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Differences between Chemical Elements of Thyroid Benign Nodules and Those of Thyroid Tissue adjacent to Nodules investigated using Neutron Activation Analysis

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ABSTRACT

Thyroid benign nodules (TBNs) are the most common diseases of this endocrine gland and are common worldwide. The etiology and pathogenesis of TBNs must be considered as multifactorial. The present study was performed to clarify the role of some chemical elements (ChEs) in the etiology of these thyroid disorders. For this purpose, thyroid tissue levels of calcium (Ca), chlorine (Cl), iodine (I), potassium (K), magnesium (Mg), manganese (Mn), and sodium (Na) were prospectively evaluated in nodular tissue and tissue adjacent to nodules of 79 patients with TBNs. Measurements were performed using neutron activation analysis Results of the study were additionally compared with previously obtained data for the same ChEs in "normal" thyroid tissue.

It was found that mass fractions of Cl and Na in "adjacent" group of samples were approximately 2.7 times and 60% higher, respectively, than in "normal" thyroid. Contents of Ca, Cl, K, Mg, Mn, and Na found in the "nodular" and "adjacent" groups of thyroid tissue samples were very similar, while level of I in "adjacent" group of samples was over 2 times higher than in nodular tissue and almost equals the normal value. From results obtained, it was possible to conclude that the main characteristics of tissue adjacent to TBNs were elevated levels of Cl and Na in comparison with these ChEs contents in "normal" thyroid. The little reduced level of I content in nodular tissue could possibly be explored for differential diagnosis of TBNs and thyroid cancer.

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Introduction

Thyroid benign nodules (TBNs) are universally encountered and frequently detected by palpation during a physical examination, or incidentally, during clinical imaging procedures. TBNs include non-neoplastic lesions, for example, colloid goiter and thyroiditis, as well as neoplastic lesions such as thyroid adenomas [1-3]. For over 20th century, there was the dominant opinion that TBNs is the simple consequence of iodine deficiency. However, it was found that TBNs is a frequent disease even in those countries and regions where the population is never exposed to iodine shortage [4]. Moreover, it was shown that iodine excess has severe consequences on human health and associated with the presence of TBNs [5-8]. It was also demonstrated that besides the iodine deficiency and excess many other dietary, environmental, and occupational factors are associated with the TBNs incidence [9-11]. Among these factors a disturbance of evolutionary stable input of many chemical elements (ChEs) in human body after industrial revolution plays a significant role in etiology of TBNs [12].

Besides iodine, many other ChEs have also essential physiological functions [13]. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of ChEs depend on tissue-specific need or tolerance, respectively [13]. Excessive accumulation or an imbalance of the ChEs may disturb the cell functions and may result in cellular proliferation, 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 iodine and other ChEs contents in the normal and pathological thyroid [16-22]. Iodine level in the normal thyroid was investigated in relation to age, gender and some non-thyroidal diseases [23,24]. After that, variations of many ChEs content with age in the thyroid of males and females were studied and age- and gender-dependence of some ChEs was observed [25-41]. Furthermore, a significant difference between some ChEs contents in colloid goiter, thyroiditis, and thyroid adenoma in comparison with normal thyroid was demonstrated [42-46]. To date, the etiology and pathogenesis of TBNs must be considered as multifactorial. The present study was performed to find out differences in ChEs contents between the group of nodular tissues and tissue adjacent to nodules, as well as to clarify the role of some ChEs in the etiology of TBNs. Having this in mind, the aim of this exploratory study was to examine differences in the content of calcium (Ca), chlorine (Cl), iodine (I), potassium (K), magnesium (Mg), manganese (Mn), and sodium (Na) in nodular and adjacent to nodules tissues of thyroids with TBNs using a non-destructive instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR), and to compare the levels of these ChEs in two groups (nodular and adjacent to nodules tissues) of the cohort of TBNs samples. Moreover, for understanding a possible role of ChEs in etiology and pathogenesis of TBNs results of the study were compared with previously obtained data for the same ChEs in "normal" thyroid tissue [42-46].

Material and Methods

All 79 patients suffered from TBNs (46 patients with colloid goiter, mean age M±SD was 48±12 years, range 30-64; 19 patients with thyroid adenoma, mean age M±SD was 41±11 years, range 22-55; and 14 patients with thyroiditis, mean age M±SD was 39±9 years, range 34-50) were hospitalized in the Head and Neck Department of the Medical Radiological Research Centre (MRRC), Obninsk. The group of patients with thyroiditis included 8 persons with Hashimoto's thyroiditis and 6 persons with Riedel's Struma. 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 ChEs contents. For all patients the diagnosis has been confirmed by clinical and morphological/histological results obtained during studies of biopsy and resected materials. "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 studies were approved by the Ethical Committees of MRRC. 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. Informed consent was obtained from all individual participants included in the study. All tissue samples were divided into two portions using a titanium scalpel [47]. One was used for morphological study while the other was intended for ChEs analysis. After the samples intended for ChEs analysis were weighed, they were freeze-dried and homogenized [48]. The pounded samples weighing about 10 mg (for biopsy) and 100 mg (for resected materials) were used for ChEs 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 [49]. 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 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 ChEs contents in human thyroid, scalp hair, and prostate [27,28,50-52].

A dedicated computer program for INAA-SLR mode optimization was used [53]. All thyroid samples for ChEs analysis were prepared in duplicate, and mean values of ChEs contents were used in final calculation. Using Microsoft Office Excel software, 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 ChEs contents in nodular and adjacent tissue of thyroids with TBNs. Data for "normal" thyroid were taken from our previous publications [42-46]. The difference in the results between three groups of samples ("normal", "nodular", and "adjacent") was evaluated by the parametric Student's t-test and non-parametric Wilcoxon-Mann-Whitney U-test.

Results

Table 1 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 Ca, Cl, I, K, Mg, Mn, and Na mass fraction in "normal", "nodular", and "adjacent" groups of thyroid tissue samples.

Table 1: Some statistical parameters of Ca, Cl, I, K, Mg, Mn, and Na mass fraction (mg/kg, dry mass basis) in "normal" thyroid and thyroid benign nodules (nodular and adjacent tissue)

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Normal thyroid	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
Thyroid benign nodules (nodular tissue)	Ca	1237	902	138	52.0	4333	1108	116	3536
	Cl	8231	3702	772	1757	16786	8326	2543	15157
	I	992	901	103	29.0	3906	695	84.8	3629
	K	6190	2360	352	797	12222	6185	1438	10297
	Mg	331	180	26	13.0	844	311	15.0	745
	Mn	1.80	1.38	0.21	0.100	5.54	1.45	0.367	5.48
	Na	10207	3786	558	2319	22381	9802	3689	16969
Thyroid benign nodules (adjacent tissue)	Ca	1532	1700	380	418	6466	994	442	6312
	Cl	9203	6033	1384	2881	23731	8161	3294	22429
	I	2158	1436	214	343	7912	1917	527	5441
	K	6793	4044	862	3406	18255	5607	3500	18077
	Mg	316	275	59	15.0	987	292	15.0	890
	Mn	1.77	1.66	0.36	0.100	5.83	1.10	0.100	5.67
	Na	10850	5541	1209	4663	31343	9642	5548	23981

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 ratios of means and the comparison of mean values of Ca, Cl, I, K, Mg, Mn, and Na mass fractions in pairs of sample groups such as "normal" and "nodular", "normal" and "adjacent", and also "adjacent" and "nodular" are presented in Table 2, 3, and 4, respectively.

Table 2: Differences between mean values (M±SEM) of Ca, Cl, I, K, Mg, Mn, and Na mass fraction (mg/kg, dry mass basis) in "normal" thyroid (NT) and thyroid benign nodules (TBNs) (nodular tissue)

Element	Thyroid tissue				Ratio
	NT	TBNs (nodular)	Student's t-test, *p*≤	U-test, *p*	nodular/NT
Ca	1692±109	1237±138	**0.0082**	**≤0.05**	0.73
Cl	3400±174	8231±772	**0.0000025**	**≤0.01**	2.42
I	1841±107	992±103	**0.00000005**	**≤0.01**	0.54
K	6071±306	6190±352	0.798	>0.05	1.02
Mg	285±17	331±26	0.140	>0.05	1.16
Mn	1.35±0.07	1.80±0.21	**0.048**	**≤0.01**	1.33
Na	6702±1785	10207±558	**0.00000018**	**≤0.01**	1.52

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

Table 3: Differences between mean values (M±SEM) of Ca, Cl, I, K, Mg, Mn, and Na mass fraction (mg/kg, dry mass basis) in "normal" thyroid (NT) and thyroid benign nodules (TBNs) (adjacent tissue)

Element	Thyroid tissue				Ratio
	NT	TBNs (nodular)	Student's t-test, *p*≤	U-test, *p*	nodular/NT
Ca	1692±109	1532±380	0.655	>0.05	0.91
Cl	3400±174	9203±1384	**0.00056**	**≤0.01**	2.71
I	1841±107	2158±214	0.188	>0.05	1.17
K	6071±306	6793±862	0.437	>0.05	1.12
Mg	285±17	316±59	0.610	>0.05	1.11
Mn	1.35±0.07	1.77±0.36	0.271	>0.05	1.31
Na	6702±1785	10850±1209	**0.0028**	**≤0.01**	1.62

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

Table 4: Differences between mean values (M±SEM) of Ca, Cl, I, K, Mg, Mn, and Na mass fraction (mg/kg, dry mass basis) in nodular and adjacent tissue of thyroid benign nodules (TBNs)

Element	Thyroid tissue				Ratio
	Adjacent	Nodular	Student's t-test, *p*≤	U-test, *p*	Nodular/Adjacent
Ca	1532±380	1237±138	0.473	≤0.05	0.81
Cl	9203±1384	8231±772	0.545	>0.05	0.89
I	2158±214	992±103	**0.0000065**	**≤0.01**	0.46
K	6793±862	6190±352	0.523	>0.05	0.91
Mg	316±59	331±26	0.820	>0.05	1.05
Mn	1.77±0.36	1.80±0.21	0.950	>0.05	1.02
Na	10850±1209	10207±558	0.633	>0.05	0.94

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

Discussion

As was shown before good agreement of the Ca, Cl, I, K, Mg, Mn, and Na contents in CRM IAEA H-4 samples analyzed by INAA-SLR with the certified data of this CRM indicates acceptable accuracy of the results obtained in the study of thyroid tissue samples presented in Tables 1-4[27,28,50-52].

The Cl, Mn, and Na contents in "nodular" tissue were higher, while Ca and I content was lower in comparison with contents of these ChEs in normal gland (Table 2). Significant differences between ChEs contents of "normal" thyroid and ChEs contents of thyroid tissue adjacent to nodules were found only for Cl and Na. Mass fractions of Cl and Na in "adjacent" group of samples were approximately 2.7 times and 60% higher, respectively, than in "normal" thyroid (Table 3). In a general sense Ca, Cl, K, Mg, Mn, and Na contents found in the "nodular" and "adjacent" groups of thyroid tissue samples were very similar (Table 4). However, level of I in "adjacent" group of samples was over 2 times higher than in nodular tissue (Table 4) and almost equals the normal value (Table 3).

Characteristically, elevated or reduced levels of ChEs observed in thyroid nodules are discussed in terms of their potential role in the initiation and promotion of these thyroid lesions. In other words, using the low or high levels of the ChEs in affected thyroid tissues researchers try to determine the role of the deficiency or excess of each ChEs in the etiology and pathogenesis of thyroid diseases. In our opinion, abnormal levels of many ChEs in TBNs could be and cause, and also effect of thyroid tissue transformation. From the results of such kind studies, it is not always possible to decide whether the measured decrease or increase in ChEs level in pathologically altered tissue is the reason for alterations or vice versa. According to our opinion, investigation of ChEs contents in thyroid tissue adjacent to nodules and comparison obtained results with ChEs levels typical of "normal" thyroid gland may give additional useful information on the topic because this data show conditions of tissue in which TBNs were originated and developed.

Chlorine and sodium

Cl and Na are ubiquitous, extracellular electrolytes essential to more than one metabolic pathway. In the body, Cl and Na mostly present as sodium chloride. Therefore, as usual, there is a correlation between Na and Cl contents in tissues and fluids of human body. Because Cl is halogen like I, in the thyroid gland the biological behavior of chloride has to be similar to the biological behavior of iodide. The main source of natural Cl for human body is salt in food and chlorinated drinking water. Environment (air, water and food) polluted by artificial nonorganic Cl-contained compounds, for example such as sodium chlorate ($NaClO_3$), and organic Cl-contained compounds, for example such as polychlorinated biphenyls (PCBs) and dioxin, is other source. There is a clear association between using chlorinated drinking water, levels $NaClO_3$, PCBs and dioxin in environment and thyroid disorders, including cancer [54-58]. Thus, on the one hand, the accumulated data suggest that Cl level in thyroid tissue might be responsible for TBNs development. However, on the other hand, it is well known that Cl and Na mass fractions in human tissue samples depend mainly on the extracellular water volume [59]. Nodular and adjacent to nodules thyroid tissues can be more vascularized and can contain more relative volume of colloid than normal thyroid. Because blood and colloid are extracellular liquids, it is possible to speculate that it could be the reason for elevated levels of Cl and Na in TBNs and adjacent tissue. If that is the case, the equilibrium between Cl and Na increases has to be, however, in comparison with "normal" thyroid the change of Cl level in TBNs and adjacent tissue is significantly higher than change of Na level. Thus, it is possible to assume that an excessive accumulation of Cl in thyroid tissue is involved in TBNs etiology. Overall, the elevated levels of Cl in thyroid tissue could possibly be explored as risk factor of TBNs.

Iodine

To date, it was well established that I deficiency or excess has severe consequences on human health and associated with the presence of TBNs [5-8,54,60]. However, in present study neither reduced nor elevated levels of I in thyroid tissue adjacent to nodules in comparison with "normal" thyroid tissue were not found.

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. As was shown in present study, benign nodular transformation is probably accompanied by a partial loss of tissue-specific functional features, which leads to a modest reduction in I content associated with functional characteristics of the human thyroid tissue. Little reduced level of I content in nodular tissue could possibly be explored for differential diagnosis of TBNs and thyroid cancer, because, as was found in our ealier studies, thyroid malignant trasformation is accompanied by a drastically loss of I accumulation [18, 61-63].

This study has several limitations. Firstly, analytical techniques employed in this study measure only seven ChE (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 ChEs investigated in "normal" thyroid and in pathologically altered tissue. Secondly, the sample size of TBNs group was relatively small and prevented investigations of ChEs contents in this group using differentials like gender, histological types of TBNs, nodules functional activity, stage of disease, and dietary habits of patients with TBNs. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on TBNs-specific tissue Cl, I, and Na level alteration and shows the necessity to continue ChEs research of thyroid benign nodules.

Conclusion

In this work, ChEs analysis was carried out in the tissue samples of TBNs using INAA-SLR. It was shown that INAA-SLR is an adequate analytical tool for the non-destructive determination of Ca, Cl, I, K, Mg, Mn, and Na content in the tissue samples of human thyroid in norm and pathology, including needle-biopsy specimens. It was observed that mass fractions of Cl and Na in "adjacent" group of samples were approximately 2.7 times and 60% higher, respectively, than in "normal" thyroid. In a general sense Ca, Cl, K, Mg, Mn, and Na contents found in the "nodular" and "adjacent" groups of thyroid tissue samples were very similar. However, level of I in "adjacent" group of samples was over 2 times higher than in nodular tissue and almost equals the normal value.

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