RESEARCH ARTICLE

Distinguish Thyroid Malignant From Benign Alterations Using X-Ray Fluorescence And Neutron Activation Analysis Of Chemical Element Contents In Nodular Tissue

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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 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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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 [42-49].

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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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
	Co	0.0615	0.0332	0.0046	0.0083	0.159	0.0579	0.0152	0.141
	Cr	0.966	0.844	0.121	0.075	3.65	0.673	0.109	2.76
	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
	I	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
	Co	0.055	0.0309	0.006	0.0042	0.143	0.0497	0.0159	0.129
	Cr	0.835	0.859	0.157	0.039	3.5	0.46	0.0941	3.05
	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	Thyroid nodules				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
Co	0.0615 ± 0.0046	0.0550 ± 0.0060	0.37	>0.05	0.89
Cr	0.966 ± 0.121	0.835 ± 0.157	0.511	>0.05	0.86
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
I	991 ± 105	71.8 ± 10.0	1E-09	≤0.01	0.072
K	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, Statistically significant values are in bold

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	Published data [Reference]			This work
		Med of Means	Min of Means	Max of Means	
		(n)*	M or M ± 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
	Co	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
	I	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
	Co	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
	I	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

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