



Research Article

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Distinguish Thyroid Malignant from Benign Alterations using Neutron Activation Analysis of Trace Element Contents in Nodular Tissue

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Abstract

Background: Thyroid benign (TBN) and malignant (TMN) nodules are a common thyroid lesion. The differentiation of TMN often remains a clinical challenge and further improvements of TMN diagnostic accuracy are warranted. The aim of present study was to evaluate possibilities of using differences in trace elements (TEs) contents in nodular tissue for diagnosis of thyroid malignancy.

Methods: Contents of TEs such as silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), iodine (I), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), and zinc (Zn) were prospectively evaluated in nodular tissue of thyroids with TBN (79 patients) and to TMN (41 patients). Measurements were performed using non-destructive instrumental neutron activation analysis.

Results: It was observed that in TMN tissue the mean mass fractions of I and Se are approximately 14 and 1.35 times, respectively, lower while the mean mass fraction of Rb 34% higher those in TBN tissue. Contents of Al, Co, Cr, Fe, Hg, Sb, Sc, and Zn found in the TBN and TMN groups of nodular tissue samples were similar.

Conclusions: It was proposed to use the I mass fraction and I/Rb mass fraction ratio in a needle-biopsy of thyroid nodules as a potential tool to diagnose thyroid malignancy. Further studies on larger number of samples are required to confirm our findings and proposals.

Keywords

Thyroid, Thyroid malignant and benign nodules, Trace elements, Neutron activation analysis

Introduction

Nodules are a common thyroid lesion, particularly in women. Depending on the method of examination and general population, thyroid nodules (TNs) have an incidence of 19-68% [1]. In clinical practice, TNs are classified into benign (TBN) and malignant (TMN), and among all TNs approximately 10% are TMN [2]. It is appropriate mention here that the incidence of TMN is increasing rapidly (about 5% each year) worldwide [2]. Surgical treatment is not always necessary for TBN whereas surgical treatment is required in TMN. Thus, differentiated TBN and TMN have a great influence on thyroid therapy.

Ultrasound (US) examination widely use as the primary method for early detection and diagnosis of the TNs. However, there are many similarities in the US characteristics of both TBN and TMN. For misdiagnosis prevention some computer-diagnosis systems based on the analysis of US images were developed, however as usual these systems for the diagnosis of TMN showed accuracy, sensitivity, and specificity nearly

80% [2,3]. Therefore, when US examination shows suspicious signs, an US-guided fine-needle aspiration biopsy is advised. Despite the fine needle aspiration biopsy has remained the diagnostic tool of choice for evaluation of US suspicious thyroid nodules, the differentiation of TMN often remains a diagnostic and clinical challenge since up to 30% of nodules are categorized as cytologically "indeterminate" [4]. Thus, to

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improve diagnostic accuracy of TMN, new technologies have to be developed for clinical applications. However, a recent systematic review and meta-analysis of molecular tests in the preoperative diagnosis of indeterminate TNs shown that at the current time there is no perfect biochemical, immunological, and genetic biomarkers to discriminate malignancy [5]. Therefore, further improvements of TMN diagnostic accuracy are warranted [6-8].

During the last decades it was demonstrated that besides the iodine deficiency and excess many other dietary, environmental, and occupational factors are associated with the TNs incidence [3,9-11]. 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 TNs [12]. Besides iodine, many other TEs have also essential physiological role and involved in thyroid 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, thyroid adenoma, and cancer in comparison with normal thyroid and thyroid tissue adjacent to TNs was demonstrated [42-48].

The present study had two aims. The main objective was to assess the silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), iodine (I), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), and zinc (Zn) contents in nodular tissue of patients who had either TBN or TMN using a combination of non-destructive instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) and long-lived radionuclides (INAA-LLR). The second aim was to compare the levels of TEs in TBN and TMN and to evaluate possibilities of using TEs differences for diagnosis of thyroid malignancy.

Material and Methods

All patients suffered from TBN ($n = 79$, mean age $M \pm SD$ was 44 ± 11 years, range 22-64) and from TMN ($n = 41$, mean age $M \pm SD$ 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 TEs 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 TBN were: 46 colloid goiter, 19 thyroid adenoma, 8 Hashimoto's thyroiditis, and 6 Riedel's Struma, whereas for TMN were: 25 papillary adenocarcinomas, 8 follicular adenocarcinomas, 7 solid carcinomas, and 1 reticulosarcoma. Samples of nodular tissue for INAA-SLR and INAA-LLR were taken from both biopsy and resected materials.

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 TBN and TMN were divided into two portions using a titanium scalpel to prevent contamination by TEs of stainless steel [49]. 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 [50].

To determine contents of the TEs by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [51]. 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 were treated and analyzed in the same conditions that thyroid samples to estimate the precision and accuracy of results.

The content of I were determined by INAA-SLR using a horizontal channel equipped with the pneumatic rabbit system of the WWR-c research nuclear reactor (Branch of Karpov Institute, Obninsk). Details of used nuclear reaction, radionuclide, gamma-energies, spectrometric unit, sample preparation, and the quality control of results were presented in our earlier publications concerning the INAA-SLR of I contents in human thyroid [27,28] and scalp hair [52].

A vertical channel of the same 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-LLR of TEs contents in human thyroid [29,30], scalp hair [52], and prostate [53,54].

A dedicated computer program for INAA-SLR and INAA-LLR mode optimization was used [55]. All thyroid samples for TEs analysis were prepared in duplicate, and mean values of TEs contents were used in final calculation. 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 two groups of nodular tissue (TBN and TMN). The difference

in the results between two groups of samples was evaluated by the parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test.

Results

(Table 1) depicts 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, I, Rb, Sb, Sc, Se, and Zn mass fraction in thyroid intact tissue samples of two groups of samples - TBN and TMN.

The ratios of means and the comparison of mean values of Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn mass fractions in pair of sample groups such as TBN and TMN is presented in (Table 2).

The comparison of our results with published data for Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn mass fraction in TBN [56-66] and TMN [59-61,64,66-70] is shown in (Table 3). A number of values for TEs 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%) [71] and ash (4.16% on dry mass basis) [72] contents in thyroid of adults.

Discussion

As was shown before [27-30,52-54] good agreement of the TEs contents in CRM IAEA H-4 and CRM IAEA HH-1 samples analyzed by two methods of instrumental neutron activation analysis with the certified data of these CRM

indicates acceptable accuracy of the results obtained in the study of TBN and TMN groups of tissue samples presented in (Tables 1 and Table 2 and Table 3).

From Table 2, it is observed that in TMN tissue the mass fractions of I and Se are approximately 14 and 1.35 times, respectively, lower while the mass fraction of Rb 34% higher than in TBN tissue. In a general sense Al, Co, Cr, Fe, Hg, Sb, Sc, and Zn contents found in the TBN and TMN groups of tissue samples were similar (Table 2).

Mean values obtained for Ag, Fe, I, Rb, Se, and Zn contents in TBN (Table 3) agree well with median of mean values reported by other researches [56,57,59,61-66]. Mean mass fractions of Co and Hg in TBN obtained in present study were almost one and two order of magnitude, respectively, lower medians of means for these TEs in published articles [58-60]. Mean mass fraction of Cr in TBN obtained in present study was approximately 4 times lower the median of published means [56,60]. No published data referring Sb and Sc contents of TBN were found (Table 3).

Mean values obtained for Fe, I, Rb, Se, and Zn contents in TMN (Table 3) agree well with median of mean values reported by other researches [59-61,64,66,68-70]. Mean mass fraction obtained for Co and Hg in TMN were approximately two order of magnitude and 37 times, respectively, lower median of previously reported means [59,60,67]. Mean mass fraction of Cr founded in TMN was 3.3 times lower the median of published means [60,66]. No published data referring Ag, Sb, and Sc contents of TMN were found (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 thyroid benign (TBN) and malignant (TMN) nodules.

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
TBN N = 79	Ag	0.226	0.219	0.031	0.002	0.874	0.179	0.0022	0.808
	Co	0.0615	0.0332	0.0046	0.0083	0.159	0.0579	0.0152	0.141
	Cr	0.966	0.844	0.121	0.075	3.65	0.673	0.109	2.76
	Fe	332	332	40	52.3	1407	186	59.9	1346
	Hg	0.924	0.649	0.088	0.0817	3.01	0.856	0.104	2.12
	I	992	901	103	29	3906	695	84.8	3629
	Rb	9.55	4.37	0.52	1	22.1	8.9	2.48	19.6
	Sb	0.137	0.116	0.016	0.0024	0.466	0.101	0.0112	0.423
	Sc	0.0144	0.0217	0.003	0.0002	0.091	0.0058	0.0002	0.0878
	Se	2.75	2.13	0.29	0.72	12.6	2.31	1.05	10
TMN N = 41	Zn	117.7	50	5.9	47	278	107	48.8	256
	Ag	0.193	0.215	0.041	0.0075	1.02	0.147	0.008	0.705
	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
	I	71.8	62	10	2	261	62.1	2.93	192
	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.

Table 2: 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 thyroid benign (TBN) and malignant (TMN) nodules.

Element	Thyroid nodules				Ratio TMN / TBN
	TBN	TMN	Student's t-test, <i>p</i> £	U-test, <i>p</i>	
Ag	0.226 ± 0.031	0.193 ± 0.041	0.515	> 0.05	0.85
Co	0.0615 ± 0.0046	0.0550 ± 0.0060	0.37	> 0.05	0.89
Cr	0.966 ± 0.121	0.835 ± 0.157	0.511	> 0.05	0.86
Fe	332 ± 40	248 ± 28	0.094	> 0.05	0.75
Hg	0.924 ± 0.088	0.824 ± 0.149	0.567	> 0.05	0.89
I	992 ± 103	71.8 ± 10.0	0.00000001	≤ 0.01	0.072
Rb	9.55 ± 0.52	12.8 ± 0.8	0.00098	≤ 0.01	1.34
Sb	0.137 ± 0.016	0.124 ± 0.015	0.572	> 0.05	0.91
Sc	0.0144 ± 0.0030	0.0077 ± 0.0020	0.105	> 0.05	0.53
Se	2.75 ± 0.29	2.04 ± 0.18	0.039	≤ 0.01	0.74
Zn	117.7 ± 5.9	95.1 ± 5.9	0.111	> 0.05	0.81

M - Arithmetic mean, SEM - Standard error of mean, statistically significant values are in bold.

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

Tissue	Element	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)**	
Goiter	Ag	0.16 (4)	0.098 ± 0.042 (19) [56]	1.20 ± 2.28 (51) [57]	0.23 ± 0.22
	Co	0.86 (13)	0.110 ± 0.003 (64) [58]	62.8 ± 22.4 (11) [59]	0.062 ± 0.033
	Cr	4.0 (6)	0.72 (51) [56]	146 ± 14 (4) [60]	0.97 ± 0.84
	Fe	207 (9)	54.6 ± 36.1 (5) [61]	4848 ± 3056 (11) [59]	332 ± 332
	Hg	79.2 (1)	79.2 ± 8.0 (4) [60]	79.2 ± 8.0 (4) [60]	0.924 ± 0.649
	I	812 (55)	77 ± 14 (66) [62]	2800 (4) [63]	992 ± 901
	Rb	7.5 (2)	7,0 (10) [64]	864 ± 148 (11) [59]	9.55 ± 4.37
	Sb	-	-	-	0.137 ± 0.116
	Sc	-	-	-	0.014 ± 0.022
	Se	1.97 (9)	0.248 (41) [65]	174 ± 116 (11) [59]	2.75 ± 2.13
	Zn	104 (30)	22.4 (130) [65]	1236 ± 560 (2) [66]	118 ± 50
Cancer	Ag	-	-	-	0.19 ± 0.21
	Co	71.6 (3)	2.48 ± 0.85 (18) [67]	94.4 ± 69.6 (3) [59]	0.055 ± 0.031
	Cr	2.74 (2)	1.04 ± 0.52 (4) [66]	119 ± 12 (4) [60]	0.84 ± 0.86
	Fe	316 (8)	69 ± 51 (3) [61]	5588 ± 556 (4) [60]	248 ± 173
	Hg	30.8 (1)	30.8 ± 3.2 (4) [60]	30.8 ± 3.2 (4) [60]	0.824 ± 0.844
	I	78.8 (12)	< 23 ± 10 (8) [68]	800 (1) [69]	71.8 ± 62.0
	Rb	14.7 (2)	11,5 (10) [64]	17.8 ± 9.7 (5) [64]	12.8 ± 4.9
	Sb	-	-	-	0.124 ± 0.081
	Sc	-	-	-	0.008 ± 0.013
	Se	2.16 (7)	1.00 ± 0.24 (3) [66]	241 ± 296 (3) [59]	2.04 ± 1.02
	Zn	112 (13)	48 ± 8 (5) [70]	494 ± 37 (2) [66]	95.1 ± 78.9

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, I, Rb, Se, and Zn level reported in the literature for TBN and TMN vary widely (Table 3). This can be explained by a dependence of TEs content on many factors, including age, gender, ethnicity, mass of the TNs, and the stage of diseases. 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 TEs are lost as a result of this treatment. That concerns not only such volatile element as Hg, but also other TEs investigated in the study [73-75]. On the other hand, when destructive analytical techniques are used the tissue samples may be contaminated by TEs contained in chemicals using for digestion.

Trace elemental analysis of affected thyroid tissue could become a powerful diagnostic tool. To a large extent, the resumption of the search for new methods for early diagnosis

of TMN was due to experience gained in a critical assessment of the limited capacity of the US-examination [2,3]. In addition to the US test and morphological study of needle-biopsy of the TNs, the development of other highly precise testing methods seems to be very useful. Experimental conditions of the present study were approximated to the hospital conditions as closely as possible. In all cases we analyzed a part of the material obtained from a puncture biopsy of the TNs. Therefore, our data allow us to evaluate adequately the importance of TEs content information for distinguish TMN from TBN.

Tissue content of I, Rb, and Se are different in most TMN as compared to TBN (Tables 2). Level of I in nodular tissue has very promising prospects as a biomarker of malignancy because there is a great difference between content of this TE in TBN and TMN (Tables 2). It is very interest a potential possibilities of using the I/Rb ratio as cancer biomarker, because during the thyroid malignant transformation contents of these TEs in nodular tissue change in different directions – a drastically decrease of I and an increase of Rb (Tables 2). Thus, the results of study show that nondestructive analysis of TEs contents in biopsy of TNs may serve as a potential tool for detection of TMN.

This study has several limitations. Firstly, analytical techniques employed in this study measure only eleven TEs (Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn) mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of TEs investigated in TBN and TMN. Secondly, the sample size of TBN and TMN group was relatively small and prevented investigations of TEs contents in this group using differentials like gender, functional activity of nodules, stage of disease, and dietary habits of patients with TNs. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on significant TEs level alteration in thyroid nodular tissue and shows the necessity to continue TEs research as potential biomarkers of thyroid malignant transformation.

Conclusion

In this work, trace elemental analysis was carried out in the nodular tissue samples of thyroid with TBN and TMN using instrumental neutron activation analysis. It was shown that instrumental neutron activation analysis is an adequate analytical tool for the non-destructive determination of Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn content in the tissue samples of human thyroid, including needle-biopsy material. It was observed that in TMN tissue the mean mass fractions of I and Se are approximately 14 and 1.35 times, respectively, lower while the mean mass fraction of Rb 34% higher those in TBN tissue. Contents of Al, Co, Cr, Fe, Hg, Sb Sc, and Zn found in the TBN and TMN groups of nodular tissue samples were similar. In our opinion, the drastically decrease in level I and abnormal increase in Rb level in thyroid nodular tissue could be a specific consequence of malignant transformation. It was proposed to use the I mass fraction and I/Rb mass fraction ratio in a needle-biopsy of thyroid nodules as a potential tool to diagnose thyroid malignancy. Further studies on larger

number of samples are required to confirm our findings and proposals.

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Conflict of Interest

The author has not declared any conflict of interests.

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