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Research Article

COMPARISON BETWEEN BROMINE, CALCIUM, CHLORINE, IODINE, POTASSIUM, MAGNESIUM, MANGANESE, AND SODIUM CONTENTS IN MACRO- AND MICRO-FOLLICULAR COLLOID GOITER

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ABSTRACT

Objective: Colloid nodular goiter (CNG) is the most common disease of the thyroid, even in non-endemic regions, but the etiology of CNG is unclear. It is known that not merely iodine (I) but other chemical elements (ChE) are involved in goitrogenesis. The current study was performed to clarify the preferential accumulation of some ChE either in the colloid or in cells of the thyroid gland.

Methods: Eight ChE: Bromine, calcium, chlorine (Cl), I, potassium, magnesium, manganese, and sodium (Na) in the thyroid tissues with diagnosed CNG were prospectively evaluated in 16 patients with macrofollicular CNG and 13 patients with microfollicular CNG. The control group included thyroid tissue samples from 105 healthy individuals. Measurements were conducted using non-destructive instrumental neutron activation analysis with high-resolution spectrometry of short-lived radionuclides.

Results: It was found that in macrofollicular CNG, the mass fraction of Cl and Na was 2.57 and 1.82 times, respectively, higher than in tissues of the normal thyroid. In microfollicular CNG, the mass fraction of I was 59% lower, whereas the mass fraction of Na was 67% higher than in tissues of the normal thyroid. The level of I in macrofollicular goiter was 2.08 times higher than in microfollicular goiter

Conclusion: There are substantial changes in ChE contents in the goitrous transformed tissue of the thyroid, which depend on the histology of the goiter.

Keywords: Macro- and micro-follicular colloid nodular goiter of thyroid, Intact thyroid, Chemical elements, Instrumental neutron activation analysis.

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INTRODUCTION

Colloid nodular goiter (CNG) is the most common thyroid disease, even in non-endemic regions [1]. CNG is clinically identified in about 4% of people older than 30 years [1]. CNG is a benign lesion; however, during clinical examination, it can imitate malignant tumors. Furthermore, the origination of CNG can stipulate the beginning of the malignant transformation of the thyroid gland [2]. Up to now, the etiology of CNG is unclear, and it is probably multifactorial [3]. There is an opinion that CNG occurs when the thyroid is not able to meet the metabolic demands of the body with adequate hormone production. The thyroid gland compensates by enlarging, which usually overcomes mild deficiencies of thyroid hormones. For over the 20th century, there was the governing opinion that NG is the straightforward sequel of iodine (I) deficiency. Although, it was found that NG is a frequent disease even in those countries and regions where the inhabitants are never exposed to I shortage [4]. Moreover, it was found that I excess has severe effects on human health and is associated with the presence of thyroidal dysfunctions and autoimmunity, NG and diffuse goiter, benign and malignant tumors of the 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 disruption of evolutionary stable input of many chemical elements (ChE) in the human body after the industrial revolution plays a significant role in the etiology of thyroidal disorders [12].

In addition to I, many other ChE is involved in essential physiological functions [13]. Crucial or toxic (goitrogenic, mutagenic, and carcinogenic) properties of ChE depend on tissue-specific need or tolerance, respectively [13]. Deficiency, overload, or an imbalance of the ChE may result in cellular dysfunction, degeneration, death, and benign or malignant transformation [13-15].

In our earlier 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]. I level in the normal thyroid was scrutinized in relation to age, gender, and some non-thyroidal diseases [23,24]. Hereafter, variations of ChE content with age in the thyroid of males and females were studied, and age and gender dependence of some ChE was perceived [25-41]. In addition, a significant difference between some ChE contents in normal and cancerous thyroid was demonstrated [42-47].

Histologically, the CNG is cellular hyperplasia of the thyroid acini. There are two histological types of CNG: Macro- and micro-follicular. It is clear that these two types of CNG have different volume ratios, "colloid to cells."

The present study was executed to elucidate the preferential accumulation of some ChE either in the colloid or in cells of the thyroid gland. Having this in mind, we focused on assessing the bromine (Br), calcium (Ca), chlorine (Cl), I, potassium (K), magnesium (Mg), manganese (Mn), and sodium (Na) contents in macro- and micro-follicular CNG tissue using non-destructive instrumental neutron activation analysis with high-resolution spectrometry of short-lived radionuclides (INAA-SLR). A further objective was to compare the levels of these ChE in the macro- and micro-follicular CNG separately with those in intact (normal) gland of apparently healthy persons, as well as to find differences between the levels of these ChE in the macro- and micro-follicular CNG.

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre (MRRC), 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.

METHODS

All patients who suffered from CNG (n=29, mean age M±SD was 47±14 years, range 30–64) were hospitalized in the Head and Neck Department of the MRRC. A 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 ChE contents. The diagnosis has been confirmed for all patients by clinical and morphological results acquired throughout studies of biopsy and resected materials. The histological conclusion for all thyroidal lesions was the macrofollicular CNG (n=16) and microfollicular CNG (n=13).

Normal thyroids for the control group samples were drawn out 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, also to confirm the absence of micronodules and latent cancer.

All tissue samples were divided into two parts using a titanium scalpel [48]. One was used for morphological study, while the other was 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.

Details of sample preparation, activation by neutrons of nuclear reactor, gamma-spectrometry, calibration with biological synthetic standards, and quality insurance using certified reference material (CRM) of International Atomic Energy Agency IAEA H-4 (animal muscle) were presented in our earlier publications concerning the INAA-SLR of ChE contents in human thyroid, scalp hair, and prostate [18,27,28,50].

A dedicated computer program for INAA-SLR mode optimization was used [51]. All the thyroid samples were prepared in duplicate, and mean values of ChE contents were used in the final calculation. Using Microsoft Office Excel, a summary of the statistics, including arithmetic mean, standard deviation, standard error of the mean, minimum and maximum values, median, and percentiles with 0.025 and 0.975 levels, was calculated for ChE contents. The distinction in the results between normal thyroid and two groups of CNG (separately macro- and micro-follicular), as well as between two groups of CNG, was evaluated by the parametric Student's t-test and non-parametric Wilcoxon-Mann–Whitney U-test.

IRESULTS

Table 1 presents certain statistical parameters (arithmetic mean, standard deviation, standard error of the mean, minimal and maximal values, median, and percentiles with 0.025 and 0.975 levels) of the Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction in normal thyroid (n=105), macrofollicular CNG (n=16), and microfollicular CNG (n=13).

The comparison of Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction in normal thyroid with those in macro- and micro-follicular CNG is shown in Tables 2 and 3, respectively.

The ratios of means and the distinction between mean values of Br, Ca, Cl, I, K, Mg, Mn, and Na mass fractions in macro- and micro-follicular CNG are presented in Table 4.

DISCUSSION

Precision and accuracy of results

Previously found 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 [18,27,28,50] indicates an acceptable accuracy of the results obtained in the study of ChE of the thyroid samples presented in Tables 1-4.

The mean values and all chosen statistical parameters were calculated for eight ChE (Br, Ca, Cl, I, K, Mg, Mn, and Na) mass fractions (Table 1). The mass fraction of Br, Ca, Cl, I, K, Mg, Mn, and Na was measured in all or a major portion of normal thyroid and CNG samples.

Effect of goitrous transformation on ChE contents

From Table 2, it is observed that in macrofollicular CNG, the mass fraction of Cl and Na is 2.57 and 1.82 times, respectively, higher than

Table 1: Some statistical parameters of Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction (mg/kg, dry mass basis) in normal thyroid and colloid nodular goiter of different histology (macro- and micro-follicular)

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Normal n=105	Br	16.3	11.6	1.3	1.90	66.9	13.6	2.57	51.0
	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	14,300	5477	2541	13,285
	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	13,453	6690	3855	10,709
Macro n=16	Br	42.2	23.3	10.4	12.0	65.3	40.3	13.6	65.3
	Ca	1455	999	258	209	4333	1264	278	3632
	Cl	8749	4089	1546	4226	16,786	8191	4487	15,880
	I	1587	1087	302	300	3715	1206	322	3686
	K	6254	1801	465	3801	9936	6185	3917	9641
	Mg	345	158	41	13.0	531	374	30.5	531
	Mn	1.35	0.68	0.18	0.370	2.70	1.20	0.432	2.63
	Na	12,211	4164	1075	7229	22,381	11,056	7326	20,493
Micro n=13	Br	19.4	7.1	3.5	13.7	29.6	17.1	13.9	28.7
	Ca	1152	610	249	288	2101	1092	358	2025
	Cl	9977	3939	2274	5462	12,712	11,756	5777	12,664
	I	762	600	173	141	1936	586	173	1929
	K	6932	2783	1052	3353	10,318	6461	3423	10,193
	Mg	328	134	51	122	497	371	134	486
	Mn	2.40	1.70	0.69	0.450	5.50	1.93	0.619	5.16
	Na	11,167	2472	1009	8065	14,584	11,518	8153	14,329

M: Arithmetic mean, SD: Standard deviation, SEM: Standard error of the 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: 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 thyroid and macrofollicular colloid nodular goiter

Element	Thyroid tissue			U-test p	Ratio
	Normal thyroid n=105	Macrofollicular goiter n=16	Student's t-test p≤		Goiter to norm
Br	16.3±1.3	42.2±10.4	0.067	>0.05	2.59
Ca	1692±109	1455±258	0.371	>0.05	0.86
Cl	3400±174	8749±1546	**0.013**	**≤0.01**	2.57
I	1841±107	1587±302	0.439	>0.05	0.86
K	6071±306	6254±465	0.745	>0.05	1.03
Mg	285±17	345±41	0.184	>0.05	1.21
Mn	1.35±0.07	1.35±0.18	0.966	>0.05	1.00
Na	6702±1785	12,211±1075	**0.00015**	**≤0.01**	1.82

M: Arithmetic mean, SEM: Standard error of the mean, significant values are in **bold**

Table 3: 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 thyroid and microfollicular colloid nodular goiter

Element	Thyroid tissue			U-test p	Ratio
	Normal thyroid n=105	Microfollicular goiter n=13	Student's t-test p≤		Goiter to norm
Br	16.3±1.3	19.4±3.5	0.464	>0.05	1.19
Ca	1692±109	1152±249	0.078	>0.05	0.68
Cl	3400±174	9977±2274	0.101	**≤0.05**	2.93
I	1841±107	762±173	**0.00003**	**≤0.01**	0.41
K	6071±306	6932±1052	0.458	>0.05	1.14
Mg	285±17	328±51	0.436	>0.05	1.15
Mn	1.35±0.07	2.40±0.69	0.192	>0.05	1.78
Na	6702±1785	11,167±1009	**0.0063**	**≤0.01**	1.67

M: Arithmetic mean, SEM: Standard error of the 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 macro- and micro-follicular colloid nodular goiter

Element	Thyroid tissue			U-test p	Ratio
	Macrofollicular goiter n=16	Microfollicular goiter n=13	Student's t-test p≤		Macro to Micro
Br	42.2±10.4	19.4±3.5	0.094	>0.05	2.18
Ca	1455±258	1152±249	0.411	>0.05	1.26
Cl	8749±1546	9977±2274	0.678	>0.05	0.88
I	1587±302	762±173	**0.028**	**≤0.01**	2.08
K	6254±465	6932±1052	0.571	>0.05	0.90
Mg	345±41	328±51	0.801	>0.05	1.05
Mn	1.35±0.18	2.40±0.69	0.194	>0.05	0.56
Na	12,211±1075	11,167±1009	0.489	>0.05	1.09

M: Arithmetic mean, SEM: Standard error of mean, significant values are in **bold**

in tissues of the normal thyroid. From Table 3, it is observed that in microfollicular CNG, the mass fraction of I is 59% lower, whereas the mass fraction of Na is 67% higher than in tissues of the normal thyroid. Thus, if we accept the ChE contents in thyroid glands in the control group as a norm, we have to conclude that the Cl, I, and Na level in thyroid tissue can be notably changed with a goitrous transformation.

Association between ChE levels and relative volume of colloid and cells

Comparison mass fraction of Br, Ca, Cl, I, K, Mg, Mn, and Na in macro- and micro-follicular CNG shown that level of I in macrofollicular goiter is 2.08 times higher than in microfollicular goiter (Table 4). Because the relative volume of colloid in the macrofollicular CNG is higher than in the microfollicular CNG, it is possible to conclude that I increasingly associated with colloid.

Comparison with published data

The published data on ChE contents in the CNG compared to normal levels are very sparse and contradictory. For example, information about Cl content in CNG was not found. Merely, one paper with results on Na level in normal thyroid and CNG was published in 1963 by Kamenev [52], but changes of this electrolyte level in goitrous thyroid were not shown. A relative good agreement there is only for I, since most of the published studies showed a significant decrease of I content in the CNG [53-56].

Information on the ChE contents in macro- or micro-follicular CNG, also about the association between ChE level and relative volume of colloid and cells in goitrous tissue, was not found.

Limitations

This study has some limitations. First, analytical techniques used in this study measure merely eight ChE (Br, Ca, Cl, I, K, Mg, Mn, and Na) mass fractions. Future studies should be aimed toward using other analytical methods which will elongate the list of ChE investigated in normal and goitrous thyroid. Second, the sample size of macro- or micro-follicular CNG groups was relatively small and averted investigations of ChE contents in CNG group using differentials such as gender, stage of disease, and dietary habits of healthy persons and patients with CNG. Finally, the generalization of our outcomes may be bounded to the Russian population. Despite these constraints, this study provides evidence on goiter-specific tissue Cl, I, and Na level alteration demonstrates associations between I and relative volume of colloid in CNG, and shows the necessity to continue ChE research of CNG of different histology.

CONCLUSION

In this work, ChE analysis was carried out in the tissue samples of normal and goitrous 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 content in the tissue samples of human thyroid in norm and pathology, including needle biopsy cores. It was perceived the considerable changes in ChE contents in the goitrous transformed tissue of thyroid, which depends on the histology of goiter. It was found that I predominately accumulates in colloid of CNG.

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