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Comparison of trace element contents in normal thyroid and thyroid with Hashimoto's thyroiditis using neutron activation analysis

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Abstract

Role of trace elements (TE) in etiology and pathogenesis of Hashimoto's thyroiditis (HT) is unclear. The aim of this exploratory study was to assess whether there were significant changes in thyroid tissue levels of ten TE (Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn) are present in the autoimmune transformed thyroid. Ten TE of thyroid tissue were determined in 8 patients with HT and 105 healthy populations. The measurements were performed using non-destructive instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides. Elevated mean values of Ag, Hg, and Rb content were found in thyroid with HT in comparison with normal level (in approximately 21, 30, and 1.6 times, respectively). Because the considerable changes in some TE contents in tissue of thyroid with HT were found, it is reasonable to assume that the levels of these TE in thyroid tissue can be used as HT markers. However, this topic needs additional studies.

Keywords: Hashimoto's thyroiditis; Thyroid; Trace elements; Neutron activation analysis

1. Introduction

Hashimoto's thyroiditis (HT), also called chronic lymphocytic or autoimmune thyroiditis, is part of the spectrum of chronic autoimmune thyroid diseases [1]. Hashimoto's disease is associated with thyroid autoantibodies production like the most common, thyroid peroxidase and thyroglobulin antibodies, and with lymphocytic infiltration [1]. Although the HT was described over 100 years ago the exact mechanism of progressive thyroid tissue destruction as a result of HT is still not sufficiently elucidated. Clinical differentiation between HT, Riedel's struma and other thyroid benign and malignant nodules is often difficult [2,3]. We hypothesized that disbalance of trace elements (TE) contents in thyroid tissue may play a significant role in etiology and pathogenesis of HT. Furthermore, specific levels of TE contents in autoimmune transformed thyroid tissue may be used as HT biomarkers.

For over 20th century, there was the dominant opinion that all thyroid nodules (TN), including HT, are the elementary consequence of iodine (I) deficiency. However, TN have been found to be a frequent disease even in those countries and regions where the population is never exposed to I deficiency [4]. Moreover, it was shown that iodine excess has severe effects on human health and associated with the development of thyroidal disfunctions and autoimmunity, nodular 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 HT 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].

In addition to I, many other TE are involved in essential physiological functions [13]. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of TE depend on tissue-specific need or tolerance, respectively [13]. Deficiency,

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overload or an imbalance of the TE may result in cellular dysfunction, 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 employed for the investigation of I and other TE levels in the normal and pathological thyroid gland [16-22]. Level of I in the normal gland was studied in relation to age, gender and some non-thyroidal diseases [23,24]. After that, variations of many other TE content with age in the thyroid of males and females were investigated and age- and gender-dependence of some TE was observed [25-41]. Furthermore, a significant difference between some TE mass fractions in normal and malignant thyroid was demonstrated [42-47].

So far, the etiology and pathogenesis of HT has to be considered as multifactorial. The present study was performed to clarify the role of some TE in the HT etiology. With 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 normal thyroid tissue (NT) and HT affected gland 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 TE in the NT and HT groups of samples.

2. Material and methods

All patients with HT (n=8, 7 females and 1 male, mean age $M \pm SD$ was 40 ± 10 years, range 34-55) were hospitalized in the Head and Neck Department of the MRRRC. Thick-needle puncture biopsy of suspicious lesion of the gland was performed for every persons, to allow morphological examination of affected thyroid tissue and to determine 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 HT.

Normal thyroid 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. Histological examination was used in the NT group to match the age criteria, as well as to confirm the absence of micro-nodules and underlying cancer.

All thyroid samples were divided into two parts using a titanium scalpel [48]. One was used for morphological study while the other was for TE evaluation. All samples for TE analysis were weighed, freeze-dried and homogenized [49]. 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.

Details of sample preparation, activation by neutrons of nuclear reactor, gamma-spectrometry, calibration with biological synthetic standards, and quality insurance using certified reference material (CRM) of International Atomic Energy Agency (IAEA) CRM IAEA H-4 (animal muscle) were presented in our earlier publications concerning the INAA-LLR of TE contents in human thyroid, prostate and hair [18,29,30,50-52].

A dedicated computer program for INAA-LLR mode optimization was used [53]. All the thyroid samples were prepared in duplicate, and mean values of TE contents were used in the final calculation. Using Microsoft Office Excel, a summary of the statistics, including arithmetic mean, standard deviation, standard error of the mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for TE contents in NT and HT groups of tissue samples. The difference in the results between two groups (NT and HT) 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 of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction in normal thyroid and thyroid with Hashimoto's thyroiditis.

Comparison of values obtained for Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in the NT and HT samples with median of means reported by other researches [9,54-68] depicts in Table 2.

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 thyroid and thyroid with Hashimoto's thyroiditis are presented in Table 3.

Table 1 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 thyroid with Hashimoto's thyroiditis

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Normal	Ag	0.0151	0.0140	0.0016	0.0012	0.0800	0.0121	0.0017	0.0454
thyroid	Co	0.0399	0.0271	0.0030	0.0046	0.140	0.0327	0.0134	0.124
n=105	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
Hashimoto's	Ag	0.319	0.116	0.067	0.187	0.408	0.361	0.196	0.406
thyroiditis	Co	0.0499	0.0172	0.0099	0.0321	0.0664	0.0512	0.0331	0.0656
n=8	Cr	0.404	0.546	0.315	0.0750	1.03	0.103	0.0764	0.987
	Fe	165	129	46	94.0	478	112	95.4	423
	Hg	1.27	0.39	0.23	0.894	1.68	1.23	0.911	1.66
	Rb	11.4	5.2	1.9	3.80	19.3	11.8	4.36	19.0
	Sb	0.0946	0.0493	0.0280	0.0377	0.126	0.1200	0.0418	0.126
	Sc	0.0214	0.0294	0.0170	0.00020	0.0550	0.0091	0.00065	0.0527
	Se	1.63	0.47	0.27	1.15	2.09	1.64	1.18	2.06
	Zn	97.6	28.0	9.9	50.0	140	97.3	55.1	138

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.

Table 2 Median, minimum and maximum value of means Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in normal thyroid and thyroid with Hashimoto's thyroiditis according to data from the literature in comparison with our results (mg/kg, dry mass basis)

Tissue	El	Published data [Reference]			This work M±SD
		Median of means (n)*	Minimum of means M or M±SD, (n)**	Maximum of means M or M±SD, (n)**	
Normal	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
Hashimoto's	Ag	0.110 (1)	0.11±0.05 (19) [67]	0.11±0.05 (19) [67]	0.319±0.116
thyroiditis	Co	-	-	-	0.050±0.017
	Cr	-	-	-	0.404±0.546
	Fe	-	-	-	165±129
	Hg	-	-	-	1.27±0.39
	Rb	-	-	-	11.4±5.2
	Sb	-	-	-	0.095±0.049
	Sc	-	-	-	0.021±0.029
	Se	1.03 (4)	0.408±0.209 (51) [9]	3.88±1.76 (7) [68]	1.63±0.47
	Zn	54.9 (4)	22.4 (31) [9]	86.4±38.8 (14) [67]	97.6±28.0

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

Table 3 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 thyroid and thyroid with Hashimoto's thyroiditis

Element	Thyroid tissue				Ratio Hashimoto's thyroiditis to normal thyroid
	Normal thyroid n=105	Hashimoto's thyroiditis n=8	Student's t-test <i>p</i> ≤	U-test <i>p</i>	
Ag	0.0151±0.0016	0.319±0.067	0.0456	≤ 0.01	21.1
Co	0.0399±0.0030	0.0499±0.0099	0.420	>0.05	1.25
Cr	0.539±0.032	0.404±0.315	0.711	>0.05	0.75
Fe	225±11	165±46	0.238	>0.05	0.73
Hg	0.0421±0.0041	1.27±0.23	0.0328	≤ 0.01	30.2
Rb	7.37±0.44	11.4±1.9	0.0675	≤ 0.05	1.55
Sb	0.111±0.008	0.0946±0.0280	0.634	>0.05	0.85
Sc	0.0046±0.0008	0.0214±0.0170	0.425	>0.05	4.65
Se	2.32±0.14	1.63±0.27	0.103	>0.05	0.70
Zn	97.8±4.5	97.6±9.9	0.981	>0.05	1.00

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

4. Discussion

As was shown before [18,29,30,50-52] 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 indicates acceptable accuracy of the results obtained in the study of TE of the thyroid samples presented in Tables 1-3. The mean values and all selected statistical parameters were calculated for all ten TE (Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn) mass fractions in NT and HT groups of tissue samples (Table 1)..

In a general sense values obtained for Br, Cu, Fe, Rb, Sr, and Zn contents in the NT samples (Table 2) agree well with median of mean values reported by other researches [54-66]. A number of values for TE mass fractions in literature

were not expressed on a dry mass basis. However, we calculated these values using published data for water (75%) [69] and ash (4.16% on dry mass basis) [70] contents in thyroid of adults.

Data cited in Table 2 for NT 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.

The data on TE levels in thyroid with HT are very limited (Table 2). Results for Se obtained in the present study agree well with published data, while our value for Ag and Zn are some higher than the upper limit of means from literature. Information on Co, Cr, Fe, Hg, Rb, Sb, and Sc contents in thyroid with HT was not found.

The range of means of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn level reported in the literature for NT tissue vary widely (Table 2). 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, mass of the gland, and its functional activity. Not all these factors were strictly controlled in cited studies. However, in our opinion, the main reason for the inter-observer discrepancy can be attributed to the accuracy of the analytical techniques, sample preparation methods, and the inability to take standardized samples from 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 [71,72].

From Table 3, it is observed that in HT samples the mass fraction of Ag, Hg, and Rb are approximately 21, 30, and 1.6 times, respectively, higher than in NT. Thus, if we accept the TE contents in the NT group as a norm, we have to conclude that with an autoimmune transformation the Ag, Hg, and Rb level in thyroid tissue significantly changed.

Characteristically, elevated or reduced levels of TE observed in affected tissues are discussed in terms of their potential role in the initiation and promotion of TN. In other words, using the low or high levels of the TE in TN researchers try to determine the role of the deficiency or excess of each TE in the TN etiology. In our opinion, abnormal levels of many TE in TN, including HT, 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 TE level in pathologically altered tissue is the reason for alterations or vice versa. Nevertheless the differences between TE levels in normal and affected thyroid tissue could be used as HT markers.

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 TE investigated in NT and HT. Secondly, the sample size of HT group was relatively small and prevented investigations of TE contents in HT group using differentials like gender, thyroid functional activity, stage of disease, dietary habits of healthy persons and patients with HT. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on autoimmune-specific tissue Ag, Hg, and Rb level alteration and shows the necessity to continue TE research of HT.

5. Conclusion

In this work, TE measurements in tissue samples from NT and HT were performed 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 tissue samples from healthy and affected human thyroid, including needle biopsy samples. It was observed that in HT contents of Ag, Hg, and Rb were significantly higher than in normal tissues. In our opinion, the presented study data strongly suggest that TE plays an important role in thyroid health, as well as in the etiology and pathogenesis of HT. It was assumed that the differences in TE levels in affected thyroid tissue could be used as HT markers.

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.

Statement of informed consent

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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