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Original Research Article

Comparison of Chemical Element Contents in Thyroid Goiter, Adenoma, and Thyroiditis investigated using X-Ray fluorescence and Neutron Activation Analysis

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Abstract

Thyroid benign nodules (TBNs) are the most common lesions of this endocrine gland and are prevalent diseases around the world. Among TBNs the colloid goiter (CG) and thyroid adenoma (TA) are very frequent diseases. An evaluation of the variant of TBNs is clinically important for subsequent therapeutic interventions, as well as for more clear understanding the etiology of these disorders. The aim of this exploratory study was to examine differences in the content of silver (Ag), bromine (Br), calcium (Ca), chlorine (Cl), cobalt (Co), chromium (Cr), cooper (Cu), iron (Fe), mercury (Hg), iodine (I), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), rubidium (Rb), ammonium (Sb), scandium (Sc), selenium (Se), strontium (Sr), and zinc (Zn) in tissues of CG, TA, and T. Thyroid tissue levels of twenty chemical elements (ChE) were prospectively evaluated in 46 patients with CG, 19 patients with TA, and 12 patients with T. Measurements were performed using non-destructive energy-dispersive X-Ray fluorescent analysis combined with instrumental neutron activation analysis with high resolution spectrometry of short- and long-lived radionuclides. Tissue samples were divided into two portions. One was used for morphological study while the other was intended for ChE analysis. It was observed that in CG, TA and T tissues content of Ag, Br, and Hg was significantly higher, while level of I was lower than in normal thyroid tissue. In addition to these ChE, in CG and TA samples content of Cl, Cr, and Na was higher than in normal gland. ChE composition of T tissue differed from CG and TA tissues by elevated Rb level and reduced Se and Zn levels. The abnormal increase in Ag, Br, Hg level and decrease in I level in all TBNs might demonstrate an involvement of these ChE in etiology and pathogenesis of TBNs. In addition, elevated levels of such ChE as Cl, Cr, and Na can be also important for goitrous and adenomatous transformation of thyroid. It was supposed that elevated level of Rb and reduced levels of Se and Zn content in thyroid with T could possibly be explored for differencial diagnosis of T among other TBNs.

Keywords: Thyroid; Thyroid colloid goiter; Thyroid adenoma; Thyroiditis; Chemical elements; Energy-dispersive X-Ray fluorescent analysis; Neutron activation analysis.

INTRODUCTION

Thyroid benign nodules (TBNs) are the most common lesions of this endocrine gland that encountered globally and frequently discovered by palpation during a physical examination, or incidentally, during clinical imaging procedures. TBNs include non-neoplastic lesions (different kinds of thyroid goiter, thyroiditis, and cysts) and neoplastic lesion such as thyroid adenoma. Among TBNs the colloid goiter (CG), thyroiditis (T), and thyroid adenoma (TA) are the most frequent diseases [1-3]. An evaluation of the variant of TBNs is clinically important for subsequent therapeutic interventions. For this reason the finding of specific characteristics of various TBNs is the barest necessity for the differential diagnosis of these thyroid disorders.

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 (ChE) in human body after industrial revolution plays a significant role in etiology of TBNs [12].

Besides iodine, many other ChE have also essential physiological functions [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 many 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 CG, TA, and T in comparison with normal thyroid was demonstrated [42-46].

To date, the etiology and pathogenesis of TBNs has to be considered as multifactorial. The present study was performed to find differences in ChE contents between CG, TA, and T group of samples, as well as to clarify the role of some ChE in the TBNs etiology. Having this in mind, our aim was to assess the silver (Ag), bromine (Br), calcium (Ca), chlorine (Cl), cobalt (Co), chromium (Cr), cooper (Cu), iron (Fe), mercury (Hg), I, potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), rubidium (Rb), ammonium (Sb), scandium (Sc), selenium (Se), strontium (Sr), and zinc (Zn) contents in CG, TA, and T tissue samples using non-destructive energy dispersive X-ray fluorescent analysis (EDXRF) combined with instrumental neutron activation analysis with high resolution spectrometry of sort-lived radionuclides (INAA-SLR) and long-lived radionuclides (INAA-LLR). A further aim was to compare the levels of these ChE in CG, TA, and T group of samples between each other.

MATERIAL AND METHODS

All patients suffered from CG (n=46, mean age M±SD was 48±12 years, range 30-64), TA (n=19, mean age M±SD was 41±11 years, range 22-55), and T (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. The group of patients with T 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 TE contents. For all patients the diagnosis has been confirmed by clinical and morphological/histological results obtained during studies of biopsy and resected materials.

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 TE analysis. After the samples intended for TE 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 ChE measurement by INAA-SLR.

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.

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) and IAEA HH-1 (human hair) 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, Cu, Fe, Rb, Sr, and Zn were determined by EDXRF. Details of the relevant facility for this method, source with ^{109}Cd radionuclide, methods of analysis and the results of quality control were presented in our earlier publications concerning the EDXRF of ChE contents in human thyroid and prostate tissue [25, 26, 50].

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). Details of used neutron flux, nuclear reactions, radionuclides, gamma-energies, spectrometric unit, sample preparation and measurement were presented in our earlier publications concerning the INAA-SLR of ChE contents in human thyroid, prostate, and scalp hair [27, 28, 51-53]

In a few days after non-destructive INAA-SLR all thyroid samples were repacked and used for INAA-LLR. A vertical channel of the WWR-c research

nuclear reactor (Branch of Karpov Institute, Obninsk).was applied to determine the content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn by INAA-LLR. Details of used neutron flux, nuclear reactions, radionuclides, gamma-energies, spectrometric unit, sample preparation and measurement were presented in our earlier publications concerning the INAA-LLR of ChE contents in human thyroid, scalp hair, and prostate [29,30,53,54]

A dedicated computer program for INAA-SLR mode optimization was used [55]. All thyroid samples were prepared in duplicate, and mean values of ChE contents were used in final calculation. Mean values of ChE contents were used in final calculation for the Br, Fe, Rb, and Zn mass fractions measured by two methods. 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 ChE contents in CG, TA, and T tissue samples. The difference in the results between three groups of samples (CG, TA, and T) 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) of the Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction in CG, TA, and T tissue samples.

The ratios of means and the comparison of mean values of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fractions in pairs of sample groups such as CG and TA, CG and T, and TA and T are presented in Table 2, 3, and 4, respectively.

Table 5 depicts the results of comparison the contents of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn in CG, TA, and T sample groups with those in normal thyroid (from data analysis of previous publications [43-46]), as well as comparison the contents of these ChE in CG, TA, and T sample groups among themselves.

DISCUSSION

As was shown before [27,28,50-54] good agreement of the Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn contents in CRM IAEA H-4 and IAEA HH-1 (human hair) samples determined by EDXRF, INAA-SLR, and INAA-LLR with the certified data of these CRMs indicates acceptable accuracy of the results obtained in the study of CG, TA, and T samples presented in Tables 1–5.

Table-1: Some statistical parameters of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction (mg/kg, dry mass basis) in thyroid colloid goiter, adenoma and thyroiditis

Element	Nodular colloid goiter			Adenoma			Thyroiditis		
	Mean	SD	SEM	Mean	SD	SEM	Mean	SD	SEM
Ag	0.226	0.236	0.042	0.211	0.201	0.056	0.274	0.131	0.066
Br	36.3	31.3	7.0	317	334	111	85	35	13
Ca	1393	855	168	1143	1135	342	694	421	188
Cl	9117	3866	1223	7722	3785	1262	7160	3541	1771
Co	0.0628	0.0287	0.0050	0.0673	0.0486	0.0140	0.0535	0.0153	0.0068
Cr	0.849	0.834	0.150	1.40	0.85	0.25	0.70	0.73	0.34
Cu	8.51	7.15	1.60	17.6	14.0	5.7	5.05	0.21	0.15
Fe	324	309	49	526	678	175	222	181	52
Hg	0.987	0.726	0.124	0.796	0.522	0.145	0.97	0.49	0.22
I	1144	943	149	962	1013	232	662	604	161
K	6518	2304	443	5137	2474	686	7268	1947	974
Mg	351	148	28	200	131	36	514	232	95
Mn	1.78	1.13	0.23	1.60	1.77	0.51	2.33	1.72	0.70
Na	11335	3597	705	9072	3952	1096	8271	3262	1332
Rb	8.28	3.68	0.57	8.37	3.19	0.82	12.8	5.2	1.5
Sb	0.146	0.121	0.021	0.149	0.124	0.036	0.096	0.036	0.016
Sc	0.0130	0.0201	0.0040	0.0174	0.0273	0.0090	0.019	0.024	0.011
Se	3.09	2.59	0.44	2.36	0.90	0.24	1.82	0.45	0.20
Sr	2.43	2.73	0.49	2.78	2.04	0.55	6.12	7.02	2.1
Zn	119	53.1	8.2	123	52	13	91.4	28.9	8.3

M – arithmetic mean, SD – standard deviation, SEM – standard error of mean.

Table-2: Differences between mean values (M±SEM) of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction (mg/kg, dry mass basis) in thyroid colloid goiter and adenoma

Element	Thyroid tissue				Ratio
	Goiter (CG)	Adenoma (TA)	Student's t-test, $p\leq$	U-tes, p	CG / TA
Ag	0.226±0.042	0.211±0.056	0.824	>0.05	1.07
Br	36.3±7.0	317±111	**0.035**	**≤0.01**	0.11
Ca	1393±168	1143±342	0.521	>0.05	1.22
Cl	9117±1223	7722±1262	0.438	>0.05	1.18
Co	0.0628±0.0050	0.0673±0.0140	0.767	>0.05	0.93
Cr	0.849±0.150	1.40±0.25	0.069	>0.05	0.61
Cu	8.51±1.60	17.6±5.7	0.178	>0.05	0.48
Fe	324±49	526±175	0.283	>0.05	0.62
Hg	0.987±0.124	0.796±0.145	0.327	>0.05	1.24
I	1144±149	962±232	0.513	>0.05	1.19
K	6518±443	5137±686	0.105	>0.05	1.27
Mg	351±28	200±36	**0.0030**	**≤0.01**	1.76
Mn	1.78±0.23	1.60±0.51	0.741	>0.05	1.11
Na	11335±705	9072±1096	0.096	>0.05	1.25
Rb	8.28±0.57	8.37±0.82	0.922	>0.05	0.99
Sb	0.146±0.021	0.149±0.036	0.949	>0.05	0.98
Sc	0.0130±0.0040	0.0174±0.0090	0.647	>0.05	0.75
Se	3.09±0.44	2.36±0.24	0.155	>0.05	1.31
Sr	2.43±0.49	2.78±0.55	0.642	>0.05	0.87
Zn	119±8.2	123±13	0.736	>0.05	0.97

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

Table-3: Differences between mean values (M±SEM) of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction (mg/kg, dry mass basis) in thyroid colloid goiter and thyroiditis

Element	Thyroid tissue				Ratio
	Goiter (CG)	Thyroiditis (T)	Student's t-test, $p\leq$	U-test, p	CG / T
Ag	0.226±0.042	0.274±0.066	0.566	>0.05	0.82
Br	36.3±7.0	85±13	**0.0093**	**≤0.01**	0.43
Ca	1393±168	694±188	**0.017**	**≤0.01**	2.01
Cl	9117±1223	7160±1771	0.398	>0.05	1.27
Co	0.0628±0.0050	0.0535±0.0068	0.300	>0.05	1.17
Cr	0.849±0.150	0.70±0.34	0.698	>0.05	1.21
Cu	8.51±1.60	5.05±0.15	**0.044**	**≤0.01**	1.69
Fe	324±49	222±52	0.162	>0.05	1.46
Hg	0.987±0.124	0.97±0.22	0.956	>0.05	1.02
I	1144±149	662±161	**0.035**	**≤0.01**	1.73
K	6518±443	7268±974	0.519	>0.05	0.90
Mg	351±28	514±95	0.153	>0.05	0.68
Mn	1.78±0.23	2.33±0.70	0.489	>0.05	0.76
Na	11335±705	8271±1332	0.076	>0.05	1.37
Rb	8.28±0.57	12.8±1.5	**0.013**	**≤0.01**	0.65
Sb	0.146±0.021	0.096±0.016	0.074	>0.05	1.52
Sc	0.0130±0.0040	0.019±0.011	0.623	>0.05	0.68
Se	3.09±0.44	1.82±0.20	**0.014**	**≤0.01**	1.70
Sr	2.43±0.49	6.12±2.1	0.118	>0.05	0.40
Zn	119±8.2	91.4±8.3	**0.026**	**≤0.01**	1.30

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

Table-4: Differences between mean values (M±SEM) of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction (mg/kg, dry mass basis) in thyroid adenoma and thyroiditis

Element	Thyroid tissue				Ratio
	Adenoma (TA)	Thyroiditis (T)	Student's t-test, $p\leq$	U-test, p	TA / T
Ag	0.211±0.056	0.274±0.066	0.486	>0.05	0.77
Br	317±111	85±13	0.071	**≤0.05**	3.73
Ca	1143±342	694±188	0.270	>0.05	1.65
Cl	7722±1262	7160±1771	0.804	>0.05	1.08
Co	0.0673±0.0140	0.0535±0.0068	0.390	>0.05	1.26
Cr	1.40±0.25	0.70±0.34	0.131	>0.05	2.00
Cu	17.6±5.7	5.05±0.15	0.080	>0.05	3.49
Fe	526±175	222±52	0.115	>0.05	2.37
Hg	0.796±0.145	0.97±0.22	0.525	>0.05	0.82
I	962±232	662±161	0.298	>0.05	1.45
K	5137±686	7268±974	0.121	>0.05	0.71
Mg	200±36	514±95	**0.019**	**≤0.01**	0.39
Mn	1.60±0.51	2.33±0.70	0.419	>0.05	0.69
Na	9072±1096	8271±1332	0.651	>0.05	1.10
Rb	8.37±0.82	12.8±1.5	**0.019**	**≤0.01**	0.65
Sb	0.149±0.036	0.096±0.016	0.204	>0.05	1.55
Sc	0.0174±0.0090	0.019±0.011	0.920	>0.05	0.92
Se	2.36±0.24	1.82±0.20	0.107	**≤0.05**	1.30
Sr	2.78±0.55	6.12±2.1	0.154	>0.05	0.45
Zn	123±13	91.4±8.3	**0.050**	**≤0.01**	1.35

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

Table-5: Comparison the contents of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn in different pathological transformation of thyroid

Comparison with:	Normal thyroid*			Colloid Goiter		Adenoma
Element	Goiter	Adenoma	Thyroiditis	Adenoma	Thyroiditis	Thyroiditis
Ag	↑	↑	↑	=	=	=
Br	↑	↑	↑	↑	↑	↓
Ca	=	=	↓	=	↓	=
Cl	↑	↑	=	=	=	=
Co	↑	=	=	=	=	=
Cr	↑	↑	=	=	=	=
Cu	↑	=	↑	=	↓	=
Fe	↑	=	=	=	=	=
Hg	↑	↑	↑	=	=	=
I	↓	↓	↓	=	↓	=
K	=	=	=	=	=	=
Mg	↑	↓	=	↓	=	↑
Mn	=	=	=	=	=	=
Na	↑	↑	=	=	=	=
Rb	=	=	↑	=	↑	↑
Sb	=	=	=	=	=	=
Sc	↑	=	=	=	=	=
Se	=	=	=	=	↓	↓
Sr	↓	↓	=	=	=	=
Zn	=	=	=	=	↓	↓

* From analysis of previous publications [43-46],↑ - element content is higher, ↓ - element content is lower, = - no difference

In a general sense variations found for Ag, Br, Hg, I, K, Mn, Sb, Se, and Zn contents in all thyroid lesions investigated (CG, TA, and T) were similar in comparison with normal thyroid tissue (Table 5). In affected tissues contents of Ag, Br, and Hg increased,

content of I decreased, whereas levels of K, Mn, Sb, Se, and Zn did not changed.

More similarity in ChE variations (Ag, Br, Ca, Cl, Cr, Hg, I, K, Mn, Na, Rb, Sb, Se, Sr, and Zn) were observed for the goitrous and adenomatous transformations in comparison with normal thyroid (Table 5). Among these ChE contents of Ag, Br, Cl, Cr, Hg, and Na in goitrous and adenomatous thyroid were higher, while I and Sr contents were lower in comparison with normal gland (Table 5). There was not found any differences between ChE contents of CG and TA, with the exception of Br and Mg (Tables 2 and 5). The Br level in TA tissue was almost 9 times higher, while the Mg content was 1.8 times lower than in CG tissue.

The variations found for Br, Ca, Cu, I, Mg, Rb, Se, and Zn in thyroid with T were some differ than the variations of these ChE in CG and TA tissues. For example, content of Br and Rb in T samples were higher, while Ca, Cu, I, Se, and Zn levels were lower than in CG tissues (Tables 3 and 5). In comparison T samples with TA samples was observed reduced Br, Se, and Zn levels but elevated contents of Mg and Rb (Tables 4 and 5). The common difference of T tissue from CG and TA tissue was elevated level of Rb and reduced levels of Se and Zn (Table 5).

Published data on comparison of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn levels in the different thyroid lesions such as CG, TA and T were not found.

Thus, from obtained results it was possible to conclude that the common characteristics of CG, TA and T samples were elevated level of Ag, Br, Hg and reduced level of I in comparison with normal thyroid and, therefore, these ChE can be involved in etiology or pathogenesis of such thyroid disorders as CG, TA and T. In addition to these ChE the common characteristics of CG and TA in comparison with normal thyroid tissue were elevated levels of Cl, Cr, and Na. Thus, these ChE can be also important in etiology or pathogenesis of CG and TA. Because significant difference between T tissue and other TBNs consist in elevated level of Rb and reduced levels of Se and Zn, these ChE can additionally play some role in etiology or pathogenesis of thyroiditis.

Silver

Ag is a TE with no recognized trace metal value in the human body [56]. Ag in metal form and inorganic Ag compounds ionize in the presence of water, body fluids or tissue exudates. The silver ion Ag^+ is biologically active and readily interacts with proteins, amino acid residues, free anions and receptors on mammalian and eukaryotic cell membranes [57]. Besides such the adverse effects of chronic exposure to Ag as a permanent bluish-gray discoloration of the skin (argyria) or eyes (argyrosis), exposure to soluble Ag compounds may produce other toxic effects, including liver and kidney damage, irritation of the eyes, skin, respiratory, and intestinal tract, and changes in blood cells [58]. In experimental studies it was shown that Ag nanoparticles may affect thyroid hormone metabolism [59]. More detailed knowledge of the Ag toxicity can lead to a better understanding of the impact on human health, including thyroid function.

Bromine

Br is one of the most abundant and ubiquitous of the recognized ChE 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 [60]. A significant age-related increase of Br content in human thyroid [25-28] correlated well with age-related prevalence of CG, TA and T [61-63]. The main source of natural Br for human body is food. Environment (air, water and food) polluted by artificial Br-contained compounds, for example such as polybrominated biphenyls (PBBs) and diphenyl ethers (PBDEs), is other source. PBBs and PBDEs impact on thyroid function and thyroid hormones metabolism [64] Thus, on the one hand, the accumulated data suggest that Br level in thyroid tissue might be responsible for CG, TA, and T development. But, on the other hand, Br compounds, especially potassium bromide (KBr), sodium bromide (NaBr), and ammonium bromide (NH_4Br), are frequently used as sedatives in Russia [65]. It may be the reason for elevated levels of Br in specimens of patients with CG, TA and T in comparison with normal thyroid. A nonuniform level of this TE in tissue of thyroid legions TA Br > T Br > CG Br may be explained by the different strength of emotional reactions of persons on the diagnosis TA, T, and CG, and, as consiquence, different dozes of Br-conteined sedatives, which were used.

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 and Br, 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 [64,66-69}. Thus, on the one hand, the accumulated data suggest that Cl level in thyroid tissue might be responsible for CG and TA 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 [70]. Goitrous and adenomatous tissues can contain more colloid that normal thyroid. Because colloid is extracellular liquid, it is possible to speculate that CG and TA are characterized by an increase of the mean value of the Cl and Na mass fractions because the relative content of colloid in these thyroid lesions is higher than that in normal thyroid tissue. Overall, the elevated levels of Cl in goutrous and adenomatous thyroids could possibly be explored for diagnosis of CG and TA.

Chromium

Cr-compounds are cytotoxic, genotoxic, and carcinogenic in nature. Some Cr forms, including hexavalent chromium (Cr^{6+}), are toxicants known for their carcinogenic effect in humans. They have been classified as certain or probable carcinogens by the International Agency for Research on Cancer [71]. The lung cancer risk is prevalent in pigment chromate handlers, ferrochromium production workers, stainless steel welders, and chrome-platers [72]. Except in Cr-related industries and associated environments, Cr intoxication from environmental exposure is not common. However, it was found, that drinking water supplies in many geographic areas contain chromium in the +3 and +6 oxidation states. Exposure of animals to Cr^{6+} in drinking water induced tumors in the mouse small intestine [73]. Many other animal experiments and in vitro studies demonstrate also that Cr can induce oxidative stress and exert cytotoxic effects [74]. Besides reactive oxygen species (ROS) generation, oxidative stress, and cytotoxic effects of Cr exposure, a variety of other changes like DNA damage, increased formation of DNA adducts and DNA-protein cross-links, DNA strand breaks, chromosomal aberrations and instability, disruption of mitotic cell division, chromosomal aberration, premature cell division, S or G2/M cell cycle phase arrest, and carcinogenesis also occur in humans or experimental test systems [72].

Mercury

Hg is one of the most dangerous environmental pollutants [75]. The growing use of this metal in diverse areas of industry has resulted in a significant increase of environment contamination and episodes of human intoxication. Many experimental and occupational studies of Hg in different chemical states shown significant alterations in thyroid hormones metabolism and thyroid gland parenchyma [76,77]. Moreover, Hg was classified as certain or probable carcinogen by the International Agency for Research on Cancer [78]. For example, in Hg polluted area thyroid cancer incidence was almost 2 times higher than in adjacent control areas [79].

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. Goitrous and adenomatous transformation, as well as thyroiditis are probably accompanied by a partial loss of tissue-specific functional features, which leads to a modest (CG and TA) or severe (T) reduction in I content associated with functional characteristics of the human thyroid tissue. Great losses of I in thyroid with T, in contract to little reduced levels of I content in thyroid with CG and TA, could possibly be explored for differencial diagnosis of T.

Rubidium

Rb was only TE, which accumulation in T tissue was higher than in normal thyroid, CG, and TA (Tables 2-5). There is very little information about Rb effects on thyroid function. Rb as a monovalent cation Rb+ is transfered through membrane by the Na+K+-ATPase pump like K+ and concentrated in the intracellular space of cells. Thus, Rb seems to be more intensivly concentrated in the intracellular space of cells during thyroiditis in comparison with normal thyroid cells, and ceels transformed by CG and TA. The sourse of Rb elevated level in T tissue may be Rb environment overload. The excessive Rb intake may result a replacement of medium potassium by Rb, which effects on iodide transport and iodoaminoacid synthesis by thyroid [80]. The sourse of Rb increase in T tissue may be not only the excessive intake of this TE in organism from the environment, but also changed Na+K+ - ATPase or H+K+ - ATPase pump membrane transport systems for monovalent cations, which can be stimulated by endocrin system, including thyroid hormones [81]. It was found also that Rb has some function in immune responce [82] and that elevated concentration of Rb could modulate proliferative responses of the cell, as was shown for bone marrow leukocytes [83].These data partially clarify the possible role of Rb in etiology and pathogenesis of thyroiditis. Moreover, the significantly elevated level of Rb in thyroid with T could possibly be explored for differential diagnosis of this thyroid disorder from other binighn thyroid nodules.

Selenium

Se as TE is essential for the functioning of many enzymes (selenoproteins) involved in the synthesis and metabolism of thyroid hormones and protection against oxidative damage (such as iodothyronine deiodinases, thioredoxin reductases and

glutathione peroxidases) [84]. In fact, compared to other organs, the thyroid gland has a high concentration of Se [84]. Se deficiency among patients with TBNs was observed in many studies [85]. In spite of the fact that low Se status correlates with risk of TBNs, it is important to point out that, like I, Se excess is also not good for health [85].

Zinc

Zn as a trace metal has structural, catalytic and regulatory roles in normal and pathophysiology. This ChE is a constituent of more than 3000 proteins and is a cofactor for over 300 enzymes [86]. Zn is an essential mediator of cell proliferation and differentiation through the regulation of DNA synthesis and mitosis. Zn also affects DNA repair pathways by regulating multiple intracellular signaling pathways and altering proteins involved in DNA maintenance [87]. This metal also maintenance the balance of a cellular redox [88]. Thus, Zn is important cofactors in diverse cellular processes. Concern the thyroid function, Zn is involved in the synthesis of TSH and important for the proper functioning of T3 because T3 nuclear receptors contain Zn ions [85]. However, high Zn concentrations are toxic to the cells and the elevated level of Zn mass fractions in thyroid tissue may contribute to harmful effects on the gland. There are good reasons for such speculations since. Experimental and epidemiological data support the hypothesis that Zn overload is a risk factor for benign and malignant tumors [87, 89-91].

Characteristically, elevated or reduced levels of ChE 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 ChE in affected thyroid tissues researchers try to determine the role of the deficiency or excess of each ChE in the etiology and pathogenesis of thyroid diseases. In our opinion, abnormal levels of many ChE 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 ChE level in pathologically altered tissue is the reason for alterations or vice versa.

Limitations

This study has several limitations. Firstly, analytical techniques employed in this study measure only twenty ChE (Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn) mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of ChE investigated in normal thyroid and in pathologically altered tissue. Secondly, the sample size of CG group and particularly of TA and T groups was relatively small and prevented investigations of ChE contents in these groups using differentials like gender, histological types of CG, TA and T, nodules functional activity, stage of disease, dietary habits of patients with CG, TA and T. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on TBNs-specific tissue Ag, Br, Cl, Cr, Hg, I, Na, Rb, Se, and Zn level alteration and shows the necessity to continue ChE research of TBNs.

CONCLUSION

In this work, ChE analysis was carried out in the tissue samples of TBNs using three non-destructive analytical methods EDXRF, INAA-SLR, and INAA-LLR. It was shown that combination of these methods is an adequate analytical tool for the non-destructive determination of twenty ChE (Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn) content in the tissue samples of human thyroid in norm and pathology, including needle-biopsy specimens. It was observed that in CG, TA and T tissues content of Ag, Br, and Hg was significantly higher, while level of I was lower than in normal thyroid tissue. In our opinion, the abnormal increase in Ag, Br, Hg level and decrease in I level in all TBNs might demonstrate an involvement of these ChE in etiology and pathogenesis of TBNs. In addition, elevated levels of such ChE as Cl, Cr, and Na can be also important for goitrous and adenomatous transformation of thyroid. It was supposed that elevated level of Rb and reduced levels of Se and Zn content in thyroid with T could possibly be explored for differencial diagnosis of T among other TBNs.

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