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Relationships between Iodine and Some Trace Elements in Normal Thyroid of Females Investigated by Energy Dispersive X-Ray Fluorescent Analysis

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Abstract

Objective: Thyroid diseases rank second among endocrine disorders, and prevalence of the diseases is higher in the elderly as compared to the younger population. Women are affected by thyroid diseases almost ten times more often than men. An excess or deficiency of trace element contents in thyroid play important role in goitro- and carcinogenesis of gland.
Study Design: The correlations with age of the seven trace element (TE) contents (Br, Cu, Fe, I, Rb, Sr, and Zn), I/Br, I/Cu, I/Fe, I/Rb, I/Sr, and I/Zn ratios, and inter relationships between TE contents and I/TE content ratios in normal thyroid of 33 females (age range 3.5-87 years) was investigated by radionuclide-induced energy dispersive X-ray fluorescent analysis.
Results: Our data reveal that the Rb and Zn content increase, while I/Cu and I/Zn content ratios decrease in the normal thyroid of female during a lifespan. Therefore, a goitrogenic and tumorogenic effect of disturbance in intrathyroidal I, Cu, and Fe relationships with increasing age may be assumed. Furthermore, it was found that the levels of Cu, Fe, Rb, Sr, and Zn in the thyroid gland are interconnected and depend on the content of I in it.
Conclusions: Because I plays a decisive role in the function of the thyroid gland, the data obtained allow us to conclude that, along with I, such TEs as Cu, Fe, Rb, Sr, and Zn, if not directly, then indirectly, are involved in the process of female thyroid hormone synthesis.

Keywords: Thyroid; Trace Elements; Age-Related Changes; Intrathyroidal Trace Elements Relationships; Trace Elements Energy Dispersive X-Ray Fluorescent Analysis

Abbreviations: CRM: Certified Reference Materials; IAEA: International Atomic Energy Agency; WHO: World Health Organization; TE: Trace Elements; EDXRF: Energy-Dispersive X-Ray Fluorescence Analysis.

Introduction

According to the World Health Organization (WHO), thyroid diseases rank second among endocrine disorders after diabetes mellitus. More than 665 million people in the world have endemic goiter or suffer from other thyroid pathologies. Women are affected by thyroid diseases almost ten times more often than men. At the same time, according to the same statistics, the increase in the number of thyroid diseases in the world is 5% per year [1]. It has been suggested that risk factors for the development of thyroid disorders may be numerous factors, including genetics, radiation, autoimmune diseases, as well as adverse environmental factors, such as an increase in the content of various chemicals in the environment [2].

Trace elements (TE) are among these various chemicals, because their levels in the environment have increased significantly over the past hundred years as a result of the industrial revolution and the tremendous technological changes that have taken place in metallurgy, chemical production, electronics, agriculture, food processing and storage, cosmetics, pharmaceuticals and medicine. In connection with these changes, the levels and ratio of trace elements entering the human body from the outside have been significantly disturbed, compared with the conditions in which human societies have lived for many millennia.

More than 50 years ago, we formulated the postulate about the somatic TE homeostasis, which is now generally recognized [3]. According to this postulate, under evolutionary environmental conditions, the mechanisms of homeostasis of organisms maintain the levels and ratios of TE in tissues and organs within certain limits. If the content of TE in the environment changes significantly, the mechanisms of somatic homeostasis may respond inadequately. Inadequate response of homeostasis mechanisms leads to changes in TE levels in tissues and organs, which, in turn, can affect their function and lead to the development of pathological conditions. The correctness of this conclusion was illustrated by us earlier on the example of the study of the role of TE in the normal and pathophysiology of the prostate [4-24]. It was shown, in particular, that a special role in the development of pathological transformations of the prostate is played by disturbances in the relationship between TE in the tissue and gland secretion. Moreover, it was found that changes in the relationship between TEs can be used as highly informative markers of various prostate diseases, including malignant tumors [25-39]. These findings stimulated our investigations of TE relationships in thyroid tissue in normal and pathological conditions.

There are many studies regarding TE content in human thyroid, using chemical techniques and instrumental methods [40-47]. However, among the published data, no works on the relationship of TE in the normal human thyroid were found.

This work had three aims. The primary purpose of this study was to determine reliable values for the bromine (Br), coper (Cu), iron (Fe), iodine (I), rubidium (Rb), strontium (Sr), and zinc (Zn) mass fractions in the normal thyroid of subjects ranging from children to elderly females using radionuclide-induced energy-dispersive X-ray fluorescence analysis and calculate individual values of I/Br, I/Cu, I/Fe, I/Rb, I/Sr, and I/Zn. The second aim was to compare the Br, Cu, Fe, Rb, Sr, and Zn mass fractions in thyroid gland obtained in the study with published data. The final aim was to estimate the inter-correlations of TE contents and I/TE content ratios in normal thyroid of females and changes of these parameters with age.

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk. All the 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 with comparable ethical standards.

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. The available clinical data were reviewed for each subject. None of the subjects were receiving medications or used any supplements known to affect thyroid TE 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 [48]. 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 [49]. To determine the contents of the TE by comparison with a known standard, aliquots of commercial, chemically pure compounds were used [50]. Ten subsamples of the Certified Reference Material (CRM) IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) were analyzed to estimate the precision and accuracy of results. The CRM IAEA H-4 and IAEA HH-1 subsamples were prepared in the same way as the samples of dry homogenized thyroid tissue.

The TE contents in thyroid samples were determined using radionuclide-induced energy-dispersive X-ray fluorescence analysis (EDXRF) with 109Cd source for Br, Cu, Fe, Rb, Sr, and Zn and 241Am source for I. The mass fraction of TE was calculated by the relative way of comparing between intensities of corresponding Kα-lines induced by radiation from radionuclide sources in tissue samples and standards.

Details of the sample preparation, the facility and method of analysis were presented in our previous publication [51-53]. 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 and I/TE content ratios. Pearson's correlation coefficient was used in Microsoft Office Excel to calculate the relationship "age – TE mass fraction", as well as to identify inter-thyroidal relationships between different TE contents and between different TE content ratios.

Results

Table 1 depicts comparison of our data for seven TE in ten sub-samples of CRM IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) with the corresponding certified values of TE contents in these materials.

Element	IAEA H-4 animal muscle	This work results	IAEA HH-1 human hair	This work results
Br	4.1±1.1a	5.0±09	4.2±2.1b	3.9±1.6
Cu	4.0±1.0a	3.9±1.1	10.2±3.2a	-
Fe	49.1±6.5a	47.0±1.0	23.7±3.1a	25.1±4.3
I	0.08±0.10b	<1.0	20.3±8.9b	19.1±6.2
Rb	18.7±3.5a	22±4	0.94±0.09b	0.89±0.17
Sr	-	<1	0.82±0.16b	1.24±0.57
Zn	86.3±11.5a	91±2	174±9a	173±17

M-arithmetical mean, SD-standard deviation, a-certified values, b-information values.
Table 1: EDXRF data of Br, Cu, Fe, I, Rb, Sr, and Zn 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).

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, I, Rb, Sr, and Zn mass fractions, as well as I/Br, I/Cu, I/Fe, I/Rb, I/Sr, and I/Zn mass fraction ratios in normal thyroid of females.

Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Br	20.4	13.4	2.6	1.4	54.1	16.3	4.52	52.2
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
I	1752	1180	225	110	5345	1504	296	4201
Rb	6.64	2.47	0.48	2.2	12.8	6.38	3.08	11.7
Sr	4.67	3.11	0.78	0.7	10.9	4.4	0.82	10.8
Zn	89	43	8.4	6.1	166	88.1	6.16	156
I/Br	117	115	23	3.9	608	104	4.62	356
I/Cu	545	674	174	19	2756	418	44.4	2160
I/Fe	10.6	10.7	2.2	1.3	42.4	7.53	1.42	37.5
I/Rb	292	223	46	50	811	181	63.7	762
I/Sr	642	954	246	18	3259	245	37.1	3005
I/Zn	30.7	44.2	9	3.4	222	18.3	5.6	141

Mean – 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 2: Some statistical parameters of Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction (mg/kg, dry tissue) as well as I/Br, I/Cu, I/Fe, I/Rb, I/Sr, and I/Zn mass fraction ratios in normal thyroid of female (n=33).

The comparison of our results with published data for the Br, Cu, Fe, I, Rb, Sr, and Zn contents in the human thyroid is shown in Table 3.

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 N=105
Br	18.1 (11)	5.12 (44) [40]	284±44 (14) [41]	20.4±13.4
Cu	6.1 (57)	1.42 (120) [42]	220±22 (10) [43]	4.18±1.72
Fe	252 (21)	56 (120) [42]	2444±700 (14) [41]	223±104
I	1888 (95)	159±8 (23) [44]	5772±2708 (50) [45]	1752±1180
Rb	12.3 (9)	≤0.85 (29) [46]	294±191 (14) [41]	6.64±2.47
Sr	0.73 (9)	0.55±0.26 (21) [47]	46.8±4.8 (4) [43]	4.67±3.11
Zn	118 (51)	32 (120) [42]	820±204 (14) [41]	89.0±43.0

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

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

To estimate the effect of female age on the TE contents and I/TE content ratios Pearson's correlation coefficient was used (Table 4).

Element	Br	Cu	Fe	I	Rb	Sr	Zn
r	0.25	0.18	0.3	0.18	0.39a	-0.1	0.67c
Ratio	I/Br	I/Cu	I/Fe	I/Rb	I/Sr	I/Zn	-
r	-0.2	-0.52a	0.1	-0.1	-0.08	-0.57b	-

Statistically significant values: a ≤0.05, b ≤0.01.

Table 4: Correlations between age (years) and trace element content (mg/kg, dry tissue), as well as between age and I/trace element mass fraction ratios in the normal thyroid of females (r – coefficient of correlation).

The data of inter-thyroidal correlations (values of r – Pearson's coefficient of correlation) including all TE and TE ratios identified by us are presented in Tables 5 & 6 respectively.

Element	Br	Cu	Fe	I	Rb	Sr	Zn
Br	1	0.17	0.26	0.17	0.06	-0.2	0.02
Cu	0.17	1	0.05	-0.30a	0.01	0.25	0.02
Fe	0.26	0.05	1	-0.16	0.19	-0.35a	0.21
I	0.17	-0.30a	-0.16	1	0.01	-0.36a	0.23
Rb	0.06	0.01	0.18	0.01	1	-0.12	0.48b
Sr	-0.2	0.25	-0.35a	-0.36a	-0.12	1	-0.01
Zn	0.02	0.02	0.21	0.23	0.48b	-0.01	1

Statistically significant values: a ≤0.05, b ≤0.01.

Table 5: Intercorrelations of the trace element mass fractions in the normal thyroid of female (r – coefficient of correlation).

Ratio	I/Br	I/Cu	I/Fe	I/Rb	I/Sr	I/Zn
I/Br	1	-0.06	0.17	0.15	0.09	-0.05
I/Cu	-0.1	1	0.31	0.3	0.24	0.94c
I/Fe	0.17	0.3	1	0.81c	0.70b	0.17
I/Rb	0.15	0.3	0.81c	1	0.74b	0.25
I/Sr	0.09	0.24	0.70b	0.74b	1	0.06
I/Zn	-0.1	0.94c	0.17	0.25	0.06	1

Statistically significant values: a ≤0.05, b ≤0.01, c ≤0.001.

Table 6: Intercorrelations of the I/trace element mass fraction ratios in the normal thyroid of female (r – coefficient of correlation).

Discussion

Good agreement of the Br, Cu, Fe, I, Rb, Sr, and Zn contents analyzed by EDXRF with the certified data of CRMs IAEA H-4 and IAEA HH-1 (Table 1) indicates an acceptable accuracy of the results obtained in the study for TE contents and I/TE content ratios in the normal male thyroid presented in Tables 2-5.

The content of TE was determined in all or most of the examined samples, which made it possible to calculate the main statistical parameters: the mean value of the mass fraction (M), standard deviation (SD), standard error of the mean (SEM), minimum (Min), maximum (Max), median (Med), and percentiles with levels of 0.025 (P 0.025) and 0.975 (P 0.975), of the Br, Cu, Fe, I, Rb, Sr, and Zn mass fractions, as well as I/Br, I/Cu, I/Fe, I/Rb, I/Sr, and I/Zn mass fraction ratios in normal thyroid of males (Table 2). The values of M, SD, and SEM can be used to compare data for different groups of samples only under the condition of a normal distribution of the results of determining the content of TE in the samples under study. Statistically reliable identification of the law of distribution of results requires large sample sizes, usually several hundred samples, and therefore is rarely used in biomedical research. In the conducted study, we could not prove or disprove the "normality" of the distribution of the results obtained due to the insufficient number of samples studied. Therefore, in addition to the M, SD, and SEM values, such statistical characteristics as Med, range (Min-Max) and percentiles P 0.025 and P 0.975 were calculated, which are valid for any law of distribution of the results of TE content in thyroid tissue.

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 [40-47]. 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%) [54] and ash (4.16% on dry mass basis) [55] contents in thyroid of adults. No published data referring to I/Br, I/Cu, I/Fe, I/Rb, I/Sr, and I/Zn mass fraction ratios in human thyroid was found.

With age, the Rb and Zn content increase, while I/Cu and I/Zn content ratios decrease (Table 4). These characteristics can be used to estimate the "biological age" of the female thyroid gland.

A significant strong direct correlation between the Rb and Zn mass fractions, as well as an inverse weak correlation between I and Cu, I and Sr, and Fe and Sr mass fractions was seen in female thyroid (Table 5). Since no correlations were found between I and other TE, except for an inverse weak correlation between I and Cu and I and Sr, it would appear that the content of Br, Rb, and Zn in the thyroid gland is independent of its content of I. However, this is not quite so. If we bring the content of the studied TE to the content of I (I/TE ratio), then there are close strong relationships between I/Zn and I/Cu (r=0.94, p<0.001), I/Fe and I/Rb (r=0.94, p<0.001),), I/Fe and I/Sr (r=0.70, p<0.01), and I/Rb and I/Sr), I/Fe and I/Rb (r=0.74, p<0.01) (Table 6). From this it follows that, at least, the levels of Cu, Fe, Rb, Sr, and Zn in the thyroid gland are interconnected and depend on the content of I in it. Because I plays a decisive role in the function of the thyroid gland, the data obtained allow us to conclude that, along with I, such TEs as Cu, Fe, Rb, Sr, and Zn, if not directly, then indirectly, are involved in the process of thyroid hormone synthesis.

Conclusion

The 109Cd and 241Am radionuclide-induced energy-dispersive X-ray fluorescence analysis is a useful analytical tool for the non-destructive determination of TE contents in the thyroid tissue samples. This method allows determine means for Br, Cu, Fe, I, Rb, Sr, and Zn (seven TE).

Our data reveal that the Rb and Zn content increase, while I/Cu and I/Zn content ratios decrease in the normal thyroid of female during a lifespan. Therefore, a goitrogenic and tumorogenic effect of disturbance in intrathyroidal I, Cu, and Fe relationships with increasing age may be assumed. Furthermore, it was found that the levels of Cu, Fe, Rb, Sr, and Zn in the thyroid gland are interconnected and depend on the content of I in it. Because I plays a decisive role in the function of the thyroid gland, the data obtained allow us to conclude that, along with I, such TEs as Cu, Fe, Rb, Sr, and Zn, if not directly, then indirectly, are involved in the process of female thyroid hormone synthesis.

Conflict of Interest

Author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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