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ABSTRACT

Background: Thyroid Benign (TBN) and Malignant (TMN) Nodules is 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 Chemical Elements (ChEs) contents in nodular tissue for diagnosis of thyroid malignancy.

Methods: Contents of nineteen ChEs including silver (Ag), calcium (Ca), chlorine (Cl), cobalt (Co), chromium (Cr), cooper (Cu), iron (Fe), mercury (Hg), iodine (I), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), rubidium (Rb), ammonium (Sb), scandium (Sc), selenium (Se), 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 a combination of non-destructive nuclear analytical methods: X-ray fluorescence and instrumental neutron activation analysis.

Results: It was observed that in TMN tissue means of Br, Fe, I, Se, and Zn mass

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)

fractions are approximately 3.0, 1.6, 14, 1.4, and 1.3 times, respectively, lower, while the means of Ca, K, Mg, and Rb mass fraction are 94%, 56%, 36%, and 62%, respectively, higher those in TBN tissue. Mean contents of Ag, Cl, Co, Cr, Cu, Hg, Mn, Na, Sb, Sc, and Sr found in the TBN and TMN groups of nodular tissue samples were similar.

Conclusion: It was proposed to use the I mass fraction as well as I/Ca, I/K, I/Mg, and I/Rb 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

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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 silver (Ag), calcium (Ca), chlorine (Cl), cobalt (Co), chromium (Cr), cooper (Cu), iron (Fe), mercury (Hg), iodine (I), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), rubidium (Rb), ammonium (Sb), scandium (Sc), selenium (Se), strontium (Sr), and zinc (Zn) contents in nodular tissue of patients who had either TBN or TMN using a non-destructive Energy-Dispersive X-Ray Fluorescent analysis (EDXRF) combined with Instrumental Neutron Activation Analysis With High Resolution Spectrometry Of Short- And Long-Lived Radionuclides (INAA-SLR and INAA-LLR, respectively). 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.

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MATERIALS AND METHODS

All patients suffered from TBN (n=79, mean age M \pm SD was 44 \pm 11 years, range 22-64) and from TMN (n=41, mean age M \pm SD was 46 \pm 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]. To determine contents of the ChE by comparison with a known standard, Biological Synthetic Standards (BSS) prepared from phenolformaldehyde resins were used [52]. 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) and IAEA HH-1 (Human Hair) 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 Cu, Fe, Rb, Sr, and Zn were determined by EDXRF. Details of the relevant facility for this method, source with 109Cd radionuclide, methods of analysis and the results of quality control were presented in our earlier publications concerning the EDXRF of ChE contents in human thyroid [25,26] and prostate tissue [53].

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 neutron flux, nuclear reactions, radionuclides, gamma-energies, spectrometric unit, sample preparation and measurement were presented in our earlier publications concerning the INAA-SLR of ChE contents in human thyroid [27,28], prostate [54,55], and scalp hair [56].

In a few days after non-destructive INAA-SLR all thyroid samples were repacked and used for INAA-LLR. A vertical channel of the WWR-c research nuclear reactor (Branch of Karpov Institute, Obninsk).was applied to determine the content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn by INAA-LLR. Details of used neutron flux, nuclear reactions, radionuclides, gamma-energies, spectrometric unit, sample preparation and measurement were presented in our earlier publications concerning the INAA-LLR of ChE contents in human thyroid [29,30], scalp hair [56], and prostate [57]. A dedicated computer program for INAA-SLR and INAA-LLR mode optimization was used [58]. 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 Fe, Rb, and Zn mass fractions measured by two methods. 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 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 Ag, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, 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, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, 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, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction in TBN [59-76] and TMN [61,64,66,75-82] 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%) [83] and ash (4.16% on dry mass basis) [84] contents in thyroid of adults.

DISCUSSION

As was shown before [25-30,53-57] good agreement of the Ag, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn contents in CRM IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) samples determined by EDXRF, INAA-SLR, and INAA-LLR 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, Fe, I, Se, and Zn mass fractions are approximately 3.0, 1.6, 14, 1.4, and 1.3 times, respectively, lower, while the means of Ca, K, Mg, and Rb mass fraction are 94%, 56%, 36%, and 62%, respectively, higher those in TBN tissue. In a general sense means of Ag, Cl, Co, Cr, Cu, Hg, Mn, Na, Sb, Sc, and Sr contents found in the TBN and TMN groups of tissue samples were similar (Table 2).

Mean values obtained for Ag, Br, Ca, Cu, Fe, I, Mn, Rb, Se, Sr, and Zn contents in TBN (Table 3) agree well with median of mean values reported by other researches [59-64,66-70,73-76]. Mean mass fractions of Cl and Na in TBN obtained in present study were almost one order of magnitude and 3 times, respectively, higher those in only published article on Cl [64] and Na [74]. Mean mass fractions of Co, Cr, and Hg in TBN obtained in present study were approximately 13, 4.1, and 86 times, respectively, lower medians of reported means for these ChEs. Mean mass fraction of K in TBN obtained in present study was a little higher the upper value of range of published means [63], while mass fraction of Mg was a little lower the lowest value of range of published means [71]. No published data referring Sb and Sc contents in TMN were found (Table 3).

Table 1: Some statistical parameters of Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, 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	Ag	0.226	0.219	0.031	0.002	0.874	0.179	0.0022	0.808
n=79	Br	412	682	98	3.2	2628	64.5	8.35	2336
-	Ca	1237	902	138	52	4333	1108	116	3536
	Cl	8231	3702	772	1757	16786	8326	2543	15157
	Со	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
	Cu	10.2	9.2	1.7	2.9	35.2	6	3.04	34.9
	Fe	387	475	56	52,3	2563	188	60	1739
	Hg	0.924	0.649	0.088	0.0817	3.01	0.856	0.104	2.12
	Ι	991	906	105	29	3906	690	84.7	3632
	K	6191	2360	352	797	12222	6185	1438	10297
	Mg	331	180	26	13	844	311	15	745
	Mn	1.8	1.38	0.21	0.1	5.54	1.45	0.367	5.48
	Na	10207	3786	558	2319	22381	9802	3689	16969
	Rb	9.16	4.21	0.5	1	20.3	8.6	2.48	17.9
	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
	Sr	4.48	6.84	0.88	0.42	32	1.9	0.769	27.5
	Zn	115.3	49.6	5.9	47	270	105	48.8	248
TMN	Ag	0.193	0.215	0.041	0.0075	1.02	0.147	0.008	0.705
n=41	Br	139	203	36	6.2	802	50.2	7.75	802
	Ca	2397	2368	558	452	8309	1302	467	7428
	Cl	7699	2900	703	4214	14761	7216	4240	13619
	Со	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
	Cu	14.5	9.4	2.6	4	32.6	10.9	4.21	31.4
	Fe	243	177	29	55.1	887	200	58.2	679
	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
	K	9655	4444	970	1660	19225	8746	3381	19035
	Mg	450	232	51	122	1033	408	126	931
	Mn	1.9	1.41	0.32	0.1	5.79	1.59	0.1	5.37
	Na	8556	2959	646	4083	17284	7264	4704	14543
	Rb	12.6	4.6	0.7	5.5	27.4	11.2	5.84	19.8
	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
	Sr	6.25	7.83	1.63	0.93	30.8	3	0.985	25
	Zn	89.7	57.6	10.8	36.7	326	67.7	37.7	324

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, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn mass fraction (mg/kg, dry mass basis) in Thyroid Benign (TBN) and Malignant (TMN) nodules.

Element		Ratio			
	TBN	TMN	Student's t-test, p£	U-test, p	TMN/TBN
Ag	0.226 ± 0.031	0.193 ± 0.041	0.515	>0.05	0.85
Br	412 ± 98	139 ± 36	0.012	≤0.01	0.33
Ca	1237 ± 138	2397 ± 558	0.058	≤0.05	1.94
Cl	8231 ± 772	7699 ± 703	0.614	>0.05	0.94
Со	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
Cu	10.2 ± 1.7	14.5 ± 2.6	0.176	>0.05	1.42
Fe	387 ± 56	243 ± 29	0.026	≤0.01	0.63
Hg	0.924 ± 0.088	0.824 ± 0.149	0.567	>0.05	0.89
Ι	991 ± 105	71.8 ± 10.0	1E-09	≤0.01	0.072
К	6191 ± 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
Rb	9.16 ± 0.50	12.6 ± 0.7	0.00022	≤0.01	1.38
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	
Sr	4.48 ± 0.88	6.25 ± 1.63	0.348	>0.05	1.4	
Zn	115.3 ± 5.9	89.7 ± 10.8	0.042	≤0.01	0.78	
M. Arithmetic Mean, SEM, Standard Error of Mean. Statictically significant values are in hold						

M: Arithmetic Mean; SEM: Standard Error of Mean, Statistically significant values are in bole

Table 3 : Median, minimum and maximum value of means Ag, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, 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).

Nodule	El		This work		
		Med of Means	Min of Means	Max of Means	
		(n)*	M or M \pm SD, (n)**	M or M ± SD, (n)**	M ± SD
TBN _	Ag	0.16 (4)	0.098 ± 0.042 (19) [59]	1.20 ± 2.28 (51) [60]	0.226 ± 0.219
	Br	528 (5)	20.2 ± 11.3 (5) [61]	1277 (1) [62]	412 ± 682
	Ca	1664 (10)	1080 (2) [61]	8010 ± 1290 (-) [63]	1237 ± 902
	Cl	864 (1)	864 ± 84 (4) [64]	864 ± 84 (4) [64]	8231 ± 3702
_	Со	0.86 (13)	0.110 ± 0.003 (64) [65]	62.8 ± 22.4 (11) [66]	0.0615 ± 0.0332
_	Cr	4.0 (6)	0.72 (51) [59]	146 ± 14 (4) [64]	0.966 ± 0.844
	Cu	9.84 (38)	0.84 (1) [67]	462 (101) [68]	10.2 ± 9.2
_	Fe	296 (9)	54.6 ± 36.1 (5) [61]	4848 ± 3056 (11) [66]	387 ± 475
	Hg	79.2 (1)	79.2 ± 8.0 (4) [64]	79.2 ± 8.0 (4) [64]	0.924 ± 0.649
_	Ι	812 (55)	77 ± 14 (66) [69]	2800 (4) [70]	991 ± 906
_	K	3100 (6)	72,8 ± 7,2 (4) [64]	6030 ± 620 (-) [63]	6191 ± 2360
_	Mg	834 (4)	588 ± 388 (13) [71]	1616 (70) [72]	331 ± 180
_	Mn	2.36 (21)	0.40 ± 0.22 (64) [73]	57.6 ± 6.0 (4) [64]	1.80 ± 1.38
	Na	3520(1)	3520 (25) [74]	3520 (25) [74]	10207 ± 3786
_	Rb	7.5 (2)	7,0 (10) [75]	864 ± 148 (11) [66]	9.16 ± 4.21
_	Sb	-	-	-	0.137 ± 0.116
_	Sc	-	-	-	0.0144±0.0217
_	Se	1.97 (9)	0.248 (41) [73]	174 ± 116 (11) [66]	2.75 ± 2.13
_	Sr	1.64 (3)	1.32 (25) [74]	27.2 ± 2.4 (4) [64]	4.48 ± 6.84
	Zn	104 (30)	22.4 (130) [74]	1236 ± 560 (2) [76]	115.3 ± 49.6
TMN	Ag	-	-	-	0.193 ± 0.215
_	Br	15.7 (4)	9.6 (1) [77]	160 ± 112 (3) [66]	139 ± 203
	Ca	1572 (6)	390 (1) [61]	3544 (1) [77]	2397 ± 2368
_	Cl	940 (1)	940 ±92 (4) [64]	940 ± 92 (4) [64]	7699 ± 2900
	Со	71.6 (3)	2.48 ± 0.85 (18) [78]	94.4 ± 69.6 (3) [66]	0.0550 ± 0.0309
	Cr	2.74 (2)	1.04 ± 0.52 (4) [76]	119± 12 (4) [64]	0.835 ± 0.839
	Cu	6.8 (11)	4.7 ± 1.8 (22) [79]	51.6 ± 5.2 (4) [64]	14.5 ± 9.4
	Fe	316 (8)	69 ±51 (3) [61]	5588 ± 556 (4) [64]	243 ± 177
	Hg	30.8 (1)	30.8 ± 3.2 (4) [64]	30.8 ± 3.2 (4) [64]	0.824 ± 0.844
	Ι	78.8 (12)	< 23 ± 10 (8) [80]	800 (1) [81]	71.8 ± 62.0
_	K	6878 (4)	636 ± 64 (4) [64]	7900 (1) [61]	9655 ± 4444
	Mg	320 (2)	316 ± 84 (45) [79]	544 ± 272 (6) [79]	450 ± 232
	Mn	1.83 (4)	1.6 ± 0.8 (22) [79]	186 ± 18 (4) [64]	1.90 ± 1.41
	Na	-	-	-	8556±2959
	Rb	14.7 (2)	11,5 (10) [75]	17.8 ± 9.7 (5) [75]	12.6 ± 4.6
	Sb	-		-	0.124 ± 0.081
	Sc	-	-	-	0.0077 ± 0.0129
	Se	2.16 (7)	1.00 ± 0.24 (3) [76]	241± 296 (3) [66]	2.04±1.02
	Sr	-	-	-	6.25±7.83
	Zn	112 (13)	48 ± 8 (5) [82]	494 ± 37 (2) [76]	89.7 ± 57.6

El: Element; Med: Median; Min: Minimum; Max: Maximum; M: Arithmetic Mean; SD: Standard Deviation; (n)*: Number of all references; (n) **: Number of samples.

Mean values obtained for Ca, Cu, Fe, I, Mn, Rb, Se, and Zn contents in TMN (Table 3) agree well with median of mean values reported by other researches [61,64,66,75-77,79-82]. 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 [66,77]. Mean mass fractions of Co, Cr, and Hg in TMN obtained in present study were approximately 1300, 3.3, and 37 times, respectively, lower medians of reported means for these ChEs. Mean mass fraction of K founded in TMN was a little higher the upper value of range of published means [61], while mean mass fraction of Cl was almost one order of magnitude higher that in only published article on this ChEs content in malignant thyroid [64]. No published data referring Ag, Na, Sb, Sc, and Sr of TMN were found (Table 3).

The range of means of Ag, Ca, Co, Cr, Cu, Fe, I, K, Mg, Mn, Rb, Se, Sr, 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 [85-87]. 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, Fe, I, K, Mg, Rb, Se, and Zn are different in most TMN as compared to TBN (Table 2). It should be noted, however, that Br compounds, especially Potassium Bromide (KBr), Sodium Bromide (NaBr), and Ammonium Bromide (NH,Br), are a component of many tranquilizers and frequently used as sedatives, for example, in Russia [88]. 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 (Table 2). It is very interest a potential possibilities of using the I/Ca, I/K, I/Mg, and I/Rb 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 Rb (Table 2). Thus, the results of study show that nondestructive analysis of ChEs contents in biopsy of TNs using nuclear analytical methods 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 (Ag, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, 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, Fe, I, Se, and Zn mass

fractions are approximately 3.0, 1.6, 14, 1.4, and 1.3 times, respectively, lower, while the means of Ca, K, Mg, and Rb mass fraction are 94%, 56%, 36%, and 62%, respectively, higher those in TBN tissue. Mean contents of Ag, Cl, Co, Cr, Cu, Hg, Mn, Na, Sb, Sc, 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 Ca, K, Mg, and Rb 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/Rb 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.

COMPETING INTEREST

The author has not declared any conflict of interests.

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