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Distinguish Thyroid Malignant from Benign Alterations Using X-Ray Fluorescence Analysis of Trace Element Contents in Nodular Tissue

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Abstract

Introduction: 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.

Objective: 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 bromine (Br), copper (Cu), iron (Fe), iodine (I), rubidium (Rb), strontium (Sr), and zinc (Zn) were prospectively evaluated in nodular tissue of thyroids with TBN (79 patients) and to TMN (41 patients). Measurements were performed using energy-dispersive X-ray fluorescent analysis.

Results: It was observed that in TMN tissue the mass fractions of Br, I and Zn were approximately 2.9, 20, and 1.3 times, respectively, lower while the mass fraction of Rb 41% higher than in TBN tissue. Contents of Cu, Fe, and Sr 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; Energy-Dispersive X-Ray Fluorescent Analysis

Abbreviations: TNs: Thyroid Nodules; US: Ultrasound; MRRC: Medical Radiological Research Centre; TEs: Trace Elements.

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 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 Br, Cu, Fe, I, Rb, Sr, and Zn contents in nodular tissue of patients who had either TBN or TMN using a combination of non-destructive ^{109}Cd and ^{241}Am radionuclide-induced energy-dispersive X-ray fluorescent analysis (^{109}Cd-EDXRF and ^{241}Am-EDXRF, respectively). 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±SD was 44±11 years, range 22-64) and from TMN (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 (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 goiters, 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 ^{109}Cd-EDXRF and ^{241}Am-EDXRF analysis 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 the contents of the TEs by comparison with known data for standard, aliquots of commercial, chemically pure compounds and synthetic reference materials were used [51]. Ten subsamples of the Certified Reference Material (CRM) IAEA H-4 (animal muscle) were analyzed to estimate the precision and accuracy of results. The CRM IAEA H-4 subsamples were prepared in the same way as the samples of dry homogenized nodular tissue.

Details of the relevant facility for ^{109}Cd-EDXRF determination of Br, Cu, Fe, Rb, Sr, and Zn contents, methods of analysis and the quality control of results were presented in our earlier publications concerning the ^{109}Cd-EDXRF analysis of human thyroid and prostate tissue [25,26,47,52]. Detailed information on EDXRF determination of I contents with ^{241}Am radionuclide source, including methods of analysis and the quality control of results were presented in our earlier publication concerning the use of ^{241}Am-EDXRF analysis in human thyroid study [21].

All 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, and 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 Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction in thyroid intact tissue samples of two groups of samples - TBN and TMN.

Nodules	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
TBN n=79	Br	412	682	98	3.2	2628	64.5	8.35	2336
	Cu	10.2	9.2	1.7	2.9	35.2	6	3.04	34.9
	Fe	345	416	49	52,0	2563	185	54.3	1435
	I	1447	3313	373	47	28000	703	84.9	7175
	Rb	8.77	4.49	0.53	1	20.3	8.3	1.18	18.8
	Sr	4.48	6.84	0.88	0.42	32	1.9	0.769	27.5
	Zn	112.9	51.4	6.1	22	270	100	47.8	239
TMN n=41	Br	139	203	36	6.2	802	50.2	7.75	802
	Cu	14.5	9.4	2.6	4	32.6	10.9	4.21	31.4
	Fe	238	184	30	54	893	176	55	680
	I	71.6	72.5	11.6	2	341	64	2.19	237
	Rb	12.4	5	0.79	4.8	27.4	11.5	4.9	20
	Sr	6.25	7.83	1.63	0.93	30.8	3	0.985	25
	Zn	84.3	57.4	9.2	36.7	277	65.3	39	273

Table 1: Some statistical parameters of Br, Cu, Fe, I, Rb, Sr, and Zn 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 levels, P 0.975 – percentile with 0.975 levels.

The ratios of means and the comparison of mean values of Br, Cu, Fe, I, Rb, Sr, and Zn mass fractions in pair of sample groups such as TBN and TMN is presented in Table 2.

Element	Thyroid nodules				Ratio
	TBN	TMN	Student's t-test, *p*£	U-test, *p*	TMN / TBN
Br	412±98	139±36	**0.012**	**≤0.01**	0.34
Cu	10.2±1.7	14.5±2.6	0.176	>0.05	S1.42
Fe	345±49	238±30	0.069	>0.05	0.69
I	1447±373	71.6±11.6	**0.0004**	**≤0.01**	0.049
Rb	8.77±0.53	12.4±0.79	**0.0002**	**≤0.01**	1.41
Sr	4.48±0.88	6.25±1.63	0.348	>0.05	1.4
Zn	112.9±6.1	84.3±9.2	**0.012**	**≤0.01**	0.75

Table 2: Differences between mean values (M±SEM) of Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) in thyroid benign (TBN) and malignant (TMN) nodules.
M – Arithmetic mean, SEM – standard error of mean, Statistically significant values are in **bold**

The comparison of our results with published data for Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction in TBN [53-64] and TMN [53,57,60,62,64-69] is shown in Table 3.

Tissue	Element	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
TBN	Br	528 (5)	20.2±11.3 (5) [53]	1277 (1) [54]	412±682
	Cu	9.84 (38)	0.84 (1) [55]	462 (101) [56]	10.2±9.2
	Fe	296 (9)	54.6±36.1 (5) [53]	4848±3056 (11) [57]	345±416
	I	812 (55)	77±14 (66) [58]	2800 (4) [59]	1447±3313
	Rb	7.5 (2)	7,0 (10) [60]	864±148 (11) [57]	8.77±4.49
	Sr	1.64 (3)	1.32 (25) [61]	27.2±2.4 (4) [62]	4.48±6.84
	Zn	104 (11)	22.4 (130) [63]	1236±560 (2) [64]	113±51
TMN	Br	15.7 (4)	9.6 (1) [65]	160±112 (3) [57]	139±203
	Cu	6.8 (11)	4.7±1.8 (22) [66]	51.6±5.2 (4) [62]	14.5±9.4
	Fe	316 (8)	69±51 (3) [53]	5588±556 (4) [62]	238±184
	I	78.8 (12)	<23±10 (8) [67]	800 (1) [68]	71.6±72.5
	Rb	14.7 (2)	11,5 (10) [60]	17.8±9.7 (5) [60]	12.4±5.0
	Sr	-	-	-	6.25±7.83
	Zn	112 (13)	48±8 (5) [69]	494±37 (2) [64]	84.3±57.4

Table 3: Median, minimum and maximum value of means Br, Cu, Fe, I, Rb, Sr, 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).
M –arithmetic mean, SD – standard deviation, (n)* – number of all references, (n) ** – number of samples.

Discussion

As was shown before [21,25,26,47,52] good agreement of the Br, Cu, Fe, I, Rb, Sr, and Zn contents in CRM IAEA H-4 samples analyzed by EDXRF 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, I and Zn are approximately 2.9, 20, and 1.3 times, respectively, lower while the mass fraction of Rb 41% higher than in TBN tissue. In a general sense Cu, Fe, and Sr, contents found in the TBN and TMN groups of tissue samples were similar (Table 2).

Mean values obtained for Br, Cu, Fe, I, Rb, Sr, and Zn contents in TBN and TMN (Table 3) agree well with median of mean values reported by other researches [53-69]. The only exclusion was mean value obtained for Br in TMN, which was almost one order of magnitude higher median of previously reported means but inside the range of means (Table 3). No published data referring Sr contents of TMN were found (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%) [70] and ash (4.16% on dry mass basis) [71] contents in thyroid of adults.

The range of means of Br, Cu, Fe, I, Rb, Sr, 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 halogen as Br, but also other TEs investigated in the study [72-74]. 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 the distinguish between TBN and TMN.

Tissue content of Br, I, Rb, and Zn 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 (NH_4Br), are a component of many tranquilizers and frequently used as sedatives, for example, in Russia [75]. 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 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 EDXRF analysis of TEs contents in biopsy of TNs may serve as a potential tool for detection of TMN. Because measurements using EDXRF are fast (a few minutes) and they do not need in any samples treatment, this method can be used before morphological examination of biopsy samples.

This study has several limitations. Firstly, analytical technique employed in this study measure only seven TEs (Br, Cu, Fe, I, Rb, Sr, 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 EDXRF. It was shown that EDXRF is an adequate analytical tool for the non-destructive determination of Br, Cu, Fe, I, Rb, Sr, 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 Br, I and Zn were approximately 2.9, 20, and 1.3 times, respectively, lower while the mean mass fraction of Rb 41% higher than in TBN tissue. Contents of Cu, Fe, and Sr 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 Rb 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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