Original Research Article

Measurement of Nineteen Chemical Elements in Thyroid Malignant Nodules and Thyroid Tissue Adjacent to Nodules using Neutron Activation Analysis and Inductively Coupled Plasma Atomic Emission Spectrometry

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Abstract

Thyroid malignant nodules (TMNs) are the most common endocrine cancer and the fifth most frequently occurring type of malignancies. The etiology and pathogenesis of TMNs must be considered as multifactorial. The present study was performed to clarify the role of some chemical elements (ChEs) in the etiology of these thyroid disorders. Thyroid tissue levels of nineteen ChEs were prospectively evaluated in malignant tumor and tissue adjacent to tumor of 41 patients with TMNs. Measurements were performed using a combination of non-destructive and destructive methods: instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides and inductively coupled plasma atomic emission spectrometry. Results of the study were additionally compared with previously obtained data for the same ChEs in "normal" thyroid tissue. It was observed that in malignant tissue the mass fractions of Al, B, Ca, Cl, Cu, K, Mg, Mn, Na, P, S, and Si were 3.14, 4.64, 1.79, 2.26, 3.55, 1.57, 1.61, 1.57, 1.24, 2.45, 1.14, and 2.81 times, respectively, higher whereas mass fraction of I was 25.6 times lower than in the normal thyroid. In the "adjacent" group mean mass fractions of Cl and I were approximately 1.6 and 1.7 times, respectively, higher, while mean values of Ba, Ca and Sr content were 55%, 45%, and 70%, respectively, lower than in the "normal" group. In tumor Ca, Cl, Cu, K, and Sr contents were approximately 2.8, 1.4, 1.8, 1.7, and 5.4 times, respectively, higher, while I content was 43 times lower than in "adjacent" group of tissue samples. It was concluded that thyroid tissue adjacent to malignant nodules kept the main function of thyroid gland, while malignantly transformed thyroid cells lost its capacity to accumulate I. It was supposed that the excessive accumulation of Cl and I by thyroid tissue probably precede the TMNs origination and development. It was also supposed that elevated levels of Al, B, Ca, Cl, Cu, K, Mg, Mn, Na, P, S, and Si, as well as drastically reduced level of me in cancerous tissue could possibly be explored for differential diagnosis of benign and malignant thyroid nodules.
Keywords: Chemical elements; Inductively coupled plasma atomic emission spectrometry; Neutron activation analysis; Thyroid; Thyroid malignant nodules.

INTRODUCTION

Thyroid malignant nodules (TMNs) are the most common endocrine cancer and the fifth most frequently occurring type of malignancies [1-3]. The incidence of TMNs has increased worldwide over the past four decades. TMNs are divided into three main histological types: differentiated (papillary and follicular thyroid cancer), undifferentiated (poorly differentiated and anaplastic thyroid cancer, and medullary thyroid cancer, arising from C cells of thyroid [3]. For over 20th century, there was the dominant opinion that TMNs is the simple consequence of iodine deficiency [4]. However, it was found that TMNs is a frequent disease even in those countries and regions where the population is never exposed to iodine shortage. Moreover, it was shown that iodine excess has severe consequences on human health and associated with the presence of TMNs [5-8]. It was also demonstrated that besides the iodine deficiency and excess many other dietary, environmental, and occupational factors are associated with the TMNs incidence [9-11]. Among these factors a disturbance of evolutionary stable input of many chemical elements

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

Besides iodine, many other ChEs have also essential physiological functions [13]. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of ChEs depend on tissue-specific need or tolerance, respectively [13]. Excessive accumulation or an imbalance of the ChEs may disturb the cell functions and may result in cellular proliferation, degeneration, death, benign or malignant transformation [13-15].

In our previous studies the complex of *in vivo* and *in vitro* nuclear analytical and related methods was developed and used for the investigation of iodine and other ChEs contents in the normal and pathological thyroid [16-22]. Iodine level in the normal thyroid was investigated in relation to age, gender and some non-thyroidal diseases [23, 24]. After that, variations of many ChEs content with age in the thyroid of males and females were studied and age- and gender-dependence of some ChEs was observed [25-41]. Furthermore, a significant difference between some ChEs contents in colloid goiter and thyroid adenoma in comparison with normal thyroid was demonstrated [42-45].

To date, the etiology and pathogenesis of TMNs must be considered as multifactorial. The present study was performed to find out differences in ChEs contents between the group of cancerous tissues and tissue adjacent to tumor, as well as to clarify the role of some ChEs in the etiology of TMNs. Having this in mind, the aim of this exploratory study was to examine differences in the content of aluminum (Al), boron (B), barium (Ba), 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) in nodular and adjacent to nodules tissues of thyroids with TMNs using a combination of non-destructive instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) and destructive method such as inductively coupled plasma atomic emission spectrometry (ICP-AES), and to compare the levels of these ChEs in two groups (nodular and thyroid tissue adjacent to nodules) of the cohort of TMNs samples. Moreover, for understanding a possible role of ChEs in etiology and pathogenesis of TMNs results of the study were compared with previously obtained data for the same ChEs in "normal" thyroid tissue [42-45].

MATERIAL AND METHODS

All patients with TMNs (n=41, mean age M±SD was 46±15 years, range 16-75) were hospitalized in the Head and Neck Department of the Medical Radiological Research Centre (MRRC), Obninsk.. 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 trace element contents. In all cases the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. Histological conclusions for malignant tumors were: 25 papillary adenocarcinomas, 8 follicular adenocarcinomas, 7 solid carcinomas, and 1 reticulosarcoma. Tissue samples of tumor and visually intact tissue adjacent to tumor were taken from resected materials.

"Normal" thyroids for the control group samples were removed at necropsy from 105 deceased (mean age 44±21 years, range 2-87), who had died suddenly. The majority of deaths were due to trauma. A histological examination in the control group was used to control the age norm conformity, as well as to confirm the absence of micro-nodules and latent cancer.

All studies were approved by the Ethical Committees of MRRC. All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments, or with comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

All tissue samples obtained from tumors and visually intact tissue adjacent to tumors were divided into two portions using a titanium scalpel to prevent contamination by ChEs of stainless steel [46]. One was used for morphological study while the other was intended for ChEs analysis. After the samples intended for ChEs analysis were weighed, they were freeze-dried and homogenized [47]. The pounded samples weighing about 10 mg (for biopsy) and 100 mg (for resected materials) were used for ChE measurement by INAA-SLR. The content of Ca, Cl, I, K, Mg, Mn, and Na were determined by INAA-SLR using a horizontal channel equipped with the pneumatic rabbit system of the WWR-c research nuclear reactor (Branch of Karpov Institute, Obninsk). After non-destructive INAA-SLR investigation the thyroid samples were used for ICP-AES. The samples were decomposed in autoclaves and aliquots of solutions were used to determine the Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractions by ICP-AES using the Spectrometer ICAP-61 (Thermo Jarrell Ash, USA). Information detailing with the NAA-SLR and ICP-AES methods used and other details of the analysis were presented in our earlier publications concerning ChE contents in human thyroid [33, 34], prostate [48-52], and scalp hair [53].

To determine contents of the ChE by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [54]. 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-SLR mode optimization was used [55]. All thyroid samples for ChEs analysis were prepared in duplicate and mean values of ChEs contents were used in final calculation. Mean values of ChE contents were used in final calculation for the Ca, K, Mg, Mn, and Na 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 ChEs contents in malignant and adjacent to tumor tissue of thyroids with TMNs. Data for "normal"

thyroid were taken from our previous publications [42-45]. The difference in the results between three groups of samples ("normal", "tumor", and "adjacent") was evaluated by the parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test.

RESULTS AND DISCUSSION

Table 1 presents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Al, B, Ba, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction in "tumor" and "adjacent" groups of thyroid tissue samples.

The ratios of means and the comparison of mean values of Al, B, Ba, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractions in pairs of sample groups such as "normal" and "tumor", "normal" and "adjacent", and also "adjacent" and "tumor" are presented in Table 2, 3, and 4, respectively.

Table-1: **Some statistical parameters of Al, B, Ba, 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 thyroid cancer (tumor and "intact" thyroid tissue adjacent to tumor)**

Tissue	El	Mean	SD	SEM	Min	Max	Med	P 0.025	P 0.975
Cancer	Al	33.0	25.5	7.1	4.50	96.5	21.3	5.70	85.6
(tumor)	B	2.21	1.89	0.52	1.00	5.60	1.00	1.00	5.42
	Ba	1.42	1.30	0.35	0.220	4.09	0.945	0.259	3.93
	Ca	3013	2966	699	452	9768	1578	467	8938
	Cl	7699	2900	703	4214	14761	7216	4240	13619
	Cu	14.5	9.4	2.6	4.00	32.6	10.9	4.21	31.4
	Fe	255	168	27	60.6	880	217	74.6	673
	I	71.8	62.0	10.1	2.00	261	62.1	2.93	192
	K	10054	4018	877	1660	18814	9204	4073	17559
	Li	0.0314	0.0307	0.0090	0.0078	0.111	0.0182	0.0088	0.0995
	Mg	478	194	42	130	933	467	166	881
	Mn	2.01	1.34	0.29	0.100	5.95	1.61	0.250	5.23
	Na	8576	2433	531	4083	14048	8107	4901	12925
	P	10493	3238	866	5382	15403	9694	5767	15391
	S	9448	1605	429	7139	12591	9422	7211	12204
	Si	143	156	42	18.6	523	64.2	19.8	472
	Sr	6.26	7.61	1.59	0.93	30.8	3.00	0.985	25.0
	V	0.0904	0.0308	0.0100	0.0580	0.170	0.0870	0.0600	0.154
	Zn	96.9	80.0	12.6	28.7	375	69.8	36.3	374
Cancer	Al	19.4	11.1	5.6	7.10	32.9	18.9	7.66	32.2
(adjacent	B	5.80	9.60	4.80	1.00	20.2	1.00	1.00	18.8
thyroid	Ba	0.50	0.32	0.16	0.230	0.920	0.415	0.232	0.894
tissue)	Ca	917	524	131	81.0	1909	726	204	1822
	Cl	5339	2252	581	2526	11767	4922	2595	10201
	Cu	8.08	3.15	1.58	4.90	12.1	7.65	5.01	11.9
	Fe	256	133	26	109	752	254	112	591
	I	3183	1673	301	563	8240	2982	853	7766
	K	5847	2558	661	2097	13092	5429	2559	11220
	Li	0.0210	0.0203	0.0100	0.0096	0.0514	0.0115	0.0097	0.0485
	Mg	359	402	104	15.0	1412	247	15.0	1287
	Mn	1.77	1.60	0.40	0.410	6.78	1.25	0.429	5.54
	Na	7726	2519	630	3865	14373	7434	4250	13009

Tissue	El	Mean	SD	SEM	Min	Max	Med	P 0.025	P 0.975
	P	9724	5592	2796	4023	15979	9446	4181	15738
	S	9725	1999	999	6989	11323	10293	7176	11306
	Si	77.3	56.2	28.1	28.8	135	72.7	28.8	134
	Sr	1.16	0.29	0.14	0.830	1.40	1.20	0.843	1.40
	V	0.082	0.026	0.013	0.0630	0.120	0.0725	0.0636	0.117
	Zn	111	55	11	20.4	272	107	28.8	215

El – element, M – arithmetic mean, SD – standard deviation, SEM – standard error of mean, Min – minimum value, Max – maximum value, Med – median, P 0.025 – percentile with 0.025 level, P 0.975 – percentile with 0.975 level.

As was shown before [33,34,48-53] good agreement of the Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Mg, Mn, Na, P, S, Sr, V, and Zn mass fractions in CRM IAEA H-4, INCT-SBF-4, INCT-TL-1, and INCT-MPH-2 samples determined by both INAA-SLR and ICP-AES methods with the certified data of these CRMs indicates acceptable accuracy of the results obtained in the study of thyroid tissue samples presented in Tables 1–4.

From Table 2, it is observed that in malignant tissue the mass fraction of Al, B, Ca, Cl, Cu, K, Mg, Mn, Na, P, S, and Si are 3.14, 4.64, 1.79, 2.26, 3.55, 1.57, 1.61, 1.57, 1.24, 2.45, 1.14, and 2.81 times, respectively, higher whereas mass fraction of I is 25.6 times lower than in the normal thyroid. Thus, if we accept the ChEs contents in thyroid glands of the "normal" group as a norm, we have to conclude that with a malignant transformation the Al, B, Ca, Cl, Cu, I, K, Mg, Mn, Na, P, S, and Si contents in thyroid tissue significantly changed.

Table-2: Differences between mean values (M±SEM) of Al, B, Ba, 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 and thyroid cancer (tumor)

El	Thyroid tissue				Ratio
	Normal thyroid	Cancer (tumor)	Student's t-test $p\leq$	U-test p	Tumor/Normal
Al	10.5±1.8	33.0±7.1	**0.0083**	**≤0.01**	3.14
B	0.476±0.058	2.21±0.52	**0.0062**	**≤0.01**	4.64
Ba	1.12±0.15	1.42±0.35	0.446	>0.05	1.27
Ca	1682±106	3013±699	0.076	**≤0.05**	1.79
Cl	3400±174	7699±703	**0.000013**	**≤0.01**	2.26
Cu	4.08±0.14	14.5±2.6	**0.0017**	**≤0.01**	3.55
Fe	223±10	255±27	0.278	>0.05	1.14
I	1841±107	71.8±10.1	**0.00000000001**	**≤0.01**	0.039
K	6418±290	10054±877	**0.00060**	**≤0.01**	1.57
Li	0.0208±0.0022	0.0314±0.0090	0.265	>0.05	1.51
Mg	296±16	478±42	**0.00043**	**≤0.01**	1.61
Mn	1.28±0.07	2.01±0.29	**0.024**	**≤0.01**	1.57
Na	6928±175	8576±531	**0.0069**	**≤0.01**	1.24
P	4290±207	10493±866	**0.0000054**	**≤0.01**	2.45
S	8259±263	9448±429	**0.027**	**≤0.01**	1.14
Si	50.8±6.2	143±42	**0.047**	**≤0.01**	2.81
Sr	3.81±0.34	6.26±1.59	0.144	>0.05	1.64
V	0.102±0.005	0.0904±0.0100	0.305	>0.05	0.89
Zn	94.8±4.2	96.9±12.6	0.877	>0.05	1.02

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

In a general sense Al, B, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, V, and Zn contents found in the "normal" and "adjacent" groups of thyroid tissue samples were very similar (Table 3). However, in the "adjacent" group mean mass fractions of Cl and I were approximately 1.6 and 1.7 times, respectively, higher, while mean values of Ba, Ca and Sr content were 55%, 45%, and 70%, respectively, lower than in the "normal" group. Significant changes were found in tumor ChEs composition in comparison with thyroid tissue adjacent to tumor. In malignant tumor Ca, Cl, Cu,

K, and Sr contents were approximately 2.8, 1.4, 1.8, 1.7, and 5.4 times, respectively, higher, while I content was 43 times lower than in "adjacent" group of tissue samples (Table 4).

Characteristically, elevated or reduced levels of ChEs observed in thyroid nodules are discussed in terms of their potential role in the initiation and promotion of these thyroid lesions. In other words, using the low or high levels of the ChEs in affected thyroid tissues researchers try to determine the role of

the deficiency or excess of each ChE in the etiology and pathogenesis of thyroid diseases. In our opinion, abnormal levels of many ChEs in TMNs could be and cause, and also effect of thyroid tissue transformation. From the results of such kind studies, it is not always possible to decide whether the measured decrease or increase in ChEs level in pathologically altered tissue is

the reason for alterations or vice versa. According to our opinion, investigation of ChEs contents in thyroid tissue adjacent to malignant nodules and comparison obtained results with ChEs levels typical of "normal" thyroid gland may give additional useful information on the topic because these data show conditions of tissue in which TMNs were originated and developed.

Table-3: Differences between mean values (M±SEM) of Al, B, Ba, 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 and "intact" thyroid tissue adjacent to tumor

El	Thyroid tissue		Student's t-test $p \le$	U-test p	Ratio Adjacent/Normal
	Normal thyroid	Cancer (adjacent)			
Al	10.5±1.8	19.4±5.6	0.206	>0.05	1.85
B	0.476±0.058	5.80±4.80	0.348	>0.05	12.2
Ba	1.12±0.15	0.50±0.16	**0.017**	**≤0.01**	0.45
Ca	1682±106	917±131	**0.000055**	**≤0.01**	0.55
Cl	3400±174	5339±581	**0.0054**	**≤0.01**	1.57
Cu	4.08±0.14	8.08±1.58	0.084	>0.05	1.98
Fe	223±10	256±26	0.251	>0.05	1.15
I	1841±107	3183±301	**0.00015**	**≤0.01**	1.73
K	6418±290	5847±661	0.439	>0.05	0.91
Li	0.0208±0.0022	0.0210±0.0100	0.989	>0.05	1.01
Mg	296±16	359±104	0.558	>0.05	1.21
Mn	1.28±0.07	1.77±0.40	0.252	>0.05	1.38
Na	6928±175	7726±630	0.238	>0.05	1.12
P	4290±207	9724±2796	0.147	>0.05	2.27
S	8259±263	9725±999	0.240	>0.05	1.18
Si	50.8±6.2	77.3±28.1	0.420	>0.05	1.52
Sr	3.81±0.34	1.16±0.14	**0.000000002**	**≤0.01**	0.30
V	0.102±0.005	0.082±0.013	0.220	>0.05	0.80
Zn	94.8±4.2	111±11	0.175	>0.05	1.17

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

Thus, from results obtained, it was possible to conclude that the common characteristics of TMNs in comparison with "normal" thyroid and visually "intact" thyroid tissue adjacent to malignant tumors were elevated levels of Ca, Cl, Cu, and K, as well as drastically reduced level of I (Tables 2 and 4). The last finding meant that thyroid tissue adjacent to malignant nodules kept the main function of thyroid gland, while malignantly transformed thyroid cells lost its capacity to accumulate I. Furthermore, the ChEs composition of thyroid tissue adjacent to tumor did not equal ChEs contents of "normal" thyroid (Table 3). For example, contents of such elements as mean mass fractions of Cl and I were approximately 1.6 and 1.7 times, respectively, higher (Table 3). From this it follows that the excessive accumulation of Cl and I by thyroid tissue

is likely to precede the TMNs origination and development.

Aluminum

Al is the most widely distributed metal in the environment. Environmental media may be contaminated by Al from anthropogenic sources and through the weathering of rocks and minerals [56]. The trace element Al is not described as essential, because no biochemical function has been directly connected to it. Toxic actions of Al induce oxidative stress, immunologic alterations, genotoxicity, and other disorders, including cell membrane perturbation, apoptosis, necrosis and dysplasia [56]. Furthermore, it was shown in experimental and epidemiological studies that Al can affect thyroid iodide uptake and hormones secretion [57, 58].

Table-4: Differences between mean values (M±SEM) of Al, B, Ba, 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 thyroid cancer and "intact" thyroid tissue adjacent to tumor

El	Thyroid tissue				Ratio
	Cancer (adjacent)	Cancer (tumor)	Student's t-test $p \leq$	U-test p	Adjacent/Tumor
Al	19.4±5.6	33.0±7.1	0.157	>0.05	1.70
B	5.80±4.80	2.21±0.52	0.510	>0.05	0.38
Ba	0.50±0.16	1.42±0.35	**0.028**	**≤0.01**	2.84
Ca	917±131	3013±699	**0.0085**	**≤0.01**	3.29
Cl	5339±581	7699±703	**0.015**	**≤0.01**	1.44
Cu	8.08±1.58	14.5±2.6	**0.051**	**≤0.05**	1.79
Fe	256±26	255±27	0.982	>0.05	1.00
I	3183±301	71.8±10.1	**0.00000000002**	**≤0.01**	0.023
K	5847±661	10054±877	**0.00053**	**≤0.01**	1.72
Li	0.0210±0.0100	0.0314±0.0090	0.458	>0.05	1.50
Mg	359±104	478±42	0.301	>0.05	1.33
Mn	1.77±0.40	2.01±0.29	0.629	>0.05	1.14
Na	7726±630	8576±531	0.310	>0.05	1.11
P	9724±2796	10493±866	0.807	>0.05	1.08
S	9725±999	9448±429	0.811	>0.05	0.97
Si	77.3±28.1	143±42	0.214	>0.05	1.85
Sr	1.16±0.14	6.26±1.59	**0.0040**	**≤0.01**	5.40
V	0.082±0.013	0.0904±0.0100	0.620	>0.05	1.11
Zn	111±11	96.9±12.6	0.403	>0.05	0.87

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

Boron

Trace element B is known to influence the activity of many enzymes [59]. Numerous studies have demonstrated beneficial effects of B on human health, including anti-inflammatory stimulus - reduces levels of inflammatory biomarkers, such as high-sensitivity C-reactive protein (hs-CRP) and tumor necrosis factor α (TNF-α); as well as raises levels of antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase [60]. Why B content in TMNs is higher the normal level for thyroid and how an excess of B acts on thyroid gland are still to be cleared.

Calsium, Strontium, and Barium

It was reported that around 40% of TMNs have some type of calcification [61]. Thus, high Ca content in malignant tumor in comparison with thyroid tissue adjacent to tumor may be the result of TMNs calcification. Behavior of Sr and Ba are similar to Ca, because these trace elements are in the same group of the periodic table. Reduced levels of Ba, Ca, and Sr content in thyroid tissue adjacent to tumor in comparison with "normal" thyroid may be explain by mobilization of these ChEs from adjacent tissue for tumor needs.

Chlorine and sodium

Cl and Na are ubiquitous, extracellular electrolytes essential to more than one metabolic pathway. In the body, Cl and Na mostly present as sodium chloride. Therefore, as usual, there is a correlation between Na and Cl contents in tissues and fluids of human body. Because Cl is halogen like I, in the thyroid gland the biological behavior of chloride has to be similar to the biological behavior of iodide. The main source of natural Cl for human body is salt in food and chlorinated drinking water. Environment (air, water and food) polluted by artificial nonorganic Cl-contained compounds, for example such as sodium chlorate ($NaClO_3$), and organic Cl-contained compounds, for example such as polychlorinated biphenyls (PCBs) and dioxin, is other source. There is a clear association between using chlorinated drinking water, levels $NaClO_3$, PCBs and dioxin in environment and thyroid disorders, including cancer [62-66]. Thus, on the one hand, the accumulated data suggest that Cl level in thyroid tissue might be responsible for TMNs 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 [67]. Tumors and adjacent to tumors thyroid tissues can be more vascularized than normal thyroid. Because blood is extracellular liquid, it is possible to speculate that more intensive vascularization could be the reason for elevated levels of Cl and Na in TMNs and adjacent tissue. If that is the case, the equilibrium between Cl and Na increases has to be, however, in comparison with "normal" thyroid the change of Cl level in tumors and adjacent tissue is significantly higher than change of Na level. Thus, it is possible to assume that an excessive accumulation of Cl in thyroid tissue is involved in TMNs etiology. Overall, the elevated levels of Cl in thyroid tissue could possibly be explored as risk factor of TMNs.

Coper

Cu is a ubiquitous element in the human body which plays many roles at different levels. Various Cu-enzymes (such as amine oxidase, ceruloplasmin, cytochrome-c oxidase, dopamine-monooxygenase, extracellular superoxide dismutase, lysyl oxidase, peptidylglycineamidating monoxygenase, Cu/Zn superoxide dismutase, and tyrosinase) mediate the effects of Cu deficiency or excess. Cu excess can have severe negative impacts. Cu generates oxygen radicals and many investigators have hypothesized that excess copper might cause cellular injury via an oxidative pathway, giving rise to enhanced lipid peroxidation, thiol oxidation, and, ultimately, DNA damage [68-70]. Thus, Cu accumulation in thyroid parenchyma with age may be involved in oxidative stress, dwindling gland function, and increasing risk of goiter or cancer [25, 26, 31-34]. The significantly elevated level of Cu in thyroid malignant tumors and tissue adjacent to tumors, observed in the present study, supports this speculation. However, an overall comprehension of Cu homeostasis and physiology, which is not yet acquired, is mandatory to establish Cu exact role in the thyroid malignant tumors etiology and metabolism. Anyway, the accumulation of Cu in neoplastic thyroids could possibly be explored for diagnosis of TMNs.

Iodine

Nowadays it was well established that iodine deficiency or excess has severe consequences on human health and associated with the presence of TMNs [4-8,71-74]. In present study elevated level of I in thyroid tissue adjacent to malignant tumor and drastically reduced I mass fraction in cancerous tissue was found in comparison with "normal" thyroid.

Compared to other soft tissues, the human thyroid gland has higher levels of I, because this element plays an important role in its normal functions, through the production of thyroid hormones (thyroxin and triiodothyronine) which are essential for cellular oxidation, growth, reproduction, and the activity of the central and autonomic nervous system. As was shown in present study, malignant transformation is accompanied by a significant loss of tissue-specific functional features, which leads to a drastically reduction in I content associated with functional characteristics of the human thyroid tissue. Because the malignant part of gland stopped to produce thyroid hormones, the rest "intact" part of thyroid tries to compensate thyroid hormones deficiency and work more intensive than usual. The intensive work may explain elevated level of I in thyroid tissue adjacent to malignant tumor.

Drastically reduced level of I content in cancerous tissue could possibly be explored for differential diagnosis of benign and malignant thyroid nodules, because, as was found in our ealier studies, thyroid benign trasformation (goiter, thyroiditis, and adenoma) is accompanied by a little loss of I accumulation [42-45, 75-80].

Potassium

An uncontrollable cell proliferation characterize the malignant tumors. Therefore, morphological structures of TMNs differ from the structure of normal thyroid parenchyma. Because K is mainly an intracellular electrolyte, an elevated level of K content in cancerous tissue in comparison with "normal" and "adjacent" tissue might reflect increase of ratio "mass of transformed thyroid cell – mass of follicular colloid" in the malignant tumors. Nevertheless, the accumulation of K in neoplastic thyroids could possibly be explored for diagnosis of TMNs.

Magnesium

Mg is abundant in the human body. This element is essential for the functions of more than 300 enzymes (e.g. alkaline phosphatases, ATP-ases, phosphokinases, the oxidative phosphorylation pathway). It plays a crucial role in many cell functions such as energy metabolism, protein and DNA syntheses, and cytoskeleton activation. Moreover, Mg plays a central role in determining the clinical picture associated with thyroid disease [81]. Experimental data have shown that high doses of magnesium increase the activity of the thyroid gland [82]. Magnesium deficiency can influence bioavailability and tissue distribution of selenium which then appears diminished [83]. From these data, one can conclude that Mg is involved in the thyroid function. If so, significant reduction in Mg content cam be associate with TMNs, because malignant transformation is accompanied by a loss of thyroid-specific functional features. However, it is well known that malignant tumors have an usually higher Mg levels than do normal tissues [84-90], possibly caused by the "retention" of Mg by the tumor [91], as a result of the high Mg requirement of growing cells. In addition, cultured proliferating cells have long been known to contain more magnesium than quiescent cells, and experimental conditions that decreased magnesium availability affected cell proliferation rate [92]. Thus, the elevated levels of Mg in neoplastic thyroids could possibly be explored for diagnosis of TMNs.

Manganese

Trace element Mn is a cofactor for numerous enzymes, playing many functional roles in living organisms. The Mn-containing enzyme, manganese superoxide dismutase (Mn-SOD), is the principal antioxidant enzyme which neutralizes the toxic effects of reactive oxygen species. It has been speculated that Mn interferes with thyroid hormone binding, transport, and activity at the tissue level [93]. There is opinion that Mn deficiencies in humans are rare and humans maintain stable tissue levels of this trace element [94]. It was reported that intracellular Mn content was

positively correlated with manganese-containing superoxide dismutase (Mn-SOD), suggesting that the intracellular Mn level is associated with Mn-SOD activity [95]. However, an overall comprehension of Mn homeostasis and physiology, which is not yet acquired, is mandatory to establish Mn exact role in the TMNs etiology and metabolism. Anyway, the accumulation of Mn in neoplastic thyroids could possibly be explored for diagnosis of TMNs.

Phosphorus

P is necessary for several, various biological roles in the signal transduction of cells and energy exchange of human body. About 80%–90% of phosphorus is founded in teeth and bones in the form of hydroxyapatite. Calcium phosphates are one of the main constituents of mineral deposits in aortic wall and tissues [96]. Thus, the high P level in TMNs can be intimately linked with malignant tumor calcification [61].

Sulfur

Proteins contain between 3 and 6% of sulfur amino acids. Sulfur amino acids contribute substantially to the maintenance and integrity of the cellular systems by influencing the cellular redox state and the capacity to detoxify toxic compounds, free radicals and reactive oxygen species (ROS) [97]. ROS are generated during normal cellular activity and may exist in excess in some pathophysiological conditions, such as inflammation. Therefore exploring fundamental aspects of sulfur metabolism such as the antioxidant effects of sulfur-containing amino acids [98] may help elucidate the mechanism by which the S content increases in TMNs. Thus, it might be assumed that the elevated S level in thyroid with TMNs reflects an increase in concentration of ROS in malignant tissue.

Silicon

Si is the second most common ChE in the Earth's crust. This ChE is naturally present in foods as minerals: Si dioxide (SiO_2) and silicates. In potable water Si is mainly present as free orthosilicic acid (H_4SiO_4) [99]. Si is mainly absorbed from the diet. For European population average dietary intake of Si from uncontaminated food and water was estimated as 20–50 mg/day [100]. In such doze Si exerts beneficial effects on the human health, because as was shown in many in vitro and in vivo studies this element is involved in collagen synthesis, bone mineralization, structural integrity of nails, hair and skin [99]. However, over the last five decades Si compounds began widely use in medicine, for example, as oral supplements for improving osteoporosis, hair loss, nail, hair, and skin quality [101,102], as well as Si-containing breast implants [103]. Silicones are included in cosmetic formulations such as hair conditioners, shampoos, and facial creams [102]. Si compounds are also of high importance in food and consumer products [104]. Due to favorable properties like colloidal and chemical stability, the possibility for a covalent surface modification, and a high specific surface area, the application cases for Si- contained particles and Si-based materials have grown rapidly [104]. Thus, as a result of the above the Si intake constantly increases. There are many evidences for harmful effects of excessive Si accumulation in body tissues. For example, it was shown that reactive oxygen species (ROS) are generated on surface of SiO_2 particles distributed in the interstitium [105]. In addition, SiO_2 particles interact with macrophages, which secrete various inflammatory cytokines [106]. The elevated levels of ROS and inflammatory cytokines produce progressive inflammation [106]. Chronic inflammation induced by Si compounds can lead to allergic reaction, deficient humoral immune system, autoimmune diseases, fatal organ dysfunction, fibrosis, benign tumors, anaplastic large T cell lymphoma, and cancer [103-106].

Limitations

This study has several limitations. Firstly, analytical techniques employed in this study measure only nineteen ChE (Al, B, Ba, 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" thyroid and in pathologically altered tissue. Secondly, the sample size of TMNs group was relatively small and prevented investigations of ChEs contents in this group using differentials like gender, histological types of TMNs, tumor functional activity, stage of disease, and dietary habits of patients with TMNs. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on many ChEs level alteration in malignant tumor and thyroid tissue adjacent to tumor and shows the necessity to continue ChEs research of TMNs.

CONCLUSION

In this work, ChEs analysis was carried out in the tissue samples of TMNs using using a combination of non-destructive INAA-SLR and destructive ICP-AES methods. It was shown that this combination is an adequate analytical tool for the non-destructive determination of Al, B, Ba, 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 specimens. It was observed that in malignant tissue the mass fractions of Al, B, Ca, Cl, Cu, K, Mg, Mn, Na, P, S, and Si were 3.14, 4.64, 1.79, 2.26, 3.55, 1.57, 1.61, 1.57, 1.24, 2.45, 1.14, and 2.81 times, respectively, higher whereas mass fraction of I was 25.6 times lower than in the normal thyroid. In a general sense Al, B, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, V, and Zn contents found in the "normal" and "adjacent" groups of thyroid tissue samples were very similar. However, in the "adjacent" group mean mass fractions of Cl and I were approximately 1.6 and 1.7 times, respectively, higher, while mean values of Ba, Ca

and Sr content were 55%, 45%, and 70%, respectively, lower than in the "normal" group. Significant changes were found in tumor ChEs composition in comparison with thyroid tissue adjacent to tumor. In malignant tumor Ca, Cl, Cu, K, and Sr contents were approximately 2.8, 1.4, 1.8, 1.7, and 5.4 times, respectively, higher, while I content was 43 times lower than in "adjacent" group of tissue samples.

Thus, from results obtained, it was possible to conclude that the common characteristics of TMNs in comparison with "normal" thyroid and visually "intact" thyroid tissue adjacent to malignant tumors were elevated levels of Ca, Cl, Cu, and K, as well as drastically reduced level of I. The last finding meant that thyroid tissue adjacent to malignant nodules kept the main function of thyroid gland, while malignantly transformed thyroid cells lost its capacity to accumulate I. Furthermore, the ChEs composition of thyroid tissue adjacent to tumor did not equal ChEs contents of "normal" thyroid and, for example, contents of such elements as Cl and I were approximately 1.6 and 1.7 times, respectively, higher. From this it was supposed that the excessive accumulation of Cl and I by thyroid tissue probably precede the TMNs origination and development. It was also supposed that elevated levels of Al, B, Ca, Cl, Cu, K, Mg, Mn, Na, P, S, and Si, as well as drastically reduced level of me in cancerous tissue could possibly be explored for differential diagnosis of benign and malignant thyroid nodules

ACKNOWLEDGEMENTS

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