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RESEARCH ARTICLE

Effect of age on chemical element contents in female thyroid investigated by some nuclear analytical methods

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ABSTRACT

A prevalence of thyroid dysfunction is higher in the elderly as compared to the younger population. An excess or deficiency of chemical element contents in thyroid may play an important role in goitro- and carcinogenesis of gland. The variation with age of the mass fraction 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) in intact (normal) thyroid of 33 females (mean age 54.5 years, range 3.5-87) was investigated by energy dispersive X-ray fluorescent analysis and instrumental neutron activation analysis with high resolution spectrometry of short- and long-lived radionuclides. This work revealed that there is an increase in Br, Ca, Co, Fe, Rb, Sb, Se, and Zn mass fraction, as well as a decrease in Cl mass fraction in the normal thyroid of female during a lifespan. Therefore, a goitrogenic and carcinogenic effect of inadequate Br, Ca, Co, Fe, Rb, Sb, Se, and Zn level in the thyroid of old females with increasing age may be assumed.

Keywords: Thyroid; Chemical elements; Age-related changes; X-ray fluorescent analysis; Neutron activation analysis.

INTRODUCTION

The endocrine organs, including the thyroid gland, undergo important functional changes during aging and a prevalence of thyroid dysfunction is higher in the elderly as compared to the younger population [1, 2]. Advancing age is known to influence the formation of adenomatous goiter and thyroid cancer [3]. The prevalence of thyroid nodules is increased in the elderly, reaching a frequency of nearly 50% by the age of 65 [4]. Both prevalence and aggressiveness of thyroid cancer increase with age [2]. Women are affected by thyroid nodule and cancer two to five times more often than men [2-5].

Aging is characterized by progressive impairment of body functions caused by the accumulation of molecular damage in DNA, proteins and lipids, is also characterized by an increase in intracellular oxidative stress due to the progressive decrease of the intracellular reactive oxygen species (ROS) scavenging [6, 7]. Oxidative damage to cellular macromolecules which induce age-related diseases, including cancer, can also arise through overproduction of ROS and faulty antioxidant and/or DNA repair mechanisms [8].

Overproduction of ROS is associated with stress, inflammation, radiation, and some other factors, including overload of certain chemical elements, in both blood and certain tissues, or deficiency of other chemical elements with antioxidant properties [9-15]. The imbalance in the composition of chemical elements in cells, tissues and organs may cause different types of pathology. The importance of appropriate levels of many chemical elements is indisputable, due to their beneficial roles when present in specific concentration ranges, while on the other hand they can cause toxic effects with excessively high or low concentrations [12].

In our previous studies [16-24] the high mass fraction of iodine and some other chemical element were observed in intact human thyroid gland when compared with their levels in non-thyroid soft tissues of the human body. However, the age-dependence of chemical element mass fraction in thyroid of adult and, particularly, elderly females is still need to be evaluated. One valuable way to elucidate the situation is to compare the mass fractions of chemical elements in young adult (the control group) with those in older adult and geriatric thyroid. The findings of the excess or deficiency of chemical element contents in thyroid of adult and elderly females may indicate their roles in a higher prevalence of thyroid dysfunction in the elderly population.

The reliable data on chemical element mass fractions in normal geriatric thyroid is apparently extremely limited. There are multiple studies reporting chemical element content in human thyroid, using chemical techniques and instrumental methods [25-42]. However, majority of the analytical methods currently used and validated for the determination of major and trace elements in thyroid and other human organs are based on techniques requiring sample digestion. The most frequently used digestion procedures are the traditional dry ashing and high-pressure wet digestion that cause destruction of organic matter of the sample. Sample digestion is a critical step in elemental analysis and due to the risk of contamination and analytes loss, a digestion step contributes to the systematic uncontrolled analysis errors [43-45]. Moreover, only a few of the previous studies employed quality control using certified/standard reference materials (CRM/SRM) for determination of the chemical element mass fractions. Therefore, sample-nondestructive technique like energy dispersive X-ray fluorescent analysis (EDXRF) as well as instrumental neutron activation analysis with high resolution spectrometry of short- and long-lived radionuclides (INAA-SLR and INAA-LLR, respectively) combined with a quality assurance using CRM/SRM is good alternatives for multi-element determination in the samples of thyroid parenchyma.

There were three aims in this study. The primary purpose of the study was to determine reliable values for chemical element mass fractions in the normal (intact) thyroid of subjects ranging from children to elderly females using EDXRF, INAA-SLR, and INAA-LLR. The second aim was to compare the chemical mass fractions determined in thyroid gland of age group 2 (adults and elderly persons aged 41 to 87 years), with those of group 1 (from 3.5 to 40 years).The final aim was to find the correlations between age and chemical element contents.

All studies were approved by the Ethical Committee of the Medical Radiological Research Centre. 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 87years within 48 hours after a sudden death.All the deceased were citizens of Obninsk and had undergone routine autopsy at the Forensic Medicine Department of City Hospital, Obninsk. Subjects were divided into two age groups, group 1 with 3.5-40 years (30.9±3.1 years, M±SEM, n=11) and group 2 with 41–87 years (66.3±2.7 years, M±SEM, n=22). 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 chemical element contents. The typical causes of sudden death of most of these subjects included trauma or suicide and also acute illness (cardiac insufficiency, stroke, embolism of pulmonary artery, alcohol poisoning).

Sample preparation

All right lobes of thyroid glands were divided into two portions using a titanium scalpel [46]. One tissue portion was reviewed by an anatomical pathologist while the other was used for the chemical element 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 chemical element analysis were weighed, they were transferred to -20°C and stored until the day of transportation in the Medical Radiological Research Center, Obninsk, where all samples were freeze-dried and homogenized [47-49].

For EDXRF the pounded sample weighing about 8 mg was applied to the piece of Scotch tape serving as an adhesive fixing backing [50, 51]. 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 [52]. 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 [52].

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 photopeak in the spectra. The trace element content was calculated by the relative way of comparing between intensities of K_α-lines for samples and standards. More details of the facility and method of analysis were presented in our previous publication [50, 51].

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, intralaboratory-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 chemical element contents in human scalp hair [53].

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 $1.3 \cdot 10^{13}$ $n \cdot cm^{-2} \cdot s^{-1}$. 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 chemical element contents in human prostate and scalp hair [53, 54].

Computer programs and statistic

A dedicated computer program for INAA mode optimization was used [55]. All thyroid samples were prepared in duplicate, and mean values of chemical element contents were used in final calculation. Using Microsoft Office Excel, a summary of the statistics, including, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for chemical element contents. The difference in the results between two age groups was evaluated by the parametric Student's t-test and non-parametric Wilcoxon-Mann-Whitney U-test. For the construction of "age - chemical element mass fraction" diagrams (including lines of trend with age) and the estimation of the Pearson correlation coefficient between age and chemical element mass fraction the Microsoft Office Excel programs were also used. To identify the trend of the age dependency of chemical element contents, we applied approximation methods using exponential, linear, polynomial, logarithmic and power function. The maximum of corresponding values of R^2 parameter, reflecting the accuracy of approximation, was used for the selection of function.

RESULTS

Table 1 indicates our data for twenty chemical elements in ten sub-samples of CRM IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) in comparison with the certified values of this material.

The comparison of our results for the Br, Fe, Rb, and Zn mass fractions (mg/kg, dry mass basis) in the normal thyroid of female obtained by both EDXRF and INAA methods is shown in Table 2.

Table 3 represents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fractions in intact (normal) thyroid of females.

The comparison of our results with published data for the Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn contents in the human thyroid is shown in Table 4.

To estimate the effect of age on the chemical element contents we examined two age groups, described above (Table 5). In addition, the Pearson correlation coefficient between age and chemical element mass fraction was calculated (Table 6). Figure 1 shows the individual data sets for the Br, Ca, Co, Fe, Rb, Sb, Se, and Zn mass fraction in all samples of thyroid, and also lines of trend with age. Since the age dependency of these element contents was best described by a polynomial function, this approximation was reflected in Figure 1.

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).

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

M – arithmetic mean, SD – standard deviation, a – certified values, b – information values.

Table 2. Comparison of the mean values (M±SEM) of the chemical element mass fractions (mg/kg, dry mass basis) in the normal thyroid of female obtained by both EDXRF and INAA methods.

Element	EDXRF (1)	INAA (2)	Δ=[(M1 – M2)/M1] ·100%
Br	20.4±2.6	22.4±3.2	-9.8
Fe	223±21	232±22	-4.0
Rb	6.64±0.48	6.16±0.48	7.2
Zn	89.0±8.4	85.7±7.4	3.7

M – arithmetic mean, SEM – standard error of mean.

Table 3. Some statistical parameters of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, J, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction (mg/kg, dry mass basis) in the normal thyroid of female.

Gender	El	M	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Females n=33	Ag	0.0140	0.0093	0.0020	0.0012	0.0331	0.0130	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	0.0505	0.0322	0.0064	0.0170	0.140	0.0405	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	0.0329	0.0246	0.0051	0.0065	0.100	0.0263	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

El – element, 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 4. 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 thyroid according to data from the literature in comparison with our results (mg/kg, dry mass basis).

El	Published data [Reference]			This work
	Median of means (n)*	Minimum of means M or M±SD, (n)**	Maximum of means M or M±SD, (n)**	M±SD
Ag	0.25 (12)	0.000784 (16) [25]	1.20±1.24 (105) [26]	0.014±0.009
Br	18.1 (11)	5.12 (44) [25]	284±44 (14) [27]	21±14
Ca	1600 (17)	840±240 (10) [28]	3800±320 (29) [28]	1663±970
Cl	6800 (5)	804±80 (4) [29]	8000 (-) [30]	3317±1480
Co	0.336 (17)	0.026±0.031 (46) [31]	70.4±40.8 (14) [27]	0.051±0.032
Cr	0.69 (17)	0.105 (18) [32]	24.8±2.4 (4) [29]	0.57±0.25
Cu	6.1 (57)	1.42 (120) [33]	220±22 (10) [29]	4.2±1.7
Fe	252 (21)	56 (120) [33]	2444±700 (14) [27]	228±105
Hg	0.08 (13)	0.0008±0.0002 (10) [28]	396±40 (4) [29]	0.033±0.025
I	1888 (95)	159±8 (23) [34]	5772±2708 (50) [35]	1956±1199
K	4400 (17)	46.4±4.8 (4) [29]	6090 (17) [36]	5395±3245
Mg	390 (16)	3.5 (-) [37]	840±400 (14) [38]	212±97

El	Published data [Reference]			This work
	Median of means (n)*	Minimum of means M or M±SD, (n)**	Maximum of means M or M±SD, (n)**	M±SD
Mn	1.82 (36)	0.44±11 (12) [39]	69.2±7.2 (4) [29]	1.50±0.84
Na	8000 (9)	438 (-) [40]	10000±5000 (11) [38]	6421±1721
Rb	12.3 (9)	≤0.85 (29) [28]	294±191 (14) [27]	6.40±2.33
Sb	0.105 (10)	0.040±0.003 (-) [40]	4.0 (-) [41]	0.116±0.063
Sc	0.009 (4)	0.0018±0.0003 (17) [42]	0.0135±0.0045 (10) [28]	0.0042±0.0040
Se	2.61 (17)	0.95±0.08 (29) [28]	756±680 (14) [27]	2.22±1.19
Sr	0.73 (9)	0.55±0.26 (21) [32]	46.8±4.8 (4) [29]	4.7±3.1
Zn	118 (51)	32 (120) [33]	820±204 (14) [27]	87.4±38.7

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

Table 5. Differences between mean values (M±SEM) of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, J, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction (mg/kg, dry mass basis) in the normal female thyroid of two age groups (AG).

Element	Female thyroid tissue				Ratio
	AG1 3.5-40 years n=11	AG2 41-87 years n=22	t-test $p\leq$	U-test p	AG2 to AG1
Ag	0.0143±0.0032	0.0138±0.0027	0.909	>0.05	0.97
Br	11.8±1.7	25.8±3.7	**0.0028**	**≤0.01**	2.19
Ca	1052±65	2029±276	**0.0034**	**≤0.01**	1.93
Cl	4109±544	2965±318	0.0947	>0.05	0.72
Co	0.0328±0.0042	0.0644±0.0096	**0.0076**	**≤0.01**	1.96
Cr	0.567±0.065	0.578±0.073	0.913	>0.05	1.02
Cu	4.01±0.60	4.45±0.61	0.616	>0.05	1.11
Fe	172±23	271±28	**0.0126**	**≤0.01**	1.58
Hg	0.0275±0.0046	0.0370±0.0084	0.333	>0.05	1.35
I	1876±346	2002±288	0.782	>0.05	1.07
K	5379±1101	5408±1013	0.984	>0.05	1.01
Mg	212±39	212±31	0.994	>0.05	1.00
Mn	1.43±0.13	1.57±0.46	0.772	>0.05	1.10
Na	5969±458	6025±414	0.300	>0.05	1.01
Rb	5.13±0.56	7.33±0.58	**0.0115**	**≤0.01**	1.43
Sb	0.0880±0.0096	0.136±0.019	**0.0344**	**≤0.01**	1.55
Sc	0.0026±0.0017	0.0045±0.0014	0.438	>0.05	1.73
Se	1.86±0.27	2.48±0.34	0.169	>0.05	1.33
Sr	5.29±1.12	3.63±0.86	0.262	>0.05	0.69
Zn	62.7±9.8	105.5±8.5	**0.0033**	**≤0.01**	1.68

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. Correlations between age (years) and chemical element mass fractions (mg/kg, dry mass basis) in the normal thyroid of female (*r* – coefficient of correlation).

Element	Ag	Br	Ca	Cl	Co	Cr	Cu	Fe	Hg	I
r	0.09	0.26	0.37[a]	-0.43[a]	0.57[b]	0.20	0.18	0.27	0.04	0.08
Element	**K**	**Mg**	**Mn**	**Na**	**Rb**	**Sb**	**Sc**	**Se**	**Sr**	**Zn**
r	-0.05	-0.20	-0.04	0.18	0.48[a]	0.47[a]	0.29	0.47[a]	-0.10	0.67[c]

Statistically significant values: [a]$p \leq 0.05$, [b]$p \leq 0.01$, [c]$p \leq 0.001$.

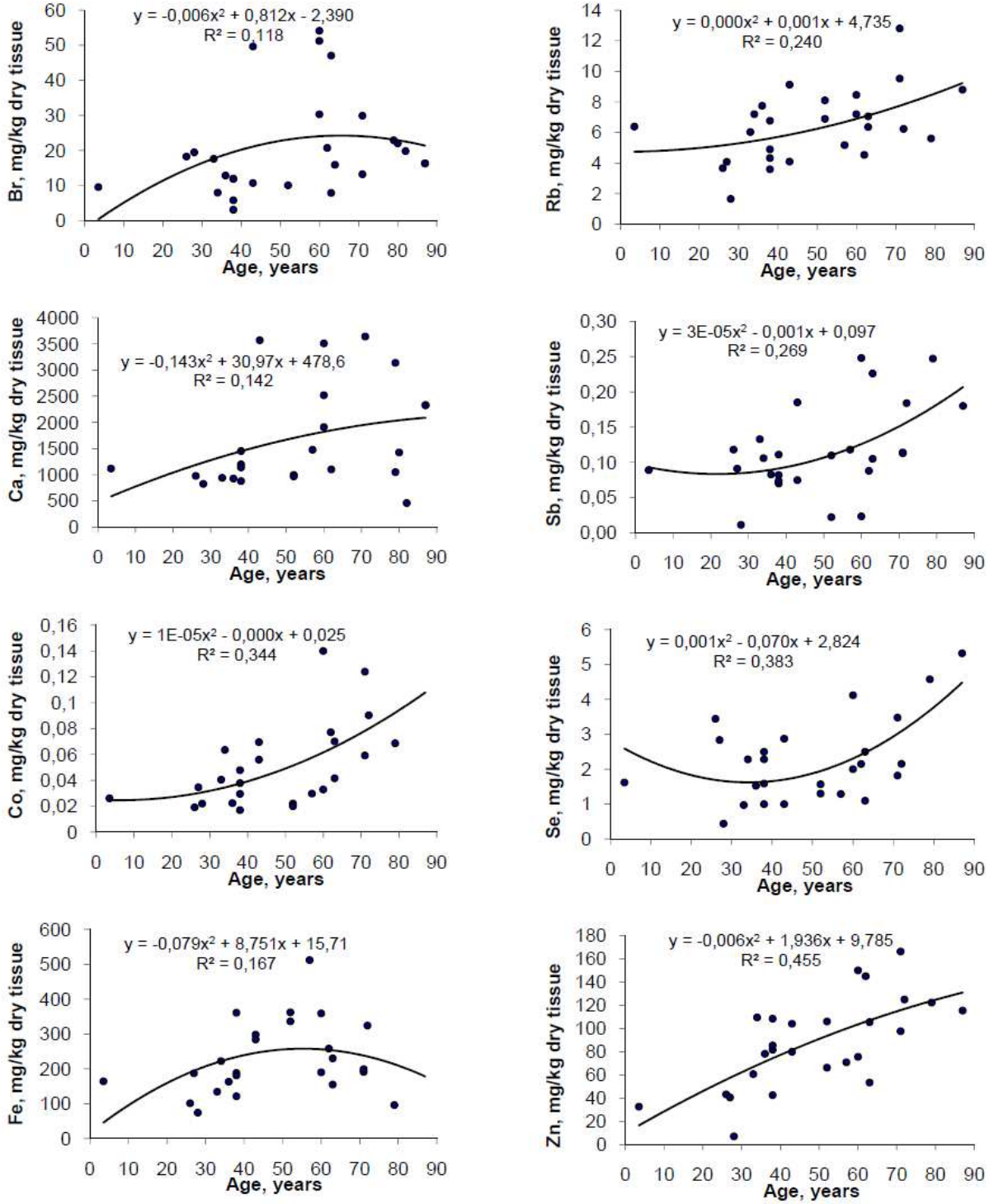

Figure 1. Data sets of individual Br, Ca, Co, Fe, Rb, Sb, Se, and Zn mass fraction values in the normal thyroid of females and their trend lines.

DISCUSSION

Precision and accuracy of results

A good agreement of our results for the Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fractions with the certified values of CRM IAEA H-4 and CRM IAEA HH-1 (Table 1) as well as the similarity of the means of the Br, Fe, Rb, and Zn mass fractions in the normal thyroid of female determined by both EDXRF and INAA methods (Table 2) demonstrates an acceptable precision and accuracy of the results obtained in the study and presented in Tables 3-6 and Figure 1.

Comparison with published data

The obtained means for Br, Ca, Cl, Cr, Cu, Fe, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, and Zn mass fraction, as shown in Table 4, 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 [25-42]. The obtained means for Ag and Co are an order of magnitude lower while the mean for Sr is an order of magnitude higher than the median of previously reported data. However, they are inside the ranges of previously reported data. 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% [56] and ash 4.16% on dry mass basis [57] contents in thyroid of adults.

Effect of age on chemical element contents

A statistically significant age-related increase in Br, Ca, Co, Fe, Rb, Sb, and Zn mass fraction was observed in thyroid of females when two age groups were compared (Table 5). In second group of females with mean age 66.3±2.7 years the mean of Br, Ca, Co, Fe, Rb, Sb, and Zn mass fraction in thyroids were 2.19, 1.93, 1.96, 1.58, 1.43, 1.55, and 1.68 times, respectively, higher than in thyroids of the first age group (mean age 30.9±3.1 years). A statistically significant increase in Ca, Co, Rb, Sb, and Zn was confirmed by the positive Pearson's coefficient of correlation between age and mass fractions of these elements (Table 6, Figure 1). In addition to these a significant increase in Se and decrease in Cl mass fraction with increasing of age was shown by the Pearson's coefficient of correlation between age and mass fractions of the elements (Table 6, Figure 1). As per author's current information, no published data referring to age-related changes of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fractions in human thyroid is available.

Role of chemical elements in malignant transformation of the thyroid

The Br is one of the most abundant and ubiquitous of the recognized trace elements in the biosphere. Inorganic bromide is the ionic form of bromine which exerts therapeutic as well as toxic effects. An enhanced intake of bromide could interfere with the metabolism of iodine at the whole-body level. In the thyroid gland the biological behavior of bromide is more similar to the biological behavior of iodide [58]. Therefore, a goitrogenic effect of excessive bromide level in the thyroid of old females may be assumed.

In addition to the elevated Br level, an age-related increase and excess in Ca mass fractions in thyroid tissue may contribute to harmful effects on the gland. There are good reasons for such speculations since many reviews and numerous papers raise the concern about role of Ca in the prostate, breast, lung and other organ malignant transformation [59-84]. Calcium ions Ca^{2+} are central to both cell proliferation and cell death [62]. Changes in cytosolic Ca^{2+} trigger events critical for tumorigenesis, such as cellular motility, proliferation and apoptosis [64]. An increased growth rate of cells is correlated with an increase in the intracellular calcium pool content [59, 60]. Moreover, increases in cytosolic free Ca2+ represent a ubiquitous signalling mechanism that controls a variety of cellular processes, including not only proliferation, but also cell metabolism and gene

transcription [63]. Indeed, an increased level of Ca content in the thyroid tissue of old females reflects an increase in the intracellular calcium pool. Thus, an increase of Ca content in tissue and organs with age is a key feature in etiology of many benign and malignant tumors, including thyroid goiter and cancer.

An age-related increase and excess in Co, Fe, Rb, Sb, and Zn mass fractions in thyroid tissue may contribute to harmful effects on the gland. There are good reasons for such speculations since many reviews and numerous papers raise the concern about toxicity and tumorigenesis of the metals [85-104]. Each of the metals is distinct in its primary mode of action. Moreover, there are several forms of synergistic action of the metals as a part of intracellular metabolism, during which several reactive intermediates and byproducts are created [85, 86, 91]. These reactive species are capable of potent and surprisingly selective activation of stress-signaling pathways, inhibition of DNA metabolism, repair, and formation of DNA crosslinks, which are known to contribute to the development of human cancers [86, 105, 106]. In addition to genetic damage via both oxidative and nonoxidative (DNA adducts) mechanisms, metals can also cause significant changes in DNA methylation and histone modifications, leading to alterations in gene expression [89, 90, 105]. In vitro and animal tumorigenic studies provided strong support for the idea that metals can also act as co-carcinogens in combination with nonmetal carcinogens [105].

The high level of Se content found just in the thyroid gland of old males cannot be regarded as pure chance. The seleno-protein characterized as Se-dependent glutathione peroxidase (Se-GSH-Px) is involved in protecting cells from peroxidative damage. This enzyme may reduce tissue concentration of free radicals and hydroperoxides. It is particular important for the thyroid gland, because thyroidal functions involve oxidation of iodide, which is incorporated into thyreoglobulin, the precursor of the thyroid hormones. For oxidation of iodide thyroidal cells produce a specific thyroid peroxidase using of physiologically generated hydrogen-peroxide (H_2O_2) as a cofactor [107]. It follows that the thyroid parenchyma must be continuously exposed to a physiological generation of H_2O_2 and in normal conditions must be a balance between levels of Se (as Se-GSH-Px) and H_2O_2. Thus, it might be assumed that the elevated level of Se in thyroid of old females reflects an increase in concentration of free radicals and hydroperoxides in female gland at age about 60 years and above.

All the samples were obtained from deceased citizens of Obninsk. Obninsk is the small nonindustrial city not far from Moscow in unpolluted area. None of the subjects include in this study had suffered from any systematic or chronic disorders before their sudden death. The normal state of thyroid gland was confirmed by morphological examination. Thus, our data on Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fractions in intact thyroid may indicate normal values for females of urban population of the Russian Central European region.

CONCLUSION

The combination of energy dispersive X-ray fluorescent analysis and instrumental neutron activation analysis with high resolution spectrometry of short- and long-lived radionuclides is a useful analytical tool for the non-destructive determination of chemical element content in the thyroid tissue samples. This combination allows determine the mean of content for 20 chemical elements: Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn.

Our data elucidate that there is a statistically significant increase in Br, Ca, Co, Fe, Rb, Sb, Se, and Zn mass fraction, as well as a decrease in Cl mass fraction in the normal thyroid of female during a lifespan. Therefore, a goitrogenic and carcinogenic effect of inadequate Br, Ca, Co, Fe, Rb, Sb, Se, and Zn level in the thyroid of old females may be assumed.

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between two authors. VZ collected thyroid samples, designed the EDXRF and INAA of samples, and carried out the statistical analysis of results. SZ managed the literature searches, wrote the first draft of the manuscript, and translated the manuscript into English. Both authors read and approved the final manuscript.

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