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Content of 31 trace elements in thyroid malignant nodules and thyroid tissue adjacent to nodules investigated using neutron activation analysis and inductively coupled plasma mass 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. Women are at particular risk for this thyroid disease The etiology and pathogenesis of TMNs must be considered as multifactorial. The present study was performed to clarify the role of some trace elements (TEs) in the etiology of these thyroid disorders. Thyroid tissue levels of silver (Ag), aluminum (Al), boron (B),, beryllium (Be), bismuth (Bi), cadmium (Cd), cerium (Ce), cobalt (Co), chromium (Cr), cesium (Cs), iron (Fe), gallium (Ga), mercury (Hg), lanthanum (La), lithium (Li), manganese (Mn), molybdenum (Mo), neodymium (Nd), nickel (Ni), lead (Pb), praseodymium (Pr), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), samarium (Sm), tin (Sn), thallium (Tl), uranium (U), yttrium (Y), and zinc (Zn) were prospectively evaluated in malignant tumor and thyroid tissue adjacent to tumor of 41 patients with TMNs. Measurements were performed using a combination of non-destructive instrumental neutron activation analysis and destructive method such as inductively coupled plasma mass spectrometry. Results of the study were additionally compared with previously obtained data for the same TEs in "normal" thyroid tissue. It was observed that main characteristics of TMNs in comparison with "normal" thyroid were significantly elevated levels of Ag, Al, B, Bi, Ce, Cr, Hg, Mn, Mo, Ni, Pb, Rb, Sm, and Tl. Furthermore, the TEs composition of thyroid tissue adjacent to tumor did not equal TEs contents of "normal" thyroid. Moreover, contents of such elements as Ag, Hg, and Se in adjacent tissue were higher than in tumor. Thus, from results obtained, it was possible to conclude that at least the excessive accumulation of Ag, Hg, and Se by thyroid tissue is likely to precede the TMNs origination and development. Elevated levels of Ag, Al, B, Bi, Ce, Cr, Hg, Mn, Mo, Ni, Pb, Rb, Sm, and Tl in nodular tissue could possibly be explored for differential diagnosis of benign and malignant thyroid nodules.

Keywords: Thyroid; Thyroid malignant nodules; Trace elements; Neutron activation analysis

1. Introduction

Thyroid malignant nodules (TMNs) are the most common endocrine cancer and the fifth most frequently occurring type of malignancies [1-3]. Women are at particular risk for this thyroid disease with 22.2/100,000 individuals affected every year [2]. 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,

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environmental, and occupational factors are associated with the TMNs incidence [9-11]. Among these factors a disturbance of evolutionary stable input of many trace elements (TEs) in human body after industrial revolution plays a significant role in etiology of TMNs [12].

Besides iodine, many other TEs have also essential physiological functions [13]. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of TEs depend on tissue-specific need or tolerance, respectively [13]. Excessive accumulation or an imbalance of the TEs 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 TEs 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 TEs content with age in the thyroid of males and females were studied and age- and gender-dependence of some TEs was observed [25-41]. Furthermore, a significant difference between some TEs contents in colloid goiter, thyroiditis, and thyroid adenoma in comparison with normal thyroid was demonstrated [42-49].

To date, the etiology and pathogenesis of TMNs must be considered as multifactorial. The present study was performed to find out differences in TEs contents between the group of cancerous tissue and thyroid visually intact tissue adjacent to tumor, as well as to clarify the role of some TEs 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), aluminum (Al), boron (B),, beryllium (Be), bismuth (Bi), cadmium (Cd), cerium (Ce), cobalt (Co), chromium (Cr), cesium (Cs), iron (Fe), gallium (Ga), mercury (Hg), lanthanum (La), lithium (Li), manganese (Mn), molybdenum (Mo), neodymium (Nd), nickel (Ni), lead (Pb), praseodymium (Pr), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), samarium (Sm), tin (Sn), thallium (Tl), uranium (U), yttrium (Y), and zinc (Zn) in tumor and adjacent to tumor tissues of thyroids with TMNs using a combination of non-destructive instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR) and destructive method such as inductively coupled plasma mass spectrometry (ICP-MS), and to compare the levels of these TEs in two groups (tumor and thyroid tissues adjacent to tumor) of the cohort of TMNs samples. Moreover, for understanding a possible role of TEs in etiology and pathogenesis of TMNs results of the study were compared with previously obtained data for the same TEs in "normal" thyroid tissue [42-49]

2. 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 TEs of stainless steel [50]. One was used for morphological study while the other was intended for TEs analysis. After the samples intended for TEs analysis were weighed, they were freeze-dried and homogenized [51].

The pounded samples weighing about 10 mg (for biopsy) and 100 mg (for resected materials) were used for TEs measurement by INAA-LLR. The content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn were determined by INAA-LLR using a vertical channel of the Water-Water-Research nuclear reactor (Branch of Karpov Institute, Obninsk). After non-

destructive INAA-LLR investigation the thyroid samples were used for ICP-MS. The samples were decomposed in autoclaves and aliquots of solutions were used to determine the Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Fe, Ga, Hg, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tl, U, Y, and Zn mass fractions by ICP-MS using an ICP-MS Thermo-Fisher "X-7" Spectrometer (Thermo Electron, USA). Information detailing with the NAA-LLR and ICP-MS methods used and other details of the analysis were presented in our earlier publications concerning TE contents in human thyroid {29,30,35], prostate [52-57], and scalp hair [58].

To determine contents of the TEs by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [59]. 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-LLR mode optimization was used [60]. All thyroid samples were prepared in duplicate, and mean values of TEs contents were used in final calculation. Mean values of TEs contents were used in final calculation for the Ag, Co, Cr, Hg, Rb, Sb, Se, and Zn mass fractions measured by INAA-LLR and ICP-MS methods. Using Microsoft Office Excel software, a summary of the statistics, including, arithmetic mean, standard deviation of mean, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for TEs contents in nodular and adjacent tissue of thyroids with TMNs. Data for "normal" thyroid were taken from our previous publications [42-49]. 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

Table 1 presents certain statistical parameters (arithmetic mean, standard deviation of mean, minimal and maximal values) of the Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Fe, Ga, Hg, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tl, U, Y, 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, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Fe, Ga, Hg, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tl, U, Y, 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 31 trace element mass fraction (mg/kg, dry mass basis) in the thyroid malignant nodules (TMN) and thyroid tissue adjacent to tumor

Element	Thyroid malignant nodules (tumor)			Thyroid tissue adjacent to tumor		
	M	SD	Range	M	SD	Range
Ag	0.139	0.141	0.00750-0.536	0.432	0.291	0.00790-1.00
Al	33.0	25.5	4.50-96.5	19.4	11.1	7.10-32.9
B	2.21	1.89	1.00-5.6	5.80	9.60	1.00-20.2
Be	0.00047	0.00013	0.000200-0.000720	<0.0002	-	-
Bi	0.067	0.083	0.00480-0.335	0.082	0.039	0.0427-0.136
Cd	1.13	1.82	0.0290-6.83	6.83	12.5	0.059-25.5
Ce	0.0277	0.0275	0.00470-0.0874	0.0085	0.0061	0.00490-0.0176
Co	0.0499	0.0292	0.00420-0.143	0.068	0.058	0.0152-0.205
Cr	0.847	0.811	0.0390-3.50	0.642	0.491	0.0512-1.58
Cs	0.0298	0.0287	0.00660-0.112	0.0166	0.0057	0.0102-0.0240
Fe	255	168	60.6-880	256	133	109-752
Ga	0.0342	0.0111	0.0200-0.0640	0.0273	0.0159	0.0100-0.0470

Hg	0.915	0.826	0.0685-3.75	2.24	1.87	0.253-7.78
La	0.0134	0.0124	0.00430-0.0443	0.0043	0.0029	0.00210-0.00760
Li	0.0315	0.0307	0.00780-0.111	0.021	0.020	0.0096-0.0514
Mn	2.01	1.34	0.100-5.95	1.77	1.60	0.410-6.78
Mo	0.292	0.112	0.0936-0.534	0.199	0.060	0.129-0.270
Nd	0.0156	0.0143	0.00330-0.0412	0.0028	0.0029	0.00100-0.00710
Ni	4.38	2.24	0.270-7.30	2.78	1.65	1.10-4.60
Pb	1.14	1.16	0.240-4.44	0.69	0.87	0.220-2.00
Pr	0.0078	0.0130	0.000920-0.0463	0.00107	0.00060	0.00051-0.00170
Rb	12.65	4.87	5.10-27.4	18.9	17.0	5.00-67.0
Sb	0.107	0.075	0.0160-0.334	0.248	0.415	0.00690-1.77
Sc	0.0077	0.0129	0.000200-0.0565	0.0059	0.0134	0.000200-0.0539
Se	2.04	1.06	0.143-4.80	3.08	1.67	0.704-6.91
Sm	0.00194	0.00174	0.000500-0.00670	<0.0004	-	-
Sn	0.0697	0.0487	0.0138-0.182	0.071	0.096	0.0126-0.214
Tl	0.00307	0.00197	0.000600-0.00700	0.0034	0.0021	0.00120-0.00600
U	0.00514	0.01109	0.000550-0.0326	0.00115	0.00092	0.000500-0.00180
Y	0.0123	0.0117	0.00230-0.0343	0.0039	0.0022	0.00230-0.00540
Zn	96.9	80.0	28.7-375	111	55	20.4-272

M – arithmetic mean, SD – standard deviation, Range – min-max values

4. Discussion

As was shown before [29,30,35,52-58] good agreement of the 50 TE mass fractions in CRM IAEA H-4, INCT-SBF-4, INCT-TL-1, and INCT-MPH-2 samples determined by both INAA-LLR and ICP-MS 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 was observed that in malignant tissue the mass fraction of Ag, Al, B, Bi, Ce, Cr, Hg, Mn, Mo, Ni, Pb, Rb, Sm, and Tl were approximately 11, 3.1, 4.6, 9.3, 3.5, 3.7, 17, 1.6, 3.5, 9.8, 4.9, 1.7, 3.8, and 3.3 times, respectively, higher, while Sc content 3.4 times lower than in normal tissues of the thyroid. In a general sense Al, B, Be, Cd, Ce, Cr, Cs, Fe, Ga, La, Li, Mn, Nd, Pb, Pr, Sb, Sm, Sn, Tl, U, Y, and Zn contents found in the "normal" and "adjacent" groups of thyroid tissue samples were similar (Table 3). However, in the "adjacent" group mean mass fractions of Ag, Bi, Co, Hg, Mo, Ni, Rb, and Se were approximately 33, 11, 1.7, 41, 2.4, 6.2, 2.5, and 1.4 times, respectively, higher, than in the "normal" group of samples. Significant reduced levels of tumor TEs in comparison with thyroid tissue adjacent to tumor were found for Ag, Hg, and Se. In malignant tumor Ag, Hg, and Se contents were approximately 3.1, 2.4, and 1.5 times, respectively, lower than in "adjacent" group of tissue samples (Table 4).

Characteristically, elevated or reduced levels of TEs 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 TEs in affected thyroid tissues researchers try to determine the role of the deficiency or excess of each TE in the etiology and pathogenesis of thyroid diseases. In our opinion, abnormal levels of many TEs 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 TEs level in pathologically altered tissue is the reason for alterations or vice versa. According to our opinion, investigation of TEs contents in thyroid tissue adjacent to malignant nodules and comparison obtained results with TEs 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 2 Differences between mean values (M±SEM) of trace element mass fractions (mg/kg, dry mass basis) in normal thyroid and thyroid malignant nodules (TMN tumor)

Element	Thyroid tissue				Ratio
	Normal thyroid (NT)	TMN tumor	Student's t-test $p\leq$	U-test p	tumor/NT
Ag	0.0133±0.0013	0.139±0.028	**0.00013**	**≤0.01**	10.5
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
Be	0.00052±0.00008	0.00047±0.00004	0.589	>0.05	0.90
Bi	0.0072±0.0022	0.067±0.023	**0.024**	**≤0.01**	9.31
Cd	2.08±0.27	1.13±0.49	0.103	>0.05	0.54
Ce	0.0080±0.0011	0.0277±0.0080	**0.025**	**≤0.01**	3.46
Co	0.0390±0.0031	0.0499±0.0050	0.082	>0.05	1.28
Cr	0.495±0.031	0.847±0.148	**0.026**	**≤0.01**	3.74
Cs	0.0245±0.0022	0.0298±0.0090	0.573	>0.05	1.22
Fe	222.8±9.6	255±27	0.270	>0.05	1.14
Ga	0.0316±0.0021	0.0342±0.0030	0.519	>0.05	1.08
Hg	0.0543±0.0043	0.915±0.146	**0.000001**	**≤0.01**	16.9
La	0.00475±0.00062	0.0134±0.0040	0.070	>0.05	2.82
Li	0.0208±0.0022	0.0315±0.0090	0.265	>0.05	1.51
Mn	1.28±0.07	2.01±0.29	**0.025**	**≤0.01**	1.57
Mo	0.0836±0.0062	0.292±0.031	**0.000017**	**≤0.01**	3.49
Nd	0.0041±0.0004	0.0156±0.0050	0.056	>0.05	3.80
Ni	0.449±0.046	4.38±0.65	**0.000079**	**≤0.01**	9.76
Pb	0.233±0.033	1.14±0.33	**0.020**	**≤0.01**	4.89
Pr	0.00107±0.00011	0.0078±0.0040	0.115	>0.05	7.29
Rb	7.54±0.39	12.65±0.76	**0.000001**	**≤0.01**	1.68
Sb	0.0947±0.0075	0.107±0.014	0.388	>0.05	1.13
Sc	0.0268±0.0060	0.0077±0.0020	**0.0053**	**≤0.01**	0.29
Se	2.22±0.14	2.04±0.19	0.457	>0.05	0.92
Sm	0.000507±0.000064	0.00194±0.00048	**0.012**	**≤0.01**	3,83
Sn	0.0777±0.0091	0.0697±0.0140	0.627	>0.05	0.90
Tl	0.00093±.0.00007	0.00307±0.00100	**0.0020**	**≤0.01**	3.29
U	0.000443±0.000059	0.00514±0.00400	0.270	>0.05	11.6
Y	0.00260±0.00032	0.0123±0.0040	0.071	>0.05	4.73
Zn	94.8±4.2	96.9±12.6	0.877	>0.05	1.02

M – Arithmetic mean, SEM – standard error of mean, statistically significant values are in bold

Table 3 Differences between mean values (M±SEM) of trace element mass fractions (mg/kg, dry mass basis) in normal thyroid (NT) and thyroid tissue adjacent to thyroid malignant nodules (TMN)

Element	Thyroid tissue				Ratio
	Normal thyroid (NT)	TMN adjacent	Student's t-test $p\leq$	U-test p	adjacent/NT
Ag	0.0133±0.0013	0.432±0.067	**0.0000064**	**≤0.01**	32.5
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
Be	0.00052±0.00008	<0.0002	-	-	-
Bi	0.0072±0.0022	0.082±0.020	**0.031**	**≤0.01**	11.4
Cd	2.08±0.27	6.83±6.2	0.501	>0.05	3.28
Ce	0.0080±0.0011	0.0085±0.0030	0.883	>0.05	1.06
Co	0.039±0.003	0.068±0.012	**0.021**	**≤0.01**	1.74
Cr	0.495±0.031	0.642±0.098	0.164	>0.05	1.30
Cs	0.0245±0.0022	0.0166±0.0030	0.057	>0.05	0.68
Fe	222.8±9.6	256±26	0.244	>0.05	1.15
Ga	0.0316±0.0021	0.0273±0.0080	0.623	>0.05	0.86
Hg	0.0543±0.0043	2.24±0.37	**0.0000049**	**≤0.01**	41.3
La	0.0048±0.0006	0.0043±0.0020	0.810	>0.05	0.90
Li	0.0208±0.0022	0.0210±0.0100	0.989	>0.05	1.01
Mn	1.28±0.07	1.77±0.40	0.252	>0.05	1.38
Mo	0.0836±0.0062	0.199±0.030	**0.028**	**≤0.01**	2.38
Nd	0.0041±0.0004	0.0028±0.0010	0.457	>0.05	0.68
Ni	0.449±0.046	2.78±0.82	0.066	**≤0.05**	6.19
Pb	0.233±0.033	0.69±0.44	0.373	>0.05	2.96
Pr	0.00107±0.00011	0.00107±0.00035	0.999	>0.05	1.00
Rb	7.54±0.39	18.9±3.3	**0.0024**	**≤0.01**	2.51
Sb	0.0947±0.0075	0.248±0.085	0.085	>0.05	2.62
Sc	0.0268±0.0060	0.0059±0.0030	**0.0029**	**≤0.01**	0.22
Se	2.22±0.14	3.08±0.33	**0.020**	**≤0.01**	1.39
Sm	0.000507±0.000064	<0.0004	-	-	-
Sn	0.078±0.009	0.071±0.048	0.897	>0.05	0.91
Tl	0.00093±.0.00007	0.00340±0.00100	0.099	>0.05	3.66
U	0.00044±0.00006	0.00115±0.00065	0.472	>0.05	2.61
Y	0.0026±0.0003	0.0039±0.0016	0.565	>0.05	1.50
Zn	94.8±4.2	111±11	0.175	>0.05	1.17

M – Arithmetic mean, SEM – standard error of mean, statistically significant values are in bold

Table 4 Differences between mean values (M±SEM) of trace element mass fractions (mg/kg, dry mass basis) in thyroid malignant nodules (TMN) and thyroid tissue adjacent to nodules

Element	Thyroid tissue				Ratio
	TMN adjacent	TMN tumor	Student's t-test $p\leq$	U-test p	tumor/ adjacent
Ag	0.432±0.067	0.139±0.028	**0.00045**	**≤0.01**	0.32
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
Be	<0.0002	0.00047±0.00004	-	-	-
Bi	0.082±0.020	0.067±0.023	0.625	>0.05	0.82
Cd	6.83±6.2	1.13±0.49	0.428	>0.05	0.17
Ce	0.0085±0.0030	0.0277±0.0080	**0.034**	**≤0.01**	3.26
Co	0.068±0.012	0.050±0.005	0.158	>0.05	0.74
Cr	0.642±0.098	0.847±0.148	0.253	>0.05	1.32
Cs	0.0166±0.0030	0.0298±0.0090	0.173	>0.05	1.80
Fe	256±26	255±27	0.982	>0.05	1.00
Ga	0.0273±0.0080	0.0342±0.0030	0.467	>0.05	1.25
Hg	2.24±0.37	0.915±0.146	**0.0024**	**≤0.01**	0.41
La	0.0043±0.0020	0.0134±0.0040	0.068	>0.05	3.12
Li	0.0210±0.0100	0.0315±0.0090	0.458	>0.05	1.50
Mn	1.77±0.40	2.01±0.29	0.629	>0.05	1.14
Mo	0.199±0.030	0.292±0.031	0.056	>0.05	1.47
Nd	0.0028±0.0010	0.0156±0.0050	**0.041**	**≤0.01**	5.57
Ni	2.78±0.82	4.38±0.65	0.169	>0.05	1.58
Pb	0.69±0.44	1.14±0.33	0.437	>0.05	1.65
Pr	0.0011±0.0004	0.0078±0.0040	0.116	>0.05	7.29
Rb	18.9±3.3	12.7±0.8	0.081	>0.05	0.67
Sb	0.248±0.085	0.107±0.014	0.118	>0.05	0.43
Sc	0.0059±0.0030	0.0077±0.0020	0.624	>0.05	1.31
Se	3.08±0.33	2.04±0.19	**0.0093**	**≤0.01**	0.66
Sm	<0.0004	0.00194±0.00048	-	-	-
Sn	0.071±0.048	0.070±0.014	0.982	>0.05	0.99
Tl	0.0034±0.00100	0.0031±0.0010	0.805	>0.05	0.91
U	0.00115±0.00065	0.00514±0.00400	0.348	>0.05	4.47
Y	0.0039±0.0016	0.0123±0.0040	0.116	>0.05	3.15
Zn	111±11	96.9±12.6	0.403	>0.05	0.87

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 main characteristics of TMNs in comparison with "normal" thyroid were significantly elevated levels of Ag, Al, B, Bi, Ce, Cr, Hg, Mn, Mo, Ni, Pb, Rb, Sm, and Tl (Table 2). Furthermore, the TEs composition of thyroid tissue adjacent to tumor did not equal TEs contents of "normal" thyroid

(Table 3). Moreover, contents of such elements as Ag, Hg, and Se in adjacent tissue were higher than in tumor (Table 4). From this it follows that at least the excessive accumulation of Ag, Hg, and Se by thyroid tissue is likely to precede the TMNs origination and development.

4.1. Silver

Ag is a trace metal with no recognized value in the human body [61]. Food is the major intake source of Ag and this metal is authorised as a food additive (E174) in the EU [62]. 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) [63]. 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 [64]. 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 [65]. Experimental studies shown that Ag nanoparticles may affect thyroid hormone metabolism [66]. More detailed knowledge of the Ag toxicity can lead to a better understanding of the impact on human health, including thyroid function.

4.2. 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 [67]. 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 [67]. Furthermore, it was shown in experimental and epidemiological studies that Al can affect thyroid iodide uptake and hormones secretion [68,69].

4.3. Boron

Trace element B is known to influence the activity of many enzymes [70]. 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 [71]. 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.

4.4. Bismuth

Trace metal Bi is the heaviest stable element. There is only limited information on Bi compounds effects and fate in the human body but Bi is seen as the least toxic heavy metal for humans. It is widely used in medical applications for its good antibacterial properties [72]. Until now Bi is not considered a human carcinogen. However, in recent publication Bi effects on thyroid function was shown [73]. Moreover, it was found that Bi replaces catalytic or structural metals such as iron, nickel and zinc in metalloproteins and the inorganic Bi derivatives can cause DNA single-strand breaks [74]. Why Bi content in TMNs is higher than normal level and how an excess of Bi acts on thyroid are still to be cleared.

4.5. Cerium

Ce is one among rare earth elements (REEs). REEs are a series of 17 chemical elements. They include scandium (Sc), yttrium (Y), lanthanum (La) and the lanthanide series from Ce to lutetium (Lu), in the periodic table. Their adverse health effects, including toxicity affected embryogenesis, fertilization, cytogenetic and redox endpoints, are well known [75,76]. However, the available information is insufficient to ascertain the mutagenicity and carcinogenicity of Ce or Ce compounds. Why Ce content in TMNs is higher than normal level and how an excess of Ce acts on thyroid are still to be cleared.

4.6. 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 [77,78]. Co is genotoxic and carcinogenic, mainly caused by oxidative DNA damage by reactive oxygen species, perhaps combined with inhibition of DNA repair [79]. 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.

4.7. 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 [80]. 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 [81]. The lung cancer risk is prevalent in pigment chromate handlers, ferrochromium production workers, stainless steel welders, and chrome-platers [82]. 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 [83]. Many other animal experiments and in vitro studies demonstrate also that Cr can induce oxidative stress and exert cytotoxic effects [84]. 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 [82].

4.8. 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 [85]. Hg is one of the most dangerous environmental pollutants [86]. 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 [87,88]. Moreover, Hg was classified as certain or probable carcinogen by the International Agency for Research on Cancer [81]. For example, in Hg polluted area thyroid cancer incidence was almost 2 times higher than in adjacent control areas [89].

4.9. 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 [90]. There is opinion that Mn deficiencies in humans are rare and humans maintain stable tissue levels of this trace element [91]. 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 [92]. However, an overall comprehension of Mn homeostasis and physiology, which is not yet acquired, is mandatory to establish Mn exact role in the thyroid malignant tumors etiology and metabolism.

4.10. Molybdenum

Mo is an essential trace element and part of a complex called molybdenum co-factor, which is required for three mammalian enzymes—xanthine oxidase, aldehyde oxidase and sulphite oxidase [93]. Mo-dependent enzymes operate in the oxidative system of thyroid epithelial cells and also play role in the release of T_3 from the thyroid gland. However, there is data that even a slight increase Mo in the diet may accelerate and/or promote the process of thyroid cell transformation, thus acting as a tumor-promoting agent rather than a carcinogen [94]. Why Mo content in TMNs is higher than normal level and how an excess of Mo acts on thyroid are still to be cleared.

4.11. Nickel

The peripheral connection between inorganic Ni and autoimmune thyroid diseases was mentioned in the literature [95]. Moreover, well known that human exposure to highly nickel-polluted environments, such as those associated with nickel refining, electroplating, and welding, has the potential to produce not only thyroid diseases but a variety of pathologic effects. Among them are skin allergies, lung fibrosis, and cancer of the respiratory tract [96]. The exact mechanisms of nickel-induced carcinogenesis are not known. However, there is data that Ni-induced oxidative stress triggers cell proliferation, a process of great significance for cancer [97].

4.12. Lead

Pb is highly cytotoxic. It affects hormonal secretion and hormonal-induced cell responses. The epidemiological evidence for an association between Pb exposures and human cancer risk has been strengthened by many studies [98]. Why Pb content in TMNs is higher than normal level and how an excess of Pb acts on thyroid are still to be cleared.

4.13. 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 [99]. 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 [100]. It was found also that Rb has some function in immune responce [101] and that elevated concentration of Rb could modulate proliferative responses of the cell, as was shown for bone marrow leukocytes [102].These data partially clarify the possible role of Rb in etiology and pathogenesis of TMNs.

4.14. Scandium and Samarium

Sc and Sm are REEs (see, **Cerium**). REEs are not described as essential for humans, because no biochemical function has been directly connected to it. At this stage of our knowledge, no doubt that REEs overload negatively impact human health [75,76]. Why Sc content in TMNs is lower while Sm content is higher than normal level and how a deficiency of Sc and an excess of Sm acts on thyroid are still to be cleared.

4.15. 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 [103]. 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.

4.16. Thallium

Tl is a ubiquitous natural metal considered as the most toxic among trace elements. Moreover, Tl is a suspected human carcinogen [104**]**. Why Tl content in TMNs is higher than normal level and how an excess of Tl acts on thyroid are still to be cleared.

Our findings show that mass fraction of Ag, Al, B, Bi, Ce, Cr, Hg, Mn, Mo, Ni, Pb, Rb, Sm, and Tl are significantly higher than in normal thyroid tissues (Table 2). Thus, it is plausible to assume that levels of these TEs in thyroid tissue can be used as tumor markers. However, this subject needs in additional studies.

4.17. Limitations

This study has several limitations. Firstly, analytical techniques employed in this study measure only 31 TEs (Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Fe, Ga, Hg, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tl, U, Y, and Zn) mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of TEs investigated in “normal” thyroid and in pathologically altered tissue. Secondly, the sample size of TMNs group was relatively small and prevented investigations of TEs 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 TEs level alteration in malignant tumor and thyroid tissue adjacent to tumor and shows the necessity to continue TEs research of TMNs.

5. Conclusion

In this work, TEs analysis was carried out in the tissue samples of TMNs (nodular and thyroid tissue adjacent to nodules) using a combination of non-destructive INAA-LLR and destructive ICP-MS methods. It was shown that this combination is an adequate analytical tool for the determination of 31 TEs 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, Al, B, Bi, Ce, Cr, Hg, Mn, Mo, Ni, Pb, Rb, Sm, and Tl were approximately 11, 3.1, 4.6, 9.3, 3.5, 3.7, 17, 1.6, 3.5, 9.8, 4.9, 1.7, 3.8, and 3.3 times, respectively, higher, while Sc content 3.4 times lower than in normal tissues of the thyroid. In the "adjacent" group mean mass fractions of Ag, Bi, Co, Hg, Mo, Ni, Rb, and Se were approximately 33, 11, 1.7, 41, 2.4, 6.2, 2.5, and 1.4 times, respectively, higher, than in the "normal" group of samples. In malignant tumor Ag, Hg, and Se contents were approximately 3.1, 2.4, and 1.5 times, respectively, lower than in "adjacent" group of tissue samples. From this it follows that at least the excessive accumulation of Ag, Hg, and Se by thyroid tissue is likely to precede the TMNs origination and development. It was supposed that elevated levels of Ag, Al, B, Bi, Ce, Cr, Hg, Mn, Mo, Ni, Pb, Rb, Sm, and Tl in nodular tissue could possibly be explored for differential diagnosis of benign and malignant thyroid nodules.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares that he has no competing interests.

Statement of ethical approval

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.

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