0.0046	0.124	0.030	0.0113	0.101
	Cr	0.520	0.286	0.041	0.130	1.30	0.414	0.152	0.980
	Cu	4.25	1.47	0.20	1.10	7.50	4.15	1.78	7.39
	Fe	222	88	11	51.0	478	224	70.4	414
	Hg	0.046	0.039	0.005	0.0091	0.180	0.032	0.0102	0.150

	I	1786	940	118	220	4205	1742	239	3808
	K	6289	2594	329	2440	14300	5670	2622	12670
	Mg	306	143	19	99.0	930	287	107	572
	Mn	1.31	0.49	0.07	0.510	2.30	1.30	0.534	2.21
	Na	6820	1781	214	3050	13453	6680	3861	11350
	Rb	8.97	5.04	0.64	2.90	29.4	8.10	3.26	22.6
	Sb	0.108	0.076	0.010	0.0047	0.308	0.0965	0.0095	0.291
	Sc	0.0051	0.0036	0.0012	0.0005	0.0118	0.0044	0.0007	0.0112
	Se	2.36	1.34	0.17	0.530	5.80	1.96	0.804	5.70
	Sr	4.52	3.27	0.43	0.100	13.7	3.55	0.443	12.4
	Zn	113	39	4.9	38.9	221	109	51.1	200
Females n=33	Ag	0.014	0.009	0.002	0.0012	0.033	0.013	0.0021	0.0321
	Br	20.6	14.3	2.7	3.10	54.1	16.3	4.86	52.2
	Ca	1663	970	198	461	3640	1170	670	3600
	Cl	3317	1480	290	1200	6000	3375	1386	5906
	Co	0.051	0.032	0.006	0.017	0.140	0.040	0.0183	0.130
	Cr	0.573	0.246	0.049	0.290	1.22	0.488	0.303	1.11
	Cu	4.18	1.72	0.43	0.50	6.50	4.05	1.18	6.50
	Fe	228	105	21	74.0	512	191	87.2	422
	Hg	0.033	0.025	0.005	0.0065	0.100	0.026	0.0079	0.100
	I	1956	1199	219	114	5061	1562	309	4662
	K	5395	3245	723	1740	13700	4835	2120	13230
	Mg	212	97	24	66.0	364	215	67.5	356
	Mn	1.50	0.84	0.22	0.550	4.18	1.37	0.603	3.41
	Na	6421	1721	320	3800	10450	6700	4122	9924
	Rb	6.40	2.33	0.46	1.66	12.8	6.38	2.87	10.8
	Sb	0.116	0.063	0.012	0.0115	0.248	0.108	0.0183	0.247
	Sc	0.0042	0.0040	0.0012	0.0002	0.0143	0.0032	0.0003	0.0124
	Se	2.22	1.19	0.23	0.439	5.32	2.07	0.773	4.85
	Sr	4.67	3.11	0.78	0.65	10.9	4.40	0.82	10.8
	Zn	87.4	38.7	7.58	7.10	166	83.5	23.0	156

Table 2: *Some statistical parameters of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction (mg/kg, dry mass basis) in normal human thyroid.*

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 mean values and all selected statistical parameters were calculated for twenty ChE (Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn) mass fractions in thyroid of female and male (Table 2).

Comparison with published data

Values obtained for Br, Ca, Cl, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn contents in the normal human thyroid (Table 3) agree well with median of mean values reported by other researches [26-43]. The obtained means for Ag and Co were almost one order of magnitude lower median of previously reported means but inside the range of means (Table 3). Data cited in Table 3 also includes samples obtained from patients who died from different non-endocrine diseases. A number of values for TE mass fractions were not expressed on a dry mass basis by the authors of the cited references. However, we calculated these values using published data for water (75%) [32] And ash (4.16% on dry mass basis) [70] contents in thyroid of adults.

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 (combined) M ± SD
Ag	0.25 (12)	0.000784 (16) [26]	1.20 ± 1.24 (105) [27]	0.0151 ± 0.0140
Br	18.1 (11)	5.12 (44) [26]	284 ± 44 (14) [28]	14.9 ± 10.9
Ca	1600 (17)	840 ± 240 (10) [29]	3800 ± 320 (29) [29]	1692 ± 1022
Cl	6800 (5)	804 ± 80 (4) [30]	8000 (-) [31]	3400 ± 1452
Co	0.336 (17)	0.026 ± 0.031 (46) [32]	70.4 ± 40.8 (14) [28]	0.0399 ± 0.0271
Cr	0.69 (17)	0.105 (18) [33]	24.8 ± 2.4 (4) [30]	0.539 ± 0.272
Cu	6.1 (57)	1.42 (120) [34]	220 ± 22 (10) [30]	4.23 ± 1.52
Fe	252 (21)	56 (120) [34]	2444 ± 700 (14) [28]	223 ± 93
Hg	0.08 (13)	0.0008 ± 0.0002 (10) [29]	396 ± 40 (4) [30]	0.0421 ± 0.0358
I	1888 (95)	159 ± 8 (23) [35]	5772 ± 2708 (50) [36]	1841 ± 1027
K	4400 (17)	46.4 ± 4.8 (4) [30]	6090 (17) [37]	6071 ± 2773
Mg	390 (16)	3.5 (-) [38]	840 ± 400 (14) [39]	285 ± 139
Mn	1.82 (36)	0.44 ± 11 (12) [40]	69.2 ± 7.2 (4) [30]	1.35 ± 0.58
Na	8000 (9)	438 (-) [41]	10000 ± 5000 (11) [39]	6702 ± 1764
Rb	12.3 (9)	≤0.85 (29) [29]	294 ± 191 (14) [28]	8.20 ± 4.54
Sb	0.105 (10)	0.040 ± 0.003 (-) [41]	4.0 (-) [42]	0.111±0.072
Sc	0.009 (4)	0.0018 ± 0.0003 (17) [43]	0.0135 ± 0.0045 (10) [29]	0.0046 ± 0.0038
Se	2.61 (17)	0.95 ± 0.08 (29) [29]	756 ± 680 (14) [28]	2.32 ± 1.29
Sr	0.73 (9)	0.55 ± 0.26 (21) [33]	46.8 ± 4.8 (4) [30]	4.55 ± 3.22
Zn	118 (51)	32 (120) [34]	820 ± 204 (14) [28]	105 ± 40

Table 3: *Median, minimum and maximum value of means Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, J, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn contents in the normal human 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. The range of means of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, J, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, 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

The ratios of means and the difference between mean values Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, 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 [61-69], 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 and 6).

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
Ag	0.0156 ± 0.0021	0.0140 ± 0.0020	0.586	>0.05	0.90
Br	12.3 ± 1.0	20.6 ± 2.7	**0.0076**	≤**0.01**	1.67
Ca	1703 ± 131	1663 ± 198	0.864	>0.05	0.98
Cl	3449 ± 219	3317 ± 290	0.718	>0.05	0.96
Co	0.0352 ± 0.0031	0.0505 ± 0.0064	**0.039**	≤**0.01**	1.43
Cr	0.520 ± 0.041	0.573 ± 0.049	0.412	>0.05	1.10
Cu	4.25 ± 0.20	4.18 ± 0.43	0.873	>0.05	0.98
Fe	222 ± 11	228 ± 21	0.807	>0.05	1.03
Hg	0.0461 ± 0.0053	0.0329 ± 0.0051	0.079	>0.05	0.71
I	1786 ± 118	1956 ± 219	0.495	>0.05	1.10
K	6289 ± 329	5395 ± 723	0.272	>0.05	0.86
Mg	306 ± 19	212 ± 24	**0.0046**	≤**0.01**	0.69
Mn	1.31 ± 0.07	1.50 ± 0.22	0.440	>0.05	1.15
Na	6820 ± 214	6421 ± 320	0.304	>0.05	0.94
Rb	8.97 ± 0.64	6.40 ± 0.46	**0.0016**	≤**0.01**	0.71
Sb	0.108 ± 0.010	0.116 ± 0.012	0.649	>0.05	1.07
Sc	0.0051 ± 0.0012	0.0042 ± 0.0012	0.590	>0.05	0.82
Se	2.36 ± 0.17	2.22 ± 0.23	0.633	>0.05	0.94
Sr	4.52 ± 0.43	4.67 ± 0.78	0.867	>0.05	1.03
Zn	113 ± 4.9	87.4 ± 7.6	**0.0077**	≤**0.01**	0.77

Table 4: *Differences between mean values (M ± SEM) of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction (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, Sstatistically significant values are in bold.

Strongly pronounced differences in Br, Co, Mg, Rb, and Zn mass fraction were observed between female and male thyroid (Table 4). The means of Br and Co mass fraction in female thyroids were almost respectively 1.7 and 1.4 time higher while the means of Mg, Rb and Zn mass fractions were respectively 31%, 29% and 23% 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.

In Age group 1 no statistically significant difference between the Br and Co content in female and male thyroids was found, but differences between their Mg, Rb and Zn contents were detected (Table 5). In Age group 1 of females with mean age 30.9 years the Mg, Rb and Zn contents in thyroid were respectively 31%, 43% and 42% lower than in thyroid of males from the same age group. Moreover, in this age group a statistically significant reduced level of Ca and Fe mass fraction in female thyroids in comparison with those in male thyroids was observed.

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
Ag	0.0160 ± 0.0031	0.0143 ± 0.0032	0.700	>0.05	0.89
Br	10.9 ± 1.1	11.8 ± 1.7	0.632	>0.05	1.08
Ca	1484 ± 90	1052 ± 65	**0.00041**	≤**0.01**	0.71
Cl	3236 ± 314	4109 ± 544	0.190	>0.05	1.27
Co	0.0374 ± 0.0046	0.0328 ± 0.0042	0.467	>0.05	0.88
Cr	0.502 ± 0.051	0.567 ± 0.065	0.441	>0.05	1.13
Cu	4.03 ± 0.24	4.01 ± 0.60	0.973	>0.05	1.00
Fe	228 ± 14	172 ± 23	0.055	≤**0.05**	0.75
Hg	0.0440 ± 0.0071	0.0275 ± 0.0046	0.058	>0.05	0.63
I	1601 ± 146	1876 ± 346	0.476	>0.05	1.17
K	6548 ± 386	5379 ± 1101	0.340	>0.05	0.82
Mg	309 ± 19	212 ± 39	**0.048**	≤**0.05**	0.69
Mn	1.46 ± 0.09	1.43 ± 0.13	0.816	>0.05	0.98
Na	6845 ± 271	5969 ± 458	0.121	>0.05	0.87
Rb	9.02 ± 0.76	5.13 ± 0.56	**0.00016**	≤**0.01**	0.57
Sb	0.109 ± 0.012	0.0880 ± 0.0096	0.174	>0.05	0.81
Sc	0.0052 ± 0.0012	0.0026 ± 0.0017	0.320	>0.05	0.50
Se	2.05 ± 0.18	1.86 ± 0.27	0.565	>0.05	0.91
Sr	4.53 ± 0.49	5.29 ± 1.12	0.548	>0.05	1.17
Zn	109 ± 6	62.7 ± 9.8	**0.00083**	≤**0.01**	0.58

Table 5: *Differences between mean values (M ± SEM) of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction (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.

For ages over 40 years (Age group 2) a statistically significant difference between the Br and Co content in female and male thyroids was observed and the mean of Br and Co content in female thyroids was respectively 1.8 and 2 times higher than that in male thyroids. In Age group 2 differences between the Ca, Fe, Mg, Rb and Zn contents in thyroids of females and males, previously found in the Age group 1, was no longer evident.

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
Ag	0.0148 ± 0.0019	0.0138 ± 0.0027	0.753	>0.05	0/93
Br	14.2 ± 2.0	25.8 ± 3.7	**0.012**	≤**0.01**	1.82
Ca	2003 ± 278	2029 ± 276	0.948	>0.05	1.01
Cl	3662 ± 305	2965 ± 318	0.122	>0.05	0.81
Co	0.0317 ± 0.0031	0.0644 ± 0.0096	**0.0051**	≤**0.01**	2.03
Cr	0.548 ± 0.070	0.578 ± 0.073	0.767	>0.05	1.05
Cu	4.59 ± 0.34	4.45 ± 0.61	0.844	>0.05	0.97
Fe	211 ± 18	271 ± 28	0.086	>0.05	1.28
Hg	0.0491 ± 0.0082	0.0370 ± 0.0084	0.311	>0.05	0.75
I	2048 ± 190	2002 ± 288	0.895	>0.05	0.98
K	5929 ± 577	5408 ± 1013	0.661	>0.05	0.91
Mg	301 ± 39	212 ± 31	0.085	>0.05	0.70
Mn	1.11 ± 0.08	1.57 ± 0.46	0.359	>0.05	1.41
Na	6784 ± 355	6025 ± 414	0.772	>0.05	0.89
Rb	8.88 ± 1.22	7.33 ± 0.58	0.263	>0.05	0.83
Sb	0.107 ± 0.018	0.136 ± 0.019	0.276	>0.05	1.27
Sc	0.0050 ± 0.0024	0.0045 ± 0.0014	0.868	>0.05	0.90
Se	2.89 ± 0.33	2.48 ± 0.34	0.396	>0.05	0.86
Sr	4.49 ± 0.82	3.63 ± 0.86	0.483	>0.05	0.81
Zn	119.1 ± 8.1	105.5 ± 8.5	0.254	>0.05	0.89

Table 6: *Differences between mean values (M ± SEM) of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction (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.

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 up to age 40 years there is a significant deficiency of Ca, Mg, Rb and Zn contents in female thyroid parenchyma, accompanied by a modest deficiency of Fe. In age over 40 deficiencies of Ca, Fe, Mg, Rb and Zn contents in female thyroid disappear and an excess of Br and Co is now seen.

Role of intra-thyroidal chemical elements in the gland function

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 [71]. 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 [72]. 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 [73-75]. 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.

Cobalt

Co is widely used in a bijouterie production. It may be one of the reasons of the higher level of this ChE 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.

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].

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 [79]. Moreover, Mg deficiency can influence bioavailability and tissue distribution of Se which then appears diminished [80]. Similar Ca, there is a significant direct correlation between serum Mg and TSH level [73]. 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.

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 [81]. Rb has some function in immune responce [82], probably by supporting cell differentiation [83]. 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.

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 [84,85]. 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.

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 multi-etiological 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 Br, Ca, Co, Fe, Mg, Rb and Zn in the etiology of female SCH may be assumed.

References

1. Krassas GE., *et al*. "Thyroid function and human reproductive health". *Endocrine Reviews* 31.5 (2010): 702-755.
2. Verma I., *et al*. "Prevalence of hypothyroidism in infertile women and evaluation of response of treatment for hypothyroidism on infertility". *International Journal of Applied and Basic Medical Research* 2.1 (2012): 17-19.
3. Bjoro T., *et al*. "Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population. The Health Study of Nord-Trondelag (HUNT)". *European Journal of Endocrinology* 143.5 (2000): 639-647.
4. Vanderpump MP and Tunbridge WM. "Epidemiology and prevention of clinical and subclinical hypothyroidism". *Thyroid* 12.10 (2002): 839-847.
5. Biondi B and Cooper DS. "The clinical significance of subclinical thyroid dysfunction". Endocrine Reviews 29 (2008): 76-131.
6. Rodondi N., *et al*. "Subclinical hypothyroidism and the risk of coronary heart disease and mortality". Journal of the American Medical Association 304.12 (2010): 1365-1374.
7. Verma I., *et al*. "Prevalence of hypothyroidism in infertile women and evaluation of response of treatment for hypothyroidism on infertility". International Journal of Applied and Basic Medical Research 2.1 (2012): 17-19.
8. Asvold BO., *et al*. "Changes in the prevalence of hypothyroidism: the HUNT Study in Norway". *European Journal of Endocrinology* 169.5 (2013): 613-620.
9. Baumgartner C., *et al*. "Thyroid function within the normal range, subclinical hypothyroidism, and the risk of atrial fibrillation". *Circulation* 136.22 (2017): 2100-2116.
10. Khan MA., *et al*. "Subclinical Hypothyroidism: Frequency, clinical presentations and treatment indications". *Pakistan Journal of Medical Sciences* 33.4 (2017): 818-822.
11. Battikhi MN. "Correlation between AMH and TSH in infertile women". EC Gynaecology 7.1 (2018): 12-15.
12. Zaichick V and Choporov Yu. "Determination of the natural level of human intra-thyroid iodine by instrumental neutron activation analysis". *Journal of Radioanalytical and Nuclear Chemistry* 207.1 (1996): 153-161.
13. Zaichick V and Zaichick S. "Normal human intrathyroidal iodine". *Science of the Total Environment* 206.1 (1997): 39-56.
14. Zaichick V. "Iodine excess and thyroid cancer". *Journal of Trace Elements in Experimental Medicine* 11.4 (1998): 508-509.
15. Zaichick V. "In vivo and in vitro application of energy-dispersive XRF in clinical investigations: experience and the future". *Journal of Trace Elements in Experimental Medicine* 11.4 (1998): 509-510.
16. Zaichick V and Iljina T. "Dietary iodine supplementation effect on the rat thyroid 131I blastomogenic action". In: Die Bedentung der Mengen- und Spurenelemente. 18. Arbeitstangung. Friedrich-Schiller-Universität, Jena (1998): 294-306.
17. Zaichick V and Zaichick S. "Energy-dispersive X-ray fluorescence of iodine in thyroid puncture biopsy specimens". *Journal of Trace and Microprobe Techniques* 17.2 (1999): 219-232.
18. Zaichick V. "Human intrathyroidal iodine in health and non-thyroidal disease". In: New aspects of trace element research. Smith-Gordon and Nishimura, London and Tokyo (1999): 114-119.
19. Zaichick V. "Relevance of, and potentiality for in vivo intrathyroidal iodine determination". In: In Vivo Body Composition Studies". *Annals of the New York Academy of Sciences* 904 (2000): 630-632.
20. Usha Menon V., *et al*. "High prevalence of undetected thyroid disorders in an iodine sufficient adult south Indian population". *Journal of the Indian Medical Association* 107.2 (2009): 72-77.

21. Zimmermann MB and Boelaert K. "Iodine deficiency and thyroid disorders". *Lancet Diabetes and Endocrinology* 3.4 (2015): 286-295.
22. Guo Y., *et al*. "Iodine nutrition status and thyroid disorders: a cross-sectional study from the Xinjiang Autonomous Region of China". *European Journal of Clinical Nutrition* 70.11 (2016): 1332-1336.
23. Shan Z., *et al*. "Iodine status and prevalence of thyroid disorders after introduction of mandatory universal salt iodization for 16 years in China: A Cross-Sectional Study in 10 cities". *Thyroid* 26.8 (2016):1125-1130.
24. Zaichick V. *et al*. "Trace elements and thyroid cancer". *Analyst* 120 (1995): 817-821.
25. Zaichick V. "Medical elementology as a new scientific discipline". *Journal of Radioanalytical and Nuclear Chemistry* 269 (2006): 303-309.
26. Zhu H., *et al*. "Element contents in organs and tissues of Chinese adult men". *Health Physics* 98.1 (2010): 61-73.
27. Vlasova ZA. "Dynamics of trace element contents in thyroid gland in connection with age and atherosclerosis". *Proceedings of the Leningrad Institute of Doctor Advanced Training* 80 (1969): 135-144.
28. Salimi J., *et al*. "Investigation of heavy trace elements in neoplastic and non-neoplastic human thyroid tissue: A study by proton – induced X-ray emissions". *Iranian Journal of Radiation Research* 1.4 (2004): 211-216.
29. Boulyga SF., *et al*. "Determination of microelements in thyroids of the inhabitants of Belarus by neutron activation analysis using the k0-method". *Journal of Radio analytical and Nuclear Chemistry* 222.1-2 (1997): 11-14.
30. Reddy SB., *et al*. "Trace elemental analysis of adenoma and carcinoma thyroid by PIXE method". *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 196.3-4 (2002): 333-339.
31. Woodard HQ and White DR. "The composition of body tissues". *British Journal of Radiology* 708 (1986): 1209-1218.
32. Katoh Y., *et al*. "Determination of multielement concentrations in normal human organs from the Japanese". *Biological Trace Element Research* 90.1-3 (2002): 57-70.
33. Tipton IH and Cook MJ. "Trace elements in human tissue. Part II. Adult subjects from the United States". *Health Physics* 9.2 (1963): 103-145.
34. Ataulchanov IA. "Age-related changes of manganese, cobalt, coper, zinc, and iron contents in the endocrine glands of females". *Problemy Endocrinologii* 15.2 (1969): 98-102.
35. Neimark II and Timoschnikov VM. "Development of carcinoma of the thyroid gland in person residing in the focus of goiter endemic". *Problemy Endocrinologii* 24.3 (1978): 28-32.
36. Zabala J., *et al*. "Determination of normal human intrathyroidal iodine in Caracas population". *Journal of Trace Elements in Medicine and Biology* 23.1 (2009): 9-14.
37. Forssen A. "Inorganic elements in the human body". *Annales Medicinae Experimentalis et Biologiae Fenniae* 50.3 (1972): 99-162.
38. Kortev AI., *et al*. "Bio-elements in human pathology". *Sverdlovsk, Middle-Ural Publishing-House* (1972).
39. Soman SD., *et al*. "Studies of major and trace element content in human tissues". *Health Physics* 19.5 (1970): 641-656.
40. Teraoka H. "Distribution of 24 elements in the internal organs of normal males and the metallic workers in Japan". *Archives of Environmental & Occupational Health* 36.4 (1981): 155-165.
41. Boulyga SF., et al. "Application of ICP-MS for multi element analysis in small sample amounts of pathological thyroid tissue". *Microchimica Acta* 134.3-4 (2000): 215-222.
42. Fuzailov YuM. "Reaction of human and animal thyroids in the conditions of antimony sub-region of the Fergana valley". In: IX All-Union Conference on Trace Elements in Biology. Kishinev (1981): 58-62.
43. Kvicala J., *et al*. "Determination of some trace elements in the thyroid gland by INAA". *Journal of Radioanalytical and Nuclear Chemistry* 149.2 (1991): 267-274.
44. Zaichick V. "Sampling, sample storage and preparation of biomaterials for INAA in clinical medicine, occupational and environmental health". *In: Harmonization of Health-Related Environmental Measurements Using Nuclear and Isotopic Techniques. IAEA, Vienna* (1997): 123-133.

45. Zaichick V. "Losses of chemical elements in biological samples under the dry Ashing process". *Trace Elements in Medicine* 5 (2004): 17-22.
46. Zaichick V and Zaichick S. "INAA applied to halogen (Br and I) stability in long-term storage of lyophilized biological materials". *Journal of Radio analytical and Nuclear Chemistry* 244.2 (2000): 279-281.
47. Zaichick V and Zaichick S. "Instrumental effect on the contamination of biomedical samples in the course of sampling". *Journal of Analytical Chemistry* 51.12 (1996): 1200-1205.
48. Zaichick V and Tsislyak YuV. "A simple device for bio-sample lyophilic drying". *Laboratornoe Delo* 2 (1978): 109-110.
49. Zaichick V and Tsislyak YuV. "A modified adsorptive and cryogenic lyophilizer for biosample concentrations". *Laboratornoe Delo* 2 (1981):100-101.
50. Zaichick V and Zaichick S. "A search for losses of chemical elements during freeze-drying of biological materials". *Journal of Radioanalytical and Nuclear Chemistry* 218.2 (1997): 249-253.
51. Zaichick S and Zaichick V. "Method and portable facility for energy-dispersive X-ray fluorescent analysis of zinc content in needle-biopsy specimens of prostate". *X-Ray Spectrometry* 39.2 (2010): 83-89.
52. Zaichick S and Zaichick V. "The Br, Fe, Rb, Sr, and Zn contents and interrelation in intact and morphologic normal prostate tissue of adult men investigated by energy-dispersive X-ray fluorescent analysis". *X-Ray Spectrometry* 40.6 (2011): 464-469.
53. Zaichick V. "Applications of synthetic reference materials in the medical Radiological Research Centre". *Fresenius' Journal of Analytical Chemistry* 352 (1995): 219-223.
54. Zaichick S, Zaichick V. "The effect of age and gender on 37 chemical element contents in scalp hair of healthy humans". *Biological Trace Element Research* 134.1 (2010): 41-54.
55. Zaichick S and Zaichick V. "The scalp hair as a monitor for trace elements in bio monitoring of atmospheric pollution". *International Journal of Environment and Health* 5.1/2 (2011): 106-124.
56. Zaichick S and Zaichick V. "INAA application in the age dynamics assessment of Br, Ca, Cl, K, Mg, Mn, and Na content in the normal human prostate". *Journal of Radio analytical and Nuclear Chemistry* 288.1 (2011): 197-202.
57. Zaichick S, Zaichick V. "The effect of age on Ag, Co, Cr, Fe, Hg, Sb, Sc, Se, and Zn contents in intact human prostate investigated by neutron activation analysis". *International Journal of Applied Radiation and Isotopes* 69 (2011): 827-833.
58. Zaichick S and Zaichick V. "Trace elements of normal, benign hypertrophic and cancerous tissues of the human prostate gland investigated by neutron activation analysis". *International Journal of Applied Radiation and Isotopes* 70.1 (2012): 81-87.
59. Zaichick V and Zaichick S. "INAA application in the assessment of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction in pediatric and young adult prostate glands". *Journal of Radio analytical and Nuclear Chemistry* 298.3 (2013): 1559-1566.
60. Korelo AM and Zaichick V. "Software to optimize the multielement INAA of medical and environmental samples". *In: Activation Analysis in Environment Protection. Joint Institute for Nuclear Research, Dubna* (1993): 326-332.
61. Zaichick V and Zaichick S. "Age-related changes of some trace element contents in intact thyroid of females investigated by energy dispersive X-ray fluorescent analysis". *Trends in Geriatric Healthcare* 1.1 (2017): 31-38.
62. Zaichick V and Zaichick S. "Age-related changes of Br, Ca, Cl, I, K, Mg, Mn, and Na contents in intact thyroid of females investigated by neutron activation analysis". *Current Updates in Aging* 1 (2017): 51
63. Zaichick V and Zaichick S. "Age-related changes of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in intact thyroid of females investigated by neutron activation analysis". *Gerontology and Geriatric Medicine* 3 (2017): 015
64. Zaichick V and Zaichick S. "Effect of age on chemical element contents in female thyroid investigated by some nuclear analytical methods". *Micro Medicine* 6.1 (2018): 47-61.
65. Zaichick V and Zaichick S. "Variation with age of chemical element contents in females' thyroids investigated by neutron activation analysis and inductively coupled plasma atomic emission spectrometry". *Journal of Biochemical and Analytical Studies* 3.1 (2018):1-10.

66. Zaichick V and Zaichick S. "Age-related changes of some trace element contents in intact thyroid of males investigated by energy dispersive X-ray fluorescent analysis". *MOJ Gerontology and Geriatrics* 1.5 (2017): 00028.
67. Zaichick V and Zaichick S. "Age-related changes of Br, Ca, Cl, I, K, Mg, Mn, and Na contents in intact thyroid of males investigated by neutron activation analysis". *Journal of Aging and Age Related Diseases* 1.1 (2017): 1002
68. Zaichick V and Zaichick S. "Association between Age and Twenty Chemical Element Contents in Intact Thyroid of Males". *SM Gerontology and Geriatric Research* 2.1 (2018): 1014
69. Zaichick V and Zaichick S. "Associations between age and 50 trace element contents and relationships in intact thyroid of males". *Aging Clinical and Experimental Research* 30.9 (2018): 1059–1070.
70. Schroeder HA., *et al*. "Trace metals in man: strontium and barium". *Journal of Chronic Diseases* 25.9 (1972): 491-517.
71. Maschkovsky MD. "The sedatives". In: The Medicaments. 15th Ed. Novaya Volna, Moscow (2005): 72-86.
72. Pavelka S. "Radiometric determination of thyrotoxic effects of some xenobiotics". *Radiation Applications* 1.2 (2016): 155-158.
73. Schwarza C., *et al*. "Thyroid function and serum electrolytes". *Swiss Medical Weekly* 142 (2012): w13669.
74. Ashmaik AS., *et al*. "Assessment of Serum Levels of Calcium and Phosphorous in Sudanese Patients with Hypothyroidism". *Asian Journal of Biomedical and Pharmaceutical Sciences* 03.25 (2013): 21-26.
75. Sidhu GK., *et al*. "Assessment of variations in serum Phosphorus, Calcium, Sodium and Potassium levels in hypothyroid patients". *International Journal Internal Medicine Research* 3.3 (2016): 26-29.
76. Leyssens L., *et al*. "Cobalt toxicity in humans—A review of the potential sources and systemic health effects". *Toxicology* 387 (2017): 43-56.
77. Yu R. "Cobalt Toxicity, An overlooked Cause of Hypothyroidism". *Journal of Endocrinology and Thyroid Research* 1.3 (2017): 1-4.
78. Simonsen LO., *et al*. "Cobalt metabolism and toxicology--a brief update". *Science of the Total Environment* 432 (2012): 210-215.
79. Moncayo R and Moncayo H. "A post-publication analysis of the idealized upper reference value of 2.5 m IU/L for TSH: Time to support the thyroid axis with magnesium and iron especially in the setting of reproduction medicine". *BBA Clinical* 7 (2017): 115-119.
80. Jiménez A. "Changes in bioavailability and tissue distribution of selenium caused by magnesium deficiency in rats". *Journal of the American College of Nutrition* 16.2 (1997): 175-180.
81. Johnson GT., *et al*. "Acute toxicity of cesium and rubidium compounds". *Toxicology and Applied Pharmacology* 32.2 (1975): 239-245.
82. Jones JM., *et al*. "Effects of lithium and rubidium on immune responses of rats". *Toxicology Letters* 52 (1990): 163-168.
83. Petrini M., *et al*. "Rubidium is a possible supporting element for bone marrow leukocyte differentiation". *Haematologica* 75 (1990): 27-31.
84. Betsy A., *et al*. "Zinc deficiency associated with hypothyroidism: An overlooked cause of severe alopecia". *International Journal of Trichology* 5.1 (2013): 40-42.
85. Baltaci AK and Mogulkoc R. "Leptin, NPY, melatonin and zinc levels in experimental hypothyroidism and hyperthyroidism: the relation to zinc". *Biochemical Genetics* 55.3 (2017): 223-233.