AGE-RELATED CHANGES OF IODINE/TRACE ELEMENT CONTENT RATIOS IN INTACT THYROID OF MALES INVESTIGATED BY ENERGY DISPERSIVE X-RAY FLUORESCENT ANALYSIS

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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. Women are affected by thyroid nodule and cancer two to five times more often than men, but in age over 65 years a prevalence of thyroid cancer is higher in men [2–4,5].

In our previous studies the high mass fraction of iodine (I) and some other trace element (TE) were observed in intact human thyroid gland when compared with their levels in non-thyroid soft tissues of the human body [6–14]. However, some questions about the age-dependence of TE mass fraction and their relationships in thyroid of adult and, particularly, elderly males still remain unanswered. The findings of the excess or deficiency of TE contents in thyroid and the perturbations of their relative proportions in glands of adult and elderly males, may give an indication of their role in a higher prevalence of thyroid cancer in the elderly males.

The reliable data on TE mass fractions in normal geriatric thyroid is apparently extremely limited. There are many studies regarding TE content in human thyroid, using chemical techniques and instrumental methods [15–20]. 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 [21–23]. Moreover, only a few of these studies employed quality control using certified/standard reference materials (CRM/SRM) for determination of the TE mass fractions.

In present study TE contents and the effect of age on TE contents in intact thyroid of apparently healthy male 2–80 year old was investigated. Mass fractions of Br, Fe, Cu, I, Rb, Sr, and Zn in thyroid tissue samples were determined using two methods of non-destructive energy-dispersive X-ray fluorescence analysis (EDXRF). In these methods X-ray fluorescence were induced by radionuclide ^{241}Am for I measurement and by radionuclide ^{109}Cd for Br, Fe, Cu, Rb, Sr, and Zn measurement.

All studies were approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk.

Experimental

Samples of the human thyroid were obtained from randomly selected autopsy specimens of 71 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, 2.0-35 years (22.5±1.4 years, M±SEM, n=36) and group 2, 36–80 years (52.4±2.4 years, M±SEM, n=36). 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 illness (cardiac insufficiency, stroke, embolism of pulmonary artery, alcohol poisoning). All right lobes of thyroid glands were divided into two portions using a titanium scalpel [24]. 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 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 [25]. The pounded sample was applied to the piece of Scotch tape serving as an adhesive fixing backing [26,27].

To determine the contents of the TE by comparison with a known standard, aliquots of commercial, chemically pure compounds were used [28]. The microliter standards were placed on disks made of thin, ash-free filter papers fixed on the Scotch tape pieces and dried in a vacuum. Ten subsamples of the Certified Reference Material (CRM) produced by the International Atomic Energy Agency (IAEA) CRM IAEA H-4 (animal muscle) were analyzed to estimate the precision and accuracy of results. The CRM IAEA H-4 subsamples were prepared in the same way as the samples of dry homogenized thyroid tissue.

Details of the sample preparation, the facility and method of analysis were presented in our previous publication on TE content investigation in human thyroid and prostate [12,26,27].

All thyroid samples were prepared in duplicate, and mean values of TE 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 TE contents. The reliability of 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 estimation of the Pearson correlation coefficient between age and different TE content the Microsoft Office Excel programs were also used.

Results and Discussion

Table 1 depicts our data for seven TE in ten sub- samples of CRM IAEA H-4 (animal muscle) and the certified values of this material. Of 4 (Br, Fe, Rb, and Zn) TE with certified values for the CRM IAEA H-4 (animal muscle) we determined contents of all certified elements (Table 1). Mean values (M±SD) for Br, Fe, Rb, and Zn were in the range of 95% confidence interval. Good agreement of the TE contents analyzed by ^{109}Cd radionuclide-induced EDXRF with the certified data of CRM IAEA H-4 (Table 1) indicate an acceptable accuracy of the results obtained in the study and presented in Tables 2-4

Table 1. EDXRF data Br, Cu, Fe, I, Rb, Sr, and Zn contents in the IAEA H-4 (animal muscle) reference material compared to certified values (mg/kg, dry mass basis)

Element	Certified values			This work results
	Mean	95% confidence interval	Type	Mean±SD
Br	4.1	3.5 – 4.7	C	5.0±1.2
Cu	4.0	3.6 – 4.3	C	3.9±1.1
Fe	49	47 – 51	C	48±9
I	0.08	–	N	<5.0
Rb	18	17 – 20	C	22±4
Sr	0.1	–	N	<1.0
Zn	86	83 – 90	C	90±5

Mean – arithmetical mean, SD – standard deviation, C- certified values, N – non-certified values

Table 2 represents certain statistical parameters of the Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) as well as I/Br, I/Cu, I/Fe, I/Rb, I/Sr, and I/Zn mass fraction ratios in intact (normal) thyroid of males.

Table 2. Some statistical parameters of Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) as well as I/Br, I/Cu, I/Fe, I/Rb, I/Sr, and I/Zn mass fraction ratios in normal thyroid of male

Gender	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Males n=72	Br	10.8	10.0	1.3	1.90	54.4	8.05	2.33	42.0
	Cu	4.25	1.48	0.20	1.10	7.50	4.15	1.78	1.39
	Fe	221	102	13	47.1	502	224	58.4	419
	I	1574	974	123	112	4829	1582	220	3542
	Rb	10.1	6.96	0.89	1.80	42.9	8.60	2.65	27.5
	Sr	4.52	3.27	0.43	0.100	13.7	3.55	0.443	12.4
	Zn	122	41	5.2	35.4	221	115	57.2	201
	I/Br	226	183	24	4.26	902	191	13.1	645
	I/Cu	425	393	57	30.9	2055	312	34.8	1363
	I/Fe	11.0	12.5	1.7	0.223	59.4	5.97	0.749	41.3
	I/Rb	221	229	31	11.1	1036	162	19.1	839
	I/Sr	1046	2484	351	13.2	16570	390	49.8	5353
	I/Zn	14.0	9.0	1.2	0.679	36.7	12.0	1.43	27.3

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 obtained means for Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction agree well with the medians of mean values cited by other researches for the human thyroid, including samples received from persons who died from different non-thyroid diseases [15–20].

To estimate the effect of age on the TE contents two age groups were examined (Table 3) and values of *r* – coefficient of correlation between age and all TE identified by us were calculated (Table 4) .

Table 3. Differences between mean values (M±SEM) of I/Br, I/Cu, I/Fe, I/Rb, I/Sr, and I/Zn mass fraction ratios in normal male thyroid of two age groups (AG)

Ratio	Male thyroid tissue				Ratio
	AG1 2.0-35 years n=36	AG2 36-80 years n=36	t-test *p*≤	U-test p	AG2 to AG1
I/Br	246±40	208±30	0.447	>0.05	0.85
I/Cu	413±72	437±88	0.829	>0.05	1.06
I/Fe	10.1±1.9	12.1±3.0	0.587	>0.05	1.20
I/Rb	201±39	244±50	0.503	>0.05	1.21
I/Sr	820±246	1291±686	0.524	>0.05	1.57
I/Zn	15.2±1.8	12.7±1.6	0.317	>0.05	0.84

M – arithmetic mean, SEM – standard error of mean, *t*-test - Student's *t*-test, U-test - Wilcoxon-Mann-Whitney *U*-test.

Table 4. Correlations between age and I/Br, I/Cu, I/Fe, I/Rb, I/Sr, and I/Zn mass fraction ratios in the normal thyroid of male (*r* – coefficient of correlation)

Ratio	I/Br	I/Cu	I/Fe	I/Rb	I/Sr	I/Zn
r	0.130	0.206	0.315[a]	0.183	0.076	0.091

Statistically significant values: [a] $p \leq 0.05$.

A significant direct correlation between age and I/Fe ratio was found in male thyroid (Table 4).

Conclusions

The developed methods of radionuclide-induced EDXRF are an efficient technique for the determination of many important TE in thyroid tissue. The methods are simple, fast, multielemental, and non-destructive. Our results for Fe, Cu, I, Rb, Sr, and Zn mass fractions in intact thyroid tissue may serve as indicative normal values for males of the Russian Central European region.

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