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Age-Related Changes of Some Trace Element Contents in Intact Thyroid of Females Investigated by Energy Dispersive X-Ray Fluorescent Analysis

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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 trace element contents in thyroid play important role in goitro-and carcinogenesis of gland. The variation with age of the mass fraction of six trace elements (Br, Cu, Fe, Rb, Sr, and Zn) in intact (normal) thyroid of 33 females (mean age 54.5 years, range 3.5-87) was investigated by109 Cd radionuclide-induced energy dispersive X-ray fluorescent analysis. Mean values ± standard error of mean for mass fractions (mg/kg, on dry-mass basis) of the trace elements studied were: Br 20.4 ± 2.6, Cu 4.18 ± 0.43, Fe 223 ± 21, Rb 6.64 ± 0.48, Sr 4.67 ± 0.78, and Zn 89.0 ± 8.4. This work revealed that there is a significant tendency for an increase in Rb and Zn mass fraction in normal female thyroid from age 41 years to the nine decade. Therefore, a goitrogenic and carcinogenic effect of excessive Rb and Zn level in the thyroid of old females may be assumed.

Keywords

Thyroid, Trace elements, Age-related changes, Energy dispersive X-ray fluorescent analysis

Abbreviations

109 Cd EDXRF: 109 Cd Radionuclide-Induced Energy-Dispersive X-Ray Fluorescence Analysis; CRM/SRM: Certified/Standard Reference Materials; IAEA: International Atomic Energy Agency

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 a complex process involving biochemical and morphologic changes in single cells, in organs, and in the whole organism. One of the most generally accepted explanations of how aging occurs at the molecular level is the oxidative stress hypothesis [6]. Reactive Oxygen Species (ROS) are widely considered to be a causal factor not only in aging but in a number of pathological conditions, including carcinogenesis. Aging, considered as an impairment of body functions over time, 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 de-

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crease of the intracellular ROS scavenging [7]. Oxidative damage to cellular macromolecules which induce cancer can also arise through overproduction of ROS and faulty antioxidant and/or DNA repair mechanisms [8]. Overproduction of ROS is associated with inflammation, radiation, and some other factors, including overload of some trace elements, in both blood and certain tissues, or deficiency of other trace elements with antioxidant properties [9-15]. Studies have shown that the imbalance in the composition of trace elements may cause different types of pathology. The importance of appropriate levels of many trace elements is indisputable, due to their beneficial roles when 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 I and some other trace element were observed in intact human thyroid gland when compared with their levels in non-thyroid soft tissues of the human body. However, some questions about the age-dependence of trace element mass fraction in thyroid of adult and, particularly, elderly females still remain unanswered. One valuable way to elucidate the situation is to compare the mass fractions of trace elements in young adult (the control group) with those in older adult and geriatric thyroid. The findings of the excess or deficiency of trace element contents in thyroid and the perturbations of their relative proportions in glands of adult and elderly females, may give an indication of their role in a higher prevalence of thyroid dysfunction in the elderly.

The reliable data on trace element mass fractions in normal geriatric thyroid is apparently extremely limited. There are many studies regarding trace element content in human thyroid, using chemical techniques and instrumental methods [25-30]. However, the majority of these data are based on measurements of processed tissue and in many studies tissue samples are shed 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 trace elements are lost as a result of such treatment [31-33]. Moreover, only a few of these studies employed quality control using Certified/Standard Reference Materials (CRM/SRM) for determination of the trace element mass fractions.

This work had three aims. The primary purpose of this study was to determine reliable values for the Bromine (Br), Copper (Cu), Iron (Fe), Rubidium (Rb), Strontium (Sr), and Zinc (Zn) mass fractions in the normal (intact) thyroid of subjects ranging from children to elderly females using 109 Cd radionuclide-induced energy-dispersive X-ray fluorescence analysis (109 Cd EDXRF). The second aim was to compare the Br, Cu, Fe, Rb, Sr, and Zn mass fractions 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), and the final aim was to estimate the inter-correlations of trace elements in normal thyroid of females. All studies were approved by the Ethical Committee of the Medical Radiological Research Center.

Materials and Methods

Samples of the human thyroid were obtained from randomly selected autopsy specimens of 33 females (European-Caucasian) aged 3.5 to 87 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, 3.5-40 years (30.9 ± 3.1 years, M ± SEM, n = 11) and group 2, 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 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 [34]. One tissue portion was reviewed by an anatomical pathologist while the other was used for the trace 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 [35]. The pounded sample weighing about 8 mg was applied to the piece of Scotch tape serving as an adhesive fixing backing [36,37].

To determine the contents of the elements by comparison with a known standard, aliquots of commercial, chemically pure compounds were used [38]. The micro liter 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) IAEA H-4 (animal muscle) weighing about 8 mg 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.

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. 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 Kα-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. Details of the sample preparation, the facility and method of analysis were presented in our previous publication [36,37].

All thyroid samples were prepared in duplicate and mean values of trace element contents were used in final calculation. 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 trace element 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 construction of "age-trace element mass fraction" diagrams and the estimation of the Pearson correlation coefficient between age and trace element mass fraction as well as between different trace elements the Microsoft Office Excel programs were also used.

Table 1: EDXRF data Br, Fe, 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	3.6-4.3	C	3.9 ± 1.1
Fe	49	47-51	C	48 ± 9
Rb	18	17-20	C	22 ± 4
Sr	0.1	-	N	< 1
Zn	86	83-90	C	90 ± 5

Mean: Arithmetical mean; SD: Standard Deviation; C: Certified values; N: Non-certified values.

Results

Table 1 depicts our data for 5 trace elements in ten sub-samples of CRM IAEA H-4 (animal muscle) and the certified values of this material.

Table 2 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 Br, Cu, Fe, Rb, Sr, and Zn mass fractions in intact (normal) thyroid of females.

The comparison of our results with published data for the Br, Cu, Fe, Rb, Sr, and Zn contents in the human thyroid is shown in Table 3. To estimate the effect of age on the trace element contents we examined two age groups, described above (Table 4).

Table 2: Some statistical parameters of Br, Cu, Fe, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) in intact thyroid of female.

Gender	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Females	Br	20.4	13.4	2.6	1.4	54.1	16.3	4.52	52.2
n = 33	Cu	4.18	1.72	0.43	0.5	6.5	4.05	1.18	6.5
	Fe	223	104	21	84	512	191	87.6	442
	Rb	6.64	2.47	0.48	2.2	12.8	6.38	3.08	11.7
	Sr	4.67	3.11	0.78	0.65	10.9	4.4	0.82	10.8
	Zn	89	43	8.4	6.1	166	88.1	6.16	156

M: Arithmetic mean; SD: Standard Deviation; SEM: Standard Error of Mean; Min: Minimum value; Max: Maximum value; P 0.025: Percentile with 0.025 level; P 0.975: Percentile with 0.975 level.

Table 3: Median, minimum and maximum value of means Br, Cu, Fe, Rb, Sr, and Zn contents in normal thyroid according to data from the literature in comparison with our results (mg/kg, dry mass basis).

Element	Published data [Reference]			This work
	Median of means (n)*	Minimum of means M or M ± SD, (n)**	Maximum of means M or M ± SD, (n)**	M ± SD
Br	18.1 (11)	5.12 (44) [25]	284 ± 44 (14) [26]	20 ± 13
Cu	6.1 (57)	1.42 (120) [27]	220 ± 22 (10) [28]	4.2 ± 1.7
Fe	252 (21)	56 (120) [27]	2444 ± 700 (14) [26]	223 ± 104
Rb	12.3 (9)	≤ 0.85 (29) [29]	294 ± 191 (14) [26]	6.6 ± 2.5
Sr	0.73 (9)	0.55 ± 0.26 (21) [30]	46.8 ± 4.8 (4) [28]	4.7 ± 3.1
Zn	118 (51)	32 (120) [27]	820 ± 204 (14) [26]	89 ± 43

M: Arithmetic mean; SD: Standard Deviation; (n)*: Number of all references; (n) **: Number of samples.

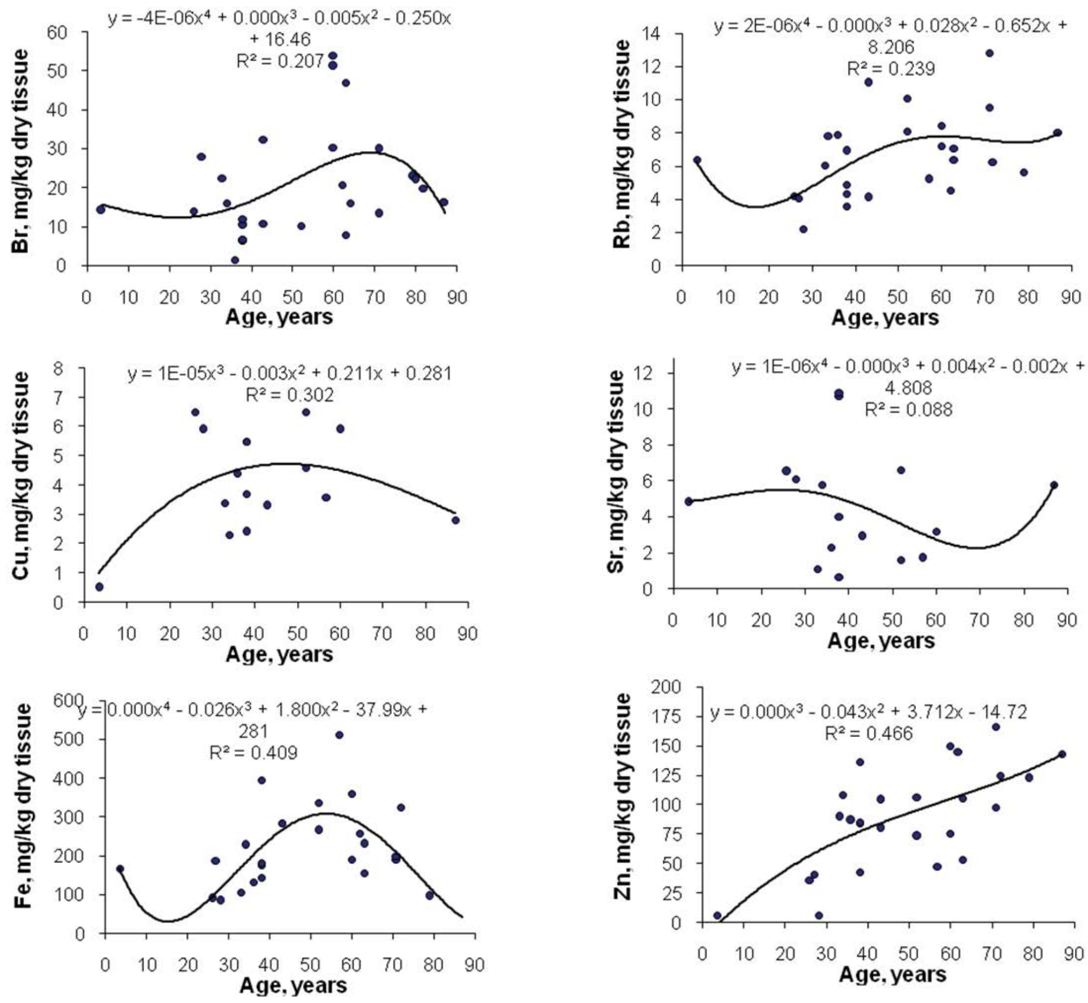

Figure 1: Data sets of individual Br, Cu, Fe, Rb, Sr, and Zn mass fraction values in intact thyroid of females and their trend lines.

Table 4: Differences between mean values (M ± SEM) of Br, Cu, Fe, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) in 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 ≤	U-test p	AG2 to AG1
Br	13.1 ± 2.5	24.7 ± 3.5	**0.0112**	**≤ 0.01**	1.89
Cu	4.01 ± 0.60	4.45 ± 0.61	0.616	> 0.05	1.11
Fe	172 ± 26	263 ± 27	**0.0240**	**≤ 0.01**	1.53
Rb	5.30 ± 0.55	7.62 ± 0.64	**0.0112**	**≤ 0.01**	1.44
Sr	5.29 ± 1.12	3.63 ± 0.86	0.262	> 0.05	0.69
Zn	65.6 ± 12.7	106.2 ± 9.3	**0.0176**	**≤ 0.01**	1.62

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 5: Correlations between age and chemical element mass fractions in the intact thyroid of female (r-coefficient of correlation).

Element	**Br**	**Cu**	**Fe**	**Rb**	**Sr**	**Zn**
Age	0.246	0.184	0.27	0.393[a]	-0.095	0.670[c]

Statistically significant values: [a]$p \le 0.05$; [c]$p \le 0.001$.

In addition, the Pearson correlation coefficient between age and trace element mass fraction was calculated (Table 5). Figure 1 shows the individual data sets for the Br, Cu, Fe, Rb, Sr, and Zn mass fraction in all samples of thyroid, and also lines of trend with age.

Table 6: Inter correlations of the chemical element mass fractions in the intact thyroid of female (r-coefficient of correlation).

Element	Br	Cu	Fe	Rb	Sr	Zn
Br	**1.00**	0.173	0.255	0.062	-0.200	0.023
Cu	0.173	**1.00**	0.048	0.009	0.249	0.024
Fe	0.255	0.048	**1.00**	0.185	-0.349[a]	0.213
Rb	0.062	0.009	0.185	**1.00**	-0.116	0.481[b]
Sr	-0.200	0.249	-0.349[a]	-0.116	**1.00**	-0.001
Zn	0.023	0.024	0.213	0.481[b]	-0.001	**1.00**

Statistically significant values: [a]$p \leq 0.05$; [b]$p \leq 0.01$.

The data of inter-correlation calculations (values of r-coefficient of correlation) including all trace elements identified by us are presented in Table 6.

Discussion

A set of existing international CRM prepared from the soft tissues of humans and animals is extremely limited. As is was previously discussed [36] 97% of the self-absorption in the dry sample of human tissue is due to the content of bulk elements (C, N, O, P, S) and main electrolytes (Ca, Cl, Na). The content of these elements and the mass density of muscle and thyroid in humans are virtually identical [39]. Accordingly, the use of CRM IAEA H-4 as a CRM for the analysis of samples of thyroid tissue can be seen as quite acceptable. Good agreement of the Br, Cu, Fe, Rb, Sr, and Zn contents analyzed by EDXRF with the certified data of CRM IAEA H-4 (Table 1) indicates an acceptable accuracy of the results obtained in the study of trace elements of the thyroid presented in Table 2, Table 3, Table 4 and Table 5.

The obtained means for Br, Cu, Fe, Rb, Sr, and Zn mass fraction, as shown in Table 3, 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 [24-30]. A number of values for chemical element 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%) [40] and ash (4.16% on dry mass basis) [41] contents in thyroid of adults.

A strongly pronounced tendency of age-related increase in Br mass fraction was observed in thyroid (Table 4). In second group of females with mean age 66.3 years the mean Br mass fraction in thyroids was almost 2 times higher than in thyroids of the first age group (mean age 30.9 years). A modest tendency of age-related increase in Fe, Rb, and Zn mass fractions was also observed in thyroid (Table 4). The Fe, Rb, and Zn mass fraction growth in thyroid tissue for ages between 30.9 (age group 1) and 66.3 (age group 2) years averaged 53%, 44%, and 62%, respectively. There were no statistically significant differences between the Cu and Sr mass fractions within different age-groups.

Age-dependence of Br and Fe mass fractions found using the comparison between results for two age groups was not confirmed when the Pearson correlation coefficient between age and mass fractions of these elements was calculated (Table 5). Thus, the questions about the Br and Fe mass fraction dependence on age stay open and need a similarly future study with larger sample size. No published data referring to age-related changes of Br, Cu, Fe, Rb, Sr, and Zn mass fractions in female thyroid was found.

A significant direct correlation between the Zn and Rb mass fractions ($p \pm 0.01$, $r = 0.48$) and an inverse correlation between Fe and Sr mass fractions ($p \pm 0.05$, $r = 0.35$) was seen in female thyroid. The interpretation of observed correlations requires further study for a more complete understanding. No correlation was demonstrated between any other chemical elements (Table 5). No published data referring to inter-correlations of Br, Cu, Fe, Rb, Sr, and Zn mass fractions in thyroid of females was found.

An age-related increase and excess in Rb 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 [10,11,42-74]. 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 [42,43,48]. These reactive species are capable of potent and surprisingly selective activation of stress-signaling pathways, inhibition of DNA metabolism, repair, and formation of DNA cross links, which are known to contribute to the development of human cancers [43,75,76]. 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 [44,46,75]. *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 [75].

All the deceased were citizens of Obninsk. Obninsk is the small nonindustrial city not far from Moscow in unpolluted area. None of those who died a sudden death had suffered from any systematic or chronic disorders before. The normal state of thyroid was confirmed by morphological study. Thus, our data for Br, Cu, Fe, Rb, Sr, and Zn mass fractions in intact thyroid may serve as indicative normal values for females of urban population of the Russian Central European region.

Conclusion

The 109 Cd radionuclide-induced energy-dispersive X-ray fluorescence analysis is a useful analytical tool for the non-destructive determination of trace element content in the thyroid tissue samples. This method allows determine means for Br, Cu, Fe, Rb, Sr, and Zn (6 trace elements).

Our data reveal that there is strongly pronounced tendency of increase in Rb and Zn mass fraction in the normal thyroid of female during a lifespan. Therefore, a goitrogenic and tumorogenic effect of excessive Rb and Zn level in the thyroid of old females may be assumed.

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