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Research Article

Trace Element Contents in Thyroid Cancer Investigated by Instrumental Neutron Activation Analysis

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Abstract

Background: Thyroid cancer is an internationally important health problem. The aim of this exploratory study was to evaluate whether significant changes in the thyroid tissue levels of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn exist in the malignantly transformed thyroid.

Methods: Thyroid tissue levels of ten trace elements were prospectively evaluated in 41 patients with thyroid malignant tumors and 105 healthy inhabitants. Measurements were performed using non-destructive instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides. Tissue samples were divided into two portions. One was used for morphological study while the other was intended for trace element analysis.

Results: It was found that contents of Ag, Co, Cr, Hg, and Rb were significantly higher (approximately 12.8, 1.4, 1.6, 19.6, and 1.7 times, respectively) in cancerous tissues than in normal tissues.

Conclusions: There are considerable changes in trace element contents in the malignantly transformed tissue of thyroid.

Keywords: Thyroid Malignant Tumors; Intact Thyroid; Trace Elements; Instrumental Neutron Activation Analysis

Introduction

Thyroid cancer (TC) is the most common endocrine malignancy. TC incidence has dramatically increased in the recent decades [1]. During the same period no other cancer has increased as much as TC. With the worldwide increase in the incidence of TC, it has become the fifth most common cancer in women [2-4]. In some countries, the incidence of TC has increased extremely fast, and it has been the most common cancer for the last years [5].

Although the etiology of TC is unknown, several risk factors including deficiency or excess of such micronutrient as iodine (I) have been well identified [6-17]. It was also reported that incidence of TC and mortality from this disease increases progressively with advancing age [18-19]. For example, a 37-fold increase in hazard ratio from age <40 years to age >70 years was showed in the study of 3664 TC patients that received surgery and adjuvant treatment at Memorial Sloan Kettering Cancer Center from the years 1985 to 2010 [19].

Besides I involved in thyroid function, other trace elements have also essential physiological functions such as maintenance and regulation of cell function, gene regulation, activation or inhibition of enzymatic reactions, and regulation of membrane function. Essential or toxic (mutagenic, carcinogenic) properties of trace elements depend on tissue-specific need or tolerance, respectively [20]. Excessive accumulation or an imbalance of the trace elements may disturb the cell functions and may result in cellular degeneration, death or malignant transformation [20-22].

In our previous study a significant positive correlation between age and some trace element contents in the thyroid was observed [23-28]. For example, a strongly pronounced tendency of age-related increase in cobalt (Co), iron (Fe), rubidium (Rb), antimony (Sb), and zinc (Zn) mass fraction in female thyroid, as well as an increase in selenium (Se) in male thyroid was demonstrated by us using non-destructive instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR) [24-25]. In addition, a significant positive correlation was seen between the contents of Zn and Co, Zn and Rb, Zn and Sb, Zn and scandium (Sc), Zn and Se in female thyroid [25], as well as between Zn and chromium (Cr), Zn and Fe, and also Zn and Sc in male thyroid [24]. It was concluded that high intra-thyroidal Zn concentrations are probably one of the main factors acting in both initiation and promotion stages of thyroid carcinogenesis [24-25], as it was earlier shown by us for a prostate gland [29-34]. Moreover, it seems fair to suppose that besides Zn, such trace elements as Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, and Se also play a role in the pathophysiology of the thyroid.

This work had two aims. The first was to assess the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in TC tissue using INAA-LLR. The second aim was to compare the levels of trace elements in the malignant thyroid with those in intact (normal) gland of apparently healthy persons.

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk.

Material and Methods

All patients suffered from TC (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. 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.

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

All tissue samples were divided into two portions using a titanium scalpel [35]. One was used for morphological study while the other was intended for trace element analysis. After the samples intended for trace element analysis were weighed, they were freeze-dried and homogenized [36]. The pounded sample weighing about 5-10 mg (for biopsy) and 50 mg (for resected materials) was used for trace element measurement by INAA-LLR. The samples for INAA-LLR were wrapped separately in a high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [37]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. Ten certified reference material IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) sub-samples weighing about 50 mg were treated and analyzed in the same conditions that thyroid samples to estimate the precision and accuracy of results..

A vertical channel of nuclear reactor was applied to determine the content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn by INAA-LLR. The quartz ampoule with thyroid samples, standards, and certified reference material was soldered, positioned in a transport aluminum container and exposed to a 24-hour neutron irradiation in a vertical channel with a neutron flux of $1.3 \cdot 10^{13}$ n·cm-2·s-1. Ten days after irradiation samples were reweighed and repacked.

The samples were measured for period from 10 to 30 days after irradiation. The duration of measurements was from 20 min to 10 hours subject to pulse counting rate. The gamma spectrometer included the 100 cm^3 Ge (Li) detector and on-line computer-based MCA system. The spectrometer provided a resolution of 1.9 keV on the ^{60}Co 1332 keV line. Details of used nuclear reactions, radionuclides, and gamma-energies were presented in our earlier publications concerning the INAA chemical element contents in human prostate and scalp hair [38-39].

A dedicated computer program for INAA mode optimization was used [40]. All thyroid samples were prepared in duplicate, and mean values of trace element contents were used in final calculation. Using Microsoft Office Excel, a summary of the statistics, including, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for trace element contents. The difference in the results between two age groups was evaluated by the parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test.

Results

(Table 1) depicts our data for Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions in ten sub-samples of IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) certified reference material and the certified values of this material.

(Table 2) 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 Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction in normal and cancerous thyroid tissue.

The comparison of our results with published data for Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction in normal and cancerous thyroid [41-55] is shown in (Table

3). The ratios of means and the difference between mean values of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions in normal and cancerous thyroid are presented in (Table 4).

Table (1): INAA-LLR data of trace element contents in certified reference material IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) compared to certified values (mg/kg, dry mass basis)

Element	IAEA H-4 animal muscle	This work results	IAEA HH-1 human hair	This work results
	95% confidence interval	M ± SD	95% confidence interval	M ± SD
Ag	-	0.033 ± 0.008	0.19[b]	0.18 ± 0.05
Co	0.0027[b]	0.0034 ± 0.0008	5.97 ± 0.42[a]	5.4 ± 1.1
Cr	0.06[b]	0.071 ± 0.010	0.27[b]	≤ 0.3
Fe	49.1 ± 6.5[a]	47.0 ± 1.0	23.7 ± 3.1[a]	25.1 ± 4.3
Hg	0.014[b]	0.015 ± 0.004	1.70 ± 0.09[a]	1.54 ± 0.14
Rb	18.7 ± 3.5[a]	23.7 ± 3.7	0.94[b]	0.89 ± 0.17
Sb	0.0056[b]	0.0061 ± 0.0021	0.031[b]	0.033 ± 0.009
Sc	0.0059[b]	0.0015 ± 0.0009	-	-
Se	0.28 ± 0.08[a]	0.281 ± 0.014	0.35 ± 0.02[a]	0.37 ± 0.08
Zn	86.3 ± 11.5[a]	91 ± 2	174 ± 9[a]	173 ± 17
M – arithmetical mean, SD – standard deviation, a – certified values, b – information values.				

Discussion

Precision and accuracy of results

Good agreement of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents analyzed by INAA-LLR with the certified data of CRM IAEA H-4 and IAEA HH-1 (Table 1) indicates an acceptable accuracy of the results obtained in the study of trace elements of the thyroid presented in (Tables 2–4).

The mean values and all selected statistical parameters were calculated for ten trace elements (Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn) mass fractions (Table 2). The mass fraction of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn were measured in all, or a major portion of normal and cancerous tissue samples.

Table (2): Some statistical parameters of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction (mg/kg, dry mass basis) in normal and cancerous thyroid

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Normal	Ag	0.0151	0.014	0.0016	0.0012	0.08	0.0121	0.0017	0.0454
n=105	Co	0.0399	0.0271	0.003	0.0046	0.14	0.0327	0.0134	0.124
	Cr	0.539	0.272	0.032	0.13	1.3	0.477	0.158	1.08
	Fe	225	100	11	51	512	217	67.4	456
	Hg	0.0421	0.0358	0.0041	0.0065	0.18	0.0304	0.0091	0.15
	Rb	7.37	4.1	0.44	1.11	29.4	6.49	2.6	16.7
	Sb	0.111	0.072	0.008	0.0047	0.308	0.103	0.0117	0.28
	Sc	0.0046	0.0038	0.0008	0.0002	0.0143	0.0042	0.00035	0.0131
	Se	2.32	1.29	0.14	0.439	5.8	2.01	0.775	5.65
	Zn	97.8	42.3	4.5	8.1	221	91.7	34.8	186
Cancer	Ag	0.193	0.215	0.041	0.0075	1.02	0.147	0.008	0.705
n=41	Co	0.055	0.0309	0.006	0.0042	0.143	0.0497	0.0159	0.129
	Cr	0.835	0.859	0.157	0.039	3.5	0.46	0.0941	3.05
	Fe	248	173	28	55.1	880	209	62.2	678
	Hg	0.824	0.844	0.149	0.0685	3.75	0.475	0.0689	2.85
	Rb	12.8	4.9	0.8	5.5	27.4	12.2	6.38	21.8
	Sb	0.124	0.081	0.015	0.016	0.381	0.108	0.0174	0.315
	Sc	0.0077	0.0129	0.002	0.0002	0.0565	0.0023	0.0002	0.0447
	Se	2.04	1.02	0.18	0.143	4.7	1.8	0.663	4.33
	Zn	95.1	78.9	12.6	36.5	375	67	36.7	374

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.

Comparison with published data

Values obtained for Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in the normal human thyroid (Table 3) agree well with median of mean values reported by other researches [41-55]. The obtained means for Ag and Co were almost one order of magnitude lower median of previously reported means but inside the range of means (Table 3). Data cited in Table 3 also includes samples obtained from patients who died from different non-endocrine diseases. A number of values for trace element mass fractions were not expressed on a dry mass basis by the authors of the cited references. However, we calculated these values using published data for water (75%) [43] and ash (4.16% on dry mass basis) [56] contents in thyroid of adults.

In cancerous tissues (Table 3) our results were comparable with published data for Fe, Rb, Se, and Zn contents. The obtained

means for Co and Cr were approximately three and one, respectively, order of magnitude lower median of previously reported means and inside the range of these means (Table 3). The obtained mean for Hg was almost two order of magnitude lower the only reported result (Table 3). No published data referring Ag, Sb, and Sc contents of cancerous thyroid tissue were found.

Table (3): Median, minimum and maximum value of means Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in normal and cancerous thyroid according to data from the literature in comparison with our results (mg/kg, dry mass basis)

Tissue	El	Published data [Reference]			This work
		Median of means (n)*	Minimum of means M or M ± SD, (n)**	Maximum of means M or M ± SD, (n)**	M ± SD
Normal	Ag	0.25 (12)	0.000784 (16) [41]	1.20 ± 1.24 (105) [42]	0.015 ± 0.014
	Co	0.34 (17)	0.026 ± 0.031 (46) [43]	70.4 ± 40.8 (14) [44]	0.040 ± 0.027
	Cr	0.69 (17)	0.105 (18) [45]	24.8 ± 2.4 (4) [64]	0.54 ± 0.27
	Fe	252 (21)	56 (120) [47]	2444 ± 700 (14) [44]	225 ± 100
	Hg	0.08 (13)	0.0008 ± 0.0002 (10) [48]	396 ± 40 (4) [46]	0.042 ± 0.036
	Rb	12.3 (9)	≤ 0.85 (29) [48]	294 ± 191 (14) [44]	7.37 ± 4.10
	Sb	0.11 (10)	0.040 ± 0.003 (-) [49]	4.0 (-) [50]	0.111 ± 0.072
	Sc	0.009 (4)	0.0018 ± 0.0003 (17)[51]	0.014 ± 0.005 (10) [48]	0.005 ± 0.004
	Se	2.61 (17)	0.95 ± 0.08 (29) [48]	756 ± 680 (14) [44]	2.32 ± 1.29
	Zn	118 (51)	32 (120) [47]	820 ± 204 (14) [44]	97.8 ± 42.3
Cancer	Ag	-	-	-	0.19 ± 0.21
	Co	71.6 (3)	2.48 ± 0.85 (18) [52]	94.4 ± 69.6 (3) [44]	0.055 ± 0.031
	Cr	2.74 (2)	1.04 ± 0.52 (4) [53]	119 ± 12 (4) [46]	0.84 ± 0.86
	Fe	316 (8)	69 ± 51 (3) [54]	5588 ± 556 (4) [46]	248 ± 173
	Hg	30.8 (1)	30.8 ± 3.2 (4) [46]	30.8 ± 3.2 (4) [46]	0.824 ± 0.844
	Rb	14.7 (2)	11,5 (10) [51]	17.8 ± 9.7 (5) [51]	12.8 ± 4.9
	Sb	-	-	-	0.124 ± 0.081
	Sc	-	-	-	0.008 ± 0.013
	Se	2.16 (7)	1.00 ± 0.24 (3) [53]	241 ± 296 (3) [44]	2.04 ± 1.02
	Zn	112 (13)	48 ± 8 (5) [55]	494 ± 37 (2) [53]	95.1 ± 78.9

El - element, M –arithmetic mean, SD – standard deviation, (n)* – number of all references, (n)** – number of samples.

The range of means of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn level reported in the literature for normal and for untreated cancerous thyroid vary widely (Table 3). This can be explained by a dependence of trace element content on many factors, including the region of the thyroid, from which the sample was taken, age, gender, ethnicity, mass of the gland, and the cancer stage. Not all these factors were strictly controlled in cited studies. Another and, in our opinion, leading cause of inter-observer variability can be attributed to the accuracy of the analytical techniques, sample preparation methods, and inability of taking uniform samples from the affected tissues. It was insufficient quality control of results in these studies. In many reported papers tissue samples were ashed or dried at high temperature for many hours. In other cases, thyroid samples were treated with solvents (distilled water, ethanol, formalin etc). There is evidence that by use of these methods some quantities of certain trace elements are lost as a result of this treatment That concern not only such volatile halogen as Br, but also other trace elements investigated in the study [57-59].

Effect of malignant transformation on chemical element contents

From (Table 4), it is observed that in cancerous tissue the mass fraction of Ag and Hg are approximately 13 and 20 times, respectively, higher and also mass fractions of Co, Cr, and Rb are almost in 38%, 55%, and 74%, respectively, higher than in normal tissues of the thyroid. Thus, if we accept the trace element contents in thyroid glands in the control group as a norm, we have to conclude that with a malignant transformation the levels of Ag, Co, Cr, Hg, and Rb in thyroid tissue significantly increased.

Table (4): Differences between mean values (M±SEM) of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction (mg/kg, dry mass basis) in normal and cancerous thyroid

Element	Thyroid tissue				Ratio
	Norm n = 105	Cancer n = 41	Student's t-test $p \leq$	U-test p	Cancer to Norm
Ag	0.0151 ± 0.0016	0.193 ± 0.041	**0.00022**	**≤ 0.01**	12.8
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
Fe	225 ± 11	248 ± 28	0.445	> 0.05	1.1
Hg	0.0421 ± 0.0041	0.824 ± 0.149	**0.000011**	**≤ 0.01**	19.6
Rb	7.37 ± 0.44	12.8 ± 0.8	**0.000000084**	**≤ 0.01**	1.74
Sb	0.111 ± 0.008	0.124 ± 0.015	0.422	> 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
Zn	97.8 ± 4.5	95.1 ± 5.9	0.839	> 0.05	0.97
M – arithmetic mean, SEM – standard error of mean, Statistically significant values are in **bold**					

Role of trace elements in malignant transformation of the thyroid

Characteristically, elevated or reduced levels of trace elements observed in cancerous tissues are discussed in terms of their potential role in the initiation and promotion of thyroid cancer. In other words, using the low or high levels of the trace element in cancerous tissues researchers try to determine the carcinogenic role of the deficiency or excess of each trace element in investigated organ. In our opinion, abnormal levels of many trace elements in tumor could be and cause, and also effect of malignant transformation. From the results of such kind studies, it is not always possible to decide whether the measured decrease or increase in trace element level in pathologically altered tissue is the reason for alterations or vice versa.

Silver: Ag is a chemical element with no recognized trace metal value in the human body [60]. 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 [61]. 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 [62]. More detailed knowledge of the Ag toxicity can lead to a better understanding of the impact on human health, including thyroid function. Anyway, a drastically elevated level of Ag in malignant thyroid tumors could possibly be explored for diagnosis of TC.

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 [63-64]. Co is genotoxic and carcinogenic, mainly caused by oxidative DNA damage by reactive oxygen species, perhaps combined with inhibition of DNA repair [65]. In our previous studies it was found a significant age-related increase of Co content in female thyroid [25]. Therefore, a goitrogenic and, probably, carcinogenic effect of excessive Co level in the thyroid of old females was assumed. Elevated level of Co in TC tissues, observed in the present study, supports this conclusion. Anyway, the accumulation of Co in malignant thyroid tumors could possibly be explored for diagnosis of TC.

Chromium: Cr-compounds are cytotoxic, genotoxic, and carcinogenic in nature. Some Cr forms, including hexavalent chromium (Cr^{6+}), are toxicants known for their carcinogenic effect in humans. They have been classified as certain or probable carcinogens by the International Agency for Research on Cancer [66]. The lung cancer risk is prevalent in pigment chromate handlers, ferrochromium production workers, stainless steel welders, and chrome-platers [67]. 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 [68]. Many other animal experiments and in vitro studies demonstrate also that Cr can induce oxidative stress and exert cytotoxic effects [69]. 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 [67]. Anyway, the accumulation of Cr in malignant thyroid tumors could possibly be explored for diagnosis of TC.

Mercury: Hg is one of the most dangerous environmental pollutants [70]. 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. Hg damages the central nervous system and has irreparable effects on the kidneys [71]. Hg may also harm a developing fetus and decrease fertility in men and women [72]. Besides these effects, Hg has been classified as certain or probable carcinogen by the International Agency for Research on Cancer [66]. For example, in Hg polluted area thyroid cancer incidence was almost 2 times higher than in in adjacent control areas [73].

Negative effects of Hg are due to the interference of this metal in cellular signaling pathways and protein synthesis during the period of development. Since it bonds chemically with the sulfur hydride groups of proteins, it causes damage to the cell membrane and decreases the amount of RNA [74]. Moreover, it was shown that Hg may be involved in four main processes that lead to genotoxicity: generation of free radicals and oxidative stress, action on microtubules, influence on DNA repair mechanisms and direct interaction with DNA molecules [75]. . Anyway, a drastically elevated level of Hg in malignant thyroid tumors could possibly be explored for diagnosis of TC.

Rubidium: As for Rb, there is very little information about its effects in organisms. No negative environmental effects have been reported. Rb is only slightly toxic on an acute toxicological basis and would pose an acute health hazard only when ingested in large quantities [76]. Rb has some function in immune responce [77], probably by supporting cell differentiation [78].

Potassium (K) and Rb are in the first group of the periodic table. Rb, like K, seems to be concentrated in the intracellular space and transfered through membrane by the Na+K+-ATPase pump. An overload of Rb could modulate proliferative responses of the cell, as was shown for bone marrow leukocytes [78]. In our previous studies it was found a significant age-related increase of Rb content in female thyroid [25,28]. Therefore, a goitrogenic and, probably, carcinogenic effect of excessive Rb level in the thyroid of old females was assumed. Elevated level of Rb in TC tissues, observed in the present study, supports this conclusion. Anyway, the accumulation of Rb in malignant thyroid tumors could possibly be explored for diagnosis of TC.

Our findings show that mass fraction of Ag, Co, Cr, Hg, and Rb are significantly different in TC as compared to normal thyroid tissues (Tables 4). Thus, it is plausible to assume that levels of these trace elements in thyroid tissue can be used as tumor markers. However, this subjects needs in additional studies.

Limitations: This study has several limitations. Firstly, analytical techniques employed in this study measure only ten trace element (Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn) mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of chemical elements investigated in normal and cancerous thyroid tissue. Secondly, the sample size of TC group was relatively small. It was not allow us to carry out the investigations of trace element contents in TC group using differentials like gender, histological types of tumors, stage of disease, and dietary habits of healthy persons and patients with TC. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on cancer-specific tissue Ag, Co, Cr, Hg, and Rb level alteration and shows the necessity the need to continue trace element research of malignant thyroid tumors.

Conclusion

In this work, trace elemental analysis was carried out in the tissue samples of normal thyroid and malignant tumors of thyroid using INAA-LLR. It was shown that INAA-LLR is an adequate analytical tool for the non-destructive determination of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn content in the tissue samples of human thyroid, including needle-biopsy cores. It was observed that in cancerous tissues content of Ag, Co, Cr, Hg, and Rb were significantly higher than in normal tissues. In our opinion, the increase in levels of Ag, Co, Cr, Hg, and Rb in cancerous tissue might demonstrate an involvement of these elements in etiology and pathogenesis of malignant thyroid tumors. It was supposed that elevated levels of Ag, Co, Cr, Hg, and Rb in thyroid tissue can be used as tumor markers.

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