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Research Article

Distinguish Thyroid Malignant From Benign Alterations Using Chemical Element Contents in Nodular Tissue Determined By Neutron Activation and Inductively Coupled Plasma Atomic Emission Spectrometry

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Abstract

Objectives: 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.

Methods: Contents of nineteen ChEs including aluminum (Al), boron (B), barium (Ba), calcium (Ca), chlorine (Cl), coper (Cu), iron (Fe), I (iodine), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), sulfur (S), silicon (Si), strontium (Sr), vanadium (V), and zinc (Zn) were prospectively evaluated in nodular tissue of thyroids with TBN (79 patients) and to TMN (41 patients). Measurements were performed using a combination of non-destructive and destructive methods: instrumental neutron activation analysis and inductively coupled plasma atomic emission spectrometry.

Results: that in TMN tissue means of Br, I, Na, S, and V mass fractions are approximately 2.9, 15, 1.25, 1.15, and 1.7 times, respectively, lower, while the means of Ca, K, Mg, and P mass fraction are 2.3, 1.4, 1.4, and 2.0 times, respectively, higher those in TBN tissue. Mean contents of Al, B, Ba, Cl, Cu, Fe, Li, Mn, Si, Sr, 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 as well as I/Ca, I/K, I/Mg, and I/P 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.

Keywords: 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 [3,9-11]. 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 [42-49].

The present study had two aims. The main objective was to assess the aluminum (Al), boron (B), barium (Ba), calcium (Ca), chlorine (Cl), coper (Cu), iron (Fe), I, potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), sulfur (S), silicon (Si), strontium (Sr), vanadium (V), 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 destructive method such as inductively coupled plasma atomic emission spectrometry (ICP-AES). 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±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 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 ChEs 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 ChEs of stainless steel [50]. 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 [51]. The pounded samples weighing about 10 mg (for biopsy) and 100 mg (for resected materials) were used for ChE measurement by INAA-SLR. The content of 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). After non-destructive INAA-SLR investigation the thyroid samples were used for ICP-AES. The samples were decomposed in autoclaves and aliquots of solutions were used to determine the Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractions by ICP-AES using the Spectrometer ICAP-61 (Thermo Jarrell Ash, USA). Information detailing with the NAA-SLR and ICP-AES methods used and other details of the analysis were presented in our earlier publications concerning ChE contents in human thyroid [33,34], prostate [52-56], and scalp hair [57,58].

To determine contents of the ChE by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [59]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. Ten sub-samples of certified reference material (CRM) IAEA H-4 (animal muscle) and five sub-samples of CRM of the Institute of Nuclear Chemistry and Technology (INCT, Warszawa, Poland) INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2 Mixed Polish Herbs were treated and analyzed in the same conditions that thyroid samples to estimate the precision and accuracy of results

A dedicated computer program for INAA-SLR mode optimization was used [60]. All thyroid samples for ChEs analysis were prepared in duplicate and mean values of ChEs contents were used in final calculation. Mean values of ChE contents were used in final calculation for the Ca, K, Mg, Mn, and Na mass fractions measured by two methods. 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 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 Al, B, Ba, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction in thyroid intact tissue samples of two groups of samples - TBN and TMN.

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
TBN	Al	27.3	23.6	4.2	6.60	95.1	19.5	7.07	82.2
n=79	B	1.97	1.69	0.31	0.810	7.30	1.00	0.875	5.63
	Ba	1.70	2.42	0.43	0.180	11.7	0.960	0.265	0.988
	Br	412	682	98	3.20	2628	64.5	8.35	2336
	Ca	1313	860	131	52.0	4333	1169	120	3536
	Cl	8231	3702	772	1757	16786	8326	2543	15157
	Cu	10.2	9.2	1.7	2.90	35.2	6.00	3.04	34.9
	Fe	332	332	39	52.3	1360	197	59.9	1285
	I	1086	1219	139	29.0	8260	700	84.8	3734
	K	7051	3955	577	797	23007	6298	1577	19908
	Li	0.0295	0.0151	0.0030	0.0073	0.0680	0.0253	0.0096	0.0669
	Mg	344	155	23	15.0	844	326	66.8	745
	Mn	1.81	1.41	0.21	0.100	6.12	1.44	0.454	5.48
	Na	10675	4434	647	2319	28481	10118	3690	18451
	P	5145	1719	304	2890	9637	5030	2933	9091
	S	10909	2177	385	5591	16706	10719	7353	15361
	Si	90.4	68.3	12.3	7.80	346	83.7	13.4	223
	Sr	5.35	7.09	0.99	0.420	32.0	2.52	0.788	29.3
	V	0.152	0.066	0.012	0.0430	0.370	0.150	0.0606	0.289
	Zn	117.7	48.7	5.8	47.0	264	110	49.8	253
TMN	Al	33.0	25.5	7.1	4.50	96.5	21.3	5.70	85.6
n=41	B	2.21	1.89	0.52	1.00	5.60	1.00	1.00	5.42
	Ba	1.42	1.30	0.35	0.220	4.09	0.945	0.259	3.93
	Br	139	203	36	6.20	802	50.2	7.75	802
	Ca	3013	2966	699	452	9768	1578	467	8938
	Cl	7699	2900	703	4214	14761	7216	4240	13619
	Cu	14.5	9.4	2.6	4.00	32.6	10.9	4.21	31.4
	Fe	255	168	27	60.6	880	217	74.6	673
	I	71.8	62.0	10.1	2.00	261	62.1	2.93	192
	K	10054	4018	877	1660	18814	9204	4073	17559
	Li	0.0314	0.0307	0.0090	0.0078	0.111	0.0182	0.0088	0.0995
	Mg	478	194	42	130	933	467	166	881
	Mn	2.01	1.34	0.29	0.100	5.95	1.61	0.250	5.23
	Na	8576	2433	531	4083	14048	8107	4901	12925
	P	10493	3238	866	5382	15403	9694	5767	15391
	S	9448	1605	429	7139	12591	9422	7211	12204
	Si	143	156	42	18.6	523	64.2	19.8	472
	Sr	6.26	7.61	1.59	0.93	30.8	3.00	0.985	25.0
	V	0.0904	0.0308	0.0100	0.0580	0.170	0.0870	0.0600	0.154
	Zn	96.9	80.0	12.6	28.7	375	69.8	36.3	374

Table 1: *Some statistical parameters of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, 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 level, P 0.975 – percentile with 0.975 level.

The ratios of means and the comparison of mean values of Al, B, Ba, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractions in pair of sample groups such as TBN and TMN is presented in Table 2.

Element	Thyroid tissue				Ratio
	TBN	TMN	Student’s t-test, *p*≤	U-test, *p*	TMN / TBN
Al	27.3±4.2	33.0±7.1	0.497	>0.05	1.21
B	1.97±0.31	2.21±0.52	0.696	>0.05	1.12
Ba	1.70±0.43	1.42±0.35	0.608	>0.05	0.84
Br	412±98	139±36	**0.012**	**≤0.01**	0.34
Ca	1313±131	3013±699	**0.028**	**≤0.01**	2.29
Cl	8231±772	7699±703	0.614	>0.05	0.94

Cu	10.2±1.7	14.5±2.6	0.176	>0.05	1.42
Fe	332±39	255±27	0.108	>0.05	0.77
I	1086±139	71.8±10.1	**0.00000001**	**≤0.01**	0.066
K	7051±577	10054±877	**0.00068**	**≤0.01**	1.43
Li	0.0295±0.0030	0.0314±0.0090	0.832	>0.05	1.06
Mg	344±23	478±42	**0.0083**	**≤0.01**	1.39
Mn	1.81±0.21	2.01±0.29	0.589	>0.05	1.11
Na	10675±647	8576±531	**0.015**	**≤0.01**	0.80
P	5145±304	10493±866	**0.000024**	**≤0.01**	2.04
S	10909±385	9448±429	**0.016**	**≤0.01**	0.87
Si	90.4±12.3	143±42	0.247	>0.05	1.58
Sr	5.35±0.99	6.26±1.59	0.627	>0.05	1.17
V	0.152±0.012	0.0904±0.0100	**0.00042**	**≤0.01**	0.59
Zn	117.7±5.8	96.9±12.6	0.141	>0.05	0.82

Table 2: *Differences between mean values of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction (mg/kg, dry mass basis) in thyroid benign (TBN) and malignant (TMN) nodules El – element, Ned – median, Min – minimum, Max – maximum, M –arithmetic mean, SD – standard deviation, (n)* – number of all references, (n)** – number of samples.*

The comparison of our results with published data for Al, B, Ba, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction in TBN [61-77] and TMN [64,67,70,73,75,77-81] 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%) [82] and ash (4.16% on dry mass basis) [83] contents in thyroid of adults.

Tissue	El	Published data [Reference]			This work
		Med of Means (n)*	Min of Means M or M±SD, (n)**	Max of Means M or M±SD, (n)**	M±SD
TBN	Al	3.84 (5)	2.45 (123) [61]	840 (25) [62]	27.3±23.6
	B	-	-	-	1.97±1.69
	Ba	4.92 (1)	4.92±4.56 (51) [63]	4.92±4.56 (51) [63]	1.70±2.42
	Br	528 (5)	20.2±11.3 (5) [64]	1277 (1) [65]	412±682
	Ca	1664 (10)	1080 (2) [64]	8010±1290 (-) [66]	1313±860
	Cl	864 (1)	864±84 (4) [67]	864±84 (4) [67]	8231±3702
	Cu	9.84 (38)	0.84 (1) [68]	462 (101) [69]	10.2±9.2
	Fe	296 (9)	54.6±36.1 (5) [64]	4848±3056 (11) [70]	332±332
	I	812 (55)	77±14 (66) [71]	2800 (4) [72]	1086±1219
	K	3100 (6)	72,8±7,2 (4) [67]	6030±620 (-) [66]	7051±3955
	Li	-	-	-	0.0295±0.0151
	Mg	834 (4)	588±388 (13) [73]	1616 (70) [74]	344±155
	Mn	2.36 (21)	0.40±0.22 (64) [75]	57.6±6.0 (4) [67]	1.81±1.41
	Na	3520 (1)	3520 (25) [62]	3520 (25) [62]	10675±4434
	P	8200 (1)	8200±280 (-) [66]	8200±280 (-) [66]	5145±1719
	S	10300 (1)	10300±340 (-) [66]	10300±340 (-) [66]	10909±2177
	Si	64 (1)	45 (122) [76]	114 (122) [76]	90.4±68.3
	Sr	1.64 (3)	1.32 (25) [62]	27.2±2.4 (4) [67]	5.35±7.09
	V	10.4 (2)	3.92±8.84 (51) [63]	16.8±1.6 (4 [67]	0.152±0.066
	Zn	104 (30)	22.4 (130) [75]	1236±560 (2) [77]	117.7±48.7
TMN	Al	-	-	-	33.0±25.5
	B	-	-	-	2.21±1.89
	Ba	-	-	-	1.42±1.30
	Br	15.7 (4)	9.6 (1) [78]	160±112 (3) [70]	139±203
	Ca	1572 (6)	390 (1) [64]	3544 (1) [78]	3013±2966
	Cl	940 (1)	940±92 (4) [67]	940±92 (4) [67]	7699±2900
	Cu	6.9 (17)	1.34 (40) [75]	51.6±5.2 (4) [67]	14.5±9.4
	Fe	316 (8)	48.5 (2) [64]	5588±556 (4) [67]	255±168
	I	78.8 (12)	<23±10 (8) [79]	800 (1) [80]	71.8±62.0
	K	6878 (4)	636±64 (4) [67]	7900 (1) [64]	10054±4018
	Li	-	-	-	0.0314±0.0307
	Mg	320 (2)	316±84 (45) [81]	544±272 (6) [73]	478±194
	Mn	1.95 (9)	10.54 (40) [75]	186±18 (4) [67]	2.01±1.34
	Na	-	-	-	8576±2433

	P	-	-	-	10493±3238
	S	-	-	-	9448±1605
	Si	-	-	-	143±156
	Sr	-	-	-	6.26±7.61
	V	81.2 (1)	81.2±8.4 (4) [67]	81.2±8.4 (4) [67]	0.0904±0.0308
	Zn	92.4 (20)	22.6 (85) [75]	494±37 (2) [77]	96.9±80.0

Table 3: *Median, minimum and maximum value of means Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction in thyroid benign (TBN) and malignant (TMN) nodules according to data from the literature in comparison with our results (mg/kg, dry mass basis)*

El – element, Ned – median, Min – minimum, Max – maximum, M –arithmetic mean, SD – standard deviation, (n) – number of all references, (n)** – number of samples*

Discussion

As was shown before [33,34,52-58] good agreement of the Al, B, Ba, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn contents in CRM IAEA H-4, INCT-SBF-4, INCT-TL-1, and INCT-MPH-2 samples determined by both INAA-SLR and ICP-AES methods with the certified data of these CRMs 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 means of Br, I, Na, S, and V mass fractions are approximately 2.9, 15, 1.25, 1.15, and 1.7 times, respectively, lower, while the means of Ca, K, Mg, and P mass fraction are 2.3, 1.4, 1.4, and 2.0 times, respectively, higher those in TBN tissue. In a general sense means of Al, B, Ba, Cl, Cu, Fe, Li, Mn, Si, Sr, and Zn contents found in the TBN and TMN groups of tissue samples were similar (Table 2).

Mean values obtained for Al, Ba, Br, Ca, Cu, Fe, I, Mn, S, Si, Sr, and Zn contents in TBN (Table 3) agree well with median of mean values reported by other researches [61-67,68-72,75-77]. Mean mass fractions of Cl and Na in TBN obtained in present study were almost one order of magnitude and 3 times, respectively, higher while mean mass fraction of P was 1.6 time lower those in only published article on Cl [67], Na [62], and P [66]. Mean mass fraction of K in TBN obtained in present study was a little higher the upper value of range of published means {66], whereas mean mass fraction of Mg was a little lower the lowest value of range of published means {73]. Mean mass fraction obtained for V in TMN was almost two order of magnitude lower median of previously reported means 63,67] No published data referring B and Li contents in TMN were found (Table 3).

Mean values obtained for Ca, Cu, Fe, I, Mg, Mn, and Zn contents in TMN (Table 3) agree well with median of mean values reported by other researches [64,67,73,75,77-81]. 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 [78,70]. Mean mass fraction of K founded in TMN was a little higher the upper value of range of published means {64], while mean mass fraction of Cl was almost one order of magnitude higher that in only published article on this ChE content in malignant thyroid [67]. Mean mass fractions of V in TMN obtained in present study was approximately three orders of magnitude lower the only reported value [67]. No published data referring Al, B, Ba, Li, Na, P, S, Si, and Sr of TMN were found (Table 3). .

The range of means of Al, Ca, Cu, Fe, I, K, Mg, Mn, Sr, V, and Zn level reported in the literature for TNs 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 [84-86]. 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, Na, P, S, and V 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 [87]. 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, I/Mg, and I/P 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, Mg, and P (Tables 2). Thus, the results of study show that determination of ChEs contents in biopsy of TNs may serve as a potential tool for accurate detection of TMN.

This study has several limitations. Firstly, analytical technique employed in this study measure only nineteen ChEs (Al, B, Ba, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn) 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 ChEs contents in these groups 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 a combination of nuclear analytical methods. It was shown that a combination of three methods such as EDXRF, INAA-SLR and INAA-LLR is an adequate analytical tool for the non-destructive determination of Ag, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn content in the tissue samples of human thyroid, including needle-biopsy.

It was observed that in TMN tissue means of Br, I, Na, S, and V mass fractions are approximately 2.9, 15, 1.25, 1.15, and 1.7 times, respectively, lower, while the means of Ca, K, Mg, and P mass fraction are 2.3, 1.4, 1.4, and 2.0 times, respectively, higher those in TBN tissue. Mean contents of Al, B, Ba, Cl, Cu, Fe, Li, Mn, Si, Sr, 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 level Ca, K, Mg, and P in thyroid nodular tissue could be a specific consequence of malignant transformation. It was proposed to use the I mass fraction as well as I/Ca, I/K, I/Mg, and I/P 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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Conflict of Interest

The author has not declared any conflict of interests.

References

1. Fresilli D, David E, Pacini P, Gaudio GD, Dolcetti V, Lucarelli GT et al(2021). Thyroid Nodule Characterization: How to Assess the Malignancy Risk. Update of the Literature. Diagnostics (Basel).11(8):1374.
2. Jin Z, Zhu Y, Zhang S, Xie F, Zhang M, Zhang Y, Tian X, Zhang J, Luo Y, Cao J(2020). Ultrasound Computer-Aided Diagnosis (CAD) based on the Thyroid Imaging Reporting and Data System (TI-RADS) to distinguish benign from malignant thyroid nodules and the diagnostic performance of radiologists with different diagnostic experience. Med Sci Monit; 26: e918452.
3. Trimboli P, Castellana M, Piccardo A, Romanelli F, Grani G, Giovanella L, Durante C(2021). The ultrasound risk stratification systems for thyroid nodule have been evaluated against papillary carcinoma. A meta-analysis. Rev Endocr Metab Disord.22(2):453-460.
4. Patel SG, Carty SE, Lee AJ(2021). Molecular testing for thyroid nodules including its interpretation and use in clinical practice. Ann Surg Oncol ;28(13):8884-8891.
5. Silaghi CA, Lozovanu V, Georgescu CE, Georgescu RD, Susman S(2021), and microRNA Panels versus previous molecular tests in the preoperative diagnosis of indeterminate thyroid nodules: a systematic review and meta-analysis. Front Endocrinol (Lausanne).12:649522.
6. Zaichick V(1998). Iodine excess and thyroid cancer. J Trace Elem Exp Med;11(4):508-509.
7. Zaichick V, Iljina T(1998). Dietary iodine supplementation effect on the rat thyroid ^{131}I blastomogenic action. In: Die Bedentung der Mengen- und Spurenelemente. 18. Arbeitstangung. Jena: Friedrich-Schiller-Universität. p. 294-306.
8. Kim K, Cho SW, Park YJ, Lee KE, Lee D-W, Park SK(2021). Association between iodine intake, thyroid function, and papillary thyroid cancer: A case-control study. Endocrinol Metab (Seoul);36(4):790-799.
9. Stojsavljević A, Rovčanin B, Krstić D, Borković-Mitić S, Paunović I, Diklić A, Gavrović-Jankulović M, Manojlović D(2019). Risk assessment of toxic and essential trace metals on the thyroid health at the tissue level: The significance of lead and selenium for colloid goiter disease. Expo Health .
10. Fahim YA, Sharaf NE, Hasani IW, Ragab EA, Abdelhakim HK(2020). Assessment of thyroid function and oxidative stress state in foundry workers exposed to lead. J Health Pollut 2020;10(27):200903.
11. Liu M, Song J, Jiang Y, Lin Y, Peng J, Liang H et al(2021). A case-control study on the association of mineral elements exposure and thyroid tumor and goiter. Ecotoxicol Environ Saf.
12. Zaichick V (2006). Medical elementology as a new scientific discipline. *J Radioanal Nucl Chem*
13. Moncayo R, Moncayo H. A post-publication analysis of the idealized upper reference value of 2.5 mIU/L for TSH: Time to support the thyroid axis with magnesium and iron especially in the setting of reproduction medicine. BBA Clin;7:115–119.
14. Beyersmann D, Hartwig A.(2017) Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol 2008;82(8):493-512.
15. Martinez-Zamudio R, Ha HC(2011). Environmental epigenetics in metal exposure. Epigenetics 6(7):820-827.
16. Zaĭchik V, Raibukhin YuS, Melnik AD, Cherkashin VI(1970). Neutron-activation analysis in the study of the behavior of iodine in the organism. Med Radiol (Mosk).15(1):33-36.
17. Zaĭchik V, Matveenko EG, Vtiurin BM, Medvedev VS(1982). Intrathyroid iodine in the diagnosis of thyroid cancer. Vopr Onkol ,28(3):18-24.
18. Zaichick V, Tsyb AF, Vtyurin BM(1995). Trace elements and thyroid cancer. Analyst 1995;120(3):817-821.
19. Zaichick V, Choporov YuYa (1996). Determination of the natural level of human intra-thyroid iodine by instrumental neutron activation analysis. J Radioanal Nucl Chem,207(1):153-161.
20. Zaichick V(1998). *In vivo* and *in vitro* application of energy-dispersive XRF in clinical investigations: experience and the future. *J Trace Elem Exp Med*;11(4):509-510.
21. Zaichick V, Zaichick S(1999). Energy-dispersive X-ray fluorescence of iodine in thyroid puncture biopsy specimens. *J Trace Microprobe Tech*; 17(2):219-232.
22. Zaichick V(2000). Relevance of, and potentiality for in vivo intrathyroidal iodine determination. Ann N Y Acad Sci;904:630-632.
23. Zaichick V, Zaichick S(1997).Normal human intrathyroidal iodine. Sci Total Environ 206(1):39-56.
24. Zaichick V(1999). Human intrathyroidal iodine in health and non-thyroidal disease. In: New aspects of trace element research (Eds: M.Abdulla, M.Bost, S.Gamon, P.Arnaud, G.Chazot). London: Smith-Gordon; and Tokyo: Nishimura; p.114-119.
25. Zaichick V, Zaichick S(2017). Age-related changes of some trace element contents in intact thyroid of females investigated by energy dispersive X-ray fluorescent analysis. Trends Geriatr Healthc .1(1):31-38.

26. Zaichick V, Zaichick S(2017). Age-related changes of some trace element contents in intact thyroid of males investigated by energy dispersive X-ray fluorescent analysis. MOJ Gerontol Ger;1(5):00028.
27. Zaichick V, Zaichick S(2017). Age-related changes of Br, Ca, Cl, I, K, Mg, Mn, and Na contents in intact thyroid of females investigated by neutron activation analysis. Curr Updates Aging;1:5.1.
28. Zaichick V, Zaichick S(2017). Age-related changes of Br, Ca, Cl, I, K, Mg, Mn, and Na contents in intact thyroid of males investigated by neutron activation analysis. J Aging Age Relat Dis;1(1):1002.
29. Zaichick V, Zaichick S(2017). Age-related changes of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in intact thyroid of females investigated by neutron activation analysis. J Gerontol Geriatr Med 2017;3:015.
30. Zaichick V, Zaichick S(2017). Age-related changes of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in intact thyroid of males investigated by neutron activation analysis. Curr Trends Biomedical Eng Biosci;4(4):555644.
31. Zaichick V, Zaichick S(2018). Effect of age on chemical element contents in female thyroid investigated by some nuclear analytical methods. MicroMedicine;6(1):47-61.
32. Zaichick V, Zaichick S(201;). Neutron activation and X-ray fluorescent analysis in study of association between age and chemical element contents in thyroid of males. Op Acc J Bio Eng Bio Sci 2(4):202-212.
33. Zaichick V, Zaichick S(2018). Variation with age of chemical element contents in females' thyroids investigated by neutron activation analysis and inductively coupled plasma atomic emission spectrometry. J Biochem Analyt Stud;3(1):1-10.
34. Zaichick V, Zaichick S(2018). Association between age and twenty chemical element contents in intact thyroid of males. SM Gerontol Geriatr Res;2(1):1014.
35. Zaichick V, Zaichick S(2018). Associations between age and 50 trace element contents and relationships in intact thyroid of males. Aging Clin Exp Res;30(9):1059–1070.
36. Zaichick V, Zaichick S(2018). Possible role of inadequate quantities of intra-thyroidal bromine, rubidium and zinc in the etiology of female subclinical hypothyroidism. EC Gynaecology 7(3):107-115.
37. Zaichick V, Zaichick S(2018). Possible role of inadequate quantities of intra-thyroidal bromine, calcium and magnesium in the etiology of female subclinical hypothyroidism. Int Gyn and Women's Health.1(3):IGWHC.MS.ID.000113.
38. Zaichick V, Zaichick S(2018). Possible role of inadequate quantities of intra-thyroidal cobalt, rubidium and zinc in the etiology of female subclinical hypothyroidism. *Womens Health Sci J;*2(1):000108.
39. Zaichick V, Zaichick S(2018). Association between female subclinical hypothyroidism and inadequate quantities of some intra-thyroidal chemical elements investigated by X-ray fluorescence and neutron activation analysis. Gynaecology and Perinatology;2(4):340-355.
40. Zaichick V, Zaichick S(2018). Investigation of association between the high risk of female subclinical hypothyroidism and inadequate quantities of twenty intra-thyroidal chemical elements. Clin Res: Gynecol Obstet;1(1):1-18.
41. Zaichick V, Zaichick S(2018). Investigation of association between the high risk of female subclinical hypothyroidism and inadequate quantities of intra-thyroidal trace elements using neutron activation and inductively coupled plasma mass spectrometry. Acta Scientific Medical Sciences.2(9):23-37.
42. Zaichick V(2021). Evaluation of Twenty Chemical Elements in Thyroid with Hashimoto's thyroiditis using X-Ray Fluorescent and Neutron Activation Analysis. Journal of Medical Research and Health Sciences.2 (10):1500-1510.
43. Zaichick V(2021). Determination of twenty chemical element contents in macro- and micro-follicular colloid goiter using neutron activation analysis and inductively coupled plasma atomic emission spectrometry. International Journal of Medical and All Body Health Research.2(2):5-18.
44. Zaichick V(2021). Comparison of twenty chemical element contents in normal thyroid tissue and hypertrophic thyroid tissue. Universal Journal of Pharmaceutical Research.6(4):32-42.
45. Zaichick V(2021). Evaluation of twenty chemical element contents in thyroid adenomas using neutron activation analysis and inductively coupled plasma atomic emission spectrometry**.** World Journal of Advanced Research and Reviews 11(03):242-257.
46. Zaichick V(2021). Are there any differences between chemical element contents of goitrous and adenomatous thyroid? Journal of Medical Research and Health Sciences.4 (11):1576-1587.
47. Zaichick V, Zaichick S(2018). Levels of chemical element contents in thyroid as potential biomarkers for cancer diagnosis (a preliminary study). J Cancer Metastasis Treat;4:60.
48. Zaichick V. Contents of Nineteen Chemical Elements in Thyroid Malignant Nodules and Thyroid Tissue adjacent to Nodules using Neutron Activation Analysis and Inductively Coupled Plasma Atomic Emission Spectrometry. Saudi Journal of Biomedical Research 2022; 7(1): 45-56.
49. Zaichick V(2022). Comparison of Nineteen Chemical Elements in Thyroid Tissue adjacent to Thyroid Malignant and Benign Nodules using Neutron Activation Analysis and Inductively Coupled Plasma Atomic Emission Spectrometry. International Journal of Multidisciplinary and Current Educational Research;4(1):219-229.
50. Zaichick V, Zaichick S(1996). Instrumental effect on the contamination of biomedical samples in the course of sampling. The Journal of Analytical Chemistry;51(12):1200-1205.
51. Zaichick V, Zaichick S(1997). A search for losses of chemical elements during freeze-drying of biological materials. J Radioanal Nucl Chem 218(2):249-253.
52. Zaichick S, Zaichick V(2011). INAA application in the age dynamics assessment of Br, Ca, Cl, K, Mg, Mn, and Na content in the normal human prostate. J Radioanal Nucl Chem 288:197-202.
53. Zaichick V, Zaichick S(2013). The effect of age on Br, Ca, Cl, K, Mg, Mn, and Na mass fraction in pediatric and young adult prostate glands investigated by neutron activation analysis. J Appl Radiat Isot;82:145-151.
54. Zaichick V, Nosenko S, Moskvina I(2012). The effect of age on 12 chemical element contents in intact prostate of adult men investigated by inductively coupled plasma atomic emission spectrometry. Biol Trace Elem Res;147:49-58.
55. Zaichick V, Zaichick S(2013). NAA-SLR and ICP-AES Application in the assessment of mass fraction of 19 chemical elements in pediatric and young adult prostate glands. Biol Trace Elem Res;156:357-366.
56. Zaichick V, Zaichick S(2014). Determination of trace elements in adults and geriatric prostate combining neutron activation with inductively coupled plasma atomic emission spectrometry. Open Journal of Biochemistry 1(2):16-33.
57. Zaichick S, Zaichick V(2010). The effect of age and gender on 37 chemical element contents in scalp hair of healthy humans. Biol Trace Elem Res .134(1):41-54.

58. Zaichick S, Zaichick V(2011). The scalp hair as a monitor for trace elements in biomonitoring of atmospheric pollution. Int J Environment and Health .5(1/2):106-124.
59. Zaichick V(1995). Applications of synthetic reference materials in the medical Radiological Research Centre. Fresenius J Anal Chem; 352:219-223.
60. Korelo AM, Zaichick V(1993). Software to optimize the multielement INAA of medical and environmental samples. In: Activation Analysis in Environment Protection. Dubna, Russia: Joint Institute for Nuclear Research,326-332.
61. Yanchur NM, Elenevskaya NS, But-Gusain AM, Nikhamkin LP(1967). Contents of manganese, aluminum, copper, and zinc in bloos and thyroid of patients with goiter. Clinicheskaya Hirurgiya 1967;4:27-20. Янчур Н.М., Еленевская Н.С., Бут-Гусаин А.М., Нихамкин Л.П. Содержание марганца, алюминия, меди и цинка в крови и щитовидной железе больных зобом. Клиническая хирургия, 1967;4:27-20.
62. Kamenev VF(1963). About trace element contents in thyroid of adults. In: Trace Elements in Agriculture and Medicine. Buryatia publishing-house, Ulan-Ude, , pp.12-16.
63. Antonova MV, Elinova VG, Voitekhovskaya YaV(1966). Some trace element contents in thyroid and water in endemic goiter region. Zdravookhranenie BSSR 1966;9:42-44.
64. Maeda, K, Yokode, Y, Sasa, Y, Kusuyama, H., Uda, M(1987). Multielemental analysis of human thyroid glands using particle induced X-ray emission (PIXE)**.** Nuclear Inst and Methods in Physics Research, B;22(1-3):188-190.
65. Turetskaia ES(1961). Studies on goitrous thyroid glands for iodine and bromine content. Probl Endokrinol Gormonoter;7(2):75-80.
66. Borodin AE, Sokolova II, Gogolev VG, Makarova Mya(1967). About goitrous thyroid chemical composition. In: Goiter in Amur region. Khabarovsk publishing-house, Blagoveshchensk, Russia, , pp.21-29.
67. Reddy SB, Charles MJ, Kumar MR, Reddy B, Anjaneyulu Ch., Raju GJN, Sundareswar B, Vijayan V(2002). Trace elemental analysis of adenoma and carcinoma thyroid by PIXE method. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms .196(3-4):333-339.
68. Remiz AM(, 1962). Endemic goiter and trace elements in Kabardino-Balkaria ASSR. In: The V meeting of chirurgeons of Northern Caucasia. Rostov-on-Don, pp.276-278.
69. Aingorn NM, Chartorizhskaya NA(1966). Comparative characteristics of trace element contents under thyroid disorders. In: Trace Elements in Agriculture and Medicine. Buryatia publishing-house, Ulan-Ude, 1966, pp.113-114.
70. Salimi J, Moosavi K, Vatankhah S, Yaghoobi A(2004). Investigation of heavy trace elements in neoplastic and non-neoplastic human thyroid tissue: A study by proton – induced X-ray emissions. Iran J Radiat Res 1(4):211-216.
71. Błazewicz A, Orlicz-Szczesna G, Szczesny P, Prystupa A, Grzywa-Celinska A, Trojnar M(2011). A comparative analytical assessment of iodides in healthy and pathological human thyroids based on IC-PAD method preceded by microwave digestion. Journal of Chromatography B;879:573-578.
72. Braasch JW, Abbert A, Keating FR, Black BM(1955). A note of the iodinated constituents of normal thyroids and of exophthalmic goiters. J Clin Endocrinol Metab;15(4):732-738.
73. Kaya G, Avci H, Akdeniz I, Yaman M(2009). Determination of Trace and Minor Metals in Benign and Malign Human Thyroid Tissues. Asian Journal of Chemistry 2009;21(7):5718-5726.
74. Li AA, Brechov EE(1973). Somr features of Ca and Mg methabolism in thyroid with toxical goiter. In: Proceedings of scientific conference. Moscow, , pp.129-131.
75. Stojsavljević A, Rovčanin B, Krstić D, Borković-Mitić S, Paunović I, Kodranov I, Gavrović-Jankulović M, Manojlović D(2019). Evaluation of trace metals in thyroid tissues: Comparative analysis with benign and malignant thyroid diseases. Ecotoxicol Environ Saf.183:109479.
76. Bredichin LM, Soroka VP(1969). Trace element metabolism in patients with thyroid goiter under treatment. Vrachebnoe Delo;51(6):81-84.
77. Zagrodzki P, Nicol F, Arthur JR, Słowiaczek M, Walas S, Mrowiec H, Wietecha-Posłuszny R(2010). Selenoenzymes, laboratory parameters, and trace elements in different types of thyroid tumor. *Biol Trace Elem Res* ;134 (1):25-40.
78. Jundt FC, Purser KH, Kubo H, Schenk EA(1974). Proton-induced X-ray analysis of trace elements in tissue sections. J Histochem Cytochem;22(1):1-6.
79. Nishida M, Sakurai H, Tezuka U, Kawada J, Koyama M, Takada J(1990). Alterations in manganese and iodide contents in human thyroid tumors; a correlation between the contents of essential trace elements and the states of malignancy. Clinica Chimica Acta 1990;187(2):181-187.
80. Tardos TG, Maisey MN, Ng Tang Fui SC, Turner PC(1981). The iodine concentration in binign and malignant thyroid nodules measured by X-Ray fluorescence. Brit J Radiol;54:626-629.
81. Al-Sayer H, Mathew TC, Asfar S, Khourshed M, Al-Bader A, Behbehani A, Dashti H(2004). Serum changes in trace elements during thyroid cancers. Molecular and Cellular Biochemistry;260(1):1-5.
82. Katoh Y, Sato T, Yamamoto Y(2002). Determination of multielement concentrations in normal human organs from the Japanese. Biol Trace Elem Res;90(1-3):57-70.
83. Schroeder HA, Tipton IH, Nason AP(1972). Trace metals in man: strontium and barium. J Chron Dis;25(9):491-517.
84. Zaichick V(1997). Sampling, sample storage and preparation of biomaterials for INAA in clinical medicine, occupational and environmental health. In: Harmonization of Health-Related Environmental Measurements Using Nuclear and Isotopic Techniques. IAEA, Vienna, p.123-133.
85. Zaichick V, Zaichick S(1997). A search for losses of chemical elements during freeze-drying of biological materials. J Radioanal Nucl Chem;218(2):249-253.
86. Zaichick V(2004). Losses of chemical elements in biological samples under the dry aching process. Trace Elements in Medicine;5(3):17–22.
87. Maschkovsky MD(2005). The sedatives. In: The Medicaments, 15th Ed., Novaya Volna, Moscow, pp.72-86.