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Original Research Article

Comparison of Trace Element Contents in Normal and Adenomatous Thyroid investigated using Instrumental Neutron Activation Analysis

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Abstract

Thyroid adenomas (TA) are benign tumors, but there is a 20% possibility of malignant transformation. The distinguishing between the TA and thyroid cancer (TC) is tricky, therefore new TA biomarkers are needed. Furthermore, the role of trace elements (TE) in etiology and pathogenesis of TA is unclear. The aim of this exploratory study was to examine the content of ten trace elements (TE): silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), and zinc (Zn) in the normal and adenomatous thyroid. Thyroid tissue levels of TE were prospectively evaluated in 46 patients with TA 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 TE analysis. It was found that in adenomatous thyroid content of Ag, Cr, Hg, and Zn were significantly higher than in normal gland tissues. Thus, it is possible to suppose that the considerable changes in TE contents in the adenomatous transformed tissue of thyroid can be used as TA biomarkers.

Keywords: Thyroid, Thyroid adenoma, Trace elements, Instrumental neutron activation analysis, Biomarkers.

INTRODUCTION

Thyroid adenomas (TA) are homogenous, solitary, encapsulated benign tumors, more common in females, and have a good prognosis [1]. However, because there is a 20% possibility of malignant transformation, TA should be differentiated from other thyroid nodular diseases such as nodular goiter (NG) and thyroid cancer (TC). The distinguishing between the TA and TC is tricky, therefore new differential diagnostics and TA biomarkers are needed [2, 3].

For over 20th century, there was the dominant opinion that NG, including TA, is the simple consequence of iodine deficiency. However, it was found that NG is a frequent disease even in those countries and regions where the population is never exposed to iodine shortage [4]. Moreover, it was shown that iodine excess has severe consequences on human health and associated with the presence of thyroidal disfunctions and autoimmunity, NG and diffuse goiter, benign and malignant tumors of gland [5-8]. It was also demonstrated that besides the iodine deficiency and excess many other dietary, environmental, and occupational factors are associated with the NG incidence [9-11]. Among them a disturbance of evolutionary stable input of many chemical elements in human body after industrial revolution plays a significant role in etiology of thyroidal disorders [12].

Besides iodine involved in thyroid function, other trace elements (TE) 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 [13]. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of TE depend on tissue-specific need or tolerance, respectively [13]. Excessive accumulation or an imbalance of the TE may disturb the cell functions and may result in cellular degeneration, death, benign or malignant transformation [13-15].

In our previous studies the complex of in vivo and in vitro nuclear analytical and related methods was developed and used for the investigation of iodine and other TE 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 TE content with age in the thyroid of males and females were studied and age- and gender-dependence of some TE was observed [25-41]. Furthermore, a significant difference between some TE contents in normal and cancerous thyroid was demonstrated [42-47].

To date, the etiology and pathogenesis of TA has to be considered as multifactorial. The present study was performed to clarify the role of some TE in the TA etiology. Having this in mind, our aim was to assess the silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), and zinc (Zn) contents in TA tissue using non-destructive instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR). A further aim was to compare the levels of these ten TE in the adenomatous thyroid with those in intact (normal) gland of apparently healthy persons.

MATERIAL AND METHODS

All patients suffered from TA (n=19, 16 females and 3 males, mean age M±SD was 41±11 years, range 22-55) 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 TE contents. For all patients the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. Histological conclusion for all thyroidal lesions was the TA.

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 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.

All tissue samples were divided into two portions using a titanium scalpel [48]. One was used for morphological study while the other was intended for TE analysis. After the samples intended for TE analysis were weighed, they were freeze-dried and homogenized [49]. The pounded sample weighing about 10 mg (for biopsy) and 50 mg (for resected materials) was used for TE measurement by INAA-LLR.

To determine contents of the TE by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [50]. 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. Details of used nuclear reactions, radionuclides, gamma-energies, spectrometric unit, sample preparation and procedure of measurement were presented in our earlier publications concerning the INAA of TE contents in human thyroid, prostate and scalp hair [29, 30, 51, 52].

A dedicated computer program for INAA mode optimization was used [53]. All thyroid samples were prepared in duplicate, and mean values of TE 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 TE contents. The difference in the results between two groups (normal thyroid and TA) 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 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.

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 adenomatous thyroid.

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 adenomatous thyroid [54-71] 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 adenomatous thyroid are presented in Table 4.

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 thyroid and adenomatous thyroid

Tissue	El	Mean	SD	SEM	Min	Max	Median	P0.025	P0.975
Normal	Ag	0.0151	0.0140	0.0016	0.0012	0.0800	0.0121	0.0017	0.0454
n=105	Co	0.0399	0.0271	0.0030	0.0046	0.140	0.0327	0.0134	0.124
	Cr	0.539	0.272	0.032	0.130	1.30	0.477	0.158	1.08
	Fe	225	100	11	51.0	512	217	67.4	456
	Hg	0.0421	0.0358	0.0041	0.0065	0.180	0.0304	0.0091	0.150
	Rb	7.37	4.10	0.44	1.11	29.4	6.49	2.60	16.7
	Sb	0.111	0.072	0.008	0.0047	0.308	0.103	0.0117	0.280
	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	5.80	2.01	0.775	5.65
	Zn	97.8	42.3	4.5	8.10	221	91.7	34.8	186
Adenoma	Ag	0.211	0.201	0.056	0.0115	0.679	0.198	0.0124	0.627
n=19	Co	0.0673	0.0485	0.0140	0.0083	0.159	0.0478	0.0104	0.149
	Cr	1.40	0.85	0.25	0.259	2.79	1.25	0.265	2.70
	Fe	417	419	112	52.3	1407	316	53.3	1357
	Hg	0.796	0.522	0.145	0.149	1.72	0.817	0.162	1.65
	Rb	8.73	3.26	0.84	2.40	16.4	8.60	3.42	15.0
	Sb	0.149	0.124	0.036	0.0449	0.466	0.105	0.0449	0.419
	Sc	0.0174	0.0273	0.0090	0.0003	0.0900	0.0060	0.0003	0.0759
	Se	2.36	0.90	0.24	0.720	3.57	2.25	0.929	3.52
	Zn	128	51	13	48.0	251	135	52.9	225

El – element, 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.

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 TE of the thyroid presented in Tables 2–4.

The mean values and all selected statistical parameters were calculated for ten TE (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 adenomatous samples.

Comparison with published data

In general, 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 [54-66]. 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). A number of values for TE 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%) [72] and ash (4.16% on dry mass basis) [73] contents in thyroid of adults.

Data cited in Table 3 for normal thyroid also includes samples obtained from patients who died from different non-endocrine diseases. In our previous study it was shown that some non-endocrine diseases can effect on TE contents in thyroid [24]. Moreover, in many studies the "normal" thyroid means a visually non-affected tissue adjacent to benign or malignant thyroidal nodules. However, there are no data on a comparison between the TE contents in such kind of samples and those in thyroid of healthy persons, which permits to confirm their identity.

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

T	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
N	Ag	0.21 (12)	0.000784 (16) [54]	1.20±1.24 (105) [55]	0.015±0.014
	Co	0.306 (25)	0.016 (66) [56]	70.4±40.8 (14) [57]	0.040±0.027
	Cr	0.69 (17)	0.088 (83) [58]	24.8±2.4 (4) [59]	0.54±0.27
	Fe	252 (21)	56 (120) [60]	3360 (25) [61]	225±100
	Hg	0.08 (13)	0.0008±0.0002 (10) [62]	396±40 (4) [59]	0.042±0.036
	Rb	7.8 (9)	≤0.85 (29) [62]	294±191 (14) [57]	7.37±4.10
	Sb	0.15 (10)	0.040±0.003 (-) [63]	≤ 12.4(-) [64]	0.111±0.072
	Sc	0.009 (4)	0.0018±0.0003 (17) [56]	0.014±0.005 (10) [62]	0.005±0.004
	Se	2.32 (21)	0.436 (40) [65]	756±680 (14) [57]	2.32±1.29
	Zn	110 (56)	2.1 (-) [66]	820±204 (14) [57]	97.8±42.3
A	Ag	0.110 (1)	0.110±0.045 (19) [67]	0.110±0.045 (19) [67]	0.211±0.201
	Co	46.4 (1)	46.4±4.8 (4) [59]	46.4±4.8 (4) [59]	0.067±0.049
	Cr	76 (2)	6.00±5.32 (9) [68]	146±14 (4) [59]	1.40±0.85
	Fe	566 (3)	54.6±36.1 (5) [69]	2100±208 (4) [59]	417±419
	Hg	79.2 (1)	79.2±8.0 (4) [59]	79.2±8.0 (4) [59]	0.796±0.522
	Rb	7.0 (1)	7.0 (10) [65]	7.0 (10) [65]	8.73±3.26
	Sb	-	-	-	0.149±0.124
	Sc	-	-	-	0.017±0.027
	Se	1.88 (4)	0.316 (46) [70]	3,16±2,88 (9) [68]	2.36±0.90
	Zn	68.5 (8)	21.0 (130) [71]	330±282 (9) [68]	128±51

T - tissue**,** N – normal tissue, A - adenoma, El - element, M –arithmetic mean, SD – standard deviation, (n)* – number of all references, (n)** – number of samples.

Our results for TA tissues were comparable with published data for Ag, Fe, Rb, Se, and Zn contents (Table 3). The obtained means for Co, Cr, and Hg were approximately 693, 54, and 100, respectively, times lower median of previously reported means, herewith, mean for Cr was outside the range of means (Table 3). For Ag, Co, Hg, and Rb only one article for each element was extracted from literature. No published data referring Sb and Sc contents of adenomatous thyroid were found.

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 adenomatous thyroid vary widely (Table 3). This can be explained by a dependence of TE content on many factors, including "normality" of thyroid samples (see above), the region of the thyroid, from which the sample was taken, age, gender, ethnicity, and mass of the gland, as well as the TA stage, histology, and functional activity. Not all these factors were strictly controlled in cited studies. However, in our opinion, the leading causes 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 scientific reports, 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 during ashing, drying and digestion at high temperature some quantities of certain TE are lost as a result of this treatment. That concerns not only such volatile halogen as Br, but also other TE investigated in the study [74, 75].

Effect of adenomatous transformation on ChE contents

From Table 4, it is observed that in adenomatous tissues the mass fractions of all TE investigated Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn are 14.0, 1.69, 2.60, 1.85, 18.9, 1.18, 1.34, 3.78, 1.02, and 1.31 times, respectively, higher than in normal tissues of the thyroid. However, the changes for Ag, Cr, Hg, and Zn are just statistically significant. Thus, if we accept the TE contents in thyroid glands in the control group as a norm, we have to conclude that with an adenomatous transformation the Ag, Cr, Hg, and Zn level in thyroid tissue significantly changed.

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 adenomatous thyroid

Element	Thyroid tissue				Ratio
	Norm n=105	Adenoma n=19	Student's t-test $p\leq$	U-test p	Adenoma to Norm
Ag	0.0151±0.0016	0.211±0.056	**0.0044**	**≤0.01**	14.0
Co	0.0399±0.0030	0.0673±0.0140	0.080	>0.05	1.69
Cr	0.539±0.032	1.40±0.25	**0.048**	**≤0.05**	2.60
Fe	225±11	417±112	0.111	>0.05	1.85
Hg	0.0421±0.0041	0.796±0.145	**0.00022**	**≤0.01**	18.9
Rb	7.37±0.44	8.73±0.84	0.168	>0.05	1.18
Sb	0.111±0.008	0.149±0.036	0.320	>0.05	1.34
Sc	0.0046±0.0008	0.0174±0.0090	0.171	>0.05	3.78
Se	2.32±0.14	2.36±0.24	0.876	>0.05	1.02
Zn	97.8±4.5	128±13	**0.044**	**≤0.01**	1.31

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

Role of ChE in adenomatous transformation of the thyroid

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

Silver

Ag is a TE with no recognized trace metal value in the human body [76]. 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 [77]. 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 [78]. More detailed knowledge of the Ag toxicity can lead to a better understanding of the impact on human health, including thyroid function.

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

Mercury

Hg is one of the most dangerous environmental pollutants [83]. 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 [84]. Hg may also harm a developing fetus and decrease fertility in men and women [85]. Besides these effects, Hg has been classified as certain or probable carcinogen by the International Agency for Research on Cancer [79]. For example, in Hg polluted area thyroid cancer incidence was almost 2 times higher than in in adjacent control areas [86].

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 [87]. 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 [88].

Zinc

Zn as a trace metal plays an important role in normal and pathophysiology. This TE is a constituent of more than 3000 proteins and is a cofactor for over 300 enzymes [89]. Zn is an essential mediator of cell proliferation and differentiation through the regulation of DNA synthesis and mitosis. Zn also affects DNA repair pathways by regulating multiple intracellular signaling pathways and altering proteins involved in DNA maintenance [90]. This metal also maintenance the balance of a cellular redox [91]. Thus, Zn is important cofactors in diverse cellular processes, but its high concentrations are toxic to the cells. The elevated level of Zn mass fractions in thyroid tissue may contribute to harmful effects on the gland. There are good reasons for such speculations since. experimental and epidemiological data support the hypothesis that Zn overload is a risk factor for benign and malignant tumors [90, 92-94].

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

Limitations

This study has several limitations. Firstly, analytical techniques employed in this study measure only ten TE (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 adenomatous thyroid. Secondly, the sample size of TA group was relatively small and prevented investigations of TE contents in TA group using differentials like gender, histological types of adenoma and its functional activity, stage of disease, dietary habits of healthy persons and patients with TA. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on adenoma -specific tissue Ag, Cr, Hg, and Zn level alteration and shows the necessity to TE research of TA.

CONCLUSION

In this work, TE analysis was carried out in the tissue samples of normal thyroid and TA 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 adenomatous thyroid content of Ag, Cr, Hg, and Zn were significantly higher than in normal gland tissues. In our opinion, the increase in levels of Ag, Cr, Hg, and Zn in adenomatous tissue might demonstrate an involvement of these TE in etiology and pathogenesis of TA. It was supposed that elevated levels of Ag, Cr, Hg, and Zn in affected thyroid tissue can be used as TA markers.

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