

Comparison Between Trace Element Contents in Macro and Micro Follicular Colloid Goiter using Energy Dispersive X-Ray Fluorescent Analysis

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ABSTRACT

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

Methods: Seven TE: bromine (Br), copper (Cu), iron (Fe), I, rubidium (Rb), strontium (Sr), and zinc (Zn) in the thyroid tissues with diagnosed colloid CNG were prospectively evaluated in 16 patients with macro-follicular CNG and 13 patients with micro-follicular CNG. Control group included thyroid tissue samples from 105 healthy individuals. Measurements were performed using ^{109}Cd and ^{241}Am radionuclide-induced energy-dispersive X-ray fluorescent analysis (EDXRF).

Results: It was found that I predominately accumulate in colloid, whereas Rb and Zn in thyroid cells.

Conclusions: There are considerable changes in TE contents in the goitrous transformed tissue of thyroid, which depend on the histology of goiter.

Keywords: Macro and micro follicular colloid nodular goiter of thyroid, Intact thyroid, Trace elements, Energy-dispersive X-ray fluorescent analysis

Abbreviations: CNG: Colloid Nodular Goiter; TE: Trace Elements; EDXRF: Energy-Dispersive X-Ray Fluorescent Analysis; CRM: Certified Reference Material

INTRODUCTION

Colloid nodular goiter (CNG) is the most common disease of the thyroid, even in non-endemic regions [1]. CNG is clinically detected in about 4% of people older than 30 years [1]. CNG is benign lesion; however, during clinical examination, it can mimic malignant tumors. Furthermore, the origination of CNG can indicate the beginning of malignant transformation of the thyroid gland [2].

Up to now, an etiology of CNG is unclear and probably it is multifactorial [3]. There is opinion that CNG occurs when the thyroid is unable to meet the metabolic demands of the body with sufficient hormone production. The thyroid gland compensates by enlarging, which usually overcomes mild deficiencies of thyroid hormones. For over 20th century, there was the dominant hypothesis that CNG is the simple consequence of iodine (I) deficiency, because I is an essential part of thyroid hormones. However, it was found that CNG 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, CNG and diffuse goiter, benign and malignant tumors of gland [5-8]. It was also demonstrated that besides I deficiency and excess many other dietary, environmental, and occupational factors are associated with the CNG incidence [9-11]. Among them a disturbance of evolutionary stable input of many trace elements (TE) in human body after industrial revolution plays a significant role in etiology of thyroidal disorders [12].

Besides I involved in thyroid function, other TE has also essential physiological functions such as maintenance and regulation of cell function, gene regulation, activation or

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inhibition of enzymatic reactions, and regulation of membrane 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 TE depend on tissue-specific need or tolerance, respectively [13]. Excessive accumulation or an imbalance of the TE may disturb the cell functions and may 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 iodine and other TE 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 TE content with age in the thyroid of males and females were studied and age- and gender-dependence of some TE was observed [25-41]. Furthermore, a significant difference between some TE 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 obvious that these two types of CNG have different volume ratios "colloid to cells".

The present study was performed to clarify the preferential accumulation of some TE either in the colloid or in cells of the thyroid gland. Having this in mind, our aim was to assess the bromine (Br), copper (Cu), iron (Fe), I, rubidium (Rb), strontium (Sr), and zinc (Zn) contents in macro- and micro-follicular CNG tissue using ^{109}Cd and ^{241}Am radionuclide-induced energy-dispersive X-ray fluorescent analysis (EDXRF). A further aim was to compare the levels of these TE 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 TE 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.

MATERIALS AND METHODS

All patients suffered from CNG ($n=29$, mean age $M\pm SD$ was 47 ± 14 years, range 30-64) 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 macro-follicular CNG ($n=16$) and micro-follicular CNG ($n=13$).

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 TE analysis. After the samples intended for TE analysis were weighed, they were freeze-dried and homogenized [49]. The pounded sample weighing about 8 mg was applied to the piece of Scotch tape serving as an adhesive fixing backing.

To determine the contents of the TE by comparison with known data for standard, aliquots of commercial, chemically pure compounds and synthetic reference materials were used [50]. The microliter standards were placed on disks made of thin, ash-free filter papers fixed on the Scotch tape pieces and dried in a vacuum. Ten subsamples of the Certified Reference Material (CRM) IAEA H-4 (animal muscle) weighing about 8 mg were analyzed to estimate the precision and accuracy of results. The CRM IAEA H-4 subsamples were prepared in the same way as the samples of dry homogenized thyroid tissue.

Details of the relevant facility for EDXRF with ^{109}Cd radionuclide source for Br, Cu, Fe, Rb, Sr, and Zn determination, and facility for EDXRF with ^{241}Am radionuclide source for I determination, as well as the results of quality control were presented in our earlier publications [21,25,26,51].

All thyroid samples were prepared in duplicate, and mean values of TE 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 TE contents in normal and CNG tissue. The difference 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.

RESULTS

Table 1 depicts our data for seven TE in ten sub-samples of CRM IAEA H-4 (animal muscle) and the certified values of this material.

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, Cu, Fe, Rb, Sr, Zn mass fraction in normal thyroid (n=105), macro-follicular CNG (n=16), and micro-follicular CNG (n=13).

The comparison of Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction in normal thyroid with those in macro- and micro-follicular CNG is shown in **Tables 3 & 4**, respectively.

The ratios of means and the difference between mean values of Br, Cu, Fe, I, Rb, Sr, Zn mass fractions in macro- and micro-follicular CNG are presented in **Table 5**.

Table 1. EDXRF data Br, Cu, Fe, I, Rb, Sr, and Zn 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	C	5.0±1.2
Cu	4.0	3.6 - 4.3	C	3.9±1.1
Fe	49	47 - 51	C	48±9
I	0.08	-	N	<3.0
Rb	18	17 - 20	C	22±4
Sr	0.1	-	N	<1
Zn	86	83 - 90	C	90±5

Mean: arithmetical mean; SD: standard deviation; C: certified values; N: non-certified values

Table 2. Some statistical parameters of Br, Cu, Fe, I, Rb, Sr, and Zn 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	13.9	12.0	1.3	1.4	54.4	10.0	2.23	50.8
	Cu	4.23	1.52	0.18	0.50	7.50	4.15	1.57	7.27
	Fe	222	102	11	47.1	512	204	65.7	458
	I	1618	1041	108	110	5150	1505	220	3939
	Rb	9.03	6.17	0.66	1.80	42.9	7.81	2.48	25.5
	Sr	4.55	3.22	0.37	0.10	13.7	3.70	0.48	12.3
	Zn	112	44.0	4.7	6.10	221	106	35.5	188
Macro n=16	Br	42.2	23.3	10.4	12.0	65.3	40.3	11.6	65.3
	Cu	6.15	2.07	0.63	2.90	10.3	5.90	3.23	9.95
	Fe	368	332	83	54.9	1052	211	60.2	994
	I	1499	1079	299	300	3617	1206	332	3606
	Rb	6.69	4.30	1.08	1.00	15.9	5.70	1.08	14.7
	Sr	3.34	3.99	1.15	0.80	13.7	1.75	0.91	12.5
	Zn	117	36	9.3	82.9	215	103	83.1	196
Micro n=13	Br	19.4	7.06	3.5	13.7	29.6	17.1	13.9	28.7
	Cu	18.6	11.2	5.6	10.0	34.8	14.8	10.2	33.4
	Fe	218	125	39	55.2	427	178	70.9	415
	I	667	473	136	141	1912	586	173	1702
	Rb	9.36	2.91	0.88	6.0	16.6	9.30	6.13	15.2
	Sr	1.84	1.74	0.62	0.80	5.30	0.80	0.80	5.04
	Zn	163	45	14	98.1	235	165	102	227

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 3. Differences between mean values ($M \pm SEM$) of Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid and macro-follicular colloid nodular goiter.

Element	Thyroid tissue				Ratio
	Normal thyroid n=105	Macro-follicular goiter n=16	Student's t-test $p \leq$	U-test p	Goiter to Norm
Br	13.9±1.3	42.2±10.4	0.052	>0.05	3.04
Cu	4.23±0.18	6.15±0.63	0.013	≤0.01	1.45
Fe	222±11	368±83	0.101	>0.05	1.66
I	1618±108	1499±299	0.713	>0.05	0.93
Rb	9.03±0.66	6.69±1.08	0.075	>0.05	0.74
Sr	4.55±0.37	3.34±1.15	0.335	>0.05	0.73
Zn	112±5	117±9.3	0.648	>0.05	1.04

M: arithmetic mean; *SEM*: standard error of mean; Statistically significant values are in **bold**.

Table 4. Differences between mean values ($M \pm SEM$) of Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid and micro-follicular colloid nodular goiter.

Element	Thyroid tissue				Ratio
	Normal thyroid n=105	Micro-follicular goiter n=13	Student's t-test $p \leq$	U-test p	Goiter to Norm
Br	13.9±1.3	19.4±3.5	0.220	>0.05	1.40
Cu	4.23±0.18	18.6±5.6	0.082	>0.05	4.40
Fe	222±11	218±39	0.937	>0.05	0.98
I	1618±108	667±136	0.000008	≤0.01	0.41
Rb	9.03±0.66	9.36±0.88	0.767	>0.05	1.04
Sr	4.55±0.37	1.84±0.62	0.002	≤0.01	0.40
Zn	112±5	163±14	0.006	≤0.01	1.37

M: arithmetic mean; *SEM*: standard error of mean; Statistically significant values are in **bold**.

Table 5. Differences between mean values ($M \pm SEM$) of Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) in macro- and micro-follicular colloid nodular goiter.

Element	Thyroid tissue				Ratio
	Macro-follicular goiter n=16	Micro-follicular goiter n=13	Student's t-test $p \leq$	U-test p	Macro- to Micro-
Br	42.2±10.4	19.4±3.5	0.094	>0.05	2.18
Cu	6.15±0.63	18.6±5.6	0.112	>0.05	0.33
Fe	368±83	218±39	0.120	>0.05	1.69
I	1499±299	667±136	0.021	≤0.01	2.25
Rb	6.69±1.08	9.36±0.88	0.066	≤0.05	0.71
Sr	3.34±1.15	1.84±0.62	0.268	>0.05	1.82
Zn	117±9.3	163±14	0.015	≤0.01	0.72

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, Cu, Fe, I, Rb, Sr, and Zn contents analyzed by EDXRF with the certified data of CRM IAEA H-4 (Table 1) indicates an acceptable accuracy of the results obtained in the study of TE of the thyroid samples presented in Tables 2-5.

The mean values and all selected statistical parameters were calculated for seven TE (Br, Cu, Fe, I, Rb, Sr, and Zn) mass fractions (Table 2). The mass fraction of Br, Cu, Fe, I, Rb, Sr, and Zn were measured in all, or a major portion of normal thyroid and CNG samples.

Effect of goitrous transformation on TE contents

From Table 3, it is observed that in macro-follicular CNG the mass fraction of Cu is 45% higher than in tissues of the normal thyroid. From Table 4, it is observed that in micro-follicular CNG the mass fraction of I and Sr is 59% and 60%, respectively, lower, whereas the mass fraction of Zn is 37% higher than in tissues of the normal thyroid. Thus, if we accept the TE contents in thyroid glands in the control group as a norm, we have to conclude that with a goitrous transformation the Cu, I, Sr, and Zn level in thyroid tissue can be significantly changed.

Association between TE levels and relative volume of colloid and cells

Comparison mass fraction of Br, Cu, Fe, I, Rb, Sr, and Zn in macro- and micro-follicular CNG shown that level of I in macro-follicular goiter is 2.25 times higher, whereas level of Rb and Zn is 29% (only U-test) and 28%, respectively, lower than in micro-follicular goiter. Because the relative volume of colloid in the macro-follicular CNG is higher than

in the micro-follicular CNG, it is possible to conclude that I increasingly associated with colloid. On the contrary, because the relative volume of cells in the micro-follicular CNG is higher than in the macro-follicular CNG, it is possible to conclude that Rb and Zn increasingly associated with thyroid cells.

Comparison with published data

The published data on TE contents in the CNG in comparison with normal levels are very scanty and contradictory. For example, Kasanen and Viitanen [52] found elevated levels of Cu in the CNG, but Stojavljević [9] did not. A significant decrease of the Zn content during goitrous transformation was shown by Błazewicz [53], but in the recent study this change was not confirmed [9]. A relatively good agreement there is only for I, since most of published studies showed the significant decrease of, I content in the CNG [53-56].

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

LIMITATIONS

This study has several limitations. Firstly, analytical techniques employed in this study measure only seven TE (Br, Cu, Fe, I, Rb, Sr, and Zn) mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of chemical elements investigated in normal and goitrous thyroid. Secondly, the sample size of macro- or micro-follicular CNG groups was relatively small and prevented investigations of TE contents in CNG group using differentials like gender, stage of disease, and dietary habits of healthy persons and patients with CNG. Lastly,

generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on goiter-specific tissue Cu, I, Rb, Sr, and Zn level alteration, demonstrates associations between some TE and relative volume of colloid and cells in CNG, and shows the necessity to continue TE research of CNG of different histology.

CONCLUSION

In this work, TE analysis was carried out in the tissue samples of normal and goitrous thyroid using ^{109}Cd and ^{241}Am radionuclide-induced EDXRF. It was shown that ^{109}Cd and ^{241}Am radionuclide-induced EDXRF is an adequate analytical tool for the non-destructive determination of Br, Cu, Fe, I, Rb, Sr, and Zn content in the tissue samples of human thyroid in norm and pathology, including needle-biopsy cores. It was observed the considerable changes in TE contents in the goitrous transformed tissue of thyroid, which depend on the histology of goiter. It was found that I predominately accumulate in colloid, whereas Rb and Zn in thyroid cells.

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CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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