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Research Article

Evaluation of Bromine, Calcium, Chlorine, Iodine, Potassium, Magnesium, Manganese, and Sodium Content in the Thyroid Adenomas using Neutron Activation Analysis

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ABSTRACT

The aim of this exploratory study was to examine the content of Bromine (Br), Calcium (Ca), Chlorine (Cl), Iodine (I), Potassium (K), Magnesium (Mg), Manganese (Mn), and Sodium (Na) in the normal and adenomatous Thyroid (TA). Thyroid tissue levels of eight Chemical Elements (ChE) were prospectively evaluated in 19 patients with TA and 105 healthy inhabitants. Measurements were performed using non-destructive instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides. Tissue samples were divided into two portions. One was used for morphological study while the other was intended for ChE analysis. A reduced content of I and Mg, as well as an elevated content of Br, Cl and Na in TA in comparison with normal thyroid was found. The study showed that the adenomatous transformation was accompanied by considerable changes in ChE contents of affected thyroid tissue.

Keywords: Thyroid adenomas; Intact thyroid; Chemical elements; Instrumental neutron activation analysis

ABBREVIATIONS

TA: Thyroid Adenoma; ChE: Chemical Element; NG: Nodular Goiter; TC: Thyroid Cancer; INAA-SLR: Instrumental Neutron Activation Analysis with high resolution spectrometry of Short-Lived Radionuclides; BSS: Biological Synthetic Standards; CRM: Certified Reference Material; IAEA: International Atomic Energy Agency

INTRODUCTION

Thyroid adenomas (TA) are homogenous, solitary, encapsulated benign tumors, more common in females, and have a good prognosis [1]. However, because there is a 20% possibility of malignant transformation, TA should be differentiated from other thyroid nodular diseases such as Nodular Goiter (NG) and Thyroid Cancer (TC). The distinguishing between the TA and TC is tricky, therefore new differential diagnostics and TA biomarkers are needed [2,3].

For over 20th century, there was the dominant opinion that NG, including TA, is the simple consequence of Iodine (I) deficiency. However, it was found that NG is a frequent disease even in those countries and regions where the population is never exposed to I shortage [4]. Moreover, it was shown that I excess has severe consequences on human health and associated with the presence of thyroidal disfunctions and autoimmunity, nodular and diffuse goiters, adenomas and malignant tumors of gland [5-8]. It was also demonstrated that besides the I deficiency and excess many other dietary, environmental, and occupational factors are associated with the NG incidence [9-11]. Among them a disturbance of evolutionary stable input of many Chemical Elements (ChE) in human body after industrial revolution plays a significant role in etiology of thyroidal disorders [12].

Besides I involved in thyroid function, other ChE have also essential physiological functions such as maintenance and regulation of cell function, gene regulation, activation or inhibition of enzymatic reactions, and regulation of membrane function [13]. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of ChE depend on tissue-specific need or tolerance, respectively [13]. Excessive accumulation or an imbalance of the ChE may disturb the cell functions and may

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result in cellular degeneration, death, benign or malignant transformation [13-15].

In our previous studies the complex of *in vivo* and *in vitro* nuclear analytical and related methods was developed and used for the investigation of I and other ChE contents in the normal and pathological thyroid [16-22]. Level of I in the normal thyroid was investigated in relation to age, gender and some non-thyroidal diseases [23,24]. After that, variations of many ChE content with age in the thyroid of males and females were studied and age- and gender-dependence of some ChE was observed [25-41]. Furthermore, a significant difference between some ChE contents in normal and cancerous thyroid was demonstrated [42-47].

To date, the etiology and pathogenesis of TA has to be considered as multifactorial. The present study was performed to clarify the role of some TE in the TA etiology. The aim of this work was to assess the Bromine (Br), Calcium (Ca), Chlorine (Cl), Iodine (I), Potassium (K), Magnesium (Mg), Manganese (Mn), and Sodium (Na) contents in TA tissue using instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) and also to compare the levels of these ChE in the adenomatous thyroid with those in intact (normal) gland of apparently healthy persons.

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

All patients suffered from TA (n=19, 16 females and 3 males, mean age M ± SD was 41 ± 11 years, range 22-55) were hospitalized in the Head and Neck Department of the Medical Radiological Research Centre. Thick-needle puncture biopsy of suspicious nodules of the thyroid was performed for every patient, to permit morphological study of thyroid tissue at these sites and to estimate their TE contents. For all patients the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. Histological conclusion for all thyroidal lesions was the TA.

Normal thyroids for the control group samples were removed at necropsy from 105 deceased (mean age 44 ± 21 years, range 2-87), who had died suddenly. The majority of deaths were due to trauma. A histological examination in the control group was used to control the age norm conformity, as well as to confirm the absence of micro-nodules and latent cancer.

All tissue samples were divided into two portions using a titanium scalpel [48]. One was used for morphological study while the other was intended for ChE analysis. After the samples intended for ChE analysis were weighed, they were freeze-dried and homogenized [49]. The pounded samples weighing about 10 mg (for biopsy) and 100 mg (for resected materials) were used for ChE measurement by INAA-SLR.

To determine contents of the ChE by comparison with a known standard, Biological Synthetic Standards (BSS) prepared from phenol-formaldehyde resins were used [50]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. Ten sub-samples of Certified Reference Material (CRM) of the International Atomic Energy Agency (IAEA) IAEA H-4 (animal muscle) weighing about 100 mg were treated and analyzed in the same conditions as 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 (Branch of Karpov Institute, Obninsk). Details of used nuclear reactions, radionuclides, gamma-energies, spectrometric unit, sample preparation, and the quality control of results were presented in our earlier publications concerning the INAA-SLR of ChE contents in human thyroid, scalp hair, and prostate [19,51-53].

A dedicated computer program for INAA mode optimization was used [54]. All thyroid samples were prepared in duplicate and mean values of ChE 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 ChE contents. The difference in the results between two groups (normal thyroid and TA) was evaluated by the parametric Student's t-test and non-parametric Wilcoxon-Mann-Whitney U-test.

RESULTS

Table 1 depicts our data for eight ChE (Br, Ca, Cl, I, K, Mg, Mn, and Na) in ten sub-samples of CRM IAEA H-4 (animal muscle) and the certified values of this material.

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 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).

Table 2 presents 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, Ca, Cl, I, K, Mg, Mn, and Na mass fraction in normal and adenomatous thyroid.

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Normal	Br	16.3	11.6	1.3	1.90	66.9	13.6	2.57	51.0
n=105	Ca	1692	1022	109	414	6230	1451	460	3805
	Cl	3400	1452	174	1030	6000	3470	1244	5869
	I	1841	1027	107	114	5061	1695	230	4232
	K	6071	2773	306	1740	14300	5477	2541	13285
	Mg	285	139	16.5	66.0	930	271	81.6	541
	Mn	1.35	0.58	0.07	0.510	4.18	1.32	0.537	2.23
	Na	6702	1764	178	3050	13453	6690	3855	10709
Adenoma	Br	286	330	104	3.2	871	133	5.09	841
n=19	Ca	1143	1135	342	52	3582	650	110	3353
	Cl	7722	3785	1262	1757	13824	9085	2043	13179
	I	961	1013	232	131	3906	476	170	3591
	K	5137	2474	686	797	8436	5741	937	8216
	Mg	200	131	36	15.0	397	269	15.0	376
	Mn	1.60	1.77	0.51	0.100	5.54	9.65	0.210	5.08
	Na	9072	3952	1096	2319	16414	9100	2728	15822

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 2: Some statistical parameters of Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction (mg/kg, dry mass basis) in normal and adenomatous thyroid.

The comparison of our results with published data for Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction in normal and adenomatous thyroid [9,55-70] is shown in Table 3.

Tissue	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
Normal	Br	18.1 (11)	5.12 (44) [55]	284±44(14) [56]	16.3± 11.6
	Ca	1600 (17)	840 ± 240 (10) [57]	3800± 320 (29) [57]	1692±1022
	Cl	6800 (5)	804 ± 80 (4) [58]	8000(-) [59]	3400± 1452
	I	1888 (95)	159 ± 8 (23) [60]	5772± 2708 (50) [61]	1841± 1027
	K	4400 (16)	46.4 ± 4.8 (4) [58]	6090 (17) [62]	6071± 2773
	Mg	390 (16)	3.5(-) [63]	1520 (20) [64]	285 ± 139
	Mn	1.62 (40)	0.076(83) [65]	69.2 ± 7.2 (4) [58]	1.35 ± 0.58
	Na	8000 (9)	438 (-) [66]	10000 ± 5000 (11) [67]	6702± 1764
Adenoma	Br	38 (4)	11 (5) [68]	777 (1) [69]	286 ± 330
	Ca	2298(4)	900 (1) [57]	3500 (1) [57]	1143 ± 1135
	Cl	864 (1)	864 ± 84 (4) [58]	864 ± 84 (4) [58]	7722± 3785
	I	640(13)	80 (1) [68]	2800 (1) [70]	961 ± 1013
	K	3650 (3)	72,8 ± 7,2 (4) [58]	5600 (1) [68]	5137± 2474
	Mg	-	-]	-	200 ± 131
	Mn	1.28 (4)	0.40 (46) [9]	57,6 ± 6,0 (4) [58]	1.60 ± 1.77
	Na	-	-	-	9072± 3952

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, Ca, Cl, I, K, Mg, Mn, and Na contents in normal and adenomatous thyroid according to data from the literature in comparison with our results (mg/kg, dry mass basis).

The ratios of means and the difference between mean values of Br, Ca, Cl, I, K, Mg, Mn, and Na mass fractions in normal and adenomatous thyroid are presented in Table 4.

Element	Thyroid tissue				Ratio
	Norm n=105	Adenoma n=19	Student's t-test p≤	U-test p	Adenoma to Norm
Br	16.3 ± 1.3	286 ± 104	**0.0295**	**≤ 0.01**	17.5
Ca	1692± 109	1143± 342	0.139	>0.05	0.68
Cl	3400± 174	7722±1262	**0.0089**	**≤ 0.01**	2.27

Normal Br 18.1 (11) 5.12 (44)

I	1841± 107	961 ± 232	**0.0020**	**≤0.01**	0.52
K	6071± 306	5137± 686	0.213	>0.05	0.85
Mg	285 ± 17	200 ± 36	**0.049**	**≤0.05**	0.70
Mn	1.35±0.07	1.60±0.51	0.647	>0.05	1.19
Na	6702± 1785	9072±1096	0.053	**≤0.05**	1.35

M: Arithmetic mean; SEM: Standard Error of Mean; Significant values are in bold

Table 4: Differences between mean values (M ± SEM) of Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction (mg/kg, dry mass basis) in normal and adenomatous thyroid.

DISCUSSION

Precision and accuracy of results

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 ChE of the thyroid samples presented in Tables 2-4.

The mean values and all selected statistical parameters were calculated for eight ChE (Br, Ca, Cl, I, K, Mg, Mn, and Na) mass fractions (Table 2). The mass fraction of Br, Ca, Cl, I, K, Mg, Mn, and Na were measured in all, or a major portion of normal and adenomatous thyroid samples.

Comparison with published data

In general, values of means obtained in present study for Br, Ca, Cl, I, K, Mg, Mn, and Na contents in the normal human thyroid (Table 3) agree well with median of means reported by other researchers [55-67]. A number of values for ChE 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%) and ash (4.16% on dry mass basis) contents in thyroid of adults [71,72].

Data cited in Table 3 for normal thyroid also includes samples obtained from patients who died from different non-endocrine diseases. In our previous study it was shown that some non-endocrine diseases can effect on ChE contents in thyroid [24]. Moreover, in many studies the "normal" thyroid means a visually non-affected tissue adjacent to benign or malignant thyroidal nodules. However, there are no data on a comparison between the ChE contents in such kind of samples and those in thyroid of healthy persons, which permits to confirm their identity.

In adenomatous tissues (Table 3) our results were comparable with published data for Ca, I, K, and Mn contents. The obtained mean for Br was approximately 7.5 times higher than the median of previously reported means, but within the range of means (Table 3). The obtained mean for Cl was 8.9 times higher than the only reported result (Table 3). No published data referring Mg and Na contents of adenomatous thyroid tissue were found.

The range of means of levels of Br, Ca, Cl, I, K, Mg, Mn, and Na reported in the literature for normal and adenomatous thyroid vary widely (Table 3). This can be explained by a dependence of ChE content on many factors, including "normality" of thyroid samples (see above), the region of the thyroid, from which the sample was taken, age, gender, ethnicity, mass of the gland, and the adenoma stage, histology and functional activity. Not all these factors were strictly controlled in cited studies. However, in our opinion, the leading causes of inter-observer variability can be attributed to the accuracy of the analytical techniques, sample preparation methods, and inability of taking uniform samples from the affected tissues. It was insufficient quality control of results in these studies. In many scientific reports, tissue samples were ashed or dried at high temperature for many hours. In other cases, thyroid samples were treated with solvents (distilled water, ethanol, formalin etc). There is evidence that during ashing, drying and digestion at high temperature some quantities of certain ChE are lost as a result of this treatment. That concerns not only such volatile halogen as Br, but also other ChE investigated in the study [73,74].

Effect of adenomatous transformation on ChE contents

From Table 4, it is observed that in adenomatous tissues the mass fractions of I and Mg are 48% and 30%, respectively, lower, whereas mass fractions of Br, Cl, and Na are approximately 17.5, 2.27, and 1.35 times, respectively, higher than in normal tissues of the thyroid. Thus, if we accept the ChE contents in thyroid glands in the control group as a norm, we have to conclude that with an adenomatous transformation the Br, Cl, I, Mg, and Na contents in thyroid tissue significantly changed.

Role of ChE in adenomatous transformation of the thyroid

Characteristically, elevated or reduced levels of ChE observed in adenomatous tissues are discussed in terms of their potential role in the initiation and promotion of adenoma. In other words, using the low or high levels of the ChE found in adenomatous tissues, researchers try to determine the role of the deficiency or excess of each ChE in the adenomatous transformation. In our opinion, abnormal levels of many ChE in TA could be and cause, and also effect of adenomatous transformation. From the results of such kind studies, it is not always possible to decide whether the measured decrease or increase in ChE level in pathologically altered tissue is the reason for alterations or vice versa.

Bromine: This is one of the most abundant and ubiquitous of the recognized ChE 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 [75]. Moreover, many studies indicate that bromate (BrO3-) and potassium bromate (KBrO3) are carcinogens [76-82]. Bromate is formed as a drinking water ozone disinfection by-product and also used in some food and consumer product [76]. Potassium bromate is a chemical oxidizing agent that used extensively in food and cosmetic industries [77,78]. Potassium bromate is also found in drinking water as a disinfection by-product of surface water ozonation [76].

In our previous studies it was found a significant age-related increase of Br content in human thyroid [25-28]. This finding correlated with a significant age-related increase of thyroid cancer incidents. Furthermore, elevated levels of Br in cancerous thyroid and malignant tumor of prostate were indicated [42,43,46,79].

Thus, on the one hand, the accumulated data suggest that Br might be responsible for TA development. But, on the other hand, Br compounds, especially Potassium Bromide (KBr), Sodium Bromide (NaBr), and Ammonium Bromide (NH4Br), are frequently used as sedatives in Russia [80]. It may be the reason for elevated levels of Br in specimens of patients with TA. Anyway, the accumulation of Br in adenomatous thyroids could possibly be explored for diagnosis of TA.

Chlorine: Cl is a ubiquitous, extracellular electrolyte essential to many metabolic pathways. Cl exists in the form of chloride in the human body. In the body, it is mostly present as sodium chloride. Therefore, as usual, there is a correlation between Na and Cl contents in tissues and fluids of human body. It is well known that Cl mass fractions in samples depend mainly on the extracellular water volume in tissues [81]. TA tissues contain probably more colloid that normal thyroid. Because colloid is extracellular liquid, it is possible to speculate that TA, and particularly follicular adenomas, are characterized by an increase of the mean value of the Cl mass fraction because the relative content of colloid is higher than that in normal thyroid tissue. Overall, the elevated levels of Cl in adenomatous thyroids could possibly be explored for diagnosis of TA.

Iodine: Compared to other soft tissues, the human thyroid gland has higher levels of I, because this element plays an important role in its normal functions, through the production of thyroid hormones (thyroxin and triiodothyronine) which are essential for cellular oxidation, growth, reproduction, and the activity of the central and autonomic nervous system. Adenomatous transformation is probably accompanied by a partial loss of tissue-specific functional features, which leads to a reduction in I content associated with functional characteristics of the human thyroid tissue. Almost half lower mean of I content in adenomatous thyroids in comparison with normal level could possibly be explored for diagnosis of TA.

Magnesium: Mg is abundant in the human body. This element is essential for the functions of more than 300 enzymes (e.g. alkaline phosphatases, ATP-ases, phosphokinases, the oxidative phosphorylation pathway). It plays a crucial role in many cell functions such as energy metabolism, protein and DNA syntheses, and cytoskeleton activation. Moreover, Mg plays a central role in determining the clinical picture associated with thyroid disease [82]. Thus, the modest reduced levels of Mg in adenomatous thyroids could possibly be explored for diagnosis of TA.

Sodium: Na is mainly an extracellular electrolyte and its elevated level in adenomatous thyroid might link with a higher content of colloid in TA (see Chlorine). Anyway, it seems that the elevated levels of Na in adenomatous thyroids could possibly be explored for diagnosis of TA.

CONCLUSION

In this work, ChE analyses were carried out in the tissue samples of normal and adenomatous thyroid using INAA-SLR. It was shown that INAA-SLR is an adequate analytical tool for the non-destructive determination of Br, Ca, Cl, I, K, Mg, Mn, and Na contents in the tissue samples of human thyroid gland, including core needle biopsies. It was observed that in adenomatous tissues the mass fractions of I and Mg are 48% and 30%, respectively, lower, whereas mass fractions of Br, Cl, and Na are approximately 17.5, 2.27, and 1.35 times, respectively, higher than in normal tissues of the thyroid. In our opinion, the abnormal decrease in level of I and Mg, as well as the increase in levels of Br, Cl, and Na in adenomatous tissue might demonstrate an involvement of these elements in etiology and pathogenesis of TA. It was supposed that the elevated levels of Br, Cl, and Na, as well as the reduced levels of I and Mg in the affected thyroid tissue can be used as the TA markers.

LIMITATIONS

This study has several limitations. Firstly, analytical techniques employed in this study measure only eight ChE (Br, Ca, Cl, I, K, Mg, Mn, and Na) mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of ChE investigated in normal and adenomatous thyroids. Secondly, the sample size of TA group was relatively small and prevented investigations of ChE contents in TA group using differentials like gender, histological types and functional activity of TA, stage of disease, and dietary habits of healthy persons and patients with TA. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on adenoma-specific tissue Br, Cl, I, Mg, and Na level alteration and shows the necessity to continue ChE research of TA.

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