APPLICATION OF NEUTRON ACTIVATION ANALYSIS FOR THE MEASUREMENT OF Br, Ca, Cl, K, Mg, Mn, AND Na CONTENTS IN THE INTACT THYROID OF FEMALE

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Introduction

A large proportion of the world and European populations has some evidence of thyroid dysfunction. For example, the prevalence of goiter in areas of severe iodine deficiency can be as high as 80%. In Germany, an area of relative iodine deficiency, thyroid nodules or goiter were found in 33% of working adults aged 18–65 years. Another problem is thyroid cancer. In the last decades, thyroid cancer incidence has continuously and sharply increased all over the world. Moreover, thyroid cancer mortality, in spite of earlier diagnosis and better treatment, has not decreased but is rather increasing. The reasons for this increase are not well understood, but some environmental carcinogens in the industrialized lifestyle may have specifically affected the thyroid. Among potential carcinogens, the increased iodine and some other chemical elementintake isone of the most likely risk factors. Excessive accumulation or an imbalance of the chemical elements may disturb the cell functions and may result in cellular degeneration, death or, on the contrary, intensive uncontrolled proliferation, and malignancy. Questions on the role of chemical elements in etiology and pathogenesis of thyroid cancer are far from being answered. First of all, it is necessary to establish the normal level and changes of chemical element contents in thyroid tissue, and their relationships in intact gland.

In our previous studies the high mass fraction of I 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.[1-9] However, some questions about the chemical element mass fraction in thyroid of adult and, particularly, elderly females still remain unanswered

This study aimed to perform a nondestructive method to evaluate the Br, Ca, Cl, K, Mg, Mn, and Na mass fraction in thyroid parenchyma and present data on relationships of these element contents in intact thyroid of females.

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

Experimental

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 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). All right lobes of thyroid glands were divided into two portions using a titanium scalpel.[10]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.[11] The pounded 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.

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used.[12] In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. Ten certified reference material (CRM) IAEA H-4 (animal muscle) sub-samples weighing about 100 mg were treated and analyzed in the same conditions that thyroid samples to estimate the precision and accuracy of results.

The content of Br, Ca, Cl, I, K, Mg, Mn, and Na were determined by INAA-SLR using a horizontal channel equipped with the pneumatic rabbit system of the WWR-c research nuclear reactor. 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. A coaxial 98-cm3 Ge (Li) detector and a spectrometric unit (NUC 8100), including a PC-coupled multichannel analyzer, were used for measurements. The spectrometric unit provided 2.9-keV resolution at the ^{60}Co 1,332-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 scalp hair.[13,14]

A dedicated computer program for INAA mode optimization was used.[15] 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. For the estimation of the Pearson correlation coefficient between different chemical elements the Microsoft Office Excel programs was also used.

Results and discussion

Good agreement of the Br, Ca, Cl, I, K, Mg, Mn, and Na contents analyzed by INAA-SLR with the certified data of CRM IAEA H-4 (Table 1) indicates an acceptable accuracy of the results obtained in the study of chemicalelements of the thyroid presented in Tables 2–4.

The obtained means for Br, Ca, Cl, I, K, Mg, Mn, and Na 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.[16-27] 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%)[28] and ash (4.16% on dry mass basis)[29] contents in thyroid of adults.

Table 1. INAA-SLR data of chemical element 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	N	5.0±0.9
Ca	188	163 – 213	N	238±59
Cl	1890	1810 – 1970	N	1950±230
I	0.08	-	N	<1.0
K	15800	15300 – 16400	C	16200±3800
Mg	1050	990 – 1110	C	1100±190
Mn	0.52	0.48 – 0.55	C	0.55±0.11
Na	2060	1930 – 2180	C	2190±140

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

Table 2. Some statistical parameters of Br, Ca, Cl, I, K, Mg, Mn, and Na 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	22.4	16.1	3.2	5.00	66.9	16.3	5.00	59.2
n=33	Ca	1663	570	198	461	3640	1170	670	3600
	Cl	3317	1480	290	1200	6000	3375	1388	5906
	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

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 3. Median, minimum and maximum value of means Br, Ca, Cl, I, K, Mg, Mn, and Na 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) [16]	284±44 (14) [17]	22±16
Ca	1600 (17)	840±240 (10) [18]	3800±320 (29) [18]	1663±570
Cl	6800 (5)	804±80 (4) [19]	8000 (-) [20]	3317±1480
I	1888 (95)	159±8 (23) [21]	5772±2708 (50) [22]	1956±1199
K	4400 (17)	46.4±4.8 (4) [19]	6090 (17) [23]	5395±3245
Mg	390 (16)	3.5 (-) [24]	840±400 (14) [25]	212±97
Mn	1.82 (36)	0.44±11 (12) [26]	69.2±7.2 (4) [19]	1.50±0.84
Na	8000 (9)	438 (-) [27]	10000±5000 (11) [25]	6421±1721

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

A significant direct correlation between the Br-K, Br-Mn, Cl-Mg, Cl-Na, I-Na, K-Mg, and Mn-Na mass fractions as well as an inverse correlation between Ca-Cland Na-Mnmass fractions was seen in female thyroid. No correlation was demonstrated between any other chemical elements (Table 4).If some correlations between the elements were predictable (e.g., Na-Cl), the interpretation of other observed relationships would require further study.No published data referring to inter-correlations of Br, Ca, Cl, I, K, Mg, Mn, and Namass fractions in thyroid of females was found.

Table 4. Intercorrelations of the chemical element mass fractions in the intact thyroid of female (*r* – coefficient of correlation)

Element	Br	Ca	Cl	I	K	Mg	Mn	Na
Br	**1.00**	0.246	0.073	-0.053	0.398[a]	-0.179	0.727[c]	0.179
Ca	0.246	**1.00**	-0.658[c]	0.132	0.125	0.021	0.341	-0.184
Cl	0.073	-0.658[c]	**1.00**	0.159	-0.367	0.563[b]	-0.207	0.389[a]
I	-0.053	0.132	0.159	**1.00**	-0.319	0.191	-0.090	0.446[a]
K	0.398[a]	0.125	-0.367	-0.319	**1.00**	0.774[c]	-0.194	-0.152
Mg	-0.179	0.021	0.563[b]	0.191	0.774[c]	**1.00**	-0.266	0.313
Mn	0.727[c]	0.341	-0.207	-0.090	-0.194	-0.266	**1.00**	-0.410[a]
Na	0.179	-0.184	0.389[a]	0.446[a]	-0.152	0.313	0.410[a]	**1.00**

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

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, Ca, Cl, I, K, Mg, Mn, and Na mass fractions in intact thyroid may serve as indicative normal values for females of urban population of the Russian Central European region.

Conclusions

The instrumentalneutron activation analysis with high resolution spectrometry of short-lived radionuclides is a useful analytical tool for the non-destructive determination of chemical element content in the thyroid tissue samples. This method allows determine means for 8 chemical elements:Br, Ca, Cl, I, K, Mg, Mn, and Na.

The good agreement with the medians of mean values cited by other researches for Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction in the human thyroid allows accept data obtained in the study for these elements as indicative normal values for female thyroid of urban population of the Russian Central European region.

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