

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

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Abstract

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 chemical elements (ChEs) contents in nodular tissue for diagnosis of thyroid malignancy. Contents of eight ChEs including bromine (Br), calcium (Ca), chlorine (Cl), iodine (I), potassium (K), magnesium (Mg), manganese (Mn), and sodium (Na) were prospectively evaluated in nodular tissue of thyroids with TBN (79 patients) and to TMN (41 patients). Measurements were performed using instrumental neutron activation analysis. It was observed that in TMN tissue the mass fractions of Br and I are approximately 2.9 and 14 times, respectively, lower, while the mass fraction of Ca, K, and Mg are 94%, 56%, and 36%, respectively, higher than in TBN tissue. Contents of Cl, Mn, and Na found in the TBN and TMN groups of nodular tissue samples were similar. Finally, it was proposed to use the I mass fraction and I/Ca, I/K, and I/Mg mass fraction ratios 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.

key words: thyroid; thyroid malignant and benign nodules; chemical 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 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.

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 [3,6-11]. Among these factors a disturbance of evolutionary stable input of many chemical elements (ChEs) 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 ChEs depend on tissue-specific need or tolerance, respectively [13]. Excessive accumulation or an imbalance of the ChEs 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 ChEs 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 ChEs content with age in the thyroid of males and females were studied and age- and gender-dependence of some ChEs was observed [25-41]. Furthermore, a significant difference between some ChEs 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 bromine (Br), calcium (Ca), chlorine (Cl), iodine (I), potassium (K), magnesium (Mg), manganese (Mn), and sodium (Na) contents in nodular tissue of patients who had either TBN or TMN using a non-destructive instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR). The second aim was to compare the levels of ChEs in TBN and TMN and to evaluate possibilities of using ChEs 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 ChEs 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 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 ChEs of

stainless steel [49]. One was used for morphological study while the other was intended for ChEs analysis. After the samples for ChEs analysis were weighed, they were freeze-dried and homogenized [50]. The pounded samples weighing about 10 mg (for biopsy) and 100 mg (for resected materials) were used for ChEs measurement by INAA-SLR.

To determine contents of the ChE 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 sub-samples of certified reference material (CRM) of the International Atomic Energy Agency (IAEA) IAEA H-4 (animal muscle) weighing about 100 mg were treated and analyzed in the same conditions as thyroid samples to estimate the precision and accuracy of results.

The content of Br, Ca, Cl, I, K, Mg, Mn, and Na 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 reactions, radionuclides, gamma-energies, spectrometric unit, sample preparation, and the quality control of results were presented in our earlier publications concerning the INAA-SLR of ChEs contents in human thyroid [27,28], scalp hair [52], and prostate [53,54].

A dedicated computer program for INAA-SLR mode optimization was used [55]. All thyroid samples for ChEs analysis were prepared in duplicate, and mean values of ChEs contents were used in final calculation. Using Microsoft Office Excel software, 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 ChEs 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 Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction in thyroid intact tissue samples of two groups of samples - TBN and TMN.

tav	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
TBN $n=79$	Br	412	662	98	3.20	2628	64.5	8.35	2336
	Ca	1237	902	138	52.0	4333	1108	116	3536
	Cl	8231	3702	772	1757	16786	8326	2543	15157
	I	992	901	103	29.0	3906	695	84.8	3629
	K	6190	2360	352	797	12222	6185	1438	10297
	Mg	331	180	26	13.0	844	311	15.0	745
	Mn	1.80	1.38	0.21	0.100	5.54	1.45	0.367	5.48
	Na	10207	3786	558	2319	22381	9802	3689	16969
TMN $n=41$	Br	139	203	36	6.2	802	50.2	7.75	802
	Ca	2398	2368	558	452	8309	1302	467	7428
	Cl	7699	2900	703	4214	14761	7216	4240	13619
	I	71.8	62.0	10	2.00	261	62.1	2.93	192
	K	9655	4444	970	1660	19255	8746	3181	19035
	Mg	450	232	51	122	1033	408	126	931
	Mn	1.90	1.41	0.32	0.100	5.79	1.59	0.100	5.37
	Na	8556	2959	646	4083	17284	7264	4704	14543

Table 1. Some statistical parameters of Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction (mg/kg, dry mass basis) in thyroid benign (TBN) and malignant (TMN) nodules

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.

The ratios of means and the comparison of mean values of Br, Ca, Cl, I, K, Mg, Mn, and Na 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 Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction in TBN [56-65] and TMN [56,59,62,66-70] is shown in Table 3. A number of values for ChEs 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 [27,28,52-54] good agreement of the Br, Ca, Cl, I, K, Mg, Mn, and Na contents in CRM IAEA H-4 samples analyzed by INAA-SLR with the certified data of this CRM indicates acceptable accuracy of the results obtained in the study of TBN and TMN groups of tissue samples presented in Tables 1-3.

From Table 2, it is observed that in TMN tissue the mass fractions of Br and I are approximately 2.9 and 14 times, respectively, lower, while the mass fraction of Ca, K, and Mg are 94%, 56%, and 36%, respectively, higher than in TBN tissue. In a general sense Cl, Mn, and Na, contents found in the TBN and TMN groups of tissue samples were similar (Table 2).

Element	Thyroid nodules			U-test, <i>p</i>	Ratio
	TBN	TMN	Student's t-test, <i>p</i> ≤		
Br	412±98	139±36	0.012	≤ 0.01	0.34
Ca	1237±138	2398±558	0.058	≤ 0.05	1.94
Cl	8231±772	7699±703	0.614	≤0.05	0.94
I	992±103	71.8±10.0	0.00000001	≤ 0.01	0.072
K	6190±352	9655±970	0.0025	≤ 0.01	1.56
Mg	331±26	450±51	0.045	≤ 0.01	1.36
Mn	1.80±0.21	1.90±0.32	0.794	≤0.05	1.06
Na	10207±558	8556±646	0.059	≤0.05	0.84

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

Table 2. Differences between mean values ($M \pm SEM$) of Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction (mg/kg, dry mass basis) in thyroid benign (TBN) and malignant (TMN) nodules

Mean values obtained for Br, Ca, I, and Mn contents in TBN (Table 3) agree well with median of mean values reported by other researches [56-58,60,61,64]. Mean mass fractions of Cl and Na in TBN obtained in present study were almost one order of magnitude and 3 times higher those in only published article on Cl [59] and Na [65]. Mean mass fraction of K in TBN obtained in present study was a little higher the upper value of range of published means [58], while mass fraction of Mg was a little lower the lowest value of range of published means [62].

Mean values obtained for Ca, I, Mg, and Mn contents in TMN (Table 3)

agree well with median of mean values reported by other researches [56,59,62,66,68-70]. Mean mass fraction obtained for Br in TMN was almost one order of magnitude higher median of previously reported means but inside the range of means [66,67]. Mean mass fraction of K founded in TMN was a little higher the upper value of range of published means [56], while mean mass fraction of Cl was almost one order of magnitude higher than in only published article on this ChEs content in malignant thyroid [59]. No published data referring Na contents of TMN were found (Table 3).

Tissue	Element	Published data [Reference]			This work
		Median of means (n)*	Minimum of means M or $M \pm SD$, (n)**	Maximum of means M or $M \pm SD$, (n)**	
TBN	Br	585 (5)	20.2±11.3 (5) [56]	1277 (1) [57]	412±662
	Ca	1664 (10)	1080 (2) [56]	8010±1290 (-) [58]	1237±902
	Cl	864 (1)	864±84 (4) [59]	864±84 (4) [59]	8231±3702
	I	812 (55)	77±14 (66) [60]	2800 (4) [61]	992±901
	K	3100 (6)	72.8±7.2 (4) [59]	6030±620 (-) [58]	6190±2360
	Mg	834 (4)	588±388 (13) [62]	1616 (70) [63]	331±180
	Mn	2.36 (21)	0.40±0.22 (64) [64]	57.6±6.0 (4) [59]	1.80±1.38
	Na	3520 (1)	3520 (25) [65]	3520 (25) [65]	10207±3786
TMN	Br	15.7 (4)	9.6 (1) [66]	160±112 (3) [67]	139±203
	Ca	1572 (6)	390 (1) [56]	3544 (1) [66]	2398±2368
	Cl	940 (1)	940±92 (4) [59]	940±92 (4) [59]	7699±2900
	I	78.8 (12)	<23±10 (8) [68]	800 (1) [69]	71.8±62.0
	K	6878 (4)	636±64 (4) [59]	7900 (1) [56]	9655±4444
	Mg	320 (2)	316±84 (45) [70]	544±272 (6) [62]	450±232
	Mn	1.83 (4)	1.6±0.8 (22) [70]	186±18 (4) [59]	1.90±1.41
	Na	-	-	-	8556±2959

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

Table 3. Median, minimum and maximum value of means Br, Ca, Cl, I, K, Mg, Mn, and Na 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).

The range of means of Br, Ca, Cl, I, K, Mg, Mn, and Na level reported in the literature for TBN and TMN vary widely (Table 3). This can be explained by a dependence of ChEs 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 ChEs are lost as a result of this treatment. That concerns not only such volatile halogen as Br, but also other ChEs investigated in the study [73-75]. On the other hand, when destructive analytical techniques are used, the tissue samples may be contaminated by ChEs contained in chemicals using for digestion.

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 ChEs content information for distinguish TMN from TBN.

Tissue content of Br, Ca, I, K, Mg, are different in most TMN as compared to TBN (Tables 2). It should be noted, however, that Br compounds, especially potassium bromide (KBr), sodium bromide (NaBr), and ammonium bromide (NH₄Br), are a component of many tranquilizers and frequently used as sedatives, for example, in Russia [76]. Uncontrolled use of tranquilizers may be the reason for elevated levels of Br in specimens of patients with TNs. Therefore, for diagnostic purposes, data for Br content should be used with caution. 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 ChE in TBN and TMN (Tables 2). It is very interest a potential possibilities of using the I/Ca, I/K, and I/Mg ratios as cancer biomarker, because during the thyroid malignant transformation contents of these ChEs in nodular tissue change in different directions – a drastically decrease of I and an increase of Ca, K, and Mg (Tables 2). Thus, the results of study show that nondestructive INAA-SLR analysis if ChEs contents in biopsy of TNs may serve as a potential tool for accurate detection of TMN. Because measurements using INAA-SLR are fast (a few minutes) and they do not need in any samples treatment, this analytical method can be used before morphological examination of biopsy samples.

This study has several limitations. Firstly, analytical technique employed in this study measure only eight ChEs (Br, Ca, Cl, I, K, Mg, Mn, and Na) mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of ChEs investigated in TBN and TMN. Secondly, the sample size of TBN and TMN group was relatively small and prevented investigations of TE 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 ChEs level alteration in thyroid nodular tissue and shows the necessity to continue ChEs research as potential biomarkers of thyroid malignant transformation.

Conclusion

In this work, elemental analysis was carried out in the nodular tissue samples of thyroid with TBN and TMN using INAA-SLR. It was shown that INAA-SLR is an adequate analytical tool for the non-destructive determination of Br, Ca, Cl, I, K, Mg, Mn, and Na content in the tissue samples of human thyroid, including needle-biopsy material. It was observed that in TMN tissue the mass fractions of Br and I are approximately 2.9 and 14 times, respectively, lower, while the mass fraction of Ca, K, and Mg are 94%, 56%, and 36%, respectively, higher than in TBN tissue. Contents of Cl, Mn, and Na 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 level Ca, K, and Mg in thyroid nodular tissue could be a specific consequence of malignant transformation. It was proposed to use the I mass fraction and I/Ca, I/K, and I/Mg mass fraction ratios 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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