REVIEW ARTICLE

Variation in Selected Chemical Element Contents Associated with Malignant Tumors of Human Thyroid Gland

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Abstract

Thyroid cancer is an internationally important health problem. The aim of this exploratory study was to evaluate whether significant changes in the thyroid tissue levels of Br, Ca, Cl, I, K, Mg, Mn, and Na exist in the malignantly transformed thyroid. Thyroid tissue levels of eight chemical elements were prospectively evaluated in 41 patients with thyroid malignant tumors 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 chemical element analysis. It was found that contents of Br, Cl, K, Mg, and Na were significantly higher and content of I was drastically lower in cancerous tissues than in normal tissues. Thus, the study showed that the malignant transformation was accompanied by considerable changes in chemical element contents of thyroid parenchyma.

Keywords

Thyroid malignant tumors, intact thyroid, chemical elements, instrumental neutron activation analysis

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Introduction

Thyroid cancer (TC) is the most common endocrine malignancy. TC incidence has dramatically increased in the recent decades.[1] During the same period no other cancer has increased as much as TC. With the worldwide increase in the incidence of TC, it has become the fifth most common cancer in women.[2–4] In some countries, the incidence of TC has increased extremely fast, and it has been the most common cancer for the last years.[5]

Although the etiology of TC is unknown, several risk factors including deficiency or excess of such micronutrient as iodine (I) have been well identified.[6–17] It was also reported that incidence of TC and mortality from this disease increases progressively with advancing age.[18,19] For example, a 37-fold increase in hazard ratio from age <40 years to age >70 years was showed in the study of 3664 TC patients that received surgery and adjuvant treatment at Memorial Sloan Kettering Cancer Center from the years 1985 to 2010.[19]

Besides I involved in thyroid function, other chemical elements 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. Essential or toxic (mutagenic, carcinogenic) properties of chemical elements depend on tissue-specific need or tolerance, respectively.[20] Excessive accumulation or an imbalance of the chemical elements may disturb the cell functions and may result in cellular degeneration, death or malignant transformation.[20–22]

In our previous study a significant positive correlation between age and some chemical element contents in the thyroid was observed.[23–28] For example, a strongly pronounced tendency of age-related increase in bromine (Br), calcium (Ca), and I mass fractions was demonstrated by using non-destructive instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR).[23,27] In addition, a significant positive correlation was seen between the contents of I and sodium (Na) in female thyroid,[23] and also between I and Ca in male thyroid.[27] It was concluded that high intra-thyroidal I and Ca concentrations are probably one of the main factors acting in both initiation and promotion stages of thyroid carcinogenesis,[23,27] as it was earlier shown by us for Ca and some other chemical elements in prostate gland.[29-35] Moreover, it seems fair to suppose that besides I and Ca, such chemical elements as Br, chlorine (Cl), potassium (K), magnesium (Mn), manganese (Mn), and sodium (Na) also play arolein the pathophysiology of the thyroid.

The aim of this work was to assess the Br, Ca, Cl, I, K, Mg, Mn, and Na contents in TC tissue using INAA-SLR analysis and also to compare the levels of chemical elements in the malignant 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.

Material and Methods

All patients suffered from TC (n=41, mean age M±SD was 46±15 years, range 16–75) 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 chemical element contents. In all cases the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. Histological conclusions for malignant tumors were: 25 papillary adenocarcinomas, 8 follicular adenocarcinomas, 7 solid carcinomas, and 1 reticulosarcoma.

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.[36] One was used for morphological study while the other was intended for chemical element analysis. After the samples intended for chemical element analysis were weighed, they were freeze-dried and homogenized.[37] The pounded samples weighing about 5–10 mg (for biopsy) and 100 mg (for resected materials) 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.[38] 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 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). 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-cm^3 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-SLR chemical element contents in human thyroid, scalp hair, and prostate.[7,39–41]

A dedicated computer program for INAA mode optimization was used.[42] 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. The 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.

Results

Table 1 depicts our data for eight chemical elements (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.

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 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 cancerous thyroid tissue.

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 cancerous 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
Cancer	Br	139	203	36	6.2	802	50.2	7.75	802
n=41	Ca	2398	2368	558	452	8309	1302	467	7428
	Cl	7699	2900	703	4214	14761	7216	4240	13619
	I	71.8	62.0	10	2.00	261	62.1	2.93	192
	K	9655	4444	970	1660	19255	8746	3181	19035
	Mg	450	232	51	122	1033	408	126	931
	Mn	1.90	1.41	0.32	0.100	5.79	1.59	0.100	5.37
	Na	8556	2959	646	4083	17284	7264	4704	14543

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 comparison of our results with published data for Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction in normal and cancerous thyroid [43–60] is shown in Table 3.

Table 3.Median, minimum and maximum value of means Br, Ca, Cl, I, K, Mg, Mn, and Na contents in normal and cancerous thyroid according to data from the literature in comparison with our results (mg/kg, dry mass basis)

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) [43]	284±44 (14) [44]	16.3±11.6
	Ca	1600 (17)	840±240(10) [45]	3800±320(29) [45]	1692±1022
	Cl	6800 (5)	804±80(4) [46]	8000 (-) [47]	3400±1452
	I	1888 (95)	159±8 (23) [48]	5772±2708(50) [49]	1841±1027
	K	4400 (17)	46.4±4.8 (4) [46]	6090 (17) [50]	6071±2773
	Mg	390 (16)	3.5 (-) [51]	840±400 (14) [52]	285±139
	Mn	1.82 (36)	0.44±11(12) [53]	69.2±7.2 (4) [46]	1.35±0.58
	Na	8000 (9)	438 (-) [54]	10000±5000 (11) [52]	6702±1764
Cancer	Br	15.7 (4)	9.6 (1) [55]	160±112 (3) [44]	139±203
	Ca	1572 (6)	390 (1) [56]	3544 (1) [55]	2398±2368
	Cl	940 (1)	940±92 (4) [46]	940±92 (4) [46]	7699±2900
	I	78.8 (12)	<23±10 (8) [57]	800 (1) [58]	71.8±62.0
	K	6878 (4)	636±64 (4) [46]	7900 (1) [56]	9655±4444
	Mg	320 (2)	316±84 (45) [59]	544±272 (6) [60]	450±232
	Mn	1.83 (4)	1.6±0.8 (22) [59]	186±18 (4) [46]	1.90±1.41
	Na	-	-	-	8556±2959

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

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 cancerous thyroid are presented in Table 4.

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 cancerous thyroid

Element	Thyroid tissue				Ratio
	Norm n=105	Cancer n=41	Student's *t*-test *p*≤	*U*-test *p*	Cancer to Norm
Br	16.3±1.3	139±36	**0.0018**	≤0.01	8.53
Ca	1692±109	2398±558	0.243	>0.05	1.42
Cl	3400±174	7699±703	**0.000013**	**≤0.01**	2.26
I	1841±107	71.8±10.0	**0.00000000001**	**≤0.01**	0.039
K	6071±306	9655±970	**0.0017**	**≤0.01**	1.59
Mg	285±17	450±51	**0.0047**	**≤0.01**	1.58
Mn	1.35±0.07	1.90±0.32	0.107	>0.05	1.41
Na	6702±1785	8556±646	**0.011**	**≤0.01**	1.28

M – arithmetic mean, SEM – standard error of mean, statistically significant values are in bold.

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 chemical elements of the thyroid samples presented in Tables 2–4.

The mean values and all selected statistical parameters were calculated for eight chemical elements (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 cancerous tissue samples.

Comparison with published data

Values obtained for Br, Ca, Cl, I, K, Mg, Mn, and Na contents in the normal human thyroid (Table 3) agree well with median of mean values reported by other researchers.[43-54] Data cited in Table 3 also include samples obtained from patients who died from different non-endocrine diseases. A number of values for trace 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%)[61] and ash (4.16% on dry mass basis)[62] contents in thyroid of adults.

In cancerous tissues (Table 3) our results were comparable with published data for Ca, I, K, Mg, and Mn contents. The obtained mean for Br was approximately one order of magnitude higher than the median of previously reported means, but within the range of means(Table 3). The obtained mean for Cl was almost one order of magnitude higher than the only reported result (Table 3). No published data referring Na contents of cancerous 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 for untreated cancerous thyroid vary widely (Table 3). This can be explained by a dependence of chemical element content on many factors, including the region of the thyroid, from which the sample was taken, age, gender, ethnicity, mass of the gland, histological types of tumors and the cancer stage. 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 chemical elements are lost as a result of this treatment. That concern not only such volatile halogen as Br, but also other chemical elements investigated in the study.[63–65]

Effect of malignant transformation on chemical element contents

From Table 4, it is observed that in cancerous tissues the mass fraction of I is almost 26 times lower whereas mass fractions of Br, Cl, K, Mg, and Na are approximately 8.5, 2.3, 1.6, 1.6, and 1.3 times, respectively, higher than in normal tissues of the thyroid. Thus, if we accept the chemical element contents in thyroid glands in the control group as a norm, we have to conclude that with a malignant transformation the Br, Cl, I, K, Mg, and Na contents in thyroid tissue significantly changed.

Role of chemical elements in malignant transformation of the thyroid

Characteristically, elevated or reduced levels of chemical elements observed in cancerous tissues are discussed in terms of their potential role in the initiation and promotion of thyroid cancer. In other words, using the low or high levels of the chemical element found in cancerous tissues, researchers try to determine the carcinogenic role of the deficiency or excess of each chemical element in investigated organ. In our opinion, abnormal levels of many chemical elements in tumor could be and cause, and also effect of malignant transformation. From the results of such kind studies, it is not always possible to decide whether the measured decrease or increase in chemical element 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 chemical elements 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.[66]

In our previous studies, we found a significant age-related increase of Br content in human thyroid.[23,26-28] Therefore, a goitrogenic and, probably, carcinogenic effect of excessive Br levels in the thyroid of old females was assumed. On the one hand, elevated levels of Br in TC tissues, observed in the present study, supports this conclusion. But, on the other hand, bromide compounds, especially potassium bromide (KBr), sodium bromide (NaBr), and ammoniumbromide (NH4Br), are frequently used as sedatives in Russia.[67] It may be the reason for elevated levels of Br in specimens of patients with TC. Nevertheless, the accumulation of Br in neoplastic thyroid tissues could possibly be explored for diagnosis of TC.

Chlorine: Cl is a ubiquitous, extracellular electrolyte essential to more than one metabolic pathway. 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, including the blood volumes, in tissues.68 Cancerous tissues are predominantly highly vascularized lesions.[69–75] Thus, it is possible to speculate that thyroid malignant tumors are characterized by an increase of the mean value of the Cl mass fraction because the level of tumor vascularization is higher than that in normal thyroid tissue. Overall, the elevated levels of Cl in neoplastic thyroids could possibly be explored for diagnosis of TC.

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. Malignant transformation is accompanied by a loss of tissue-specific functional features, which leads to a significant reduction in I content associated with functional characteristics of the human thyroid tissue. Drastically low level of I content in neoplastic thyroids could possibly be explored for diagnosis of TC.

Potassium: An uncontrollable cell proliferation characterize the malignant tumors. Therefore, morphological structures of TC tissue differ from the structure of normal thyroid parenchyma. Because K is mainly an intracellular electrolyte, an elevated level of K content in the TC tissue might reflect increase of ratio “mass of transformed thyroid cell – mass of follicular colloid”. Nevertheless, the accumulation of K in neoplastic thyroids could possibly be explored for diagnosis of TC.

Magnesium: Mg is abundant in the human body. This element is essential for the functions of more than 300 enzymes(e.g. alkaline phosphatases, ATPases, 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.[76] Experimental data have shown that high doses of magnesium increase the activity of the thyroid gland.[77] Magnesium deficiency can influence bioavailability and tissue distribution of selenium which then appears diminished.[78] From these data, one can conclude that Mg is involved in the thyroid function. If so, significant reduction in Mg content cam be associate with TC, because malignant transformation is accompanied by a loss of thyroid-specific functional features. However, it is well known that malignant tumors have a usually higher Mg levels than do normal tissues,[79-85] possibly caused by the "retention" of Mg by the tumor,[86] as a result of the high Mg requirement of growing cells. In addition, cultured proliferating cells have long been known to contain more magnesium than quiescent cells, and experimental conditions that decreased magnesium availability affected cell proliferation rate.[87] Thus, the elevated levels of Mg in neoplastic thyroids could possibly be explored for diagnosis of TC.

Sodium: Knowledge concerning ion regulation in many normal and abnormal cell processes has had a rapid development. It was found, among other regulations, that sodium-calcium exchange is associated with the cytoskeleton and the cell membrane. A hypothesis was eventually established that a wide variety of pathological phenomena ranging from acute cell death to chronic processes, such as neoplasia, all have a common series of cellular reactions.[88] In accordance with this hypothesis, concentrations of sodium were found to be enhanced in human and animal neoplastic tissues.[89,90] Moreover, the hypothesis that physiological and biochemical changes associated with proliferating malignant tumors may cause an increase in total tissue sodium concentration was tested with non-invasive, quantitative ^{23}Na magnetic resonance imaging in patients with benign and malignant breast tumors. It was shown that elevated Na concentrations in breast lesions appears to be a cellular-level indicator associated with malignancy.[91] In addition, Na is mainly an extracellular electrolyte and its elevated level in malignant tumors might link with a high tumor vascularization (see Chlorine). Anyway, it seems that the accumulation of Na is a generic property of malignant tumors.

Limitations

This study has several limitations. Firstly, analytical techniques employed in this study measure only eight chemical elements (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 chemical elements investigated in normal and cancerous thyroid tissue. Secondly, the sample size of TC group was relatively small and prevented investigations of chemical element contents in TC group using differentials like gender, histological types of tumors, stage of disease, and dietary habits of healthy persons and patients with TC. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on cancer-specific tissue Br, Cl, I, K, Mg, and Na level alteration and shows the necessity to continue chemical element research of malignant thyroid tumors.

Conclusion

In this work, chemical elemental analyses were carried out in the tissue samples of normal and cancerous 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 glands, including core needle biopsies. It was observed that in malignant tumors content of I was drastically lower ($p<0.000001$) and contents of Br ($p<0.0018$), Cl ($p<0.000013$), K ($p<0.0017$), Mg ($p<0.0047$), and Na ($p<0.011$) were significantly higher than in normal tissues. In our opinion, the abnormal decrease in level of I, as well as the increase in levels of Br, Cl, K, Mg, and Na in cancerous tissue might demonstrate an involvement of these elements in etiology and pathogenesis of malignant thyroid tumors. It was supposed that elevated levels of Br, Cl, K, Mg, and Na, as well as reduced levels of I in thyroid tissues can be used as tumor markers.

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Conflict of Interests

The authors have not declared any conflict of interests.

Authors' Contribution

Vladimir Zaichick and Sofia Zaichick contributed equally to this work.

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