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Determination of twenty chemical element contents in macro-and micro-follicular colloid goiter using neutron activation analysis and inductively coupled plasma atomic emission spectrometry

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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 chemical elements (ChE) are involved in goitrogenesis. The present study was performed to clarify the preferential accumulation of some ChE either in the colloid or in cells of the thyroid gland.

Methods: Twenty ChE (Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn) in the thyroid tissues with diagnosed 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 a combination of non-destructive instrumental neutron activation analysis with inductively coupled plasma atomic emission spectrometry.

Results: It was found that with a goitrous transformation the Al, B, Br, Cl, Cu, Fe, I, Na, S, and Zn level in thyroid tissue can be significantly changed, and these changes depend on CNG histology. An association between I content and colloid volume, as well as between B level and cell volume of CNG was observed.

Conclusions: There are considerable changes in ChE 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; Chemical elements; Instrumental neutron activation analysis

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 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 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 ChE 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 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].

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 ChE either in the colloid or in cells of the thyroid gland. Having this in mind, our aim was to assess the aluminum (Al), boron (B), barium (Ba), bromine (Br), calcium (Ca), chlorine (Cl), coper (Cu), iron (Fe), I, potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), sulfur (S), silicon (Si), strontium (Sr), vanadium (V), and zinc (Zn) contents in macro- and micro-follicular CNG tissue using consecutively non-destructive instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) and destructive inductively coupled plasma atomic emission spectrometry (ICP-AES). A further aim 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.

Material and Methods

All patients 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 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 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 Br, Ca, Cl, I, K, Mg, Mn, and Na measurement by INAA-SLR. Details of used nuclear reactions, radionuclides, gamma-energies, spectrometric unit, and sample preparation were presented in our earlier publications concerning the INAA-SLR of ChE contents in human thyroid [18, 27, 28].

After non-destructive INAA-SLR investigation the thyroid samples were decomposed in autoclaves and used for ICP-AES. The Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractions were determined by ICP-AES using the Spectrometer ICAP-61 (Thermo Jarrell Ash, USA). The determination of the ChE content in aqueous solutions was made by the quantitative method using calibration solutions (High Purity Standards, USA) of 0.5 and 10 mg/L of each element. The calculations of the ChE content in the probe were carried out using software of a spectrometer (ThermoSPEC, version 4.1).Information detailing with the ICP-AES methods used and other details of the analysis were presented in our earlier publications concerning ChE contents in human thyroid [33, 34].

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) IAEA H-4 (animal muscle) and five sub-samples of CRM of the Institute of Nuclear Chemistry and Technology (INCT, Warszawa, Poland) INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2 Mixed Polish Herbs were treated and analyzed in the same conditions that thyroid samples to estimate the precision and accuracy of results.

A dedicated computer program for INAA mode optimization was used [51]. All thyroid samples were prepared in duplicate, and mean values of ChE contents were used. Mean values of ChE contents were used in final calculation for the Br, Fe, Rb, and Zn mass fractions measured by two methods INAA-SLR and ICP-AES. 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 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 Br, Ca, Cl, K, Mg, Mn, and Na mass fractions in ten sub-samples of CRM IAEA H-4 (animal muscle) certified reference material and the certified values of this material.

Table 2 presents our data for Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractions in five sub-samples of INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves and INCT-MPH-2 Mixed Polish Herbs certified reference materials and the certified (or informative) values of this material

The comparison of our results for the Ca, K, Mg, Mn, and Na mass fractions (mg/kg, dry mass basis) in the normal human thyroid obtained by both INAA-SLR and ICP-AES methods is shown in Table 3.

Table 4, 5, and 6 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 Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction in normal thyroid (n=105), macro-follicular CNG (n=16), and micro-follicular CNG (n=13), respectively.

The comparison of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction in normal thyroid with those in macro- and micro-follicular CNG is shown in Table 7 and 8, respectively.

The ratios of means and the difference between mean values of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractions in macro- and micro-follicular CNG are presented in Table 9.

Discussion

Precision and accuracy of results

A good agreement of our results for the Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Mg, Mn, Na, P, S, Sr, V, and Zn mass fractions with the certified values of CRM IAEA H-4, INCT-SBF-4, INCT-TL-1, and INCT-MPH-2 (Tables 1 and 2) as well as the similarity of the means of the Ca, K, Mg, Mn, and Na mass fractions in the normal human thyroid determined by both INAA-SLR and ICP-AES methods (Table 3) demonstrates an acceptable precision and accuracy of the results obtained in the study and presented in Tables 4-9.

The mean values and all selected statistical parameters were calculated for twenty ChE (Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn) mass fractions (Tables 4-6). The mass fraction of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn were measured in all, or a major portion of normal and goitrous tissue samples.

Effect of goitrous transformation on ChE contents

From Table 7, it is observed that in macro-follicular CNG the mass fraction of Al, B, Br, Cl, Cu, Fe, Na, S, and Zn is 2.53, 2.61, 2.83, 2.57, 1.51, 1.93, 1.70, 1.36, and 1.39 times, respectively, higher than in tissues of the normal thyroid. From Table 8, it is observed that in micro-follicular CNG the mass fraction of I is 59% lower, whereas the mass fraction of Al, B, Cu, Na, S, and Zn is 1.70, 3.78, 4.56, 2.0, 1.31, and 1.51 times, respectively, 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 with a goitrous transformation the Al, B, Br, Cl, Cu, Fe, I, Na, S, and Zn level in thyroid tissue can be significantly changed.

Association between ChE levels and relative volume of colloid and cells

Comparison mass fraction of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn in macro- and micro-follicular CNG shown that level of Fe and I in macro-follicular goiter is 2.03 and 2.71 times, respectively, higher, whereas B content 31% lover than in micro-follicular goiter (Table 9). 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 Fe and I increasingly associated with colloid. On the contrary, because the relative volume of cells in the macro-follicular CNG is lower than in the micro-follicular CNG, it is possible to conclude that B increasingly associated with cells of CNG.

Comparison with published data

The published data on ChE contents in the CNG in comparison with normal levels are very scanty and contradictory. For example, information about B, Cl, and Li content in CNG was not found. Only 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 was not shown. A lack of difference between ChE level in normal and goitrous thyroid was found in one study for Ba [53], S [54], and V [53], and also in two studies for Sr [52, 53]. An elevated content of Br in CNG was demonstrated in one article [55], however in two otherstudies [56, 57] this finding was not confirmed. A significant decrease of the Zn content during goitrous transformation was shown by Błazewicz *et al*. [58], but in the recent study this change was not found [9]. A relative good agreement there is only for I, since most of published studies showed the significant decrease of I content in the CNG [58-61]. Information on the ChE contents in macro- or micro-follicular CNG, as well as about the association between ChE 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 twenty ChE (Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn) mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of ChE investigated in normal and goitrous thyroid. Secondly, the sample size of macro- or micro-follicular CNG groups was relatively small and prevented investigations of ChE 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 Al, B, Br, Cl, Cu, Fe, I, Na, S, and Zn level alteration, demonstrates associations between I content and volume of colloid and between B content and volume of cells in CNG, and shows the necessity to continue ChE research of CNG of different histology.

Table 1: INAA-SLR data of chemical element contents in the IAEA H-4 (animal muscle) reference material compared to certified values (mg/kg on 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±0.9
Ca	188	163 – 213	C	238±59
Cl	1890	1810 – 1970	C	1950±230
K	15800	15300 – 16400	C	16200±3800
Mg	1050	990 – 1110	C	1100±190
Mn	0.52	0.48 – 0.55	N	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: ICP-AES data of chemical element contents in Certified Reference Materials (M±SD, mg/kg on dry mass basis)

Element	Soya Bean Flour (INCT-SBF-4)		Tea Leaves (INCT-TL-1)		Mixed Polish Herbs (INCT-MPH-2)	
	Certificate	This work result	Certificate	This work result	Certificate	This work result
Al	45.5±3.7	37.1±1.4	2290±280	2248±61	670±111	485±79
B	39.9±4.0	34.5±1.4	26[a]	24.8±1.2	-	28.8±8.1
Ba	7.30±0.23	7.38±0.23	43.2±3.9	44.7±2.6	32.5±2.5	32.2±0.6
Ca	2467±170	2737±190	5820±520	6296±360	10800±700	10250±294
Cu	14.3±0.5	14.2±0.8	20.4±1.5	19.7±1.1	7.77±0.53	8.28±0.47
Fe	90.8±4.0	80.5±6.9	432[a]	493±39	460[a]	459±33
K	24230±830	25230±1090	17000±1200	17810±1320	19100±1200	20280±870
Li	-	0.0047±0.0018	-	0.217±0.034	-	0.574±0.044
Mg	3005±82	2983±340	2240±170	2415±115	2920±180	2955±159
Mn	32.3±1.1	30.0±1.0	1570±110	1628±145	191±12	197±5
Na	-	10.2±3.4	24.7±3.2	24.2±3.5	350[a]	338±17
P	6555±355	6782±248	1800[a]	2457±150	2500[a]	3022±481
S	4245±471	4468±529	2470±250	2500±230	2410±140	2409±159
Si	-	26.7±4.8	-	325±34	-	268±64
Sr	9.32±0.46	8.76±0.21	20.8±1.7	19.8±1.0	37.6±2.7	37.4±2.1
V	-	≤0.22	2.0±0.4	1.8±0.2	0.95±0.16	0.90±0.04
Zn	52.3±1.3	54.8±6.6	34.7±2.7	36.0±3.7	33.5±2.1	32.0±6.1

M - arithmetic mean, SD - standard deviation, [a] Informative values

Table 3: Comparison of the mean values (M±SEM) of the chemical element mass fractions (mg/kg, on dry-mass basis) in the normal human thyroid (males and females combined) obtained by both NAA-SLR and ICP-AES methods

Element	NAA-SLR (M_1)	ICP-AES (M_2)	Δ, %
Ca	1692±109	1633±108	3.5
K	6071±306	6764±298	-11.4
Mg	285±17	308±17	-8.1
Mn	1.35±0.07	1.21±0.07	10.4
Na	6702±178	7154±201	-6.7

M – arithmetic mean, SEM – standard error of mean, $\Delta=[(M_1 - M_2)/M_1]\cdot 100\%$.

Table 4. Some statistical parameters of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Normal	Al	10.5	13.4	1.8	0.800	69.3	6.35	1.19	52.9
n=105	B	0.476	0.434	0.058	0.200	2.30	0.300	0.200	1.73
	Ba	1.12	1.15	0.15	0.0480	5.00	0.680	0.0838	4.48
	Br	14.9	11.0	1.2	1.90	54.1	11.6	2.56	49.3
	Ca	1682	999	106	373	5582	1454	444	4183
	Cl	3400	1452	174	1030	6000	3470	1244	5869
	Cu	4.08	1.22	0.14	0.500	7.15	4.10	1.57	6.41
	Fe	223	95	10	52.0	489	210	72.8	432
	I	1841	1027	107	114	5061	1695	230	4232
	K	6418	2625	290	1914	15293	5948	2947	13285
	Li	0.0208	0.0155	0.0022	0.0015	0.0977	0.0178	0.0041	0.0487
	Mg	296	134	16	66.0	930	284	95.8	541
	Mn	1.28	0.56	0.07	0.470	4.04	1.15	0.537	2.23
	Na	6928	1730	175	3686	13453	6835	3974	10709
	P	4290	1578	207	496	8996	4221	1360	7323
	S	8259	2002	263	644	11377	8399	3662	11208
	Si	50.8	46.9	6.2	5.70	180	36.0	7.11	174
	Sr	3.81	2.93	0.34	0.100	12.6	2.90	0.365	11.3
	V	0.102	0.039	0.005	0.0200	0.250	0.100	0.0440	0.192
	Zn	94.8	39.6	4.2	7.10	215	88.5	34.9	196

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 5. Some statistical parameters of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction (mg/kg, dry mass basis) in macro-follicular colloid nodular goiter

Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Al	26.6	25.0	7.2	6.60	76.3	17.5	6.77	76.2
B	1.24	0.46	0.13	0.900	2.00	1.00	0.928	2.00
Ba	0.898	0.606	0.175	0.180	2.10	0.765	0.224	1.96
Br	42.2	23.3	10.4	12.0	65.3	40.3	13.6	65.3
Ca	1467	950	245	407	4333	1291	423	3531
Cl	8749	4089	1546	4226	16786	8191	4487	15880
Cu	6.15	2.07	0.63	2.90	10.3	5.90	3.23	9.95
Fe	431	390	98	65.1	1210	207	72.9	1151
I	2063	2067	552	300	8260	1355	335	6783
K	6346	1745	451	3801	9936	6110	3917	9641
Li	0.0268	0.0144	0.0040	0.0073	0.0541	0.0252	0.0082	0.0535
Mg	345	116	30	63.0	530	374	110	512
Mn	1.35	0.67	0.18	0.560	2.70	1.20	0.570	2.63
Na	11784	2915	753	7229	16850	11867	7849	16615
P	4610	1352	390	2946	7386	4210	3021	7024
S	11206	1946	562	7939	14225	11124	8128	14193
Si	83.6	63.1	18.2	7.80	182	59.9	11.5	180
Sr	4.65	6.05	1.68	0.93	20.3	1.80	1.01	18.3
V	0.135	0.057	0.016	0.0430	0.230	0.140	0.0502	0.219
Zn	132	55	14	84.0	264	117	86.5	261

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 6. Some statistical parameters of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction (mg/kg, dry mass basis) in micro-follicular colloid nodular goiter

Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Al	17.9	6.7	3.0	9.80	26.0	16.4	10.2	25.7
B	1.80	0.45	0.20	1.00	2.00	2.00	1.10	2.00
Ba	1.60	1.25	0.56	0.290	3.60	1.30	0.361	3.42
Br	19.4	7.06	3.53	13.7	29.6	17.1	13.9	28.7
Ca	1246	708	289	288	2101	1092	358	2095
Cl	9977	3939	2274	5462	12712	11756	5777	12664
Cu	18.6	11.2	5.6	10.0	34.8	14.8	10.2	33.4
Fe	212	108	34	96.5	382	161	102	373
I	762	600	173	141	1936	586	173	1929
K	7087	2605	985	3353	10318	6461	3586	10193
Li	0.0273	0.0055	0.0030	0.0230	0.0355	0.0254	0.0231	0.0348
Mg	348	106	40	205	497	371	213	486
Mn	2.32	1.73	0.71	0.450	5.50	1.86	0.578	5.16
Na	13831	6971	2635	8065	28481	12338	8170	26597
P	6059	1912	855	3802	8094	5337	3926	8086
S	10847	875	391	9817	11965	10650	9866	11918
Si	46.4	35.9	17.9	16.1	95.5	36.9	16.7	92.1
Sr	9.36	12.3	5.52	1.60	31.3	4.80	1.82	28.7
V	0.187	0.106	0.047	0.0930	0.370	0.150	0.0987	0.351
Zn	143	43	13	83.0	235	137	85.6	223

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 7: Differences between mean values (M±SEM) of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction (mg/kg, dry mass basis) in) 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
Al	10.5±1.8	26.6±7.2	**0.050**	**≤0.05**	2.53
B	0.476±0.058	1.24±0.13	**0.00008**	**≤0.01**	2.61
Ba	1.12±0.15	0.898±0.175	0.340	>0.05	0.80
Br	14.9±1.2	42.2±10.4	0.058	**≤0.05**	2.83
Ca	1682±106	1467±245	0.431	>0.05	0.87
Cl	3400±174	8749±1546	**0.013**	**≤0.01**	2.57
Cu	4.08±0.14	6.15±0.63	**0.0081**	**≤0.01**	1.51
Fe	223±10	431±98	0.051	**≤0.05**	1.93
I	1841±107	2063±552	0.698	>0.05	1.12
K	6418±290	6346±451	0.894	>0.05	0.99

Li	0.0208±0.0022	0.0268±0.0040	0.221	>0.05	1.29
Mg	296±16	345±30	0.171	>0.05	1.17
Mn	1.28±0.07	1.35±0.18	0.730	>0.05	1.05
Na	6928±175	11784±753	**0.000012**	**≤0.01**	1.70
P	4290±207	4610±390	0.478	>0.05	1.07
S	8259±263	11206±562	**0.00021**	**≤0.01**	1.36
Si	50.8±6.2	83.6±18.2	0.111	>0.05	1.65
Sr	3.81±0.34	4.65±1.68	0.633	>0.05	1.22
V	0.102±0.005	0.135±0.016	0.077	>0.05	1.32
Zn	94.8±4.2	132±14	**0.017**	**≤0.01**	1.39

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

Table 8: Differences between mean values (M±SEM) of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction (mg/kg, dry mass basis) in) 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
Al	10.5±1.8	17.9±3.0	0.066	**≤0.05**	1.70
B	0.476±0.058	1.80±0.20	**0.0018**	**≤0.01**	3.78
Ba	1.12±0.15	1.60±0.56	0.451	>0.05	1.43
Br	14.9±1.2	19.4±3.53	0.301	>0.05	1.30
Ca	1682±106	1246±289	0.203	>0.05	0.74
Cl	3400±174	9977±2274	**0**.101	>0.05	2.93
Cu	4.08±0.14	18.6±5.6	0.080	**≤0.05**	4.56
Fe	223±10	212±34	0.761	>0.05	0.95
I	1841±107	762±173	**0.00003**	**≤0.01**	0.41
K	6418±290	7087±985	0.535	>0.05	1.10
Li	0.0208±0.0022	0.0273±0.0030	0.107	>0.05	1.31
Mg	296±16	348±40	0.263	>0.05	1.18
Mn	1.28±0.07	2.32±0.71	0.201	>0.05	1.81
Na	6928±175	13831±2635	**0.040**	**≤0.01**	2.00
P	4290±207	6059±855	0.107	>0.05	1.41
S	8259±263	10847±391	**0.00051**	**≤0.01**	1.31
Si	50.8±6.2	46.4±17.9	0.827	>0.05	0.91
Sr	3.81±0.34	9.36±5.52	0.372	>0.05	2.46
V	0.102±0.005	0.187±0.047	0.143	>0.05	1.83
Zn	94.8±4.2	143±13	**0.0042**	**≤0.01**	1.51

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

Table 9. Differences between mean values (M±SEM) of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction (mg/kg, dry mass basis) in) 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-follicular
Al	26.6±7.2	17.9±3.0	0.286	>0.05	1.49
B	1.24±0.13	1.80±0.20	**0.049**	≤0.01	0.69
Ba	0.898±0.175	1.60±0.56	0.286	>0.05	0.56
Br	42.2±10.4	19.4±3.53	0.094	>0.05	2.18
Ca	1467±245	1246±289	0.570	>0.05	1.18
Cl	8749±1546	9977±2274	0.678	>0.05	0.88
Cu	6.15±0.63	18.6±5.6	0.112	>0.05	0.33
Fe	431±98	212±34	**0.048**	**≤0.01**	2.03
I	2063±552	762±173	**0.039**	**≤0.01**	2.71
K	6346±451	7087±985	0.512	>0.05	0.90
Li	0.0268±0.0040	0.0273±0.0030	0.919	>0.05	0.98
Mg	345±30	348±40	0.945	>0.05	0.99
Mn	1.35±0.18	2.32±0.71	0.233	>0.05	0.58
Na	11784±753	13831±2635	0.479	>0.05	0.85
P	4610±390	6059±855	0.176	>0.05	0.76
S	11206±562	10847±391	0.608	>0.05	1.03
Si	83.6±18.2	46.4±17.9	0.177	>0.05	1.80
Sr	4.65±1.68	9.36±5.52	0.453	>0.05	0.50
V	0.135±0.016	0.187±0.047	0.333	>0.05	0.72
Zn	132±14	143±13	0.590	>0.05	0.92

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

Conclusion

In this work, ChE analysis was carried out in the tissue samples of normal and goitrous thyroid using a combination of non-destructive INAA-SLR and destructive ICP-AES. It was shown that this combination is an adequate analytical tool for the determination of twenty ChE (Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, 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 ChE contents in the goitrous transformed tissue of thyroid, which depend on the histology of goiter. It was found that I predominately accumulates in colloid, whereas B in cells of CNG.

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