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RESEARCH ARTICLE

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Contents of Nineteen Chemical Elements in Thyroid Malignant Nodules and Thyroid Tissue adjacent to Nodules investigated using X-Ray Fluorescence and Neutron Activation Analysis

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Abstract

Background: Thyroid malignant nodules (TMNs) are the most com-mon endocrine cancer and the fifth most frequently occurring type of malignancies. The etiology and pathogenesis of TMNs must be considered as multifactorial. **Objectives**: The present study was performed to clarify the role of some chemical elements (ChEs) in the etiology of these thyroid disorders. **Method**s: Thyroid tissue levels of nineteen ChEs including silver (Ag), 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), scan-dium (Sc), selenium (Se), strontium (Sr), and zinc (Zn) were prospec-tively evaluated in malignant tumor and tissue adjacent to tumor of 41 patients with TMNs. Measurements were performed using a combina-tion of non-destructive methods X-ray fluorescence and instrumental neutron activation analysis with high resolution spectrometry of short-and long-lived radionuclides. Results of the study were additionally compared with previously obtained data for the same ChEs in "normal" thyroid tissue. **Conclusions**: The excessive accumulation of Ag, Hg, I, and Se by thy-roid tissue is likely to precede the TMNs origination and development. Elevated levels of Cl and K, as well as drastically reduced level of I in cancerous tissue could possibly be explored for differential diagnosis of benign and malignant thyroid nodules **Results**: The common characteristics of TMNs in comparison with "normal" thyroid and visually "intact" thyroid tissue adjacent to malignant tumors were elevated levels of Cl and K, as well as drastically reduced level of I. Furthermore, the ChEs composition of thyroid tissue adjacent to tumor did not equal ChEs contents of "normal" thyroid. Moreover, contents of such elements as Ag, Hg, I, and Se in adjacent tissue were higher than in tumor.

Conclusions: The excessive accumulation of Ag, Hg, I, and Se by thyroid tissue is likely to precede the TMNs origination and development. Elevated levels of Cl and K, as well as drastically reduced level of I in cancerous tissue could possibly be explored for differential diagnosis of benign and malignant thyroid nodules

Keywords: Chemical elements; Neutron activation analysis; Thyroid; Thyroid malignant nodules; X-ray fluorescence

1 | 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, thyroiditis, and thyroid adenoma in comparison with normal thyroid was demonstrated (42–46).

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 silver (Ag), 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 nodular and adjacent to nodules tissues of thyroids with TMNs using a non-destructive energy-dispersive X-Ray fluorescent analysis (EDXRF) combined with instrumental neutron activation analysis with high resolution spectrometry of short- and long-lived radionuclides (INAA-SLR and INAA-LLR, respectively), and to compare the levels of these ChEs in two groups (nodular and adjacent to nodules tissues) 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 (42–46).

2 | MATERIAL AND METHODS

All patients with TMNs (n=41, mean age M±SD was 46±15 years, range 16-75) were hospitalized

Supplementary information The online version of this article (https://doi.org/10.52845/JMRHS/2022-5-1-5) contains supplementary material, which is available to authorized users.

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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 (47). 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 (48). 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 subsamples 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 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 (25, 26) and prostate tissue (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 (27, 28), prostate (51, 52), and scalp hair (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 (29, 30), scalp hair (53), and prostate (54).

A dedicated computer program for INAA-SLR and INAA-LLR 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 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 ChEs contents in

malignant and adjacent to tumor tissue of thyroids with TMNs. Data for "normal" thyroid were taken from our previous publications (42–46). 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.

3 | RESULTS

resents 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 Ag, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg,Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction in "tumor" and "adjacent" groups of thyroid tissue samples.

The ratios of means and the comparison of mean values of Ag, 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 "normal" and "tumor", "normal" and "adjacent", and also "adjacent" and "tumor" are presented in Table 2, 3, and 4, respectively.

4 | DISCUSSION

As was shown before (25–30, 50–54) good agreement of the Ag, 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 thyroid tissue samples presented in Tables 1–4.

From Table 2, it is observed that in malignant tissue the mass fraction of Ag, Cl, Co, Cr, Cu, Hg, K, Mg, Na, and Rb are approximately 13, 2.3, 1.4, 1.6, 3.4, 20, 1.6, 1.6, 1.3, and 1.5 times, respectively, higher whereas mass fraction of I is 26 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 Ag, Cl, Co, Cr, Cu, Hg, K, Mg, Na, and Rb contents in thyroid tissue significantly changed. In a general sense Cr, Cu, Fe, K, Mg, Mn, Na, Sc, 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 Ag, Cl, Co, Hg, I, Rb, and Se were approximately 33, 1.6, 1.8, 52, 1.7, 2.3, and 1.3 times, respectively, higher, while mean values of Ca and Sr content were 2 and 4 times lower than in the "normal" group.

Table 1. Some statistical parameters of Ag, 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 cancer (tumor and "intact" thyroid tissue adjacent to tumor)

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Cancer (tumor)	Ag	0.193	0.215	0.041	0.0075	1.02	0.147	0.0080	0.705
	Ca	2397	2368	558	452	8309	1302	467	7428
	Cl	7699	2900	703	4214	14761	7216	4240	13619
	Co	0.0550	0.0309	0.0060	0.0042	0.143	0.0497	0.0159	0.129
	Cr	0.835	0.859	0.157	0.0390	3.50	0.460	0.0941	3.05
	Cu	14.5	9.4	2.6	4.00	32.6	10.9	4.21	31.4
	Fe	243	177	29	55.1	887	200	58.2	679
	Hg	0.824	0.844	0.149	0.0685	3.75	0.475	0.0689	2.85
	I	71.8	62.0	10	2.00	261	62.1	2.93	192
	K	9655	4444	970	1660	19225	8746	3381	19035
	Mg	450	232	51	122	1033	408	126	931
	Mn	1.90	1.41	0.32	0.100	5.79	1.59	0.100	5.37
	Na	8556	2959	646	4083	17284	7264	4704	14543
	Rb	12.6	4.6	0.7	5.50	27.4	11.2	5.84	19.8
	Sb	0.124	0.081	0.015	0.0160	0.381	0.108	0.0174	0.315
	Sc	0.0077	0.0129	0.0020	0.0002	0.0565	0.0023	0.0002	0.0447
	Se	2.04	1.02	0.18	0.143	4.70	1.80	0.663	4.33
	Sr	6.25	7.83	1.63	0.930	30.8	3.00	0.985	25.0
	Zn	89.7	57.6	10.8	36.7	326	67.7	37.7	324
Cancer (adjacent thyroid tissue)	Ag	0.503	0.450	0.103	0.079	2.00	0.303	0.0984	1.53
	Ca	862	560	140	81.0	1909	672	149	1822
	Cl	5339	22512	581	2526	11767	4922	2595	10201
	Co	0.0707	0.0581	0.0120	0.0152	0.205	0.0455	0.0170	0.201
	Cr	0.556	0.468	0.094	0.0512	1.58	0.457	0.0589	1.56
	Cu	8.08	3.15	1.58	4.90	12.1	7.65	5.01	11.9
	Fe	244	137	27	95.2	752	213	104	591
	Hg	2.19	1.92	0.38	0.0160	7.78	1.43	0.158	6.50
	I	3183	1673	301	563	8240	2982	853	7766
	K	5717	2525	652	2097	12681	5429	2466	10953
	Mg	339	407	105	15.0	1412	199	15.0	1287
	Mn	1.72	1.63	0.41	0.410	6.78	1.15	0.429	5.54
	Na	7671	2597	649	3865	14373	7434	4169	13009
	Rb	18.8	17.0	3.3	5.00	67.0	11.9	5.69	65.6
	Sb	0.247	0.416	0.085	0.0069	1.77	0.0634	0.0159	1.38
	Sc	0.0059	0.0134	0.0030	0.0002	0.0539	0.0002	0.0002	0.0442
	Se	3.08	1.67	0.33	0.704	6.91	2.56	0.942	6.89
	Sr	1.16	0.29	0.14	0.83	1.40	1.20	0.84	1.40
	Zn	109	55	11	20.4	272	109	29.1	213

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.

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 Ag, Hg, I, and Se content 2.6, 2.6, 43, and 1.5 times, respectively, lower than in "adjacent" group of tissue samples (Table 4).

Table 2. Differences between mean values (M±SEM) of Ag, 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 normal thyroid and thyroid cancer ((tumor)

Element	Thyroid tissue				Ratio
	Normal thyroid	Cancer (tumor)	Student's t-test $p\leq$	U-test p	Tumor/Normal
Ag	0.0151±0.0016	0.193±0.041	**0.00022**	**=0.01**	12.8
Ca	1711±109	2397±558	0.243	>0.05	1.40
Cl	3400±174	7699±703	**0.000013**	**=0.01**	2.26
Co	0.0399±0.0030	0.0550±0.0060	**0.022**	**=0.01**	1.38
Cr	0.539±0.032	0.835±0.157	0.073	**=0.05**	1.55
Cu	4.23±0.18	14.5±2.6	**0.0019**	**=0.01**	3.43
Fe	223±10	243±29	0.519	>0.05	1.09
Hg	0.0421±0.0041	0.824±0.149	**0.000011**	**=0.01**	19.6
I	1841±107	71.8±10.0	**0.00000000001**	**=0.01**	0.039
K	6071±306	9655±970	**0.0017**	**=0.01**	1.59
Mg	285±17	450±51	**0.0047**	**=0.01**	1.58
Mn	1.35±0.07	1.90±0.32	0.107	>0.05	1.41
Na	6702±178	8556±646	**0.011**	**=0.01**	1.28
Rb	8.16±0.49	12.6±0.7	**0.0000029**	**=0.01**	1.54
Sb	0.111±0.008	0.124±0.015	0.423	>0.05	1.12
Sc	0.0046±0.0008	0.0077±0.0020	0.223	>0.05	1.67
Se	2.32±0.14	2.04±0.18	0.235	>0.05	0.88
Sr	4.55±0.37	6.25±1.63	0.319	>0.05	1.37
Zn	105.1±4.3	89.7±10.8	0.191	>0.05	0.85

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

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.

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 Cl 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). Moreover, contents of such elements as Ag, Hg, I, and Se in adjacent tissue were higher than in tumor (Table 4). From here, the excessive accumulation of Ag, Hg, I, and Se by thyroid tissue is likely to precede the TMNs origination and development.

Table 3. Differences between mean values (M±SEM) of Ag, 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 normal thyroid and "intact" thyroid tissue adjacent to tumor

Element	Thyroid tissue				Ratio
	Normal thyroid	Cancer (adjacent)	Student's t-test $p\leq$	U-test p	Adjacent/Normal
Ag	0.0151±0.0016	0.503±0.103	**0.00017**	**=0.01**	33.3
Ca	1711±109	862±140	**0.000028**	**=0.01**	0.50
Cl	3400±174	5339±581	**0.0054**	**=0.01**	1.57
Co	0.0399±0.0030	0.0707±0.0120	**0.016**	**=0.01**	1.77
Cr	0.539±0.032	0.556±0.094	0.860	>0.05	1.03
Cu	4.23±0.18	8.08±1.58	0.092	>0.05	1.91
Fe	223±10	244±27	0.473	>0.05	1.09
Hg	0.0421±0.0041	2.19±0.38	**0.0000093**	**=0.01**	52.0
I	1841±107	3183±301	**0.00015**	**=0.01**	1.73
K	6071±306	5717±652	0.629	>0.05	0.94
Mg	285±17	339±105	0.617	>0.05	1.19
Mn	1.35±0.07	1.72±0.41	0.391	>0.05	1.27
Na	6702±178	7671±649	0.168	>0.05	1.14
Rb	8.16±0.49	18.8±3.3	**0.0041**	**=0.01**	2.30
Sb	0.111±0.008	0.247±0.085	0.122	>0.05	2.23
Sc	0.0046±0.0008	0.0059±0.0030	0.628	>0.05	1.28
Se	2.32±0.14	3.08±0.33	**0.038**	**=0.01**	1.33
Sr	4.55±0.37	1.16±0.14	**0.00000000001**	**=0.01**	0.25
Zn	105.1±4.3	109±11	0.731	>0.05	1.04

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

Silver

Ag is a TE with no recognized trace metal value in the human body (56). Food is the major intake source of Ag and this metal is authorised as a food additive (E174) in the EU (57). Another source of

Ag is contact with skin and mucosal surfaces because Ag is widely used in different applications (e.g., jewelry, wound dressings, or eye drops) (58). 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 (59). 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 (60). Experimental studies shown that Ag nanoparticles may affect thyroid hormone metabolism (61). More detailed knowledge of the Ag toxicity can lead to a better understanding of the impact on human health, including thyroid function.

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) (63) (64) (65) (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.

Table 4. Differences between mean values (M±SEM) of Ag, 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 cancer and "intact" thyroid tissue adjacent to tumor

Element	Thyroid tissue				Ratio
	Cancer (adjacent)	Cancer (tumor)	Student's t-test $p\leq$	U-test p	Adjacent/Tumor
Ag	0.503±0.103	0.193±0.041	**0.010**	**=0.01**	0.38
Ca	862±140	2397±558	**0.015**	**=0.01**	2.78
Cl	5339±581	7699±703	**0.015**	**=0.01**	1.44
Co	0.0707±0.0120	0.0550±0.0060	0.232	>0.05	0.78
Cr	0.556±0.094	0.835±0.157	0.133	>0.05	1.50
Cu	8.08±1.58	14.5±2.6	0.051	**=0.05**	1.79
Fe	244±27	243±29	0.983	>0.05	1.00
Hg	2.19±0.38	0.824±0.149	**0.0029**	**=0.01**	0.38
I	3183±301	71.8±10.0	**0.00000000001**	**=0.01**	0.023
K	5717±652	9655±970	**0.0019**	**=0.01**	1.69
Mg	339±105	450±51	0.351	>0.05	1.33
Mn	1.72±0.41	1.90±0.32	0.729	>0.05	1.10
Na	7671±649	8556±646	0.340	>0.05	1.12
Rb	18.8±3.3	12.6±0.7	0.082	>0.05	0.67
Sb	0.247±0.085	0.124±0.015	0.166	>0.05	0.50
Sc	0.0059±0.0030	0.0077±0.0020	0.624	>0.05	1.31
Se	3.08±0.33	2.04±0.18	**0.0084**	**=0.01**	0.66
Sr	1.16±0.14	6.25±1.63	**0.0051**	**=0.01**	5.39
Zn	109±11	89.7±10.8	0.206	>0.05	0.82

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

Cobalt

Health effects of high Co occupational, environmental, dietary and medical exposure are characterized by a complex clinical syndrome, mainly including neurological, cardiovascular and endocrine deficits, including hypothyroidism (68, 69). Co is genotoxic and carcinogenic, mainly caused by oxidative DNA damage by reactive oxygen species, perhaps combined with inhibition of DNA repair (70). In our previous studies it was found a significant age-related increase of Co content in female thyroid (29). Therefore, a goitrogenic and, probably, carcinogenic

effect of excessive Co level in the thyroid of old females was assumed. Elevated level of Co in TMNs, observed in the present study, supports this conclusion. Anyway, the accumulation of Co in malignant thyroid tumors could possibly be explored for diagnosis of TMNs.

Chromium

The general population can be exposed to low levels of Cr primarily through consumption of food and to a lesser degree through inhalation of ambient air and ingestion of drinking water (71). 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 (72). The lung cancer risk is prevalent in pigment chromate handlers, ferrochromium production workers, stainless steel welders, and chrome-platers (73). 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 (74). Many other animal experiments and in vitro studies demonstrate also that Cr can induce oxidative stress and exert cytotoxic effects (75). 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 (73). Anyway, the accumulation of Cr in malignant thyroid tumors could possibly be explored for diagnosis 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 (76–78). 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.

Mercury

In the general population, potential sources of Hg exposure include the inhalation of this metal vapor in the air, ingestion of contaminated foods and drinking water, and exposure to dental amalgam through dental care (79).Hg is one of the most dangerous environmental pollutants (80). 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 (81, 82). Moreover, Hg was classified as certain or probable carcinogen by the International Agency for Research on Cancer (72). For example, in Hg polluted area thyroid cancer incidence was almost 2 times higher than in adjacent control areas (83).

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, 84–87). 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 (42–46).

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 (88). Experimental data have shown that high doses of magnesium increase the activity of the thyroid gland (89). Magnesium deficiency can influence bioavailability and tissue distribution of selenium which then appears diminished (90). 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 (91–97), possibly caused by the "retention" of Mg by the tumor (98), 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 (99). Thus, the elevated levels of Mg in neoplastic thyroids could possibly be explored for diagnosis of TMNs.

Rubidium

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. The sourse of Rb elevated level in tumor and adjacent to tumor 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 (100). The sourse of Rb increase in TMNs 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 (101). It was found also that Rb has some function in immune responce (102) and that elevated concentration of Rb could modulate proliferative responses of the cell, as was shown for

bone marrow leukocytes (103).These data partially clarify the possible role of Rb in etiology and pathogenesis of TMNs.

Calsium and Strontium

It was reported that around 40% of TMNs have some type of calcification (104). 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 is similar to Ca, because this trace element is in the same group of the periodic table.

Selinium

The high level of Se content found just in thyroid tissue adjacent to malignant tumor cannot be regarded as pure chance. The seleno-protein characterized as Se-dependent glutathione peroxidase (Se-GSH-Px) is involved in protecting cells from peroxidative damage. This enzyme may reduce tissue concentration of free radicals and hydroperoxides. It is particular important for the thyroid gland, because thyroidal functions involve oxidation of iodide, which is incorporated into thyreoglobulin, the precursor of the thyroid hormones. For oxidation of iodide thyroidal cells produce a specific thyroid peroxidase using of physiologically generated hydrogen-peroxide (H_2O_2) as a cofactor (105). It follows that the thyroid parenchyma must be continuously exposed to a physiological generation of H_2O_2 and in normal conditions must be a balance between levels of Se (as Se-GSH-Px) and H_2O_2. The elevated level of Se in thyroid tissue adjacent to malignant nodules was accompanied excessive accumulation of Ag, Co, Hg, I, and Rb in comparison with "normal" values for these elements. Moreover, contents of Ag, Co, Hg, I, and Rb in adjacent tissue were higher than in malignant nodules. Thus, it might be assumed that the elevated level of Se is reaction of adjacent tissue on an increase in concentration of free radicals and hydroperoxides in thyroid gland and that this increase preceded the TMNs origination and development.

5 | LIMITATIONS

This study has several limitations. Firstly, analytical techniques employed in this study measure only nineteen ChE (Ag, 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 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.

6 | CONCLUSION

In this work, ChEs analysis was carried out in the tissue samples of TBNs using a combination of three non-destructive methods: EDXRF, INAA-SLR and INAA-LLR. It was shown that this combination is an adequate analytical tool for the non-destructive determination of Ag, 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 malignant tissue the mass fraction of Ag, Cl, Co, Cr, Cu, Hg, K, Mg, Na, and Rb are approximately 13, 2.3, 1.4, 1.6, 3.4, 20, 1.6, 1.6, 1.3, and 1.5 times, respectively, higher whereas mass fraction of I is 26 times lower than in the normal thyroid. In a general sense Cr, Cu, Fe, K, Mg, Mn, Na, Sc, 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 Ag, Cl, Co, Hg, I, Rb, and Se were approximately 33, 1.6, 1.8, 52, 1.7, 2.3, and 1.3 times, respectively, higher, while mean values of Ca and Sr content were 2 and 4 times 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 Ag, Hg, I, and Se content 2.6, 2.6, 43, and 1.5 times, respectively, 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 Cl and K, as well as drastically reduced level of I. Furthermore, the ChEs composition of thyroid tissue adjacent to tumor did not equal ChEs contents of "normal" thyroid. Moreover, contents of such elements as Ag, Hg, I, and Se in adjacent tissue were higher than in tumor. From here, the excessive accumulation of Ag, Hg, I, and Se by thyroid tissue is likely to precede the TMNs origination and development

It was supposed that elevated levels of Cl and K, as well as drastically reduced level of I in cancerous tissue could possibly be explored for differential diagnosis of benign and malignant thyroid nodules

Declaration of Conflicting Interests

The author has not declared any conflict of interests.

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